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World Journal of Nephrology (*World J Nephrol*, *WJN*, online ISSN 2220-6124, DOI: 10.5527) is a peer-reviewed open access academic journal that aims to guide clinical practice and improve diagnostic and therapeutic skills of clinicians.

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Receptor activator of nuclear factor κ B ligand/osteoprotegerin axis and vascular calcifications in patients with chronic kidney disease

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Abstract

Vascular calcifications are commonly observed in patients with chronic kidney disease (CKD) and contri-

bute to the excessive cardiovascular morbidity and mortality rates observed in these patients populations. Although the pathogenetic mechanisms are not yet fully elucidated, recent evidence suggests a link between bone metabolism and the development and progression of vascular calcifications. Moreover, accumulating data indicate that receptor activator of nuclear factor κ B ligand/osteoprotegerin axis which plays essential roles in the regulation of bone metabolism is also involved in extra-osseous bone formation. Further studies are required to establish the prognostic significance of the above biomarkers as predictors of the presence and severity of vascular calcifications in CKD patients and of cardiovascular morbidity and mortality. Moreover, randomized clinical trials are needed to clarify whether inhibition of osteoclast activity will protect from vascular calcifications.

Key words: Arterial stiffness; Bone turnover; Chronic kidney disease; Osteoprotegerin; RANK ligand; Receptor activator nuclear factor κ B; Vascular calcifications

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Core tip: Vascular calcifications are commonly observed in chronic kidney disease patients and recently mounting evidence suggest that Receptor activator of nuclear factor κ B ligand/osteoprotegerin axis controls both bone metabolism and extra-osseous bone formation. Further studies are required to establish the role of these biomarkers as predictors of the presence and severity of vascular calcifications and of cardiovascular morbidity and mortality. Moreover, randomized clinical trials are needed to clarify whether inhibition of osteoclast activity will protect from vascular calcifications.

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INTRODUCTION

Cardiovascular disease (CVD) remains the leading cause of excessive morbidity and mortality for patients with chronic kidney disease (CKD) and particularly those with end-stage renal disease (ESRD) on renal replacement therapy with either hemodialysis or peritoneal dialysis^[1]. Vascular calcifications are also commonly observed in CKD and are now considered part of the syndrome chronic kidney disease-mineral and bone disorder (CKD-MBD), the pathogenesis of which has accumulated great research interest the last years. In CKD patients calcifications in both intimal (atherosclerotic) and medial lamina (arteriosclerotic) often coexist, appear early and follow an accelerated course. Particularly the latter is an almost ubiquitous feature of arterial tree in chronic uremia and a major contributor to the accelerated arteriosclerosis and to the increased all-cause and CVD mortality in these patients populations^[2].

The presence of vascular calcifications in CKD has been associated with a number of traditional risk factors including older age, hypertension, dyslipidemia and diabetes mellitus which are highly prevalent in this population, as well as uremia-related risk factors including chronic inflammation, oxidative stress and mineral and bone disorders which are currently under investigation. Of note, mineral alterations (hypercalcemia, hyperphosphatemia) and disorders of bone metabolism (both secondary hyperparathyroidism and adynamic bone disease) are mainly associated with the development and progression of medial but not intimal calcifications^[3].

It is now well recognized that vascular calcification is not simply a passive physicochemical process of calcium phosphate deposition but a highly regulated active process similar to normal bone modeling. Moreover, recent evidence suggests that the phenotypic trans-differentiation of vascular smooth muscle cells (VSMCs) into osteoblast-like cells is a key pathogenetic event in the osteogenesis of the vascular wall. A variety of regulatory factors and molecular pathways of this process which regulate bone turnover and/or mineralization have been identified^[3]. Although their relative importance in different disease states appear still incompletely understood emerging evidence suggest that the receptor activator of nuclear factor κ B (RANK)/RANK Ligand (RANKL)/osteoprotegerin (OPG) system that plays essential roles in the regulation of bone metabolism is also involved in extra-osseous bone formation^[4].

RANK/RANKL/OPG PATHWAY

RANK, RANKL and OPG are members of the tumor

necrosis factor (TNF) superfamily which were originally studied as factors involved in bone tissue and immune system physiology. However, recent studies revealed that they also constitute a link between bone metabolism and vascular pathophysiology by controlling simultaneously bone remodeling and vascular calcification mechanisms^[5].

RANK/RANKL/OPG signaling pathway regulates osteoclast differentiation and activation. RANKL is a transmembrane protein consisted of 316 aminoacids, which is expressed on osteoblasts, stromal and T cells in areas of bone remodeling. RANKL binds to its receptor RANK, a 616 amino acid type I transmembrane protein which is expressed on the surface of myeloid cell lineages like osteoclasts, monocytic and dendritic cells. The above activates multiple intracellular signals, including activation of the c-jun N-terminal kinase and nuclear factor κ B pathways that regulate the differentiation, function and survival of these cells. A variety of factors including hormones, cytokines and growth factors regulate its expression. Thus, parathormone (PTH), TNF- α , calcium, corticosteroids and several interleukins (IL-6,-11,-17) increase RANKL expression on osteoblasts, whereas transforming growth factor- β (TGF- β) decreases it^[3,5]. OPG is a soluble glycoprotein widely expressed in most human tissues including bone (osteoblasts, mesenchymal stem cells), immune cells (T and B cells) and vessels (endothelial and VSMCs). It acts as a decoy receptor and binds to RANKL, thereby not allowing the activation of RANK and inhibits its regulatory effects on inflammation, skeletal and vascular systems^[5]. OPG also has anti-apoptotic actions, as it binds and deactivates the TNF related apoptosis-inducing ligand (TRAIL), which is expressed by many cell types, including VSMCs, and can also lead to ectopic mineralization^[5]. OPG's expression is increased by vitamin D, TNF- α , IL-1 α , IL-6, IL-11 and IL-17, bone morphogenic protein-2, TGF- β and estrogens, whereas it is reduced by PTH, corticosteroids and prostaglandin E2^[5].

Mounting evidence suggests that the RANK/RANKL/OPG axis exerts actions simultaneously on endothelial cells and VSMCs and participate in multiple processes that regulate vascular calcification. The implication of this system in vascular pathophysiology is supported by the expression of these molecules in the normal cardiovascular system (heart, arteries and veins). Both endothelial cells and VSMCs constitutively express OPG, and their levels are particularly high in aortic and renal arteries. Furthermore, OPG is physically associated with factor VIII-von Willebrand factor complex localized in the Weibel-Palade bodies of endothelial cells, it is rapidly secreted in response to inflammatory stimuli and inhibits osteoclastogenesis and promotes endothelial cell survival^[6] through neutralization of pro-apoptotic TRAIL^[5]. In contrast, RANKL and RANK are frequently undetectable in normal vessels and non-calcified arteries or valves^[5]. However, osteoclast-like RANK(+) cells were found close to VSMCs on calcified vascular walls and

expression of both RANK and RANKL was reported on the vascular wall, in calcified areas^[7].

Several studies suggested that OPG's expression might reflect a protective mechanism against the vascular calcifications. Thus, over-expression of OPG leads to osteopetrosis, while its gene deletion, increases bone metabolism and leads to osteoporosis and medial calcifications in the aorta and the renal arteries^[8,9]. Moreover, OPG administration in laboratory animals, was reported to potently reduce both bone resorption activity and medial arterial calcifications induced by administration of toxic doses of vitamin D or warfarin^[10]. These findings, together with the fact that OPG is expressed in the vascular wall under normal circumstances, indicate that the endogenous production of OPG prevents the ossification of the vascular wall and favors bone mineralization. In accordance with the above, OPG was detected in matrix vesicles, nanoparticles that are released from VSMCs with the capacity to nucleate mineral, which directly inhibited deposition of hydroxyapatite in the vascular wall^[11].

OPG AND VASCULAR CALCIFICATIONS IN CKD PATIENTS

In the general population, studies have demonstrated that high levels of OPG are correlated with cardiovascular risk^[12]. In CKD patients, it has been shown that OPG levels significantly increase along with the decline in GFR and are reduced after a successful renal transplantation^[13]. However, studies examining the association of OPG levels with the presence and extent of cardiovascular calcifications are relatively limited and their results were sometimes inconsistent. Thus, in non-dialyzed CKD patients a cut-off value of OPG level was found to predict the presence of coronary artery calcifications (CAC) assessed by chest multidetector computed tomography^[14]. In addition, a very recent study in CKD patients reported a significant association between high OPG levels and CAC independently of other risk factors including age, gender, diabetes, body mass index and smoking habits^[15]. In transplanted patients CAC at baseline, but not 1 year after renal transplantation, was found to be independently associated with baseline OPG whereas post-transplant CAC progression was predicted by baseline CAC score^[16]. In contrast, another study in transplanted patients reported that OPG levels were significantly and independently associated with the progression of aortic calcification index (ACI) assessed by lateral lumbar x-ray during a two-year follow-up period^[17]. In adults and children with ESRD on hemodialysis, studies demonstrated a significant independent correlation between OPG levels and CAC^[18,19]. Similarly, another study showed an association between OPG and ACI assessed by computed tomography scans independently of traditional and uremia-related risk factors^[20]. In addition, high OPG levels have been found to correlate with faster progression of

aortic calcifications during a 5-year follow-up^[21]. Finally, several studies in patients with various CKD stages as well as in renal transplant recipients demonstrated that OPG levels were a significant independent predictor of all-cause and cardiovascular mortality during the follow-up period^[22-26]. However, one study in ESRD and pre-dialysis CKD patients showed that renal function rather than OPG levels were mostly associated with the progression of aortic and coronary calcifications^[27], whereas another one correlated elevated OPG levels only with moderate CAC^[28].

Regarding the association of OPG with markers of medial calcifications such as arterial stiffness and pulse wave velocity (PWV), the results are also sometimes controversial. A study in non-dialyzed CKD patients demonstrated a strong relationship between serum OPG and arterial stiffness independent of many potential confounders including traditional cardiovascular risk factors, abnormal bone and mineral metabolism, and inflammation^[29]. Similarly, in hemodialysis patients OPG levels were found to be strongly associated with aortic or carotid-to-femoral PWV independently of traditional and uremia-related risk factors including markers of inflammation^[25,26,30]. However, other studies in adults and children on hemodialysis treatment were unable to confirm the above findings^[19,31].

As it was previously noted, the exact role of OPG in the VC process remains unclear. Since OPG inhibits osteoclast activity and OPG knockout mice develop arterial calcifications, it appears reasonable to assume that it plays some regulatory and/or inhibitory role against ectopic calcifications and thus its increased levels could be interpreted as an attempt to compensate for the ongoing calcification process^[9-11]. The above speculation contradicts the reported association of high OPG levels with cardiovascular mortality^[22,23,25,26] and moreover, increase of OPG levels could be due to its production by calcified vascular cells in conditions of diffuse calcification. Thus, it remains to be clarified whether the elevated OPG levels induce arterial wall sclerosis or represent a compensatory mechanism to prevent further arterial damage or are just a marker of initiation of vascular calcification process^[4].

RANKL AND VASCULAR CALCIFICATIONS IN CKD PATIENTS

The exact role of RANKL in the development of cardiovascular calcifications and CVD remains to be identified. Some studies showed a correlation of RANKL levels with future cardiovascular events^[32], but the probable association between vascular calcifications and RANKL levels has been scarcely investigated so far. A prospective study in 3250 Framingham Study participants reported that RANKL concentrations were inversely associated with multiple cardiovascular risk factors, including smoking, diabetes, and antihypertensive treatment, but that were not related with CAC or incident CVD or mortality during

a mean follow-up of 4.6 years^[33]. However, a study in hemodialysis patients found that change in OPG levels after 1-year were an independent predictor of CAC score progression during the same period^[34]. Of note, RANKL levels, in contrast with OPG, are low in CKD patients^[35]. Considering the fact that OPG and RANKL have opposite functions in bone resorption, OPG/RANKL ratio could be considered a better biomarker of bone metabolism and consequently a better predictor of the presence and severity of vascular calcifications^[35]. In agreement with the above hypothesis, the aforementioned study in hemodialysis patients, showed that baseline OPG/RANKL ratio was significantly higher in patients whose coronary calcifications progressed during the one year follow up period^[34].

CONCLUSION

Despite the progress and the knowledge acquired within the previous years, the pathogenesis of vascular calcifications remains to be fully elucidated. Recently, mounting evidence suggest that RANKL/RANK/OPG system which controls bone metabolism plays a significant role in this process. Alterations of RANKL/OPG axis appear a promising prognostic biomarker of the initiation and progression of vascular calcifications in CKD patients and of cardiovascular morbidity and mortality. Further studies are required to establish this theory and to identify the exact role of these two biomarkers in CKD patients. Moreover, randomized clinical trials are needed to clarify whether inhibition of osteoclast activity will protect from vascular calcifications.

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Update on immunoglobulin a nephropathy. Part II : Clinical, diagnostic and therapeutical aspects

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Abstract

Immunoglobulin A nephropathy (IgAN) is characterized

by different clinical manifestations and by long-term different outcomes. Major problem for the physicians is to understanding which patients are at risk of a disease evolution and to prescribe the right therapy to the right patients. Indeed, in addition to patients with a stable disease with no trend to evolution or even with a spontaneous recovery, patients with an active disease and patients with a rapidly evolving glomerulonephritis are described. Several histopathological, biological and clinical markers have been described and are currently used to a better understanding of patients at risk, to suggest the right therapy and to monitor the therapy effect and the IgAN evolution over time. The clinical markers are the most reliable and allow to divide the IgAN patients into three categories: The low risk patients, the intermediate risk patients and the high risk patients. Accordingly, the therapeutic measures range from no therapy with the only need of repeated controls, to supportive therapy eventually associated with low dose immunosuppression, to immunosuppressive treatment in the attempt to avoid the evolution to end stage renal disease. However the current evidence about the different therapies is still matter of discussion. New drugs are in the pipeline and are described. They are object of randomized controlled trials, but studies with a number of patients adequately powered and with a long follow up are needed to evaluate efficacy and safety of these new drugs.

Key words: IgA nephropathy prevention and control; IgA nephropathy; IgA nephropathy diagnosis; IgA nephropathy prognosis; IgA nephropathy classification; IgA nephropathy therapy

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Core tip: Primary immunoglobulin A nephropathy (IgAN) is the most frequent glomerulonephritis. The IgAN is a relatively benign disease however, the long term prognosis should not be considered mild, because, after

20 years of disease evolution, 25% of the patients are going into chronic renal failure. It is essential to find out the risk factors predicting the evolution to end-stage renal disease (ESRD) and to select those patients who may benefit from immunosuppressive treatment. For all patients, it is essential to have a regular clinical control to check any disease evolution, in order to avoid or delay the disease progression to ESRD.

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INTRODUCTION

Previously^[1] we highlighted that, the diagnosis of immunoglobulin A nephropathy (IgAN) is principally based on a renal biopsy because there are different histological image results and different clinical presentation.

As the clinical presentations may be extremely different a tentative clinical classification aimed for the best therapy may be particularly useful.

Floege *et al.*^[2] categorized the clinical scenarios of IgAN into four classes: (1) patients diagnosed accidentally while looking for clinical manifestations such as reduced glomerular filtration rate (GFR), hypertension and urinary abnormalities. Such patients may be affected by IgAN and are called the silent majority; (2) patients affected by recurrent macroscopic hematuria strictly connected with acute infective diseases occurring in the upper respiratory tract. Such patients are also called typical IgAN patients and, in addition to hematuria, may be affected by proteinuria, hypertension and reduced GFR, which represent signs of disease evolution^[3]; (3) patients presenting atypical signs such as nephritic syndrome and acute or rapidly progressive renal disease; and (4) patients with IgAN recurrence after kidney transplantation.

In addition to these categories, there are patients presenting acute kidney injury (AKI) accompanying macroscopic hematuria, due to acute tubular necrosis and intratubular erythrocyte casts. The relevance of this IgAN presentation is represented by the good prognosis not characterized by a disease progression.

Finally, IgAN may affect subjects who are otherwise healthy as documented by biopsies of kidneys suitable for transplantation or by autopsies of subjects not affected by renal diseases^[4,5].

A further difficulty in decision making on therapeutic approaches is related to the fact that most subjects with IgAN may have a benign course or even disease resolution as documented by cohorts of patients followed for 10 years after diagnosis in China and Spain^[6,7].

Such extremely variable clinical presentations and disease evolution have two principal consequences: (1)

most guidelines concerning IgAN, such as the Kidney Disease Improving Global Outcomes (KDIGO)^[8], are based on a low level of evidence and are often based on opinions. As a consequence, there are few guidelines based on recommendations and the majority are only suggestions. Indeed, whether to treat and the beneficial effects of many treatments remain to be better validated^[9]; and (2) There is a need for research on histological, biological and clinical markers that are able to predict the risk of IgAN progression and to guide therapeutic decisions and monitor therapeutic results.

Indeed, only a fraction of IgAN patients require treatment to prevent disease progression, and predicting which patients are at risk of progression is of overwhelming importance. Different histological, biological and clinical markers of prognosis have been identified, and other markers will likely be validated^[10].

RESEARCH METHODOLOGY

We have analyzed the available papers on IgAN diagnosis, IgAN prognosis and IgAN therapy by a review of the currently available papers. A literature search was performed using PubMed (NCBI/NIH) with the search words "IgAN diagnosis", "IgAN prognosis", "IgAN biomarkers", "IgAN classification", and "IgAN therapy". As first line research the papers published in the last three years were examined. Paper selection has been made according to the relevance of the journal, the authors, the dimension of the study and the novelty of the findings. So doing 40 papers recently published have been selected, then we proceeded in a backward way and studies previously published have also been included. Studies currently under way were searched for in "clinical trial.gov" and the European EUDRACT register. As clinical trial.gov also includes studies that to date are either closed or have not started, we selected only randomized controlled trials (RCTs) that are active and enrolling patients. So doing we report 15 RCTs out of the 68 that may be found on clinical.trial.gov. The RCTs excluded are either terminated or closed or not enrolling patients.

DIAGNOSIS AND PROGNOSIS

Histological markers

The glomerular histopathology in the IgAN is extremely variable, and its identification and reproducibility among different observers is essential to establish any relationship between renal pathology and disease evolution^[11].

The glomerular abnormalities range from minimal abnormalities to mesangial hypercellularity, endocapillary hypercellularity, extra capillary hypercellularity, and segmental glomerulosclerosis.

The tubulointerstitial lesions may be near normal, but in some patients a tubular injury resulting in a fibro proliferative peritubular response is observed. In addition, several clinicopathological correlations have reported that the tubular atrophy is the most reliable

Table 1 Definitions of pathological variables used in the Oxford classification of immunoglobulin a nephropathy

Variable	Definition	Score
Mesangial hypercellularity	< 4 Mesangial cells/mesangial area = 0 4-5 Mesangial cells/mesangial area = 1 6-7 Mesangial cells/mesangial area = 2 > 8 Mesangial cells/mesangial area = 3	M0 < 0.5 M1 > 0.5
Segmental glomerulosclerosis	Any amount of the tuft involved in sclerosis, but not involving the whole tuft or the presence of an adhesion	S0 = absent S1 = present
Endocapillary hypercellularity	Hypercellularity due to increased number of cells within glomerular capillary lumina causing narrowing of the lumina	E0 = absent E1 = present
Tubular atrophy/interstitial fibrosis	Percentage of cortical area involved by the tubular atrophy or interstitial fibrosis, whichever is greater	0%-25% - T0 26%-50% - T1 > 50% - T2

Table 2 Summary of studies correlating the Oxford classification for immunoglobulin a nephropathy with clinical outcomes

Study	Patients (n)	End point	Univariate analysis	Multivariate analysis
Coppo <i>et al</i> ^[19]	206 A, 59 C	Rate of eGFR decline	M, E, S, T	M, E, S, T
Herzenberg <i>et al</i> ^[20]	143 A, 44 C	Rate of eGFR decline	Not done	E, S, T
Katafuchi <i>et al</i> ^[21]	702 A, C	ESRD	Not done	S, T
Zeng <i>et al</i> ^[22]	1026 A	Rate of eGFR decline	M, S, T	M, T
Shi <i>et al</i> ^[23]	410 A	ESRD	M, S, T	S, T
Edström Halling <i>et al</i> ^[24]	99 C	GFR reduction > 50%, ESRD	M, E, T	E
Shima <i>et al</i> ^[25]	161 C	eGFR < 60 mL/min per 1.73m ²	M, T	M, T
Coppo <i>et al</i> ^[26]	973 A, 174 C	Rate of eGFR decline	M, E, S, T	S, T
Alamartine <i>et al</i> ^[27]	183 A	Doubling of SCr or ESRD	E, S, T	None
El Karoui <i>et al</i> ^[28]	128 A	Rate of eGFR decline	Not done	T
Lee <i>et al</i> ^[29]	69 A	GFR reduction > 50%, ESRD	E, T	E
Kang <i>et al</i> ^[30]	197 A	GFR reduction > 50%, ESRD	T	T
Le <i>et al</i> ^[31]	218 C	eGFR reduction > 50%, ESRD	T, S	T

A: Adults; C: Children; eGFR: Estimated glomerular filtration rate; E: Endothelial hypercellularity; ESRD: End-stage renal disease; GFR: Glomerular filtration rate; M: Mesangial hypercellularity; S: Segmental sclerosis; Scr: Serum creatinine; T: Tubular atrophy/interstitial fibrosis.

marker of an adverse outcome^[11].

Several histological classifications have been proposed in an attempt to provide a valuable grading of histological damage and a clinico-pathological relationship. Until recently, the classifications from Lee *et al*^[12], Haas *et al*^[13] and Wakai *et al*^[14] has been used the most. All of these classifications have the weakness of not distinguishing between the histological markers of acute activity and chronic activity of the disease. As a consequence, they fail to provide useful information concerning therapy for the acute and evolving phase of the disease.

Later on, an international working group of over 40 pathologists and nephrologists developed an evidence-based and reproducible classification for IgAN^[15]. Data were obtained from 265 patients affected by IgAN who were followed for 5 years. Four histological variables had an independent value in predicting renal outcomes: Mesangial hypercellularity scores (M), segmental glomerulosclerosis (S), endocapillary hypercellularity (E) and tubular atrophy/interstitial fibrosis (T). This study led to the formulation of the Oxford classification (Table 1).

The Oxford classification has some limitations that should be remarked as the authors themselves recognize. The study is retrospective and the material

comes from different countries and different centers, each with a specific and different method of evaluating renal function. In addition, the median number of glomeruli with crescents was only 9% and no patient had more than 55% of glomeruli with crescents. As a consequence, as remarked by other studies^[16] in this cohort the prognostic significance of crescents is poor. Other limitations of the Oxford classification is the lack of immunohistochemical findings as the authors recognize in a further study^[17]. This lack in addition to other points claims for the need of more validation studies^[18].

Indeed, the prognostic value of the Oxford classification required validation and, since the Oxford classification was published, at least 17 validation studies have been reported. Eight of these studies were able to validate the classification (Table 2), principally highlighting the relevance of T, S and M scores^[19-26]. Five more studies apparently did not validate completely the Oxford classification^[27-31].

Validation of the Oxford classification of the IgA (VALIGA) is one of the more recent validation studies^[26]. This study involved 1147 patients from 13 European countries. The principal conclusions of the study were that M, S and T lesions independently predicted eGFR loss and lower survival rates, but the addition of M, S

Table 3 Potential biomarkers for immunoglobulin a nephropathy

Biologics	Source	Rationale
Galactose deficient IgA1	Serum	Core antigen of the pathogenic IgA1 immune complex; leads to activation of mesangial cells and glomerulonephritis
Glycan-specific IgG	Serum	Form glycan-dependent complex with galactose-deficient IgA1; alanine to serine substitution in complementary-determining region 3 of IgG heavy chain; able to differentiate IgA nephropathy patients from controls with 88% specificity and 95% sensitivity
Activated complement C3	Serum	Up-regulated level in 30% of patients; correlated with deteriorating renal function
FGF 23	Serum	FGF23 serum levels are significantly associated with IgAN progression
Soluble CD89	Serum	Low levels in patients with disease progression compared with those without disease progression
Mannose-binding lectin	Urine	Significantly higher in patients than healthy controls; associated with histopathologic aggravations such as mesangial hypercellularity, tubular atrophy, interstitial fibrosis
EGF and MCP-1	Urine	An EGF/MCP-1 ratio greater than 366.66 extends renal survival to at least 84 mo in a cohort of 44 patients
Proteomic pattern	Urine	High throughput characterization of 2000 polypeptide using capillary electrophoresis on-line coupled to a mass spectrometer
microRNA profile	Urine	Sequencing identified microRNA profiling that is specific to IgA nephropathy

IgA1: Immunoglobulin A1; IgG: Immunoglobulin G; FGF23: Fibroblast growth factor 23; IgAN: Immunoglobulin a nephritis; RNA: Ribonucleic acid; EGF: Epidermal growth factor; MCP-1: Monocyte chemotactic peptide-1.

and T lesions to clinical variables predicted progression only in patients not receiving immunosuppressive treatment.

Overall, although the studies to validate the Oxford classification system led to divergent findings, this classification offers physicians a simple tool to distinguish between active and chronic lesions^[32] and is the only classification system created in a truly evidence-based manner^[33]. In addition, the Oxford classification system should be considered a working classification, and meetings are been held to clarify the discrepancies among the different validation studies. Waiting for further results and clarifications, to date, the KDIGO guidelines^[34] do not recommend the use of pathological findings to guide therapy and predict prognosis.

The addition of clinical data to the histological findings improved the ability to predict outcomes. Indeed, in a recent study^[35], a new rule to predict the risk of developing ESRD in IgAN patients was developed and validated using clinical measures together with the Oxford classification.

Biological markers

Serum and urine biomarkers may be useful both for diagnostic and prognostic purposes.

Several authors^[36,37] have formulated the "four hits" theory to explain the IgAN pathogenesis. Accordingly, in a four steps fashion, after an increase of galactose deficient circulating IgA1 (Gd-IgA1), there is an antibody production against these Gd-IgA1. Later on immunocomplexes are formed and may deposit in the kidney. Finally an inflammatory response is activated.

According to the four hits theory of IgAN pathogenesis, the diagnostic biomarker's usefulness decreases from hit 1 to hit 4, while, on the contrary, the prognostic value increases^[38].

Table 3 summarizes the different biomarkers and their rationale in the diagnosis^[39].

Serum galactose deficient immunoglobulin A1

Galactose deficient immunoglobulin A1 (Gd-IgA1) represents a core antigen of the pathogenic IgA1 immunocomplexes and leads to activation of mesangial cells. Principally, Gd-IgA1 represents a diagnostic marker. Data from studies considering Gd-IgA1 a prognostic marker are discordant. In one study, the serum levels of Gd-IgA1 were associated with disease progression^[40]. In another study, the serum levels of Gd-IgA1 did not correlate with proteinuria and eGFR decline^[41].

Serum anti-glycan antibodies

This biomarker correlates with the urine protein/creatinine ratio^[42] and with disease progression towards ESRD^[43].

Serum breakdown of complement C3 products

Complement activation is up-regulated in 50% of patients and correlates with a decrease in renal function^[44-46]. Additionally preliminary studies have documented in IgAN the association of glomerular C4d deposition with serum creatinine, proteinuria and histological damage^[47].

Fibroblast growth factor 23

Fibroblast growth factor 23 (FGF23) is a circulating hormone involved in phosphate homeostasis. In a recent study, FGF23 levels were significantly associated with IgAN progression^[48].

CD89-IgA complexes

The deposition of CD89-IgA complexes may facilitate mesangial cell activation. A study reported that IgAN patients without disease progression had high levels of soluble CD89, whereas patients with disease progression had low levels of soluble CD89^[49].

In addition to the serum biomarkers, urinary biomarkers may also be useful both in the diagnosis of IgAN and the prognosis.

The urinary mannose-binding lectin^[50] is a biomarker for predicting IgAN progression. Indeed, it is associated with the worsening of histopathologic lesions such as mesangial hypercellularity, tubular atrophy and interstitial fibrosis.

In a small cohort^[51], a urinary epidermal growth factor/monocyte chemotactic peptide ratio greater than 366.66 was related to an improvement in the renal survival rate over the long term.

The relevance of urine proteomics as an alternative to single biomarkers has been evaluated^[52,53]. The usefulness of proteomics as a diagnostic tool has been documented, but its value as a prognostic factor remains to be evaluated.

Several studies have evaluated the role of small microRNAs in the diagnosis and the prognosis of IgAN^[54].

MicroRNAs are short, noncoding RNA molecules that regulate gene expression. Micro RNAs such as 18-5 p, 29 c, 133 a, 133 b, 148 b, 185, 192 and 200 c have been documented to exert a role in the pathogenesis of IgAN. Their level in urinary excretion may be elevated in the course of the disease and may represent a useful diagnostic tool. The prognostic value remains to be evaluated, even though the relationship between the urinary levels of miRNA 146 and miRNA 155 and proteinuria and lower GFR have recently been documented^[55].

Many biological markers have been described principally as a possible diagnostic tool. Some papers have also reported their usefulness in prognosis and have described their correlation with disease evolution. However, none of these approaches has been properly confirmed as a valuable predictor of clinical outcomes, and their superiority with respect to the clinical markers is still to be proven.

Clinical markers

To date, clinical prognostic markers remain as the most reliable predictors of IgAN evolution.

Principally, they include an impaired GFR, sustained hypertension and proteinuria^[56,57]. Longitudinal trends in blood pressure (BP) and proteinuria are both associated with disease progression^[58,59]. In a prospective study on 332 IgAN patients^[59], proteinuria > 1 g/d, and hypertension > 140/90 mmHg, when associated with severe histological lesions, allowed the calculation of a risk score predicting death or ESRD 10 years to 20 years after disease onset. Another study, based on retrospective data from 600 IgAN Chinese patients^[60], identified four baseline variables with an independent risk of ESRD evolution; *i.e.*, GFR, serum albumin, hemoglobin and systolic BP. Recently, looking for the IgAN outcome predictors, a study on a multiethnic United States cohort documented that the baseline eGFR was the strongest predictor of ESRD^[61]. High body mass index and smoking have also been identified as predictors of poorer outcomes in IgAN^[62,63]. These factors, however, are not specific for IgAN, but are common to any glomerulonephritis.

By contrast, the degree of hematuria, which is a typical manifestation of IgAN, does not have a predictive value. As already mentioned the clinical presentation with AKI accompanying macroscopic hematuria doesn't necessarily mean crescentic IgAN but may be the expression of acute tubular necrosis spontaneously resolving.

In summary, several histological, biological and clinical markers have been proposed as predictors of IgAN outcomes and, as a consequence, are useful for suggesting therapeutic measures and monitoring their effects. However, to date, neither histological nor biological markers have documented a clear superiority over the more simple clinical markers^[10].

THERAPY

From a therapeutic point of view, IgAN patients at diagnosis should be divided into three groups^[10] and the therapeutic approaches differ according IgAN groups. (1) low risk patients: These are subjects with normal GFR, no hypertension and minor urinary abnormalities (proteinuria < 0.5 g/d +/- isolated microhematuria). These patients do not require treatment but should be checked annually or biannually for at least 10 years. Monitoring is recommended to check any disease evolution. In the case of disease evolution, therapeutic measures should be adopted as described below; (2) intermediate risk patients have a proteinuria > 0.5-1 g/d that may be associated with hypertension and a reduced GFR. These patients should receive optimized supportive therapy and should be strictly monitored. A corticosteroids course and/or immunosuppressive treatment might be added if proteinuria increases or GFR declines; and (3) high risk patients show a rapid decrease in the GFR that may be associated with nephritic syndrome or crescentic glomerulonephritis. These findings may be already present at IgAN diagnosis or may develop during the disease evolution. In addition to supportive treatment, corticosteroids and immunosuppression should be considered for these high risk patients.

Supportive care

Supportive care is recommended by KDIGO guidelines^[34] for any IgAN patient at risk of disease evolution.

The supportive care includes several measures aimed to control the progression of any glomerulonephritis, among which is IgAN (Table 4)^[64].

The mainstay of supportive treatment in IgAN is the control of BP and control of the renin-angiotensin system (RAS)^[65].

A review of 11 RCTs, documented that treatment with angiotensin converting enzyme inhibitors (ACEI) or with angiotensin receptor blockers (ARB) significantly reduced proteinuria and had a renoprotective effect with respect to the controls^[66]. These data were confirmed by a meta-analysis that reviewed 6 RCTs^[67].

Table 4 Supportive therapy of immunoglobulin a nephropathy

Level 1	Control blood pressure (sitting systolic BP in the 120 s)
	ACE inhibitor or ARB therapy with up-titration of dosage or combination ACE inhibitor and ARB therapy
Level 2	Control protein intake
	Restrict NaCl intake/institute diuretic therapy
	Control each component of the metabolic syndrome
	Aldosterone antagonist therapy
	Beta-blocker therapy
	Smoking cessation
Other measures	Allopurinol therapy
	Empiric NaHCO ₃ therapy, independent of whether metabolic acidosis is present or not
	Avoid NSAIDs altogether, or no more than once or twice weekly at most
	Avoid prolonged severe hypokalemia
	Avoid phosphate cathartics
	Ergocalciferol therapy to correct vitamin D deficiency
	Control hyperphosphatemia and hyperparathyroidism

ACE: Angiotensin-converting enzyme; ARB: Angiotensin receptor blocker; NaCl: Sodium chloride; NaHCO₃: Sodium bicarbonate; NSAID: Non-steroidal anti-inflammatory drug.

More recently, the beneficial effect of Aliskiren, a direct renin inhibitor, has been documented by two studies^[68,69]. Its protective effect principally is a consequence of proteinuria reduction.

In addition, a wide Cochrane review of 56 RCTs including 2838 IgAN patients^[70] documented that antihypertensive agents, in particular the RAS inhibitors were more powerful renoprotective agents among the non-immunosuppressive therapies. Indeed, the effect of antihypertensive agents was compared with treatments such as fish oil supplementation, antiplatelets/anticoagulants agents and other treatments such as statins, phenytoin, herbal medicine, vitamin E and sodium cromoglycate.

Other controversial non-immunosuppressive treatments

Fish oil supplementation is an old therapy with varied results.

In a meta-analysis of fish oil therapies, no significant beneficial result was observed^[71]. In the largest RCT with fish oil, an improvement in disease evolution was observed in treated patients^[72], but these results were not confirmed in a more recent RCT^[73].

Antiplatelet and anticoagulant based therapy is widely used in Asia. A small study documented some efficacy with dipyridamole and warfarin, but the study did not have a control group^[74].

In a recent study^[75] a beneficial effect was observed using statins. The study was small, not controlled, and the effect of statins on IgAN remained unclear.

In summary, as documented by the above mentioned Cochrane review^[70] and after comparing the different non-immunosuppressive treatments, the only documented beneficial effect is exerted by the antihypertensive drugs, and this effect seems to be mediated by proteinuria reduction. In a recent meta-analysis^[76], combination therapy with ACEI and ARB seems to achieve more benefits, even if the long-term effects still need to be documented.

Tonsillectomy

The efficacy of tonsillectomy alone or associated with immunosuppression has been a matter of discussion, and discordant results have been reported for a long time. The rationale of tonsillectomy in IgAN prevention and/or treatment is the elimination of an important source of pathogens by removing tonsil crypts. Indeed, a recent study^[77] has indicated that palatine tonsils are probably a major site of Gd-IgA1 producing cells. In some patients these cells may be largely present in other lymphoid organs, and this fact might explain the diverging results of tonsillectomy.

Tonsillectomy associated with pulse steroids or other immunosuppressants is largely used in Japan, as documented by several retrospective studies^[78,79]. In addition, a recent meta-analysis of seven non-randomized studies (6 in Japan and 1 in China) documented an overall beneficial effect of tonsillectomy plus corticosteroids^[80]. In another meta-analysis from China, of 14 studies including 1794 patients^[81], the authors concluded that tonsillectomy may induce clinical remission, but the adjustment for confounding variables could not be performed because the majority of the studies included retrospective cohorts of patients.

Recently, the first national multicenter RCT from Japan failed to demonstrate any superior effect of tonsillectomy associated with pulse steroids over pulse steroids alone^[82].

Because other studies on Chinese^[83] and Caucasian patients^[84,85] did not confirm the tonsillectomy beneficial effect, waiting for an adequately powered RCT tonsillectomy should not be recommended. The KDIGO suggested that tonsillectomy should not be performed to treat IgAN^[8]. A retrospective study on 1147 European patients with IgAN failed to demonstrate a significant correlation between tonsillectomy and renal function decline^[86].

Corticosteroids

To date, the KDIGO guidelines^[34] suggest a 6 mo course

of corticosteroids only for those patients at intermediate risk of having persisting proteinuria > 1 g/d and with a GFR between 30 mL/min per 1.73 m² and 50 mL/min per 1.73 m², after optimization of supportive therapy. Several studies have been performed to evaluate the usefulness of corticosteroid therapy in IgAN. According to several studies^[87-90], steroids have a renoprotective effect. In some of these studies, the beneficial effect seems to be related to a long course therapy or to a higher dose. Other studies did not confirm a steroid related beneficial effect^[91] or highlight the problem of corticosteroid side effects^[92].

A Cochrane review on immunosuppressive therapy in IgAN^[93] analyzed 32 studies comprising 1781 patients. Six of these studies analyzed the effects of steroids. A renoprotective effect was observed comparing steroids vs placebo or no treatment. Unfortunately, all the aforementioned studies did not answer a number of questions such as the following: Were steroids also effective for patients with a GFR < 30 mL/min per 1.73 m²? What is the best steroid dosage and regimen to avoid side effects? RCTs that are ongoing such as the Supportive Versus Immunosuppressive Therapy of Progressive IgA Nephropathy (STOP IgAN)^[94] and the Therapeutic Evaluation of Steroids in IgA Nephropathy (TESTING) study^[95] might provide definitive evidence for a role of corticosteroids in the treatment of IgAN.

Recently, the VALIGA study retrospectively evaluated the role of corticosteroids in IgAN^[96]. The authors observed that corticosteroids reduced proteinuria and the rate of renal function decline. In addition, these benefits also involved patients with an eGFR < 50 mL/min. The results of this study should encourage nephrologists to further investigate corticosteroids efficacy in patients with low baseline GFR^[97].

Corticosteroids in association with other therapies

The already cited Cochrane review^[93] highlighted the higher efficacy of corticosteroids given in association with ARB with respect to corticosteroids alone or ARB alone.

Other studies^[80] documented the higher efficacy of tonsillectomy plus steroids with respect to tonsillectomy alone or steroid therapies alone.

The association of steroids with other immunosuppressants has been principally used for high risk patients.

Association of cyclophosphamide and corticosteroids offered different results

The association of cyclophosphamide and corticosteroids has been principally examined in studies concerning patients with progressive renal deterioration or with crescentic IgAN^[98-100]. The combined cyclophosphamide/steroid therapy may benefit patients at a high risk of renal failure. The limitation of these studies is that they are small, often retrospective, and side effects represent a serious concern. The KDIGO guidelines^[34] do not recommend such treatment for the vast majority

of IgAN patients. A possible role is suggested by the guidelines only for patients with crescentic IgAN and rapidly decreasing renal function.

Similarly, the use of azathioprine (AZA) in addition to corticosteroids is not recommended. Indeed, in two studies from Pozzi *et al.*^[101,102] the addition of AZA to corticosteroids did not provide any beneficial result in patients with ongoing severe chronic renal failure.

The aforementioned Cochrane review on immunosuppressants in the treatment of IgAN highlighted that the use of such treatments had low evidence and was not powerful to guide clinical practice. In addition, evidence on mortality, infections and cancers is sparse or of low quality.

The use of calcineurine inhibitors in addition to corticosteroids has been tested in some recent small RCTs^[103,104]. Some benefit has been reported for the reduction of proteinuria, but the addition of cyclosporine in some patients caused a serum creatinine increase and a higher infection incidence.

Other immunosuppressants

In a recent study, Kim *et al.*^[105] compared tacrolimus (TAC) with ACEI/ARB therapy. In this small study, TAC reduced proteinuria in IgAN patients, but the follow-up was too short to draw any conclusion.

Mycophenolic mofetil (MMF), in addition to its immunosuppressive action on lymphocytes, has been documented to reverse IgA1 aberrant glycosylation, up-regulating the core 1 beta 3 - GalT-specific molecular chaperone that is impaired in IgAN^[106].

The first RCT of MMF was conducted on Chinese patients with severe IgAN^[107]. The effects on proteinuria were significant at 18 mo. At the same time, two other European studies failed to document a beneficial effect of MMF^[108,109]. These data raised the possibility of a different response to MMF in different ancestral cohorts.

Later on, three other Chinese studies reported an improved outcome in IgAN patients treated by MMF^[110-112]. In addition to the improved outcomes of patients treated with MMF, the study by Tang *et al.*^[111] documented that MMF inhibited IgA binding to mesangial cells. Diverging results have also been reported in more recent studies. In an Italian study, MMF and steroids reduced proteinuria and improved outcomes in IgAN patients at risk for progression^[113]. In another study, MMF therapy was effective for IgAN children with nephritic syndrome and resistant to steroid treatment^[114].

A recent study from the United States was not able to document any MMF related beneficial effect^[115], but the study had the limitation of enrolling few patients and had a short follow-up.

A Chinese review on the efficacy and safety of MMF treatment in IgAN recognized that high quality RCTs with large sample sizes and a long follow-up are needed to evaluate the MMF efficacy in IgAN^[116]. To date, the KDIGO guidelines do not recommend the use of MMF in IgAN patients.

Therapy for recurrence of IgAN after kidney transplantation

Post-transplant recurrence of IgAN is common. As prevention and treatment of acute and chronic rejection is continuously improving, renal disease recurrence on the graft may become a relevant cause of graft loss over the long term^[117]. However, none of the current available immunosuppressive drugs are able to prevent the histological recurrence of IgAN^[118,119]. Patients with recurrent IgAN after transplantation should be given optimized supportive care. A Japanese study suggested that a preoperative tonsillectomy might not affect the recurrence of IgAN^[120].

A study from Berthoux *et al.*^[121] suggested that an induction therapy with ATG might have a protective role against IgAN recurrence, but these results have not been confirmed. Registry data from the Australia and New Zealand Dialysis and Transplant Registry documented that the corticosteroids given continuously after transplantation significantly reduced the risk of IgAN recurrence^[122]. An analysis of the United States Renal Data System similarly documented a protective effect of corticosteroids after IgAN recurrence in renal transplant patients^[123]. A retrospective study documented no benefit using MMF instead of AZA as an antimetabolite drug after transplantation^[124].

New therapies and ongoing clinical trials

New therapies: *In vitro* studies documented that peroxisome proliferator-activated receptor-gamma (PPAR-gamma) agonist attenuates inflammation in tubular epithelial cells in IgAN^[125]. The additive effect of PPAR-gamma agonist and ARB has been confirmed in an animal model of IgAN^[126].

A new enteric formulation of the locally acting glucocorticoid budesonide, designed to release the active compound in the ileo-cecal region, has been used to treat IgAN and was effective in reducing proteinuria and slightly increasing eGFR^[127]. The aim of a locally releasing compound is to limit the corticosteroid side effects. Based on these data, a multicenter phase II b trial (NEFIGAN) is currently ongoing in Europe.

Complement activation is involved in IgAN tissue injury. Rituximab has been successfully used as rescue therapy in IgAN with rapid progression^[128]. However, in another study, rituximab, given as a single dose at the beginning of the therapy, failed to reduce proteinuria and to inhibit GFR decline^[129].

Bortezomib is a proteasome inhibitor approved for the treatment of multiple myeloma and tested to decrease antibody levels in hyperimmune patients in renal transplantation. The rationale for using Bortezomib in the treatment of IgAN relies on the fact that, in IgAN, a switch from proteasome (PS) to immune PS has been observed, suggesting a hyperactivation of the PS system. In addition, an increased nuclear translocation of the p50 active subunit of NF- κ B has been observed in these patients^[130,131]. A phase III clinical trial is to date ongoing.

Spleen tyrosine kinase (SYK) is an intracellular protein tyrosine kinase involved in cell signaling downstream of the immunoreceptors. Recently, the involvement of the SYK in the inhibition of IgA1 stimulation of human mesangial cells and in the pathogenesis of IgAN has been documented^[132]. A RCT with a selective oral SYK inhibitor in patients with IgAN is currently ongoing.

Recently in China the efficacy and safety of Leflunomide given in association with steroids has been evaluated. In a first RCT the efficacy of Leflunomide was evaluated in IgAN patients affected by nephritic syndrome^[133]. In this context leflunomide resulted a safe and effective drug for the treatment of IgAN. More recently a larger number of IgAN patients were enrolled in a RCT to receive Valsartan combined with clopidogrel and/or leflunomide for the treatment of progressive IgAN^[134]. The treatment with Valsartan combined with clopidogrel and leflunomide resulted in a reduction of proteinuria and of renal function deterioration.

Ongoing clinical trials for IgAN treatment: Several clinical trials for IgAN are ongoing. As mentioned previously, only the active ongoing clinical trials that are recruiting patients will be discussed.

Clinical trials may involve old drugs given with new strategies or new drugs not yet on the market.

Two RCTs are testing the efficacy of MMF in patients with IgAN. One RCT^[135] includes patients with proteinuria > 1 g/d already in treatment with ARB. The purpose of the RCT is to evaluate the efficacy of MMF in reducing proteinuria and preserving renal function compared to corticosteroids. The other trial (MAIN)^[136] is enrolling patients with advanced IgAN. The purpose of the study is to evaluate MMF compared to losartan alone in patients treated with the maximum tolerated daily dose of losartan.

Four RCTs are evaluating the effects of corticosteroids on IgAN.

Apart from the already cited TESTING study^[95], a Chinese RCT^[137] is evaluating the efficacy and safety of steroids in IgAN patients with active pathological lesions. The TOPplus-IgAN RCT^[138] is evaluating the effects of prednisone plus cyclophosphamide in patients with advanced stage IgAN and is evaluating combination therapy with respect to corticosteroids alone. The first available data from the STOP-IgA^[94] reported that appropriate supportive care blunted the effect of immunosuppression in proteinuric IgAN patients.

The adrenocorticotrophic hormone (ACTH) has been used in RCTs for the treatment of several diseases, among which is glomerulonephritis. Indeed, ACTH seems to exert a non-specific antiproteinuric effect rather than a specific effect. Bomback *et al.*^[139] treated several proteinuric patients, among which 5 patients were affected by IgAN with proteinuria resistant to other therapies.

To date, two studies are testing the gel formulation of ACTH in the treatment of IgAN at a high risk of progression^[140].

As mentioned previously, rituximab has been used in the treatment of IgAN. To our knowledge, the only RCT on rituximab^[141] is not enrolling patients.

CCX168 is an orally administered, specific small molecule inhibitor of the C5a receptor. Trials with CCX168 are ongoing in the treatment of the atypical hemolytic uremic syndrome and antineutrophils cytoplasmic antibodies vasculitis. A phase II study is enrolling patients to evaluate CCX168 efficacy in reducing proteinuria in IgAN with persistent proteinuria despite supportive therapy with a maximally tolerated RAS blocker^[142].

Blisibimod is a selective antagonist of the B-cell activating factor and is being tested in lupus nephritis. A RCT (BRIGHT-SC) is evaluating blisibimod in a phase II / III trial in proteinuric patients affected by IgAN^[143].

The aforementioned enteric budesonide is being evaluated for the treatment of IgAN in a European multicenter RCT^[144].

A pilot study on Velcade (bortezomib)^[145] in IgAN has the purpose of investigating the ability of bortezomib to induce complete or partial remission in patients with severe IgAN.

Fostanatinib is a selective inhibitor of SYK that is involved in the pathogenesis of IgAN. A phase II RCT is, to date, ongoing with the purpose of determining whether fostanatinib is safe and effective in the treatment of IgA nephropathy^[146].

Finally, two Chinese RCTs are evaluating the efficacy of two traditional Chinese medicines; *i.e.*, Abemoschus Manihot^[147] and Tripterygium Wilfordii HOOK^[148], in the treatment of IgAN. The former RCT is comparing the study drug with losartan, and the latter with MMF.

CONCLUSION

Patients affected by IgAN may present extremely different clinical aspects at diagnosis. The disease evolution also may differ ranging from a stable course of disease with no evolution to a disease rapidly evolving to ESRD. Accordingly, the therapeutic approaches may vary from only the need for frequent controls to check for disease evolution to careful supportive care for patients with clinical signs, from urinary abnormalities, hypertension and reduced GFR to intensive treatment in patients with rapid evolution.

Because the so called "silent majority" does not have any disease evolution, the major problem is to identify those patients who will have disease evolution in the future. Histological and biological markers have been proposed in an attempt to identify such patients, but, to date, the clinical markers represent the optimal tool for monitoring IgAN patients.

Patients with stable disease with no sign of disease evolution only need to be monitored. Patients with slow evolving disease and low level proteinuria, in addition to being monitored, need optimal supportive care. In recent years, treatment with corticosteroids may be useful for such patients and is recommended by the guidelines.

Intensive treatment with corticosteroids and other immunosuppressants should only be reserved for patients with rapidly progressive disease or with a histological picture of extracapillary glomerulonephritis or with nephrotic proteinuria.

Several RCTs concerning new drugs are included in the international registries, but only some trials are enrolling patients.

In any case, these new drugs should be reserved for high risk patients and should not be used until validated in large studies for a long period of time.

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How do kinases contribute to tonicity-dependent regulation of the transcription factor NFAT5?

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Abstract

NFAT5 plays a critical role in maintaining the renal functions. Its dis-regulation in the kidney leads to or is associated with certain renal diseases or disorders, most notably the urinary concentration defect. Hypertonicity, which the kidney medulla is normally exposed to,

activates NFAT5 through phosphorylation of a signaling molecule or NFAT5 itself. Hypotonicity inhibits NFAT5 through a similar mechanism. More than a dozen of protein and lipid kinases have been identified to contribute to tonicity-dependent regulation of NFAT5. Hypertonicity activates NFAT5 by increasing its nuclear localization and transactivating activity in the early phase and protein abundance in the late phase. The known mechanism for inhibition of NFAT5 by hypotonicity is a decrease of nuclear NFAT5. The present article reviews the effect of each kinase on NFAT5 nuclear localization, transactivation and protein abundance, and the relationship among these kinases, if known. Cyclosporine A and tacrolimus suppress immune reactions by inhibiting the phosphatase calcineurin-dependent activation of NFAT1. It is hoped that this review would stimulate the interest to seek explanations from the NFAT5 regulatory pathways for certain clinical presentations and to explore novel therapeutic approaches based on the pathways. On the basic science front, this review raises two interesting questions. The first one is how these kinases can specifically signal to NFAT5 in the context of hypertonicity or hypotonicity, because they also regulate other cellular activities and even opposite activities in some cases. The second one is why these many kinases, some of which might have redundant functions, are needed to regulate NFAT5 activity. This review reiterates the concept of signaling through cooperation. Cells need these kinases working in a coordinated way to provide the signaling specificity that is lacking in the individual one. Redundancy in regulation of NFAT5 is a critical strategy for cells to maintain robustness against hypertonic or hypotonic stress.

Key words: Tonicity enhancer binding protein; Osmotic response element binding protein; Phosphorylation; Kidney; Urinary concentration; Signal transduction; Nephropathy; Hypertonicity; Hypotonicity

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Core tip: NFAT5 is critical for kidney functions. Its dysregulation results in or is associated with the renal diseases and disorders. More than a dozen of kinases have been identified to contribute to tonicity-dependent regulation of NFAT5. The present review is focused on how these kinases regulate NFAT5 activity under the context of hypertonicity or hypotonicity. Understanding these regulatory mechanisms will have therapeutic implications. A precedent example is that recognition of the cyclosporine immunosuppressive effect resulted from inhibition of the phosphatase calcineurin-dependent activation of NFAT1 allows combination use of cyclosporine with other mechanistically different immunosuppressants to improve their therapeutic efficacy and reduce their side effects.

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INTRODUCTION

Functions of NFAT5 in the kidney

The kidney medulla contributes to maintaining body fluid and electrolyte balance through concentration of urine. In order to achieve this goal, the medulla must establish two pre-requisites: Adequate water permeability alone the renal tubules and hypertonicity and hyperosmolality in the renal medullary interstitial fluid, which provide an osmolar gradient driving water absorption. NFAT5, nuclear factor of activated T cells 5^[1] also named as TonEBP^[2] and OREBP^[3], is the primary transcription factor that is activated by hypertonicity in the mammalian system and plays a pivotal role in establishing these two conditions. NFAT5 activates expression of water channels aquaporin-2 (AQP-2), which dictates the apical water permeability of the collecting ducts^[4-6] and aquaporin-1 (AQP-1), an important gene for water trafficking across the proximal tubules and descending limb of the loop of Henle^[7], and urea transporter 1 (UTA1), a critical contributor for hyperosmolality in the renal medullary interstitium^[5,6,8], and osmoprotective genes like betaine/glycine transporter 1 (BGT1), sodium-dependent myo-inositol transporter (SMIT) and aldose reductase (AR)^[1-3,9], which are essential for the kidney medulla to survive in the hypertonic environment. Expression of a dominant negative mutant of NFAT5 in the kidney epithelial cells reduces expression of AQP-2 and UTA1 and impairs urinary concentration^[5]. A majority of homozygous NFAT5 knockouts die embryonically^[5], probably due to impaired development and function of cardiomyocytes^[10]. The survived knockouts have profound renal medullary hypotrophy with reduced expression

of the osmoprotective gene^[9]. Thus, NFAT5 is tightly regulated in the kidney medulla to ensure normal process of urinary concentration. Hypokalemia, cyclosporine A and lipopolysaccharides-induced urinary concentration defect is associated with reduced NFAT5 activity in the region^[6,11,12]. Water restriction induces an increase of urinary excretion of sodium to prevent hypernatremia and rise in extracellular tonicity. In the primary rat renal medullary cells, NFAT5 is necessary for hypertonicity-induced increase of serum- and glucocorticoid-inducible kinase-dependent expression of the type A natriuretic peptide receptor^[13]. This cascade might be a mechanism for dehydration-induced natriuresis^[13].

Besides activation by hypertonicity, NFAT5 is also activated by hypoxia^[14,15]. Renal ischemia for 30 min increases the mouse medullary mRNA abundance of NFAT5, which is protective against ischemia/reperfusion-induced acute kidney injury^[14]. However, ischemia for 45 min in the rat kidney decreases NFAT5 mRNA and protein abundance in the medulla^[16], but the functional consequence of the effect remains unknown^[16]. NFAT5 mRNA is up-regulated in the kidney by unilateral ureteral obstruction^[17]. NFAT5 involves in diabetic nephropathy. Haplotype association analysis of 718 type 1 diabetic patients reveals a significant association of NFAT5 with nephropathy^[18]. High glucose increases NFAT5 transcriptional activity more in the peripheral blood mononuclear cells isolated from type 1 diabetes patients with nephropathy than in the cells isolated from the patients without nephropathy^[19].

Phosphorylation of NFAT5

NFAT5 belongs to the family of the Rel transcription factors, including NFAT1-4 and NF-κB^[1-3]. It is best known for its essential role in protecting cells from hypertonic stress. However, it has become clear that NFAT5 also has important functions outside hypertonicity^[20]. Therefore, it is not surprising that NFAT5 is also expressed in the tissues that are not normally exposed to hypertonicity^[21]. Hypertonicity activates NFAT5 by increasing its transactivation, nuclear localization and DNA binding and protein abundance^[22]. Like many other biological processes, phosphorylation of NFAT5 regulates NFAT5 activation. High NaCl rapidly increases phosphorylation of NFAT5. NFAT5 has 216 serines, 15 tyrosines, and 111 threonines, all of which could be phosphorylated^[22]. Through mass spectrometry, DNA mutation, immunocytochemistry and Western analyses, NFAT5 tyrosine 143 (Y143), threonine 135 (T135), serine 155 and 158 (S155 and S158) have been identified so far as the phosphorylation sites and play a critical role in regulation of NFAT5 activity. High NaCl increases phosphorylation of NFAT5-Y143, leading to increase of NFAT5 nuclear localization in cell culture^[23-25], and phosphorylation of NFAT5-Y143 is increased in the normal rat renal inner medulla and the Brattleboro rat inner medulla treated with vasopressin, known to increase the renal medullary tonicity^[25]. The similar

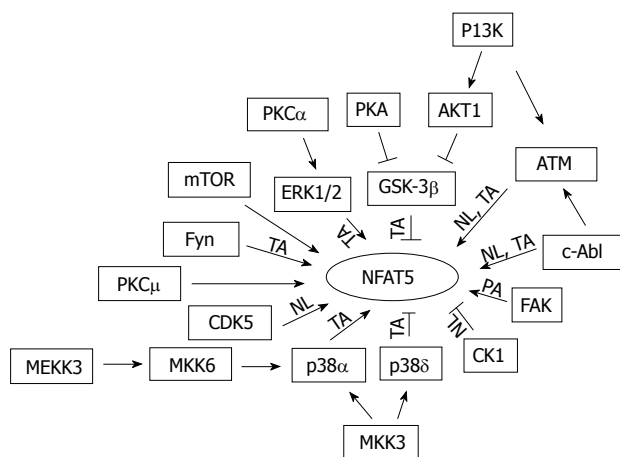


Figure 1 Summary of kinases known to regulate tonicity-dependent activation/inactivation of NFAT5 through an increase/decrease of its transactivating activity, nuclear localization and/or protein abundance. If none of these three steps appears with an arrow, this means that the mechanism is unknown. mTOR: Mammalian target of rapamycin; TA: Transactivating activity; NL: Nuclear localization; PA: Protein abundance; PKA: Protein kinase A; PKC: Protein kinase C; ERK: Extracellular signal-regulated kinase; CDK: Cyclin dependent kinase.

phenomena are also observed with NFAT5-T135^[26]. On the other hand, low NaCl increases phosphorylation of NFAT5-S155 and then S158, leading to reduced NFAT5 nuclear accumulation^[26,27]. In contrast to the demonstration of regulation of NFAT5 nuclear distribution by direct phosphorylation, how phosphorylation regulates NFAT5 transactivating activity is elusive. Although a majority of kinases contributes to tonicity-dependent increase of NFAT5 transactivation (Figure 1), none of phosphorylation sites in the transactivation domain has been definitively identified. NFAT5-S1197, S1247 and S1367 lie in the NFAT5 transactivation domain. Over expression of the alanine mutants of these serine residues in HEK293 cells or AT cells, which have inactive ATM kinase, reduces NFAT5 transcriptional activity under isotonicity and/or hypertonicity^[28]. However, whether high NaCl increases phosphorylation of these serine residues remains unknown.

Signaling regulation through coordination

Consistent with the observations that phosphorylation regulates NFAT5 activation are that more than a dozen of kinases (Figure 1) and a few phosphatases have been identified to regulate NFAT5 transcriptional activity^[22,23,29-32]. However, all of these kinases and phosphatases also regulate other cellular activities and even opposite activities. For example, p38 and ERK1/2 contribute to hypertonicity-induced activation of NFAT5, but hypotonicity, which is known to inhibit NFAT5 activity, also increases phosphorylation (activation) of these two kinases^[30]. The pleiotropic effects of these kinases and phosphatases raise a question concerning how they can selectively signal to NFAT5 in the context of hypertonicity. Another question is why many signaling molecules are needed to regulate NFAT5 activity. Signaling through

cooperation/committee might be a plausible explanation. This concept was originally put forward to describe how protein kinases and phosphatases in budding yeast capture and relay information in a coordinated way responding to a signal^[33]. This concept can be viewed as that cells have a specific committee tasked for a specific perturbation. Each member in the committee is pre-decided when, where and how to act, so that cells can respond to the perturbation in a coordinated way^[34]. The committee members are like different and redundant instrument players in an orchestra in which each one plays his/her instrument, maybe viewed as activation of a signaling molecule, in a coordinated way with other players for a specific music piece signaled by a conductor. This theory explains why each of the identified kinases is necessary for full activation of NFAT5, but none alone, is sufficient^[22], why CDK5 is only required in the early phase of NFAT5 activation^[26], and why over expression of catalytically active PKA only increases NFAT5 activity under isotonicity but not under hypertonicity^[35], because neither a single player nor an over active player can play an orchestra piece. Numerous signaling molecules are required in order to form redundancy in signaling hypertonic stress. Redundancy is a critical strategy for cells to maintain robustness against internal and external perturbations^[36], as multiple players are needed to produce desired volume from a particular instrument in orchestrating a music piece.

Potential clinical significance of this review

The present review is focused on how kinases contribute to tonicity-dependent activation/inactivation of NFAT5, since this area is the most studied one. Understanding these regulatory pathways will have therapeutic implications. A good example is the mechanism by which cyclosporine A and tacrolimus suppress immune reactions. These two medications inhibit the phosphatase calcineurin, which leads to inhibition of nuclear translocation of NFAT1, NFAT2 and NFAT4, resulting in suppression of expression of the proinflammatory cytokines^[37]. This mechanism has helped understanding both the therapeutic and side effects including renal toxic effects of the medications^[37] and is critical for the combined use of cyclosporine A and tacrolimus with other mechanistically different immunosuppressants to improve their therapeutic efficacy and reduce their side effects^[38]. Interestingly, cyclosporine A induces urinary concentration defect, which is ascribed to the decrease in NFAT5 activity in the rat kidney^[12,39]. The effect is mediated through inhibition of calcineurin remains unknown. Another example is that the anti-diabetes medication metformin was shown to induce apoptosis in the kidney medulla of both normally hydrated and dehydrated type 2 diabetic mice, probably by inhibition of NFAT5 through activation of 5'-AMP-activated protein kinase^[40]. This observation raises safety concern for metformin in the dehydrated diabetic patients^[41].

Diabetic nephropathy is one of the most severe

complications of diabetes with resultant increases of morbidity and mortality. Its treatment has posed a formidable challenge to the medical and scientific communities. Numerous novel therapeutic approaches promisingly found in animal studies have not been successfully translated into clinical practices^[42]. For example, pyridoxamine, which showed a potent effect in blocking formation of advanced glycosylated end product in animal models has failed in clinical trials^[42,43]. AR, a transcriptional target of NFAT5, is a rate-limiting enzyme of the polyol pathway, which plays a crucial role in the pathogenesis of diabetic complications including diabetic nephropathy^[44,45]. Thus, targeting the regulatory network of NFAT5 may be an alternative approach to treat diabetic nephropathy. In this regard, the extract from plant *Aralia elata* has been recently shown to prevent neuronal death by downregulating NFAT5 and AR in mice with diabetic retinopathy, although which regulatory pathway it affects remains unknown^[46].

Mitogen-activated protein kinases

Mitogen-activated protein kinases (MAPKs) have three major families: p38, extracellular signal-regulated kinases (ERKs) and c-Jun NH₂-terminal protein kinases (JNKs). Each family has multiple isoforms. They are the most studied kinases in tonicity-dependent activation of NFAT5, which was recently reviewed^[30]. The present review only summarizes the salient points of that review. p38 has four isoforms: p38 α ^[47], p38 β ^[48], p38 γ ^[49] and p38 δ ^[50]. p38 α contributes to tonicity-dependent activation of NFAT5, whereas p38 δ does the opposite^[51]. The imidazole derivatives such as SB203580 inhibit p38 α and p38 β , but not p38 δ ^[50,52]. SB203580, its analogs, p38 α dominant negative mutant or siRNAs uniformly inhibits hypertonicity-induced NFAT5 transcriptional activity^[53-63]. Because p38 α is also called p38, it has been often concluded that p38 signals hypertonicity-induced activation of NFAT5. However, this conclusion causes confusion for interpretation of the effect of the p38 upstream kinase MKK3 and phosphatase MKP-1 on NFAT5 activity. Over expression of MKK3 dominant negative mutant^[64] or MKP-1^[51] inhibits p38 without significantly affecting NFAT5 transcriptional activity. This paradox can be explained by the interpretation that inhibition of the positive effect of p38 α by SB203580, a dominant negative mutant^[54] or its siRNAs^[51] unmasks an inhibitory effect of p38 δ , whereas the dominant negative mutant of MKK3^[50,65] and MKP-1^[51] reduce both p38 α and p38 δ activities, therefore, causing no significant change in NFAT5 activity^[51,64]. Based on this theory, it is not surprising that another p38 upstream kinase MKK6^[13,66] and the MKK3 and MKK6 upstream kinase MEKK3^[67] have been demonstrated to contribute to tonicity-dependent activation of NFAT5, because although both MKK3 and MKK6 activate p38 α under hypertonicity^[68,69], MKK3 strongly activates p38 δ , whereas MKK6 does not^[70].

Although p38 is the most studied MAPKs in the

context of tonicity-dependent regulation of NFAT5, the exact mechanism underlying this effect is far from clear. Whether p38 is critical for tonicity-dependent activation of the transcription factor is even questionable. Knockdown of Rac1 or OSM by its siRNAs reduces high NaCl-induced NFAT5 transcriptional activity, but increases phosphorylation of p38 at both basal and hypertonic levels in HEK293 cells^[66]. It should be noted that an opposite effect of knockdown of Rac1 or OSM on phosphorylation of p38 in the same type of cells was reported^[68]. Although whether activation of p38 is regulated by cell volume or intracellular ionic strength remains unclear, hypotonicity, which reduces nuclear NFAT5, presumably NFAT5 activity^[27,71], also activates p38 in various types of cells^[72-75]. These observations call for more attention to which isoform of p38 when the effect of the kinase on NFAT5 is examined.

The chemical inhibitors of MEK-ERK1/2 PD98059 and U-0126 inhibit high NaCl-induced activation of NFAT5 in nucleus pulposus cells^[62], renal carcinoma cells^[55] and possibly in mIMCD3 cells^[76]. ERK2 siRNA reduces high NaCl-dependent NFAT5 transcriptional activity in nucleus pulposus^[62] and in HEK293 cells^[29]. It is reasonably concluded that ERK1/2, or at least ERK2, contributes to tonicity-dependent activation of NFAT5^[30], although it is not clear why PD98059 fails to inhibit NFAT5 transcriptional activity in the primary splenocytes^[61]. The effect of JNK on tonicity-dependent activation of NFAT5 is elusive and also least studied. Both lack of effect^[64] and a positive effect of JNK1/2^[55] on NFAT5 have been reported.

Like the effect on p38, hypotonicity also increases phosphorylation of ERK1/2 in human keratinocytes^[73], mIMCD3 cells^[77], renal epithelial A6 cells^[72], although inhibition of ERK by hypotonic stress in A6 cells was also reported^[78]. Therefore, the mechanism for how ERK1/2 contributes to tonicity-dependent activation of NFAT5 remains to be elucidated. In an overly simplified term, p38 and ERK1/2 can signal both hypertonic and hypotonic responses, depending on which committee they are in.

AGC protein kinases

Based on sequence alignments of the catalytic domains, the term AGC kinase was first used in 1995 to define a subgroup of serine/threonine protein kinases that were most related to cAMP-dependent protein kinase 1 (PKA; also known as PKAC), cGMP-dependent protein kinase (PKG; also known as CGK1 α) and protein kinase C (PKC)^[79]. It was later realized that the group of AGC protein kinases includes more than 60 protein kinases in the human genome, classified into 14 families: PDK1, AKT/PKB, SGK, PKA, PKG, PKC, PKN/PRK, RSK, NDR, MAST, YANK, DMPK, GRK and SGK494^[80]. AGC kinases regulate a wide array of important cellular functions. Therefore, their mutation and dysregulation contribute to the pathogenesis of various human diseases, including kidney diseases^[80].

(1) PKA exists as a heterotetramer composed of two regulatory subunits and two catalytic subunits. A pseudosubstrate motif in the regular subunits binds to the substrate-binding site of the catalytic domain. Upon activation, two molecules of cAMP bind to each regulatory subunit, allowing the release of active catalytic subunits^[80]. PKA is the first AGC kinase demonstrated contributing to tonicity-dependent activation of NFAT5^[35]. Hypertonicity induced by high NaCl increases PKA activity. An inhibitor of PKA (H89, 10 $\mu\text{mol/L}$) and dominant-negative PKA catalytic subunit reduce NFAT5 transcriptional activity associated with a decrease of NFAT5 transactivating activity in HepG2 cells^[35]. Further, overexpression of the catalytic subunit of PKA (PKAc) alone increases NFAT5 transactivating and transcriptional activities under the isotonic condition^[35]. Subsequent studies indicate that PKA contributes to tonicity-dependent activation of NFAT5 by suppressing the negative effect of GSK-3 β on the transcription factor through increasing the inhibitory phosphorylation of GSK-3 β at serine 9^[81]. PKA has also been suggested to contribute to tonicity-dependent activation of NFAT5 in the primary splenocytes, based on the inhibitory effect of H89^[61]. H89 at 2 $\mu\text{mol/L}$ failed to inhibit high NaCl-induced increase of protein abundance of HSP70, a transcriptional target of NFAT5^[82,83], in NIH3T3 cells^[84]. This is probably due to that the concentration of H89 is too low. Whether hypertonicity-induced activation of PKA requires cAMP is not clear. High NaCl does not significantly alter cAMP level in HepG2 cells where the effect of PKA on NFAT5 is observed^[35] or in LLC-PK1 cells^[85], but increases cAMP level in mIMCD3 cells^[86] and neutrophils^[87]. Yet, the lack of the effect of forskolin (increasing intracellular cAMP) or dibutyryl-cAMP (a mimic of cAMP) on NFAT5 transcriptional activity in HepG2 cells let investigators conclude that the effect of PKA on NFAT5 is cAMP-independent^[35]. A precedent example is that activation of NF- κ B by PKA is independent of cAMP^[35]. It is not clear why hypertonicity does not increase PKA activity in mpkCCD14 cells^[88].

(2) The PKC family has 10 isoforms and can be divided into three categories based on their structure and biochemical properties: classical or conventional PKC (cPKC), including PKC α , PKC β I, PKC β II, and PKC γ ; novel PKC (nPKC), including PKC δ , PKC ϵ , PKC η , and PKC θ ; and atypical PKC (aPKC), including PKC ζ and PKC λ . PKC is a primary target of diacylglycerol, which is produced by phospholipase C (PLC)-catalyzed hydrolysis of lipid phosphatidylinositol-(4,5)-bisphosphate [Ptd-Ins(4,5)P₂]. Diacylglycerol binds a conserved C1 domain in PKC, resulting in the plasma membrane translocation and activation of the kinase^[89]. High NaCl increases PLC γ 1 activity^[24], diacylglycerol and total PKC activity^[90]. PKC inhibitors reduce NFAT5 transcriptional activity in mIMCD3^[91] and NIH3T3 cells^[84]. We recently identified PKC α involved in regulation of NFAT5 activity^[29]. Acute hypertonic stress with high NaCl increases PKC α activity in HEK293 cells. Knockdown of PKC α by its siRNAs decreases NFAT5 transcriptional activity mediated by

reduction of NFAT5 transactivating activity, but not by NFAT5 nuclear localization or protein abundance^[29]. More interestingly, PKC α activity is elevated in the kidney inner medulla due to increase of its protein abundance. Knockout of PKC α reduces expression of NFAT5-targeted genes AR and betain/glycine transporter 1, associated with reduced expression of NFAT5 protein abundance^[29]. This is the first demonstration showing that a signaling molecule regulates NFAT5 in the kidney inner medulla. The effect of PKC α on NFAT5 is relayed by ERK1/2 in HEK293 cells and possible in the kidney inner medulla, since knockdown of PKC α attenuates high NaCl-induced phosphorylation of ERK1/2 and has no additional inhibition on NFAT5 in the presence of ERK2 siRNAs in HEK293 cells, and knockout of the kinase reduces phosphorylation of ERK1/2 in the kidney inner medulla^[29]. PKC α was previously demonstrated to contribute to regulation of urinary concentration^[92,93], possibly by increasing high NaCl-dependent phosphorylation of urea transporters^[93] and urea permeability^[94] in the inner medullary collecting ducts. Our recent observations provide a possible additional mechanism for the effect of PKC on urinary concentration^[29].

PKD1, also called PKCmu, is one of three members of PKD kinase family that is closely related to PKC. PKC activates PKD through direct phosphorylation of S744 and S748 in the activation loop of PKD^[95]. PKD is highly mobile and functions as a "communicator" between different subcellular compartments^[95]. General PKC inhibitors, Go6976 and GF109203X, and siRNA-mediated knockdown of PKD1 reduce high NaCl-induced increase of protein abundance of HSP70^[84]. The general inhibitors reduce high NaCl-induced NFAT5 mobility shift and have no significant effect on NFAT5 nuclear localization^[84]. The latter effect is consistent with the lack of effect of PKC α and ERK1/2 on NFAT5 nuclear accumulation. PKD acts upstream of ERK1/2 under certain contexts^[95]. However, it remains unclear whether PKD1 involves in PKC α -ERK1/2 signaling activation of NFAT5, since elimination of high NaCl-induced phosphorylation of ERK1/2 by PD98059 (20 micromol/L) does not reduce tonicity-dependent increase of HSP70 protein abundance^[84].

(3) The AKT protein kinase family comprises three highly related isoforms encoded by different genes. Despite the shared common, multi-step mechanism of activation downstream of class IA PI3 kinases, these isoforms play different roles in signaling, as revealed by distinct phenotypes displayed by genetically modified animals, identification of isoform-specific substrates and association with discrete subcellular locations^[96]. Inhibition of phosphorylation of AKT1-S473 by a general AKT inhibitor, triciribine, or by a PI3K inhibitor wortmannin reduces high NaCl-induced expression of AR, BGT1 and SMIT^[97]. Co-expression of the catalytically active AKT1 with GSK3 in the GSK3^{-/-} mouse embryonic fibroblasts reverses the inhibitory effect of GSK3 β on NFAT5. These data indicate that AKT1 contributes to tonicity-dependent activation of NFAT5 by attenuating the inhibitory effect of GSK3^[81]. Whether

hypertonicity/hyperosmolality activates AKT remains controversial. Hypertonicity activates AKT, including direct measurements of increased AKT activity in NIH 3T3 and CHO cells^[98]. High NaCl increases phosphorylation (activation) of AKT1-S473 in mCCD_{cl1} and HepG2 cells^[97]. Also, high NaCl increases phosphorylation of AKT-S174 in Madin-Darby canine kidney cells, so does dehydration in the rat inner medulla^[99]. In contrast, high sorbitol decreases the kinase activity in HEK293 and COS cells^[100], and high sucrose decreases the kinase activity in Swiss 3T3 cells, despite increases of phosphatidylinositol (3,4,5)-trisphosphate (PIP3) abundance and PI3K activity^[101]. In the latter study, failure of the increased PIP3 to activate AKT was ascribed to concomitant activation of an inhibitory pathway. It is worthwhile to note that these studies were done with different hypertonicity/hyperosmolality-inducers in different types of cells.

Ataxia telangiectasia-mutated, c-Abl and phosphatidylinositol 3-kinase-IA

Ataxia telangiectasia-mutated (ATM) is a DNA damage-inducible serine/threonine kinase belonging to the PI3K-like kinase family^[102]. PI3K is a family of lipid kinases that phosphorylate the 3'-position hydroxyl of the D-myo-inositol head group to generate specific phosphoinositide forms^[103]. Based on their *in vitro* lipid substrate specificity, structure, and mode of regulation, PI3Ks can be divided into three main classes. Class I, which has class IA and B, synthesizes phosphatidylinositol (3,4)-bisphosphate [PtdIns(3,4)P₂] and phosphatidylinositol (3,4,5)-trisphosphate [PtdIns(3,4,5)P₃]^[103]. It is a heterodimer composed of a p110 catalytic subunit and a p85 regulatory subunit^[103]. c-Abl belongs to a family of non-receptor tyrosine kinases, which has two members, c-Abl and Arg (Abl-related gene)^[104]. These three different types of kinases are reviewed together, because evidence already exists that they act in coordination to regulate high NaCl-induced activation of NFAT5. It has been proposed for a while that hypertonicity/hyperosmolality-induced damages interplay with hypertonicity/hyperosmolality-induced responses^[22,105,106]. The role of ATM in regulation of NFAT5 activity is an example of this theory. High NaCl damages DNA^[107]. High NaCl activates ATM, most likely through high NaCl-induced DNA damage, although it is difficult to directly approve it^[28]. ATM contributes to high NaCl-induced activation of NFAT5 through increasing NFAT5 transactivating activity^[28] and nuclear localization^[108]. Phosphatidylinositol 3-kinase-IA (PI3K-IA) contributes to tonicity-dependent activation of NFAT5 by increasing its transactivation, since over expression of a dominant negative mutant of p85 or by siRNA-mediated knockdown of p110 α reduces NFAT5 transcriptional and transactivating activities^[109]. PI3K-IA acts as an upstream kinase to mediate high NaCl- and ionizing radiation-induced activation of ATM as measured by the stimulatory phosphorylation of ATM^[109]. Since NaCl-induced increase of NFAT5 activity is reduced equally by inhibition of ATM and PI3K-IA, and the effects

are not additive, it is concluded that the effect of PI3K-IA on tonicity-dependent activation of NFAT5 is mediated by ATM^[109]. However, it is not clear why PI3K-IA is not involved in high NaCl-induced increase of nuclear NFAT5^[109]. High NaCl increases c-Abl kinase activity. Like ATM, c-Abl regulates tonicity-dependent activation of NFAT5 through increasing NFAT5 transactivating activity and nuclear localization^[25]. The effect of c-Abl on NFAT5 nuclear distribution is also mediated by direct phosphorylation of NFAT5-Y143^[25]. Over expression of a c-Abl kinase dead mutant abolishes high NaCl-induced phosphorylation (activation) of S1981 of ATM, and high NaCl-induced NFAT5 nuclear accumulation is greatly enhanced in AT cells, which lack active ATM, when wild-type ATM is transfected^[25]. These data indicate that c-Abl regulates NFAT5 activity through ATM. However, it is unlikely that the protein tyrosine kinase c-Abl directly phosphorylates ATM-S1981. Further, the relationship between PI3K-IA and c-Abl in signaling activation of NFAT5 remains unknown.

Mammalian target of rapamycin

Mammalian target of rapamycin (mTOR) is a serine-threonine kinase belonging to the phosphatidylinositol kinase-related kinase family^[110]. It has two multi-protein complex isoforms, mTORC1 and mTORC2. The mTORC1 is composed of regulatory-associated protein of mTOR (Raptor), PRAS40 (also known as Akt substrate 1) and mLST8. mTORC1 is rapamycin-sensitive. mTORC2 combines rapamycin-insensitive companion of mTOR, mSIN1, Protor and mLST8^[110]. mTOR controls cell growth and division in part through regulating ribosomal p70 S6 kinase and the eukaryotic translation initiation factor 4E binding proteins^[110]. It is well-known that mTORC1 is activated by PI3K-AKT axis^[110]. Whether this mechanism is also present in the hypertonic setting is not clear. High NaCl increases PI3K-IA kinase activity in HEK293 cells^[109] and phosphorylation (activation) of AKT1-S473 in mCCD_{cl1} or HepG2 cells^[97], but analyses of diagnostic substrates downstream mTORC1 by phosphorylated-S235/236 in the ribosomal subunit S6, and phosphorylation-dependent electrophoretic mobility shift of 4E-BP1 and mTORC2 by phosphorylation of S473 of AKT show that hypertonicity partially inhibits both complexes in the immortalized wild-type adenosine monophosphate-activated protein kinase (AMPK) mouse embryonic fibroblasts^[111]. The discrepancy could be due to different types of cells used. Nevertheless, based on the inhibitory effects of the mTOR inhibitors, torin1 and rapamycin, on high NaCl-induced expression of NFAT5-targeted genes and NFAT5 transcriptional reporter activity, it is concluded that mTOR contributes to tonicity-dependent activation of NFAT5. The effect of mTOR is probably due to facilitating a transcription-permissive condition for NFAT5 by enhancing histone H4 acetylation and the recruitment of RNA polymerase II^[111]. It should be pointed out that in human colon cancer cell lines under an isotonic condition, NFAT5 activates expression of a DNA damage-response kinase, REDD1, which in

turn inhibits mTOR signaling^[112].

Src family kinases

Src kinase is a family of non-receptor tyrosine kinases that regulate a wide variety of cellular activities such as cell adhesion and motility, carcinogenesis, immune cell function, and even learning and memory. This family has 12 members: c-Src, Fyn, Yes, Yrk, Lyn, Hck, Fgr, Blk, Lck, Brk, Srm, and Frk (with Frk/Rak and Iyk/Bsk subfamilies), 11 of which are found in humans^[113]. Src family kinases exhibit a common modular architecture dominated by so-called "SRC homology," or SH domain. SH1 is the catalytic domain. In the inactive state, a key tyrosine in this domain (Y416) blocks the substrate binding site. When autophosphorylated, this residue is displaced and substrate access is unimpeded. SH2 and SH3 are protein-protein interaction domains shared not only among the members but also with many other signaling proteins^[114]. The effects of hypertonicity on activities of Src kinases are heterogeneous. Hypertonicity increases Fyn activity and phosphorylation of its targets^[115,116], whereas it inhibits c-Src activity^[116]. The involvement of Src family kinases in regulation of NFAT5 was suggested by the observation that a Src family kinase inhibitor PP2 reduces NFAT5 transactivating activity and protein abundance in the colon cancer cells^[117]. More convincing evidence comes from studies of Fyn. Using PP2, Fyn dominant negative mutant and Fyn null cells, Ko *et al*^[54] demonstrated that Fyn contributes to hypertonicity-induced activation of NFAT5 by increasing its transactivating activity.

Focal adhesion kinase

Focal adhesion kinase (FAK) is a mechanosensitive non-receptor protein tyrosine kinase that is widely expressed. In response to integrin engagement as occurs in hypertonicity-induced cell shrinkage, FAK is autophosphorylated and activated at Y397, which entails diverse intracellular events. This function makes FAK a central signaling component downstream of integrin^[118]. FAK is abundant in the renal papilla, and furosemide, known to reduce the renal medullary interstitial tonicity, decreases phosphorylation of FAK-Y397 in the region^[32]. Hypertonicity increases time-dependent phosphorylation of FAK-Y397 in HEK293 cells^[32]. FAK contributes to hypertonicity-induced increase of NFAT5 transcriptional activity^[32]. The mechanism underlying this effect is unique, because FAK affects neither hypertonicity-induced increase of nuclear NFAT5 nor NFAT5 transactivating activity. Instead, the effect is mediated by contribution of FAK to hypertonicity-induced increase of NFAT5 protein abundance through stabilizing its mRNA, which depends on NFAT5 3'-UTR^[32]. Integrin $\alpha 1 \beta 1$ is necessary for hypertonicity-induced full activation of NFAT5 in the inner medullary collecting duct cells^[119]. Integrin $\alpha 1$ -null mice have impaired ability to accumulate organic osmolytes in the inner medulla due to decreased expression of NFAT5-targeted

osmoprotective genes and develop early tubular necrosis and increased apoptosis of renal medullary cells following dehydration^[119]. Although integrin regulates NFAT5 activity in renal cells and possible in the renal medulla^[119] and carcinoma cells^[32,117,120,121], whether the effect is through FAK remains to be determined. Besides autophosphorylation at Y397, FAK can be also phosphorylated at multiple tyrosine residues by Src family kinases^[118]. FAK is constitutively active in a renal cell carcinoma cell line Caki-1 under an isotonic condition. This is probably due to a high Src kinase activity in the cells^[55]. The high activities of Src and FAK in Caki-1 cells are in part responsible for the high basal activity of NFAT5 in the cells as compared with that in the non-cancerous proximal tubule cell line HK-2^[55].

Cyclin dependent kinases

The human kinome reveals that the serine/threonine kinase Cyclin dependent kinase (CDK) family has 26 members, of which 21 are classified as CDKs and five form a more distant group of CDK-like kinases^[122,123]. CDKs regulate the cell division cycle, apoptosis, transcription and differentiation. Each CDK serves its function by recognizing its specific substrate or other protein effector through the divergent spots located in an overall conserved architecture^[122,123]. In HEK293 cells, high NaCl activates CDK5, which directly phosphorylates NFAT5-T135. Phosphorylation of NFAT5-T135 is also increased in the rat renal inner medulla^[26]. Inhibition of CDK5 by its siRNA or an inhibitor reduces the increase in NFAT5 transcriptional activity that has occurred by 4 h after NaCl is raised, associated with inhibition of NFAT5 nuclear accumulation at that time, but does not reduce either NFAT5 activity or nuclear NFAT5 after 16 h. This is because high NaCl increases the overall abundance of NFAT5 protein at the later time, which eventually raises its effective level in the nucleus, but the early effect of high NaCl on NFAT5 nuclear localization requires CDK5^[26]. CDK5 has no significant effect on NFAT5 transactivating activity. This is special, because a majority of signaling molecules identified so far affects NFAT5 transactivation activity without altering NFAT5 nuclear localization (reviewed above). Besides CDK5, CDK9 also regulates NFAT5. The targeted proteomics shows that CDK9 is physically associated with DDX5/17, a RNA helicase important in alternative RNA splicing of NFAT5. CDK9 is necessary for DDX5 recruitment to NFAT5 as measured by chromatin immunoprecipitation^[124].

Glycogen synthase kinase 3 β , Casein kinase 1 and 5' -AMPK

In contrast to kinases reviewed above that contribute to high NaCl-induced activation of NFAT5, Glycogen synthase kinase 3 β (GSK3 β), Casein kinase 1 (CK1) and AMPK actually inhibit tonicity-dependent activation of NFAT5. Therefore, these three kinases are reviewed together. GSK3 β is a ubiquitously expressed serine/thre-

online kinase originally characterized as phosphorylating and inactivating glycogen synthase, the rate-limiting enzyme of glycogen synthesis^[125]. Since then, GSK3 β has been found to regulate a wide variety of biological processes such as function of neurons^[126], immunological responses^[127], cardiac hypertrophy^[128] and cancer^[129]. The pleiotropic effects of GSK3 β involve regulation of many transcription factors, such as cAMP response element-binding protein, neurogenin 2, SMAD1, c-Jun, β -catenin^[126] and NFAT1-4^[127]. GSK3 β is unique because unlike most other protein kinases it is most active in cells' resting state, contributing to inhibition of its target transcription factors. When the cells are stimulated, GSK3 β is inhibited, resulting in activation of its substrates. The activity of GSK3 β is inhibited by phosphorylation of serine residues, of which, serine 9 is most studied^[126]. This mechanism is not exceptional in tonicity-dependent activation of NFAT5. GSK3 β inhibits NFAT5 transcriptional activity by reducing NFAT5 transactivating activity and protein abundance under the normal tonicity. High NaCl increases phosphorylation of GSK3 β -S9 and decreases GSK3 β activity, which results in an increase of NFAT5 transcriptional activity mediated by the increment of NFAT5 transactivating activity, but not by NFAT5 nuclear localization or protein abundance^[81]. The lack of the effect of GSK3 β on NFAT5 nucleo-cytoplasmic trafficking is in contrast to its effect on NFAT1-4. GSK3 β phosphorylates the serines in serine-proline repeats, conserved in the amino terminus of NFAT1-4, resulting in promotion of nuclear exit of NFAT1-4 and inhibition of NFAT1-4 transcriptional activity^[130]. Unlike NFAT1-4, NFAT5 does not contain serine-proline repeats in its amino terminus^[2,131]. Instead, its nucleo-cytoplasmic distribution is regulated by phosphorylation of other amino acids in the terminus such as tyrosine 143^[23-25], threonine 135^[26] and serines 155 and 158^[27]. The difference in amino acid composition explains why GSK-3 β affects nuclear localization of NFAT5 differently from that of NFAT1-4.

The stimulatory effect of PKA, PI3K and AKT1 on NFAT5 is dependent on their attenuation of the GSK3 β inhibitory effect on the transcription factor^[81]. Therefore, GSK3 β integrates, at least in part, the effects of PKA, PI3K and AKT1 on NFAT5. However, GSK3 β is not involved in the effect of p38 α on NFAT5, because co-expression of p38 α and its constitutively active upstream kinase MKK6 does not increase phosphorylation of GSK3 β -S9 or reverse the inhibitory effect of GSK3 β -S9 on NFAT5^[81], despite the observations in other settings that p38 α inhibits GSK3 β activity^[132]. On the other hand, low NaCl reduces the inhibitory phosphorylation of GSK3 β -S9, which leads to reduction of NFAT5 mRNA and protein abundance in the mouse inner medullary collecting duct cells^[133]. It is worth noting that the inhibitory effect of high NaCl on GSK3 β may be cell-dependent, because high NaCl reduces the phosphorylation of GSK3 β -S9 and increases the kinase activity in several tumor cell lines^[134] and decreases the phosphorylation of GSK3 β -S9 in the renal medullary interstitial cells^[135]. It

would be interesting to know the effect of high NaCl on NFAT5 activity in these cells.

CK is a group of serine/threonine kinases that can be divided into CK1 and CK2 families based on their high homology in their catalytic domains^[136]. In vertebrates, seven CK1 isoforms (α , β , γ 1, γ 2, γ 3, δ and ϵ) and several splice variants for CK1 α , δ , ϵ and γ 3 have been identified^[136]. This family of kinases has been shown to phosphorylate key regulatory molecules involved in a wide array of cellular activities such as cell cycle, cytokinesis, chromosome and microtubule dynamics and transcription and translation^[136]. NFAT5 nucleocytoplasmic trafficking is regulated by the dual phosphorylation of serine 155 and 158^[27]. Hypotonicity increases phosphorylation of NFAT5-S155, which primes the phosphorylation of serine 158, leading to reduction of nuclear NFAT5^[27]. Unlike GSK3 β , which has no significant effect on NFAT5 cellular trafficking^[81], CK1 α 1L increases phosphorylation of NFAT5-S158, contributing to hypotonicity-induced decrease of nuclear NFAT5^[27].

The serine/threonine kinase AMPK is a major cellular energy sensor that exists as a heterotrimer composed of a catalytic α subunit and each of regulatory β and γ subunits^[137]. A high level of AMP or a low level of ATP activates AMPK through phosphorylation of the kinase, resulting in inhibition of energy consumption and stimulation of energy production, which leads to restoration of energy homeostasis^[137]. Hypertonicity inhibits the kinase as measured by phosphorylation of the enzyme in the renal medullary interstitial cells (RMIC)^[138]. Pharmacological activators of AMPK reduce high NaCl-induced NFAT5 nuclear localization and expression of NFAT5-targeted genes in the cultured RMIC and increases dehydration-induced apoptosis in the mice medulla, suggesting that AMPK inhibits tonicity-dependent activation of NFAT5^[138]. Further, the anti-diabetes medication metformin activates AMPK and inhibits NFAT5 transcriptional activity in RMIC and increases RMIC apoptosis in both normally hydrated and dehydrated type 2 diabetes mice^[40]. However, since metformin and the pharmacological activators have other effects besides activation of AMPK, whether AMPK inhibits tonicity-dependent activation of NFAT5 needs to be confirmed with a specific way of manipulating the kinase.

Summary and perspective

NFAT5 is clearly critical for kidney functions. Emerging evidence has shown that its dis-regulation results in or is associated with the renal diseases and disorders. Figure 1 summarizes currently known protein and lipid kinases that involve in regulation of tonicity-dependent activation of NFAT5. More are expected to come. Cells need these kinases working together to orchestrate a specific signal to NFAT5 in response to hypertonic or hypotonic perturbation. These kinases could fulfill their assignments by their different activation duration and strength, and their network with each other as well as with other signaling molecules and scaffolds in a

specific subcellular location and time. Further work is needed to provide direct pieces of evidence to support this hypothesis. A vast majority of these kinases were identified in cultured cells. They need to be tested directly in the kidney to determine whether they have the same functions *in vivo*. Inhibition of NFAT5 results in the urinary concentration defect, indicative of a decrease in the renal medullary interstitial tonicity^[5]. The decrease of the renal medullary tonicity inhibits NFAT5 activity^[139]. It is difficult to dissect whether the effect of knockout of a kinase, even when it is done in the kidney epithelium-specific manner, on NFAT5 is from the direct effect on the transcription factor or from an indirect effect secondary to alteration of tonicity in the renal medullary interstitium. This challenge calls for a new technology to address how NFAT5 is regulated in the kidney medulla.

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Water, electrolytes, and acid-base alterations in human immunodeficiency virus infected patients

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Abstract

The clinical spectrum of human immunodeficiency virus (HIV) infection associated disease has changed significantly over the past decade, mainly due to the wide availability and improvement of combination antiretroviral therapy regimens. Serious complications associated with profound immunodeficiency are nowadays fortunately rare in patients with adequate access to care and treatment. However, HIV infected patients, and particularly those with acquired immune deficiency syndrome, are predisposed to a host of different water, electrolyte, and acid-base disorders (sometimes with opposite characteristics), since they have a modified renal physiology (reduced free water clearance, and relatively increased fractional excretion of calcium and magnesium) and they are also exposed to infectious, inflammatory, endocrinological, oncological variables which promote clinical conditions (such as fever, tachypnea, vomiting, diarrhea, polyuria, and delirium), and may require a variety of medical interventions (antiviral medication, antibiotics, antineoplastic agents), whose combination predispose them to undermine their homeostatic capability. As many of these disturbances may remain clinically silent until reaching an advanced condition, high awareness is advisable, particularly in patients with late diagnosis, concomitant inflammatory conditions and opportunistic diseases. These disorders contribute to both morbidity and mortality in HIV infected patients.

Key words: Human immunodeficiency virus; Acquired immune deficiency syndrome; Salt; Water; Potassium; Acid-base

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Core tip: Human immunodeficiency virus infected patients, and particularly those with acquired immune

deficiency syndrome, are predisposed to different water, electrolyte, and acid-base disorders since they have a modified renal physiology and they also are exposed to infectious, inflammatory, endocrinological, oncological, and pharmacological variables whose combination undermine their homeostatic capability. We herein discuss each of these internal milieu alterations usually observed in this group.

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INTRODUCTION

The clinical spectrum of human immunodeficiency virus (HIV) infection associated disease has changed significantly over the past decade, mainly due to the wide availability and improvement of combination antiretroviral therapy regimens. Serious complications associated with profound immunodeficiency are nowadays fortunately rare in patients with adequate access to care and treatment. Currently, most complications observed in patients with HIV infection are derived from serious but non-acquired immune deficiency syndrome (AIDS) defining clinical events, which are more frequent due to the chronic inflammatory status promoted by the virus itself and are further aggravated by the use of some antiretroviral agents^[1,2].

Renal disorders have been increasingly reported in the context of human retroviral infection, particularly the decrease over time of estimated glomerular filtration rate (eGFR), nephrotic syndrome, and proximal tubular deficiency associated with the use of tenofovir disoproxil-fumarate (TDF) and some protease inhibitors such as lopinavir/ritonavir and atazanavir^[3]. Although periodic evaluation of renal function (*e.g.*, serum creatinine, eGFR) and proteinuria are routinely recommended in the care of these patients, much less is known or published about specific renal water handling abnormalities, electrolyte disturbances and alterations of acid-base balance in patients with HIV infection^[3-5].

HIV infected patients, in particular those with advanced disease may be affected by infectious, autoimmune and oncologic diseases that promote clinical conditions (such as fever, tachypnea, vomiting, diarrhea, polyuria, and delirium), and may require a variety of medical interventions (antiviral medication, antibiotics, antineoplastic agents), whose combination predispose them to develop different sort of electrolytes disorders^[6,7].

In the present report, renal water, electrolyte, and acid-base disorders in the HIV infected patients are analyzed.

RENAL WATER AND ELECTROLYTES HANDLING IN HIV PATIENTS

Renal water and electrolytes handling in HIV patients on different therapeutic regimens including with tenofovir, with non-tenofovir, and without antiretroviral drugs (naïve). These exploratory renal physiology studies (urine concentration and dilution tests) found no significant differences in sodium, potassium, chloride, phosphorus, calcium, magnesium, glucose, urea, and uric acid renal handling between healthy volunteers and stable HIV patients with normal renal function, independently whether or not they were receiving antiretroviral therapy. However, a significant reduction in maximal urine concentration - dilution capability in stable HIV patients compared to healthy volunteers was consistently documented. In this study maximum free water clearance showed values three times lower in HIV patients than in the healthy volunteers despite normal osmolar clearance^[8,9]. This finding may explain the reason why HIV patients were slightly hyponatremic during the dilution test. The urine concentration-dilution defect was attributed to a dysfunction in the thick ascending limb of the loop of Henle (TAHL). Since, HIV has been detected in renal tubular cells, this suggests that either the infection itself or the associated inflammatory process may produce direct tubular damage which appears to be independent of the presence of antiretroviral treatment. This finding also means that there may be an increased risk of developing hyponatremia in stable HIV-infected patients who undergo a water load or receive hyponatremia inducing drugs, as well as dehydration when they are exposed to settings of water loss and impaired thirst or intake of water^[8-10]. A recent study has shown that in a setting of volume expansion, where tubule reabsorption is reduced because of the high urinary flux, there was a significant reduction in serum calcium and magnesium values, as well as a concomitant and significant increase in their urinary fractional excretion in stable HIV-positive patients compared to healthy volunteers^[9]. Since calcium and magnesium are importantly reabsorbed in TAHL, and this segment show dysfunction in this population, a basal TAHL reabsorption defect worsened by the increased urinary flux was suggested. This finding means that there is an increased risk for developing hypocalcemia or hypomagnesemia in stable HIV-infected patients who undergo volume expansion or who receive hypocalcemia or hypomagnesemia-inducing drugs^[9-11].

SALT AND WATER BALANCE IN HIV INFECTION AND AIDS

Salt and water imbalances can induce abnormalities in extra-cellular volume status and/or serum sodium depending on the nature of this alteration (increase or decrease), its absolute magnitude (mild or severe), and

its relative magnitude (body sodium content relative to body water content)^[12]. A significant salt and water depletion generates real hypovolemia, and if this depletion involves an excess of hypotonic fluid loss, it can generate hypernatremia (serum sodium > 145 mmol/L), while if the loss of salt is in excess of water it may generate hyponatremia (serum sodium < 135 mmol/L)^[12].

Salt and water retention induces an increase in ECF that, depending on its pathophysiologic mechanism, it may appear either as hypervolemia and edema (*e.g.*, renal failure) or effective arterial hypovolemia and edema (*e.g.*, cirrhosis, cardiac failure, some of nephrotic syndromes). Another factor that can modify the sodium/water ratio is body potassium content since its intracellular depletion induces hyponatremia by at least two mechanisms: A shift of sodium to the intracellular space, and possibly by aberrant vasopressin release. Edelman summarized these concepts in the following equation^[13]: Serum sodium = [body (exchangeable) sodium content + body (exchangeable) potassium content]/total body water content.

Additionally, there are two infrequent causes of hyponatremia: First, a hyponatremia secondary to an overtly excessive water intake which overcomes renal capability of free water excretion and is associated with fully suppressed vasopressin secretion, especially in states of low osmolar excretion. This type of hyponatremia has been documented in AIDS patients who suffered from dementia and primary polydipsia^[14]. Second, a reset osmostat hyponatremia, usually found in malnourished chronically-ill AIDS patients^[12]. Based on the above mentioned pathophysiological mechanisms, hyponatremia is currently classified depending on patient's plasma tonicity level into: Hypertonic, normotonic, or hypotonic hyponatremia. In addition, hypotonic hyponatremia is classified depending on patient's extracellular fluid (ECF) status with low, normal or high ECF^[12]. Each type of hyponatremia in AIDS patients was described as follows:

HYPONATREMIA

Normotonic hyponatremia

Normotonic hyponatremia or pseudohyponatremia (PSH) consist of a low serum sodium value in a context of normal plasma tonicity, since it is a measurement artifact caused by an increase in the solid fraction of plasma, usually due to hyperlipidemia or hyperproteinemia^[4]. A direct ion-sensitive electrode potentiometry-based estimation can avoid this error^[15]. Also, the addition of a non-electrolyte osmoles (sorbitol, manitol, sucrose) to the extra-cellular space with redistribution of sodium-deficient water from the intracellular space can cause this finding^[14]. PSH has been described in HIV patients who have important hypergammaglobulinemia which may be related to disease progression or its response to antiretroviral therapy. Besides, polyclonal

hypergammaglobulinemia in this population it may also be secondary to a co-infection with hepatitis C. It is important to identify PSH since treating it as hypotonic hyponatremia can cause severe dehydration and even death^[14,16].

Hypertonic hyponatremia

Since in absence of renal failure, plasma osmolality (Posm) is mainly determined by serum sodium and glucose level (Calculated Posm = serum sodium × 2 + glycemia/18 + uremia/6), hypertonic hyponatremia is observed in hypertonic variety of uncontrolled diabetes mellitus with severe hyperglycemia. Hyperglycemia increases extracellular tonicity which extracts sodium-deficient water out of the intracellular space diluting the serum sodium concentration in the extracellular space, inducing hyponatremia^[12]. Other solutes, like sorbitol and manitol can behave similarly.

Hypotonic hyponatremia

Patients with cardiac, hepatic, renal, lung, intracranial, and endocrine diseases can develop hypotonic hyponatremia secondary to an excess of water consumed voluntarily or administered iatrogenically, when urine free water excretion is impaired due to a decreased circulatory delivery of fluid to diluting segments (cardiac failure), altered TALH segment function (tubulopathy), and/or (inappropriate or appropriate) vasopressin release^[12].

Since impairment of the function of the afore mentioned organs is frequent in the context of AIDS and associated complications, hyponatremia is not surprisingly the most frequent electrolyte abnormality (23.5%-75%) seen both in non-hospitalized and hospitalized patients with HIV infection and AIDS^[17-20]. Hyponatremic patients with AIDS are more prone to morbidity and mortality and frequently manifest complicating opportunistic infection-related illnesses (particularly *Pneumocystis jiroveci* and cytomegalovirus)^[19]. However, this poor prognosis has not been attributed to this electrolyte disorder since most of the patients were normonatremic at death, and their higher mortality has been attributed to the severity of their immune-compromised state: For instance, severe hyponatremia (serum sodium < 125 mmol/L) was associated to a lower CD4 T cell count than in AIDS patients who did not have hyponatremia^[8,17,21,22].

Dao *et al*^[23] also reported a higher mortality rate among women who showed hyponatremia and hypochloremia (in that context it means a serum chloride value significantly lower respect to the expected one for hyponatremia) compared with women who only had one electrolyte abnormality. This observation suggests that a combination of both disorders (hyponatremia + hypochloremia) may suggest a more profound clinical disturbance in a HIV patient, such as the one secondary to subclinical tuberculosis or cryptococcal lung or cerebral infection. Each type of hypotonic hyponatremia in AIDS patients has been described as follows:

Table 1 Causes of hypotonic hyponatremia in human immunodeficiency virus infected patients

Hyponatremia with normal ECF
SIADH: Lungs or central nervous system infection or neoplasm
Hypothyroidism: Low T3 syndrome, pituitary infections, thyroiditis and miconazole
Glucocorticoid deficiency: Glucocorticoid axis damaged
Hyponatremia with low ECF (volume depletion)
Digestive losses: vomiting, diarrhea
Renal losses: CSW, interstitial nephritis, cortisol resistance and adrenal insufficiency
Hyponatremia with high ECF (edematous states)
Non-renal causes: cirrhosis, heart failure
Renal causes: acute tubular necrosis, intra-tubular obstruction, interstitial nephritis, nephrocalcinosis, hemolytic-uremic syndrome, collapsing focal and segmental glomerulosclerosis
Hyponatremia secondary to drugs
Renal insufficiency
Interstitial nephritis
Impair maximal urinary dilution capability by direct tubular effect
Cortisol deficiency
SIADH effect

ECF: Extracellular fluid; SIADH: Syndrome of inappropriate antidiuretic hormone release; CSW: Cerebral salt wasting.

Hyponatremia with normal ECF (Table 1)

Syndrome of inappropriate antidiuretic hormone release:

This is an entity induced by free water retention secondary to an inappropriate (for the level of serum osmolality) vasopressin hormone release or an excessive response of its receptor (V2 receptor) in the collecting tubules, in the context of normal GFR, normal thyroid and adrenal gland function, and in the absence of hyponatremia inducing drugs^[12,24].

Syndrome of inappropriate antidiuretic hormone release (SIADH) may be present in up to 36% of patients with advanced HIV infection and it can be induced by infection (neurosyphilis, *etc.*) neoplasm of the lungs or central nervous system^[8,25]. SIADH must be differentiated (not always easy) from cerebral salt wasting syndrome (CSW) since both entities can appear in AIDS patients, and may present as hyponatremia with high urinary sodium, and elevated circulating natriuretic peptide and vasopressin levels. However, CSW patients show clinical signs of hypovolemia, increased serum urea:creatinine ratio, normal or high serum uric acid, lower fractional excretion of uric acid, and very high urinary sodium levels, while SIADH patients show slight hypervolemia, low urea: Creatinine ratio, low serum uric acid, higher fractional excretion of uric acid, and high urinary sodium levels^[12,26].

Central pontine myelinolysis, a severe neurological disease that may be observed in hyponatremia and its overly rapid correction, has also been documented in patients with AIDS, particularly in those with advanced HIV infection, prolonged hyponatremia, anorexia, hypoalbuminemia, chronic alcoholism, disseminated malignancy, and in those patients treated with systemic chemotherapy. The clinical presentation varies between rapidly evolving spastic paraparesis with pseudobulbar palsy, and changes in mental state such as confusion or coma^[26-28].

Hyponatremia secondary to hypothyroidism:

Several hyponatremia-inducing mechanisms have been described in patients suffering from hypothyroidism, such as reduced function of the nephron diluting segment due to low renal perfusion secondary to decrease cardiac output, inappropriate vasopressin secretion, and increased urinary salt loss^[29-36].

The most frequent cause of hypothyroidism in AIDS patients is the "low T3 syndrome" which shows a normal thyroid production of T3, but an impaired peripheral conversion of T4 to T3, since 80% of serum T3 usually comes from T4 deiodination in peripheral tissues. Another cause of reduction in thyroid function in this population is centrally-induced hypothyroidism secondary to pituitary infections caused by *Pneumocystis*, cytomegalovirus, toxoplasmosis, neurosyphilis, and HIV itself; or decrease in hypothalamic thyrotropin releasing hormone due to the wasting syndrome induced by AIDS (non-thyroidal illness syndrome)^[25,36]. Finally, hypothyroidism secondary to Hashimoto's thyroiditis (autoimmunity induced by increased B cell activation), and antifungal agents such as miconazole have been described^[36,37].

Hyponatremia secondary to glucocorticoid deficiency:

Since cortisol exerts a negative effect on neurophysiological vasopressin secretion, its deficit can promote an inappropriate vasopressin release, and consequently an increased trend to hyponatremia^[37]. The isolated cortisol deficit can be generated by any infectious, immunologic, or oncologic damage in the glucocorticoid axis^[38,39].

Hyponatremia with low ECF

The most common cause of hyponatremia in the AIDS population is one caused by volume depletion secondary to vomiting, diarrhea, or tubular disorders^[8]. Volume depletion can induce hyponatremia by stimulating the non-osmotic vasopressin release, an appropriate response for protecting the intravascular volume, in a setting of an adequate or excessive oral water (hypotonic

solution) intake^[40]. Sodium losses lead to hypovolemia and consequently induce adequate vasopressin secretion, thus hyponatremia is promoted in this case by a double mechanism: A reduction in body sodium content (sodium loss) and an increase in body water content (water retention). Negative sodium balance is worsened in settings where sodium reabsorption is ineffective, as is the case in CSW, interstitial nephritis, adrenal insufficiency^[12] (Table 1).

Gastrointestinal losses: This is the second most common cause of hyponatremia in patients with HIV infection and AIDS, particularly when it is represented by diarrhea (induced by HIV or other organisms) in a setting of low electrolyte content fluid replacement^[8,12].

CSW: CSW is an uncommon disorder characterized by hyponatremia, volume depletion and clinical response to water and salt replacement^[26]. The etiology of this entity has been attributed to a decrease in the sympathetic nervous system outflow leading to decrease sodium reabsorption in proximal tubules, inhibition of RAAS, and also release of natriuretic peptides (e.g., atrial and brain natriuretic peptides)^[26,39]. CSW occurs in patients with a central nervous system insults, and its similarity with SIADH makes crucial its recognition as water restriction, a SIADH-oriented treatment, is detrimental to patients with unrecognized CSW^[21,22]. Even though, differentiation between CSW and SIADH is not so simple, CSW tends to be characterized by the presence of clinical hypovolemia, normal or increased serum urea and uric acid levels, and polyuria with much more higher sodium excretion compared to SIADH^[39,40,41].

Interstitial nephritis: Interstitial nephritis represents another potential cause of urine loss of salt which can induce volume depletion in AIDS patients since they are exposed to polypharmacy and/or autoimmunity disorders^[38].

Adrenal insufficiency: The prevalence of adrenal insufficiency is up to 22% in AIDS patients^[42,43]. Both HIV itself as well as concomitant disseminated tuberculosis can cause suppression of hypothalamus-pituitary-adrenal axis and destruction of adrenal gland; and this may lead to adrenal insufficiency and subsequent hyponatremia. Other opportunistic organisms that can induce hypoadrenalism in HIV patients are Cytomegalovirus, *Cryptococcus neoformans*, *Mycobacterium avium-intracellulare*, *Pneumocystis jiroveci*, *Histoplasma capsulatum*, and *Blastomyces dermatitidis*. Moreover, hypoadrenalism in this population can be induced by adrenal gland damage due to Kaposi's sarcoma, lymphoma, or adrenocortical hemorrhage secondary to a coagulopathy, as well as to pharmacological intervention, as is the case of ketoconazole (inhibition of steroids synthesis), rifampicin, and phenytoin (increased cortisol metabolism)^[39-44].

Cortisol resistance: In this entity, patients suffering from advanced HIV infection present clinical features suggestive of hypoadrenalism, such as asthenia, mucocutaneous melanosis, hypovolemic hyponatremia, but serum testing reveal high serum cortisol and normal/high adrenocorticotrophic hormone levels. This particular clinical setting of cortisol resistance characteristically improves with high doses of glucocorticoids^[45-47]. In this case the presence of hyperkalemia with low potassium excretion can help to differentiate this entity from CSW^[38].

Hyponatremia with high ECF

This sort of hyponatremia is observed in severe edematous states secondary to cardiac, hepatic or renal insufficiency, as well as uncommonly in severe nephrotic syndrome.

In clinical settings of effective hypovolemia such as severe cardiac or hepatic insufficiency, and some nephrotic syndromes, hypotonic hyponatremia appears as a consequence of an impaired circulatory delivery to diluting segments, in combination with adequate vasopressin release (effective hypovolemia)^[12]. On the other hand, in clinical settings of hypervolemia such as severe renal insufficiency, hypotonic hyponatremia appears as a consequence of an impaired capability of free water excretion due to a significantly decrease in GFR (lower than 5 mL/min per 1.73 m²)^[12].

Renal insufficiency is a well-known complication in HIV positive patients, usually induced by a heterogeneous collection of miscellaneous mechanisms: Acute tubular necrosis (toxic, ischemic), intra-tubular obstruction from uric acid or phosphate (tumor destruction), different type of glomerular (glomerulonephritis, etc.), tubulointerstitial (interstitial nephritis, nephrocalcinosis, etc.), and vascular diseases (atypical hemolytic-uremic syndrome), and also a particular type of focal and segmental glomerulosclerosis only found in this population called HIV associated nephropathy often of a collapsing variant^[8,22,26,48] (Table 1).

Hyponatremia secondary to drugs

Drug induced hyponatremia is the third most common cause of hyponatremia in AIDS patients^[8].

Medication can induce hyponatremia by different mechanisms, and therefore this type of hyponatremia described here separately. AIDS patients may frequently receive medications that can induce hyponatremia by promoting water retention and/or sodium loss^[17,49,50]: (1) renal insufficiency (co-trimoxazole); (2) interstitial nephritis (trimethoprim, loop diuretics, thiazides); (3) impair maximal urinary dilution capability by direct tubular effect (thiazides); (4) cortisol deficiency (rifampin, ketoconazole, suramin); (5) SIADH effect (pyrazinamide, ethambutol, narcotics, lopinavir, ritonavir); and (6) undefined mechanism (amphotericin B, pentamidine) (Table 1).

Table 2 Causes of hypernatremia in human immunodeficiency virus infected patients

Hypernatremia
Increased insensible water losses: Fever and tachypnea
Increased digestive water losses: Vomiting, diarrhea
Increased urinary water losses: Central diabetes insipidus, nephrogenic diabetes insipidus secondary to nephrocalcinosis or tubule-interstitial damage caused by infection, tumors, drugs
Reduced water intake: Unconsciousness, adipsia: Thirst's center destruction by a vascular, neoplastic or infectious cause

HYPERNATREMIA

This disorder occurs when a large loss of free water is combined with an inadequate amount of water ingestion or insufficient iatrogenic provision of water in unconscious patients, and it was reported in up to 31% of patients with very advanced disease^[49-51]. Among the main causes of free water loss in AIDS patients are^[26,48]: (1) fever with insensible water losses through the lung and skin; (2) digestive water losses: Vomiting, diarrhea; (3) central diabetes insipidus secondary to toxoplasmosis or cytomegalovirus encephalitis; and (4) nephrogenic diabetes insipidus secondary to nephrocalcinosis, tubule-interstitial diseases caused by infections (cytomegalovirus, *Mycobacterium avium* intracellulare, systemic mycoses), tumors (lymphoma), or medication, such as rifampin, foscarnet, and amphotericin B.

Regarding hypernatremia secondary to low water intake in AIDS patients, it has been described in unconscious patients affected by a neurological disorder, or in those patients suffering from adipsia. The latter is a rare hypothalamic condition in which a conscious patient develops serum hyperosmolality secondary to reduced water intake because he/she have no thirst. This disorder commonly is associated with lack of vasopressin release, which was attributed to vascular, neoplastic, or granulomatous destruction of the osmoreceptor and thirst center^[52] (Table 2).

POTASSIUM IMBALANCE IN AIDS

Potassium is the main cation in the intracellular space, its total body content in healthy adults is around 3700 mmol, and muscle tissues represent its main body reserve. Potassium has two significant balances: The external balance between the organism and the environment, and the internal balance between the intracellular compartment and the extracellular compartment within the organism^[53-55]. The external balance depends on nutrition as well as colonic (20%) and renal (80%) potassium excretion, and this excretion depends both on GFR and potassium distal tubule secretion, which is mainly stimulated by aldosterone hormone. The internal balance depends on the potassium shifts between intracellular and extracellular compartments. Insulin and the adrenergic system are the main stimuli for its intracellular shift along with metabolic alkalosis, plasma hypotonicity and beta-adrenergic sympathetic tone, while the main stimuli for its extracellular shift are

glucagon, metabolic acidosis, plasma hypertonicity, and alpha-adrenergic sympathetic tone^[53-56].

Hypokalemia

Hypokalemia (serum potassium < 3.5 mmol/L) has been reported in about 19% of patients with AIDS^[54]. The main causes of hypokalemia in this population are gastrointestinal potassium losses, usually induced by profuse diarrhea secondary to intestinal infection, intestinal tumor, or AIDS-associated enteropathy^[8,57]. Vomiting is another important cause of hypokalemia, not only by direct potassium loss (emesis) but also increasing urinary potassium excretion by inducing hypovolemia, bicarbonaturia and consequently secondary hyperaldosteronism^[55]. Urinary potassium wasting can also accompany tubule injury secondary to direct toxic effect of nephrotoxic drugs (e.g., amphotericin B, aminoglycosides) or interstitial nephritis or secondary to some antibiotics (e.g., sulfonamides, cephalosporins) or non-steroidal anti-inflammatory (NSAIDs) drugs^[56-62]. Anorexia and low potassium intake, sarcopenia and myopathy (low potassium body reserves) observed in HIV-associated wasting syndrome exacerbate the risk of hypokalemia in this population^[8,63]. In addition, acquired tubulopathies can also induce urinary electrolytes wasting, and as a consequence hypomagnesemia, hypocalcemia, and hypophosphatemia (Fanconi syndrome) can develop in this population^[64-70]. Among the main tubulopathy inducing drugs in AIDS patients are: TDF, foscarnet, zidovudine and didanosine^[67-72] (Table 3).

Hyperkalemia

Hyperkalemia (serum potassium > 5.5 mmol/L) has been reported in 5%-53% of AIDS patients^[55,73]. Two main mechanisms of hyperkalemia have been described in these patients. First, reduced urinary potassium excretion (external balance), such as the one observed with severe renal failure (GFR < 5 mL/min per 1.73 m²), hyperkalemia inducing drugs (ACEIs, NSAIDs, trimethoprim), adrenal insufficiency, and hyporeninemic hypoaldosteronism^[21,39-43,51,74-77]. Second, increased shift of potassium from the intracellular compartment to the extracellular compartment (internal balance), such as, rhabdomyolysis, tumor lysis syndrome after chemotherapy in AIDS patients affected by malignancies, and diabetes mellitus^[77-80]. In this case plasma hypertonicity induced by severe hyperglycemia, develop hyperkalemia through osmotically induced water and potassium shifts from the intracellular compartment to

Table 3 Causes of dyskalemia in human immunodeficiency virus infected patients

Hypokalemia
Increased gastrointestinal K ⁺ losses: Diarrhea; Infection, tumor or AIDS-associated enteropathy
Increased urinary K ⁺ losses: Vomits, tubule toxicity, interstitial nephritis
Low K ⁺ body content: Low potassium intake, sarcopenia and myopathy
Hyperkalemia
Reduced urinary K ⁺ excretion: Drugs, adrenal insufficiency, hyporeninemic hypoaldosteronism
Increased K ⁺ shift to EC: Rhabdomyolysis, tumor lysis syndrome, hyperglucemia

K⁺: Potassium; EC: Extracellular compartment; AIDS: Acquired immune deficiency syndrome.

Table 4 Causes of acid-Base disorders in human immunodeficiency virus infected patients

Acidosis
Hyperchloremic metabolic acidosis: Diarrhea, tubular damage secondary to drugs, hypergammaglobulinaemia, acute tubular necrosis, interstitial nephritis, HIV
High anion gap metabolic acidosis: Uremia, diabetic ketoacidosis, lactic acidosis (type A or B)
Alkalosis
Metabolic alkalosis (volume contraction): Gastro-intestinal losses, urinary losses
Respiratory alkalosis (hyperventilation): Central nervous system alteration, altered liver function, lung opportunistic infections and malignancies

HIV: Human immunodeficiency virus.

the extracellular (intravascular) compartment^[12] (Table 3).

ACID-BASE DISORDERS

Acid-base imbalance generates different sort of internal milieu disorders such as acidosis, alkalosis, or their combination (double or triple acid-base disorders). Acidosis is the pathophysiologic process characterized by either a primary acid gain or a primary alkali loss, while acedemia indicates an increased H⁺ concentration in the blood (blood pH < 7.36). Conversely, alkalosis is the pathophysiologic process characterized by either a primary acid loss or primary alkali gain, and alkalemia indicates a decreased H⁺ concentration in the blood (blood pH > 7.44). Additionally, acidosis is usually classified depending on its pathophysiologic mechanism in respiratory acidosis (carbon dioxide retention), normochloremic or high anion-gap metabolic acidosis (bicarbonate conversion), and hyperchloremic or normal anion-gap metabolic acidosis (bicarbonate loss). On the other hand, alkalosis is usually classified depending on their pathophysiologic mechanism in respiratory alkalosis (carbon dioxide high excretion) and metabolic alkalosis (bicarbonate gain)^[81].

In AIDS patients the main cause of hyperchloremic (normal anion-gap) metabolic acidosis is bicarbonate loss through profuse diarrhea or renal tubule dysfunction induced by drugs (TDF, pentamidine, amphotericin, B, rifampicin, ethambutol, cidofovir, adefovir, abacavir or nelfinavir), hypergammaglobulinaemia, renal diseases (acute tubular necrosis, atopic or infectious interstitial nephritis), adrenal insufficiency (type IV distal tubule acidosis), and even HIV direct tubular cytopathic effect^[11,51,56,74,75,82-91].

On the other hand, normochloremic (high anion-

gap) metabolic acidosis has been documented in AIDS during severe renal failure (uremic acidosis), diabetic acidosis (ketoacidosis) secondary to pentamidine-induced pancreatic damage, and in sepsis, systemic inflammatory response syndrome, or non-Hodgkin lymphoma (hypoxic lactic acidosis: Type A)^[8,22,26,82,83]. It is worth mentioning that lactic acidosis secondary to lymphoma is considered a paraneoplastic syndrome of poor prognosis, since lactate production increases as the aggressive tumor outgrows its blood supply resulting in local hypoxia in the absence of any systemic hypoxia or hypoperfusion. As pathophysiological mechanism an increased glycolytic activity causing an increase in lactic acid generation, overexpression of the glycolytic enzyme hexokinase II or increased IGF-binding protein activity, has been proposed^[83].

A particular type of non-hypoxic lactic acidosis (type B) has been described with the use of antiretroviral drugs that are no longer recommended, such as zalcitabine, stavudine, didanosine or zidovudine. This entity is explained mainly by mitochondrial toxicity and reveals hyperlactataemia without lactic acidosis to overt life-threatening lactic acidosis^[86-97]. These antiretroviral drugs are nucleosidic inhibitors of viral reverse transcriptase which alter mitochondrial function by inhibiting the mitochondrial DNA polymerase gamma (the enzyme responsible for the replication of mitochondrial DNA). The diminution in this DNA content provokes a diminished synthesis of respiratory chain enzymes^[94]. Metabolic alkalosis is frequently induced in these patients by volume contraction secondary to gastrointestinal (vomiting, diarrhea) or urinary losses (diuretics, polyuria, etc.)^[8,84-86,90]. Opportunistic infections (*e.g.*, histoplasmosis, etc.) and malignancies affecting the respiratory tract, the central nervous system, or/and liver function can stimulate hyperventilation and as a

consequence induce respiratory alkalosis^[98,99] (Table 4).

CONCLUSION

HIV infected patients, and particularly those with AIDS, are predisposed to a host of different water, electrolyte, and acid-base disorders (sometimes with opposing effects), since they are exposed to infectious, inflammatory, oncological, and pharmacological variables whose combination undermine their homeostatic capability. As many of these disturbances may remain clinically silent until reaching an advanced condition, high awareness is advisable, particularly in patients with late diagnosis, concomitant inflammatory conditions and opportunistic diseases. These disorders contribute to both morbidity and mortality in HIV infected patients.

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Adult stem cells as a tool for kidney regeneration

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Abstract

Kidney regeneration is a challenging but promising strategy aimed at reducing the progression to end-stage renal disease (ESRD) and improving the quality of life

of patients with ESRD. Adult stem cells are multipotent stem cells that reside in various tissues, such as bone marrow and adipose tissue. Although intensive studies to isolate kidney stem/progenitor cells from the adult kidney have been performed, it remains controversial whether stem/progenitor cells actually exist in the mammalian adult kidney. The efficacy of mesenchymal stem cells (MSCs) in the recovery of kidney function has been demonstrated in animal nephropathy models, such as acute tubular injury, glomerulonephritis, renal artery stenosis, and remnant kidney. However, their beneficial effects seem to be mediated largely *via* their paracrine effects rather than their direct differentiation into renal parenchymal cells. MSCs not only secrete bioactive molecules directly into the circulation, but they also release various molecules, such as proteins, mRNA, and microRNA, in membrane-covered vesicles. A detailed analysis of these molecules and an exploration of the optimal combination of these molecules will enable the treatment of patients with kidney disease without using stem cells. Another option for the treatment of patients with kidney disease using adult somatic cells is a direct/indirect reprogramming of adult somatic cells into kidney stem/progenitor cells. Although many hurdles still need to be overcome, this strategy will enable bona fide kidney regeneration rather than kidney repair using remnant renal parenchymal cells.

Key words: Adult stem cells; Direct reprogramming; Extracellular vesicles; Mesenchymal stem cells; Paracrine factors; Indirect reprogramming

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Core tip: Although intensive studies have been performed to isolate kidney stem/progenitor cells from the mammalian adult kidney, whether stem/progenitor cells actually exist in the adult kidney is still debated. Mesenchymal stem cells seem to exert beneficial effects *via* paracrine effects rather than by direct differentiation into renal parenchymal cells. In this review, we also introduce potential roles of extracellular vesicles released

from stem cells and direct/indirect reprogramming of adult somatic cells by which kidney stem/progenitor cells will be formed in the future.

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INTRODUCTION

The kidney is a vital organ that plays various roles, such as the excretion of waste products; regulation of systemic fluid volume, electrolytes, and pH; maintenance of systemic blood pressure; and erythropoietin production. These functions are performed by the nephrons, the functional units of the kidney. If the structure and/or function of the nephrons are damaged because of diseases, such as diabetes, hypertension, and glomerulonephritis, and if such damages continue to progress, renal function gradually deteriorates. The kidney finally becomes unable to perform its critical roles, resulting in renal failure.

There are two therapeutic options for the treatment of end-stage renal disease (ESRD). One is dialysis therapy, which compromises patients' quality of life and cannot substitute for all kidney functions. Another is renal transplantation, which is limited because of the lack of sufficient donors. To explore a better treatment for ESRD, it is necessary to find strategies to regenerate the kidney. In this respect, stem cell therapy for the kidney has been intensively studied recently.

Stem cells are defined as cells that are capable of self-renewal and can differentiate into a variety of phenotypes^[1]. Adult stem cells (ASCs) are multipotent stem cells that reside in various tissues, such as the bone marrow, adipose tissue, and skeletal muscle^[2,3]. In this article, we review the possibility of kidney regeneration using ASCs.

Stem cells in the embryonic kidney

Although stem/progenitor cells in the embryonic kidney are beyond the scope of this review, we briefly describe the process of kidney organogenesis, because genetic programs that are activated during kidney organogenesis are reactivated in disease states, such as acute tubular injuries. Kidney organogenesis initiates with the interaction of the ureteric bud (UB) derived from the Wolffian duct with the metanephric mesenchyme (MM). A proportion of the MM is located adjacent to the UB, called the cap mesenchyme (CM). The CM then aggregates at the tip of the UB and differentiates into all epithelial cells of nephrons, except the collecting tubules. It is now well established that the CM contains stem/progenitor cells for kidney organogenesis^[4]. The

CM expresses unique transcription factors, such as *Pax2*, *Six2*, and *Sal1*^[5]. Although stem cells in the embryonic kidney are a promising source for kidney regeneration, their clinical use is strictly limited mainly because of the ethical concerns and small number of stem cells. Therefore, the search for stem/progenitor cells in the adult kidney has been intensively performed.

Stem/progenitor cells in the adult kidney

Four different methods have been used in an attempt to isolate stem/progenitor cells from the adult kidney (Table 1).

Label-retaining cells (LRCs): To conserve the proliferation capacity for a lifetime and to prevent genetic injuries during mitosis, stem cells cycle very slowly. Stem/progenitor cells in the kidney were isolated using this property. Cells were pulse-labeled with a dye, such as 5-bromo-2-deoxyuridine. Then, slow-cycling LRCs were detected following a chase period. Maeshima *et al*^[6] detected LRCs predominantly in the renal tubular cells of the adult rat kidney. LRCs proliferated in response to ischemia/reperfusion injury and contributed to the repair of renal tubules. In another study, Maeshima *et al*^[7] also demonstrated that LRCs were integrated into epithelial components of the nephron when transplanted into the metanephric kidney, suggesting that LRCs were multipotent stem cells. Oliver *et al*^[8] detected LRCs in the kidney papilla of adult rats. LRCs proliferated after the induction of ischemia in the kidney and migrated toward the medulla. They also injected renal papillary cells into the subcapsular area of the kidney and found that some cells were incorporated into renal tubules.

Side population cells: Because stem cells extrude dyes, such as Rhodamine 123 and Hoechst 33342, *via* the ATP-binding cassette protein^[9], they are located in a unique position on the fluorescent-assisted cell sorting scattered plot and are called side population (SP) cells. Iwatani *et al*^[10] isolated SP cells from the adult rat kidney. However, the cells did not participate in the kidney repair following experimental glomerulonephritis or gentamicin-induced nephropathy. Hishikawa *et al*^[11] isolated SP cells from the adult murine kidney. These cells expressed musclin/MyoR and improved renal function when injected systemically into mice with the induction of acute tubular injury by cisplatin administration. Furthermore, SP cells expressed reno-protective factors, such as hepatocyte growth factor (HGF), vascular endothelial growth factor, and bone morphogenetic protein 7 in a cisplatin-induced acute kidney injury (AKI) model. Challen *et al*^[12] also isolated SP cells from the adult murine kidney. These cells were located predominantly in the proximal tubules and integrated into the MM- and UB-derived structures when injected into the embryonic kidney, suggesting that they were multipotent stem cells. However, these cells were barely incorporated into the renal tissues when

Table 1 A summary of the results of isolating stem cells from the adult kidney

Species	Isolation method	Stem cell markers	Location	Incorporation into kidney tubules	Ref.
Rat	Label retaining		Proximal tubule	Yes	Maeshima <i>et al</i> ^[6]
Rat	Label retaining		Papilla	Yes	Oliver <i>et al</i> ^[8]
Rat	Side population	Sca-1, CD45	Some were derived from bone marrow	No	Iwatani <i>et al</i> ^[10]
Mouse	Side population	Sca-1	Interstitial	NE	Hishikawa <i>et al</i> ^[11]
Mouse	Side population	Sca-1	Proximal tubule	Yes (rare)	Challen <i>et al</i> ^[12]
Human	Marker	CD133	Tubules	Yes	Bussolati <i>et al</i> ^[15]
Human	Marker	CD133, CD24	Parietal epithelium in the Bowman's capsule	Yes	Sagrinati <i>et al</i> ^[16]
Mouse	Marker	Sca-1	Papilla	Yes	Dekel <i>et al</i> ^[17]
Rat	Culture	Pax2, Oct4	Proximal tubule	Yes	Gupta <i>et al</i> ^[18]

NE: Not examined.

administered to an adriamycin-induced kidney injury model, although renal function was recovered, probably because of their paracrine effect.

Cell surface markers: Cell surface markers, such as CD133, were used to isolate stem/progenitor cells from the adult kidney. Although CD133 is not a specific marker for kidney stem cells, it is a universal marker for stem cells in other tissues, such as hematopoietic stem cells, vascular endothelial progenitor cells (EPCs), and cancer stem cells^[13,14]. Bussolati *et al*^[15] isolated CD133+ cells from the adult human kidney. These cells expressed Pax2, but not CD34 or CD45, markers for hematopoietic stem cells. They could also be induced to differentiate into tubular epithelial cells and endothelial cells *in vitro*. When these cells were administered intravenously in a glycerol-induced AKI model of severe combined immune deficiency (SCID) mice, they were incorporated predominantly into the proximal and distal tubules. Sagrinati *et al*^[16] isolated CD133+ and CD24+ cells from human parietal epithelial cells in the Bowman's capsule after culturing glomeruli. When cultured in appropriate conditions, these cells were differentiated into tubular epithelial cells, osteogenic cells, adipocytes, and neuronal cells. These cells were integrated predominantly into renal tubules when they were injected intravenously in SCID mice that were treated with glycerol to induce acute tubular injuries. Dekel *et al*^[17] isolated stem cell antigen-1 (Sca1)-positive cells from the adult mouse kidney. The Sca1+ cells were located mainly in the papilla. When these cells were administered to an ischemia-induced AKI model, some of these cells were integrated into renal tubules.

Cell culture: A unique cell population was isolated during the culture of dispersed cells derived from the adult kidney. Gupta *et al*^[18] isolated progenitor-like cells from the adult rat kidney that express vimentin, CD90, Pax2, and Oct4, a marker for embryonic stem cells. These cells were incorporated into renal tubules when injected under the capsule of the kidney or intra-arterially, following ischemia-reperfusion injury of the

kidney.

Arguments against the presence of stem/progenitor cells in the mammalian adult kidney

Although aforementioned results suggest that renal stem/progenitor cells exist in the adult kidney, some reports demonstrated that differentiated renal tubular cells, but not renal stem/progenitor cells, can completely regenerate renal tubules after injury. Humphreys *et al*^[19] used genetic fate-mapping techniques in which renal epithelial cells derived from the CM (from the Bowman's capsule to the junction of the connecting segment and collecting duct) were labeled with either β -galactosidase or red fluorescent protein (RFP). After ischemia-reperfusion injury, approximately 50% of the proximal tubular cells coexpressed both RFP and Ki67, a cell proliferation marker that is expressed during the S-M phases of the cell cycle. These findings suggested that intrinsic renal tubular cells proliferate in response to injury. Furthermore, approximately 95% of tubular epithelial cells expressed RFP prior to injury, after one cycle of injury, and after two cycles of injury, indicating that no dilution of the RFP+ tubular epithelial cells occurred. These results suggested that differentiated renal epithelial cells proliferate well in response to the injury and that stem and/or progenitor cells residing in the interstitium did not participate in the regeneration of the tubules. Kusaba *et al*^[20] used a genetically modified mouse in which the tdTomato protein, which fluoresces in a red color, expressed only in differentiated proximal renal tubules. No dilution of tdTomato+ cells was observed after ischemia-reperfusion injury, suggesting that stem/progenitor cells in renal tubules did not participate in the regeneration of renal tubules. Furthermore, they observed that tdTomato+ proximal tubules expressed CD24 and CD133, markers for stem/progenitor cells. These findings suggested that renal tubules were dedifferentiated and expressed stem cell markers during their proliferation and participation in the repair of renal tubules. These results did not support that stem/progenitor cells in renal tubules and in the interstitium participated in the regeneration of renal

Table 2 Effects of Bone marrow mesenchymal stem cells on renal tissue repair

Origin of stem cells	Experimental model	Effects	Ref.
Mouse	Glycerol-induced AKI	Differentiation into tubular epithelial cells	Herrera <i>et al</i> ^[22]
Mouse	Cisplatin-induced AKI	Differentiation into tubular epithelial cells	Morigi <i>et al</i> ^[23]
Human	Glomerulonephropathy induced by anti-mesangial cell serum	Differentiation into mesangial cells	Wong <i>et al</i> ^[30]
Rat	I/R injury	Recovery of renal function No transdifferentiation into tubules	Lange <i>et al</i> ^[24]
Human	Cisplatin-induced AKI	Recovery of renal function Improved survival No transdifferentiation into tubules	Morigi <i>et al</i> ^[25]
Rat	Gentamicin-induced AKI	Recovery of renal function Conditioned medium and exosomes were effective	Reis <i>et al</i> ^[26]
Rat	Adriamycin-induced nephropathy	Reduced podocyte injury Increased VEGF production	Zoja <i>et al</i> ^[27]
Rat	Thy1.1 GN	Reduced mesangiolysis Increased glomerular cell proliferation Reduced proteinuria Production of VEGF and TGF- β	Kunter <i>et al</i> ^[28]
Mouse	CKD (Deficiency in collagen type IV, α -3 chain)	Reduced fibrosis Production of VEGF and BMP-7	Ninichuk <i>et al</i> ^[29]
Rat	5/6 nephrectomy	Improved renal function Reduced fibrosis	Semedo <i>et al</i> ^[31]
Rat	Kidney allograft	Reduced expression of IL-6 and TNF- α Improved renal function Reduced fibrosis Reduced expression of IL-6	Franquesa <i>et al</i> ^[32]

AKI: Acute kidney injury; I/R: Ischemia reperfusion; GN: Glomerulonephritis; CKD: Chronic kidney disease; VEGF: Vascular endothelial growth factor; TGF- β : Transforming growth factor- β ; BMP-7: Bone morphogenetic protein-7; IL-6: Interleukin-6; TNF- α : Tumor necrosis factor- α .

tubular cells in acute tubular injury. Nonetheless, a potential role of intrinsic stem/progenitor cells in kidney regeneration is not completely excluded from these results, because stem/progenitor cells may participate in the repair of other cell types, such as podocytes^[21]. It is difficult to distinguish renal stem/progenitor cells from dedifferentiated renal epithelial cells. Identification of specific markers that are exclusively expressed in stem/progenitor cells, but not in dedifferentiated renal epithelial cells, will be required to resolve this issue.

Mesenchymal stem cells

The MSCs reside in various organs, such as bone marrow, subcutaneous adipose tissue, and skeletal muscles. Bone marrow mesenchymal stem cells (BMMSCs) and adipose tissue-derived MSCs (ADSCs) are particularly interesting, because a large amount of MSCs can be collected with relatively less invasive procedures.

BMMSCs: Many studies have demonstrated the efficacy of BMMSCs in the treatment of kidney disease using animal models of AKI^[22-26], podocyte injury^[27], glomerulonephropathy^[28-30], a remnant kidney^[31], and kidney transplantation^[32] (Table 2). Although early studies indicated BMMSCs could be differentiated into renal epithelial cells^[22,23] and mesangial cells^[30], recent evidence suggests that the differentiation capacity of BMMSCs is limited. Thus, BMMSCs do not appear to differentiate into renal parenchymal cells *in vivo*^[33]. The beneficial effects of BMMSCs on renal

function seem to be largely mediated by paracrine factors produced by BMMSCs that have anti-apoptotic, proangiogenic, and/or immune modulatory effects^[34-36]. The transdifferentiation of BMMSCs observed in early reports may reflect cell fusion. If BMMSCs fuse with resident cells in the kidney, they acquire a phenotype of resident cells. Thus, it appears as if BMMSCs were differentiated into resident cells. Indeed, several reports have demonstrated that BMMSCs are capable of fusing with other cell types^[37,38]. A phase I clinical study evaluated the safety and efficacy of allogenic BMMSC administration for the prevention of AKI after open-heart surgery^[39]. This study enrolled 16 patients who required on-pump cardiac surgery and who were at a high risk of postoperative AKI due to underlying chronic kidney disease (CKD), advanced age, diabetes mellitus, and congestive heart failure. Allogenic BMMSCs were injected into the suprarenal aorta after surgery. The primary objective was the safety of BMMSC administration. The secondary objective was the efficacy of this treatment compared with well-matched historical controls. This treatment appeared to be both safe and effective as no adverse events related to the procedure were reported, and renal function was well preserved post-operatively, with no patients requiring hemodialysis after surgery, whereas 20% of the controls developed AKI. This is the only clinical trial published so far in which ASCs were used to treat kidney disease.

ADSCs: ADSCs are another type of MSCs residing

Table 3 Effects of adipose tissue-derived mesenchymal stem cells on renal tissue repair

Origin of stem cells	Experimental model	Effects	References
Rat	I/R injury	Recovery of renal function Reduction in oxidative stress	Chen <i>et al</i> ^[40]
Human	Cisplatin-induced AKI	Recovery of renal function Conditioned medium was effective	Kim <i>et al</i> ^[41]
Human	Folic acid-induced AKI	Recovery of renal function HGF and VEGF production	Katsuno <i>et al</i> ^[42]
Rat	Anti-GBM disease	Reduced renal injury and proteinuria Conversion of macrophages to immunoregulatory cells	Furuhashi <i>et al</i> ^[43]
Swine	Renal artery stenosis	Recovery of renal function Improved angiogenesis Increased production of VEGF and bFGF	Eirin <i>et al</i> ^[44]
Swine	Renal artery stenosis	Recovery of renal function Improved angiogenesis Decreased oxidative stress	Zhu <i>et al</i> ^[45]
Swine	Renal artery stenosis	Recovery of renal function Improved angiogenesis Increased production of VEGF	Ebrahimi <i>et al</i> ^[46]
Mouse	Renal fibrosis (unilateral clamping of the renal pedicle)	Recovery of renal function Reduced fibrosis Decreased expression of IL6 and TNF- α	Donizetti-Oliveira <i>et al</i> ^[47]

I/R: Ischemia reperfusion; AKI: Acute kidney injury; GBM: Glomerular basement membrane; HGF: Hepatocyte growth factor; VEGF: Vascular endothelial growth factor; bFGF: Basic fibroblast growth factor; IL-6: Interleukin-6; TNF- α : Tumor necrosis factor- α .

in subcutaneous adipose tissues. Because the subcutaneous adipose tissues are abundant and can be easily harvested using liposuction, ADSCs are promising stem cells for clinical use. The efficacy of ADSC administration in the treatment of kidney disease has been demonstrated in animal models of AKI^[40-42], glomerulonephropathy^[43], renal artery stenosis^[44-46], and progressive renal fibrosis^[47] (Table 3). ADSCs also seem to recover renal function largely *via* paracrine effects^[41,42].

EPCs: EPCs were originally isolated from human peripheral blood using CD34 as a marker for positive selection^[48]. The CD34+ mononuclear blood cells obtained the characteristics of vascular endothelial cells (VECs) when cultured on fibronectin-coated dishes. EPCs were reportedly incorporated in ischemic tissues *in vivo* and expressed markers for VECs such as CD31 when introduced into the circulation using a hindlimb ischemia model. The efficacy of EPC administration in the recovery of renal function was reported in animal models of AKI^[49] and renal artery stenosis^[50,51]. Interestingly, the function of EPCs was deteriorated in CKD patients^[52], suggesting that the autologous transplantation of EPCs may not be suitable for the treatment of CKD.

Umbilical cord blood-derived MSCs

Umbilical cord blood (UCB) contains MSCs, and the efficacy of UCB administration in the restoration of renal function has been reported in animal AKI models^[53,54]. Morigi *et al*^[53] injected human UCB-derived MSCs to immunodeficient mice with cisplatin-induced acute tubular injury. They demonstrated that these cells ameliorated tubular injury, resulting in the recovery

of renal function. They also cocultured UCB-derived MSCs with cisplatin-treated proximal tubular cells (HK-2 cells) and demonstrated that the expression of HGF was particularly induced and that of interleukin 1- β and tumor necrosis factor- α was significantly decreased in the coculture system. These findings suggested that the modulation of paracrine factors in the kidney was implicated in the UCB-induced recovery of renal function. Panepucci *et al*^[55] compared the gene expression profile of UCB-derived MSCs and BMMSCs. Although both MSCs expressed similar sets of genes, BMMSCs predominantly expressed a set of genes related to antimicrobial activity and osteogenesis, whereas UCB-derived MSCs predominantly expressed genes related to matrix remodeling and angiogenesis, suggesting that UCB-derived MSCs and BMMSCs may have distinct activities *in vivo*.

Amniotic fluid stem cells

Human amniotic fluid contains stem cells derived from embryos, and thus, is a promising source of stem cells. The efficacy of human amniotic fluid stem cells (HAFSCs) has been demonstrated in animal models of AKI^[56,57]. Houser *et al*^[56] compared the characteristics of HAFSCs with that of BMMSCs. They found that compared with BMMSCs, HAFSCs had a more potent anti-apoptotic activity against renal tubular cells but lesser stimulatory activity for the proliferation of renal tubular cells. They also demonstrated that HAFSCs and BMMSCs expressed distinct sets of paracrine factors, suggesting that HAFSCs and BMMSCs may have distinct activities *in vivo*.

Direct/indirect reprogramming of adult somatic cells

Another strategy for kidney regeneration is to create

pluripotent stem cells and progenitor cells, whose destination is limited to one or several cell lineages, or terminally differentiated renal parenchymal cells from adult somatic cells *via* direct/indirect reprogramming. Indirect reprogramming is a strategy in which adult somatic cells are induced to dedifferentiate into pluripotent stem cells and re-differentiate into specific cell types. Direct reprogramming involves a strategy in which adult somatic cells are induced to differentiate directly into another cell type. Since the discovery of induced pluripotent stem (iPS) cells^[58,59], it is not difficult to prepare iPS cells from various adult somatic cells. Indeed, several reports have demonstrated a successful preparation of nephrogenic intermediate mesoderm, from which the MM and the UB derive, using iPS cells^[60-62]. However, several hurdles remain to be overcome before iPS cells can be used practically to regenerate the kidney. First, the efficiency of iPS cell preparation from adult somatic cells is still low. Second, it is still challenging to prepare kidney stem/progenitor cells that exist in the CM from iPS cells. Third, even if kidney stem/progenitor cells are successfully created from iPS cells, it is difficult to continue to culture and expand those stem/progenitor cells while maintaining their unique properties. The reason for this limitation is that the niche for kidney stem/progenitor cells has not been clearly understood. Therefore, further studies will be required to use indirect reprogramming for kidney regeneration. Recently, several reports have demonstrated that a direct reprogramming method was useful for kidney regeneration. Hendry *et al.*^[63] introduced 6 transcription factors into a human adult renal proximal tubular cell line. These cells were localized in *Six2*+ and Wilm's tumor 1+ compartment in an *ex vivo* organoid culture assay. These findings suggest that these cells obtained properties similar to kidney stem/progenitor cells. Papadimou *et al.*^[64] incubated permeabilized human BMMSCs with the extracts of human proximal tubular epithelial cells and obtained a cell population similar to proximal renal tubular cells. These cells were integrated in tubular structures in an *ex vivo* organoid culture assay. Furthermore, these cells were engrafted in renal tubules when administered to a cisplatin-induced AKI model. Therefore, direct reprogramming seems to be a promising strategy for kidney regeneration. It has been reported that iPS cells derived from various cell types are not identical in their differentiation capacity^[65-67], probably because iPS cells maintain epigenetic memory of their parental cells. Thus, it may be better to use renal parenchymal cells for reprogramming than cells derived from tissues other than the kidney.

Possible roles of extracellular vesicles released from MSCs

MSCs not only secrete bioactive molecules directly into the circulation but also release extracellular vesicles (EVs)^[68], such as exosomes that contain proteins, mRNA, and microRNA^[69,70]. Several reports have demonstrated that EV administration restored the kidney

function in animal models of AKI^[26,71-73]. Bruno *et al.*^[71] isolated EVs from supernatants of human BMMSCs and examined their effects on the proliferation and apoptosis in renal epithelial cells. EVs were incorporated in renal epithelial cells *in vitro* and the incorporation depended on CD44 and β 1-interin. EVs stimulated the proliferation and inhibited apoptosis of renal epithelial cells. These effects were diminished when EVs were treated with RNase prior to administration. Furthermore, the authors administered EVs to immunodeficient mice with glycerol-induced acute tubular injury and demonstrated that EV administration restored renal function. Moreover, these beneficial effects were diminished when EVs were pretreated with RNase. Tomasoni *et al.*^[74] isolated EVs from supernatants of human BMMSCs and demonstrated that EVs contained mRNA for the insulin-like growth factor-1 receptor (IGF1R). When cisplatin-treated renal epithelial cells were incubated with EVs, proliferative capacity of renal epithelial cells increased significantly; however, the stimulatory effect was diminished when the expression of IGF1R mRNA in BMMSCs was suppressed using small interfering RNA to IGF1R prior to EV harvest. Zhou *et al.*^[75] administered EVs harvested from human UCB-derived MSCs to a rat model of cisplatin-induced AKI. EV administration significantly restored renal function and morphology. Therefore, EVs seem to contain various bioactive molecules that can be used for the treatment of kidney injury.

FUTURE DIRECTIONS

It seems that there are two major directions to improve the quality of stem cell therapy for kidney diseases. One is to analyze bioactive molecules released from stem cells in more details. If an ideal combination of bioactive proteins, mRNA, and/or microRNA is elucidated, stem cells *per se* will not be necessary in the future. Although BMMSCs have been used in the clinical setting to treat patients with cardiovascular disease^[76-80], concern about tumorigenesis still remains^[81]. Furthermore, BMMSCs isolated under uremic conditions have less capacity for the proliferation, survival, and secretion of paracrine factors compared with those isolated from normal controls^[82-84]. Patients who need stem cell therapy are probably not a suitable source of high-quality stem cells, indicating that autologous stem cell transplantation may not be effective in these patients. Therefore, cell-free therapy seems attractive. Another alternative is to explore strategies to directly and/or indirectly reprogram adult somatic cells into kidney stem/progenitor cells. If efficient and safe methods to induce direct/indirect reprogramming are explored, bona fide kidney regeneration rather than kidney repair using remnant renal parenchymal cells will be possible in the future.

CONCLUSION

Although intensive studies have been performed to isolate kidney stem/progenitor cells from the adult

kidney, it is debated whether kidney stem/progenitor cells actually exist in the adult kidney. There are no specific markers and/or assays to discriminate kidney stem/progenitor cells from dedifferentiated renal epithelial cells. MSCs are effective to repair the kidney in various animal models; however, their beneficial effects can be largely attributed to paracrine factors that are secreted from MSCs. In addition, MSCs release EVs that contain mRNA and microRNA as well as proteins. These EV-derived molecules may also play beneficial roles in the repair of the kidney. It will be necessary to elucidate ideal combinations of the molecules released from MSCs to establish a strategy for the maximal stimulation of kidney repair without using stem cells. It will also be necessary to explore strategies to directly and/or indirectly reprogram somatic cells to kidney stem/progenitor cells to regenerate the kidney.

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Baroreflex dysfunction in chronic kidney disease

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Abstract

Chronic kidney disease (CKD) patients have high cardiovascular mortality and morbidity. The presence of traditional and CKD related risk factors results in exaggerated vascular calcification in these patients. Vascular calcification is associated with reduced large arterial compliance and thus impaired baroreflex sensitivity (BRS) resulting in augmented blood pressure (BP) variability and hampered BP regulation. Baroreflex plays a vital role in short term regulation of BP. This review discusses the normal baroreflex physiology, methods to assess baroreflex function, its determinants along with the prognostic significance of assessing BRS in CKD patients, available literature on BRS in CKD patients and the probable patho-physiology of baroreflex dysfunction in CKD.

Key words: Large arterial compliance; Chronic kidney disease; Vascular calcification; Baroreflex sensitivity; Blood pressure variability

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Core tip: Cardiovascular dysfunction is an important complication and risk factor of mortality and morbidity in chronic kidney disease (CKD). Baroreflex is a functional integrator of cardiovascular homeostasis. Derangement in baroreflex function is not only a manifestation of cardiovascular pathogenesis in general and in CKD but also contribute to ongoing etio-pathogenesis. The present review discusses the physiology and dysfunction in CKD in light of the available literature.

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INTRODUCTION

Most common etiology of mortality and morbidity in chronic kidney disease (CKD) patients are cardiovascular events, rather than uremia itself. Interestingly, CKD is now recognized as an independent risk factor for cardiovascular disease^[1,2]. Practice guidelines from the National Kidney Foundation 2002 recommend that CKD be considered a coronary artery disease risk equivalent^[3].

Cardiovascular abnormalities in CKD includes both cardiomyopathy (left ventricular hypertrophy) and vasculopathy (arteriosclerosis and atherosclerosis) - which ultimately culminates to ischemic heart disease and cardiac failure^[4,5] (Figure 1).

Clinical presentation of cardiovascular disease in CKD includes hypertension, left ventricular hypertrophy, congestive heart failure, myocardial infarction and sudden death. Moreover in end stage renal disease (ESRD) patients, the prevalence of left ventricular hypertrophy and coronary artery disease are 75% and 40%, respectively. Death from cardiac causes is 10-20 times more common in patients with ESRD than in age matched segments of the general population and amounts for almost 30% to 50% of all death^[4,6-8].

Mechanism of cardiovascular dysfunction

CKD is associated with both traditional and CKD related risk factors. Furthermore, the presence of added CKD related (non-traditional) risk factors in this population accounts for the exorbitant cardiovascular risk in these patients^[9-14] as listed in Table 1 (Sarnak *et al.*^[15]).

Cumulative effect of ensemble of these risk factors results ultimately to vascular calcification in CKD patients^[9,10,16]. Central to the pathogenesis of cardiovascular dysfunction is vascular calcification^[16-19]. Reviews are available discussing the mechanism of vascular calcification in CKD patients^[16,20-29]. Vascular calcification results in stiffer arteries with reduced compliance^[18,28]. Reduction in compliance of central arteries not only results in higher afterload and diminished perfusion of heart (London *et al.*^[30]) but also impaired baroreflex sensitivity (BRS)^[31-34].

Baroreflex is a major regulatory mechanism for buffering short-term blood pressure (BP) fluctuations by modulating the heart rate and vascular tone. Baroreflex loop functioning is an important indicator of integrity of cardiovascular homeostatic regulation. Impaired baroreflex function results in loss of dampening of BP fluctuations and thus higher BP variabilities^[34,35]. Higher blood pressure variability (BPV) has been associated with end-organ damage^[36-38]. Previously a study by Kaur *et al.*^[34] proposed a model for showing the improvement in baroreflex function after renal transplantation (RT) in ESRD patients discussing the relationship between BRS, arterial stiffness and BPV and found that RT results in improvement in arterial stiffness followed by normalization in BRS and reduction in BPV. This highlights the significance of baroreflex function in CKD. The purpose of this review is to consolidate the published evidence

on baroreflex physiology, methods of assessment, its determinants and dysfunction in CKD patients.

PHYSIOLOGY OF BARORECEPTOR

REFLEX

Baroreceptor reflex (baroreflex) plays a significant role in the short term regulation of arterial BP. Pioneering works on animal models by Hering, Korner, Cowley, Guyton and others have clearly implicated its role in buffering arterial BP fluctuations induced by internal and external perturbations^[39-41]. Evidence is currently accumulating in support of the hypothesized role of baroreceptors in long term regulation of arterial BP as well^[42].

Baroreceptors are stretch sensitive receptors located in the high pressure (high pressure baroreceptors) as well as low pressure (low pressure baroreceptors) areas of the circulatory system. High pressure arterial baroreceptors located in the Carotid sinus and Aortic arch (Sinoaortic baroreceptors) are considered to play a dominant role in the moment to moment regulation of arterial BP. Considering this fact, alterations in arterial baroreflex mechanisms have been implicated in clinical disorders characterized by abnormal fluctuations in BP imposed commonly by postural variations. This section of the review would cite and discuss literature relevant to understand the physiology of arterial baroreflex and the methods of assessment of arterial BRS in human subjects and patients.

Baroreflex arc

Sino-aortic baroreceptors provide the cardiovascular regulatory centres in the brainstem with a continuous stream of information on the beat to beat fluctuations in BP. These stretch sensitive receptors are encapsulated or free nerve endings located in the tunica adventitia of carotid sinus and aortic arch that respond to the changes in dimensions of the arterial wall produced by fluctuations in transmural pressure. Afferent information from the receptors are relayed to brainstem nuclei through glossopharyngeal (afferents from carotid sinus) and vagus nerves (afferents from aortic arch) which act as the centre of the baroreflex arc (Figure 2). Baroreceptor inputs to brain stem primarily reach the nucleus tractus solitarius (NTS) located in the dorsal medulla which has intricate connections with the cardioinhibitory and vasomotor centers located in the caudal and rostral ventrolateral medulla (RVLM) and the nucleus ambiguus of vagus. RVLM projects to preganglionic sympathetic neurons located in the intermediolateral gray column of thoracic and lumbar spinal segments. Axons of the neurons in nucleus ambiguus project as pre-ganglionic parasympathetic supply to the heart. Figure 2 depicts the neuronal circuitry of the baroreflex arc.

Baroreflex activation and effector responses

NTS continuously receives a tonic input from sino-aortic baroreceptor afferents which discharge in phase with the

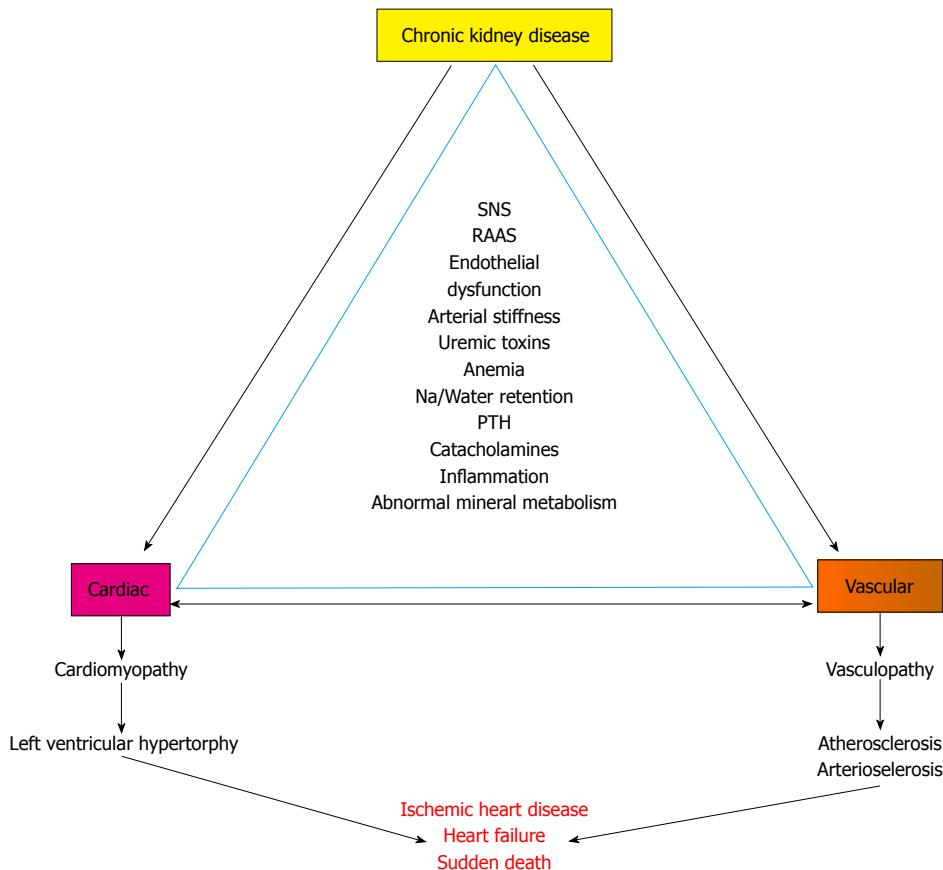


Figure 1 Cardiovascular abnormalities in chronic kidney disease. Depicts the association of chronic kidney disease related risk factors and cardiac and vascular abnormalities and outcomes in chronic kidney disease. SNS: Sympathetic nervous system; RAAS: Renin angiotensin aldosterone system; Na: Sodium; PTH: Parathyroid hormone.

arterial pressure waveform. Within the operating range, the frequency of discharge in baroreceptor afferents responds to changes in both the magnitude and slope of arterial pressure waveform. A rise in systemic mean arterial and/or pulse pressure would lead to an increased discharge in the baroreceptor afferents phase-locked with the arterial pressure waveform. Increase in baroreceptor input to NTS initiates reciprocal changes in the efferent vago-sympathetic discharge leading to increased firing of cardioinhibitory vagal neurons innervating sino-atrial node and decreased firing of sympathetic neurons controlling heart and peripheral blood vessels. This would produce a decrease in heart rate mainly through the vagal limb and a decrease in cardiac contractility, peripheral vascular resistance and venous return through the sympathetic limb. All these changes will ultimately bring the BP down, close to its set point thereby instituting negative feedback control to establish circulatory homeostasis. Thus, baroreflex arc may be considered to operate through two physiologically antagonistic efferent pathways comprising of vagal and sympathetic fibres innervating heart and peripheral blood vessels. The vagal limb is quick to act with latencies as low as 200 ms to 600 ms in comparison to the sympathetic limb which takes more than 2 s to 3 s to produce any noticeable change in the cardiac contractility or peripheral resistance. This discrepancy is

largely attributed to the obvious differences in cholinergic and adrenergic signal transduction mechanisms at the target cells.

DETERMINATION OF BRS -

METHODOLOGICAL CONSIDERATIONS

Quantification of BRS has largely been part of experimental laboratory work in animal models and human subjects until recently when clinical investigations started revealing impaired BRS as a pathophysiological entity in cardiovascular disorders^[43-45]. Moreover, BRS estimation has been attributed immense prognostic value in predicting cardiac mortality in the large multicentric autonomic tone and reflexes after myocardial infarction study^[46]. Similar observations have also been reported in a group of patients with mild to moderate heart failure, signifying the role of BRS as a prognostic indicator in the risk stratification of patients^[47].

From a physiological control system perspective, baroreceptor reflex is considered to operate in closed loop with open loop characteristics, *i.e.*, changes in BP elicit appropriate heart rate responses through open loop negative feedback mechanisms which tend to buffer the initiating change in BP through a feedforward influence of heart rate on BP that closes the loop^[48,49]. Majority of

Table 1 Cardiovascular risk factors in chronic kidney disease

Traditional risk factors	Non-traditional factors
Sympathetic hyperactivity	Albuminuria
Hyperhomocysteinemia	Inflammation
Hypertension	Oxidative stress
High LDL cholesterol	Anemia
Low HDL cholesterol	Abnormal calcium/phosphate metabolism
Diabetes	Extracellular fluid volume overload
Smoking	Electrolyte imbalance
Physical inactivity	Malnutrition
Menopause	Sleep disturbances
Family history of CVD	Endothelial dysfunction

LDL: Low density lipoprotein; HDL: High density lipoprotein; CVD: Cardiovascular disease.

the BRS assessment protocols ignore the feedforward influence considering it as inconsequential and compute BRS as the feedback gain of the open loop^[50,51].

Methodologically, BRS assessment strategies can be broadly categorized into (1) those based on artificially imposed changes in arterial BP or carotid sinus pressure including pharmacological methods, Valsalva maneuver and neck chamber techniques; (2) those based on analysis of spontaneous oscillations in BP and heart rate.

Methods based on artificially imposed changes in arterial BP or carotid sinus pressure

These methods use physiological maneuvers or pharmacological agents to impose changes in BP. The resulting baroreflex mediated changes in heart intervals are simultaneously acquired along with BP signal and subjected to appropriate analysis to derive various estimates of BRS.

Pharmacological method

Pharmacological method, also termed as the "Oxford technique" involves intravenous administration of graded bolus doses of a suitable vasoconstrictor agent to produce rise in BP that would lead to baroreflex induced bradycardia^[51-53]. Phenylephrine, a pure alpha adrenoceptor agonist is the commonly preferred vasoconstrictor agent as it is considered to have minimal extravascular effects. Many investigators prefer to administer in addition, a vasodilator agent (sodium nitroprusside infusion or amyl nitrite by inhalation) to induce fall in BP to precipitate baroreflex mediated increase in heart rate to capture responses on either side of the setpoint. Beat to beat BP and ECG signals are simultaneously recorded during the periods when BP rises above and below the resting baseline values under the influence of the vasoactive agents. Consecutive systolic BP values are plotted against the simultaneously recorded RR intervals or pulse intervals with one beat delay to fit the linear regression line between the two variables. BRS is computed as the slope of this line and expressed in ms/mm of Hg. Despite being invasive, pharmacological method is the commonly employed method to estimate

BRS for risk stratification of patients owing to its repeatability and accuracy.

Valsalva's maneuver

Valsalva's maneuver is one of the earliest known physiological maneuvers used to study the baroreflex function in humans. Performance of the maneuver involves forced expiration against a closed or partly open glottis to raise the intrathoracic and intraabdominal pressures with secondary hemodynamic effects. The maneuver physiologically imposes fall in BP due to decreased venous return during phase II and rise in BP during phases IV due to uninterrupted venous return to an already stimulated heart. The corresponding baroreflex mediated RR interval changes are acquired simultaneously with the beat to beat BP values. A linear regression analysis is commonly performed between consecutive systolic BP values and corresponding RR intervals with one beat delay during phase IV to derive BRS (also known as cardiovagal gain) as the slope of the fitted line^[51,54]. Estimation of BRS by Valsalva's manoeuvre has been reported to be non-selective for arterial baroreflex as it also engages other low pressure baroreceptors into action^[55].

Neck chamber technique

The neck chamber technique^[56,57] produces activation or deactivation of carotid baroreceptors through a graded application of negative or positive pneumatic pressure around the neck region. Negative neck pressure increases the carotid sinus transmural pressure leading to increased stretching of its wall and afferent baroreceptor firing. This would induce a fall in systemic arterial pressure consequent to baroreflex mediated changes in heart rate, cardiac contractility, peripheral resistance and venous return. BRS is computed by linear regression analysis using the transmural carotid sinus pressure and RR interval data acquired during the phases of manipulation. Neck chamber technique is the only method which selectively estimates the carotid BRS. However, it has been sparingly used in clinical investigations with most of the available literature citing its use relate to studies conducted in association with experimental laboratory work.

Methods based on analysis of spontaneous oscillations in BP and heart intervals

Since 1980s, with the invention and widespread use of non-invasive beat to beat BP monitors based on the volume clamp principle of "Penaz", there has been tremendous progress in the development of purely non-invasive methods of BRS assessment. This newer generation of techniques employs computer based analysis of spontaneous oscillations in the BP coupled with reflex changes in heart rate to derive estimates of BRS. The spontaneous BRS estimation methods can be broadly categorised into time domain and frequency domain methods.

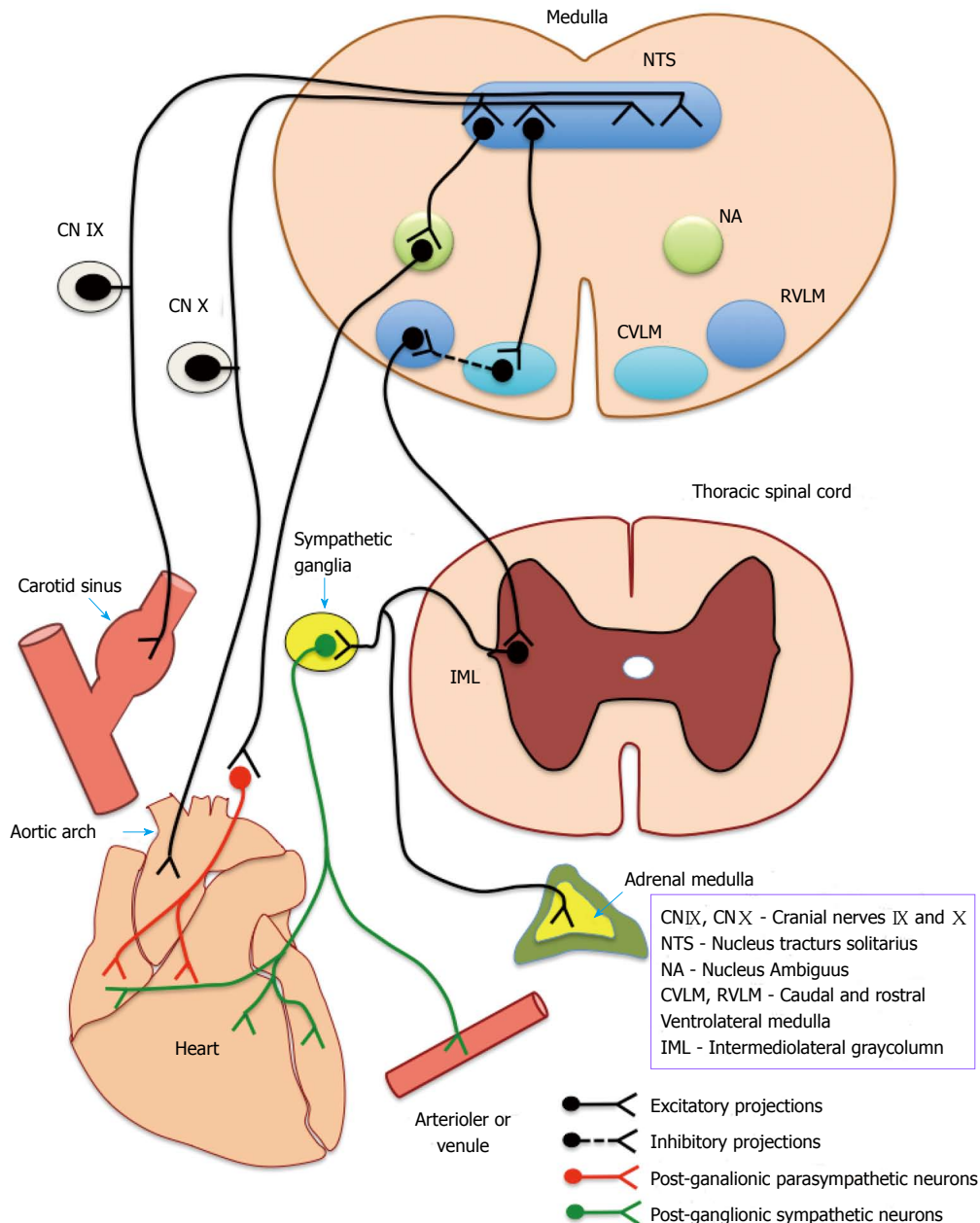


Figure 2 Neuronal circuitry of the baroreflex arc. Depicts the complete baroreflex arc - beginning from the baroreceptors (located in carotid sinus and aortic arch), afferents (IX and X cranial nerve) ascend to medullary centres and send efferents (sympathetic and parasympathetic) to end organs (heart and vasculature). CN IX and CN X: Cranial nerve IX and X; NTS: Nucleus tractus solitarius; NA: Nucleus ambiguus; CVLM: Caudal ventrolateral medulla; RVLM: Rostral ventrolateral medulla; IML: Intermediolateral gray column.

Time domain methods

Time domain methods analyse the time series data of beat to beat BP and heart intervals to estimate BRS. In the "sequence method" proposed by Parati *et al.*^[50,51,58], the algorithm automatically searches and identifies "sequences" in which BP shows a continuous increase (or decrease) for at least three consecutive beats that is accompanied by lengthening (or shortening) of consecutive RR intervals with zero to two beats delay. Sequence method considers the associated RR interval changes as baroreflex mediated response to the spontaneously emerging ascending or descending pressure ramps. A linear regression analysis between the

BP and RR interval variables will derive the slope of the best fit line as estimated BRS.

Frequency domain methods

The spectral or frequency domain methods are based on the principle that, spontaneous oscillations in BP centered around a particular frequency will lead to baroreflex mediated oscillations in heart interval in the same frequency. The ratio of the powers of the oscillations estimated by autoregressive or other methods in a particular frequency band or the modulus of the transfer function relating BP with heart interval oscillations are computed as the BRS estimates^[50,51]. Oscillations in

two frequency bands are usually being taken for the computations; a low frequency band centered around 0.1 Hz (ranging from 0.04 to 0.15 Hz) and a high frequency band of respiratory origin ranging from 0.15 to 0.4 Hz. One of the commonly used spectral methods estimates BRS as the root-squared ratio between heart interval and systolic pressure powers calculated in the LF band (α LF) or in the HF band (α HF). These spectral indices are considered to be valid when the linear correlation (coherence) between BP and heart rate oscillations in the specified frequency bands are sufficiently high. The transfer function method proposed by Robbe *et al.*^[59], computes BRS as the modulus or gain of the transfer function between variations in BP and heart interval in a specified frequency band. Transfer function is usually computed for both low frequency and high frequency bands deriving two different estimates of BRS named H_{LF} and H_{HF} respectively. Other spectral methods for estimating BRS include describing the spontaneous oscillations in BP and heart intervals using mathematical models and deriving BRS using the model coefficients.

Choice of the appropriate method of BRS assessment in clinical setting

Despite being invasive, pharmacological method is the most preferred technique for BRS estimation by most clinical investigators owing to its repeatability across different populations of patients. With the advent of non-invasive beat to beat BP monitors, impetus on the usage of spontaneous methods as replacement for the invasive pharmacological method has been steadily growing. Sequence method is considered to be the physiological replica of pharmacological method since both the techniques analyse heart interval responses to ascending or descending pressure ramps originating spontaneously or in response to vasoactive agents. However, many investigators believe spectral indices to give better and reliable estimates of BRS comparable to that obtained by invasive pharmacological methods^[59-61]. Choice of the most appropriate spontaneous method of BRS estimation is dependent on experimental factors and stationarity of the BP and heart interval signals. Reliable estimation of BRS by spectral methods is guaranteed only if the blood pressure and heart rate signals are stationary during the selected window of analysis. Sequence method is preferred over spectral indices if the stationarity of the signals cannot be ensured^[50,51]. Majority of the initial reports on baroreflex functions in CKD patients have employed pharmacological method^[62-65] to estimate BRS while a few have also used Valsalva maneuver^[66]. Spontaneous sequence and spectral methods have also been utilized in the studies conducted in the recent past^[34,67].

DETERMINANTS OF BRS

Factors determining BRS can broadly be categorized as

demographic and physiological, as reported by multiple studies conducted in healthy subjects and patients using both invasive and non-invasive methods. Age, gender, systolic and diastolic BP, resting heart rate and body mass index have been reported as the major determinants of BRS^[68,69]. The relationship between age and BRS was observed to be physiologically linked through age related changes in carotid distensibility^[70-73]. Age related decline in carotid distensibility tends to minimise the diameter changes associated with arterial pressure fluctuations, thereby reducing the transduction abilities of sino-aortic baroreceptors. This has been corroborated by direct estimation of carotid distensibility coefficients and its correlation with BRS as quantified by pharmacological method in healthy human subjects^[74]. Central arterial stiffness as measured by aortic pulse wave velocity has been reported to be an independent predictor of BRS by the Rotterdam cohort study conducted in 2083 elderly subjects^[75]. Reduction in arterial distensibility associated with stiffening of the central arteries and a consequent fall in BRS is one of the possible mechanisms implicated in baroreflex dysfunction in CKD patients.

PROGNOSTIC SIGNIFICANCE OF BAROREFLEX ASSESSMENT IN CKD

BRS is emerging as a cardinal prognostic risk factor in CKD patients. Johansson *et al.*^[76] studied BRS in hypertensive CKD patients and then followed them up prospectively for 41 +/- 15 mo and found that 69 patients died during the follow-up. Cardiovascular diseases and uremia resulted in the majority of deaths (60% and 20%, respectively), while sudden cardiac death occurred in 15 patients. Reduced BRS was found to be an independent predictor of sudden cardiac death (RR = 0.29; 95%CI: 0.09-0.86 for an increase of one standard deviation in BRS, $P = 0.022$). The authors concluded that BRS may convey important prognostic information that will have clinical implications for patients with CKD.

Reduced BRS is also associated with hemodialysis related hypotension, which results in significant mortality in hemodialysis patients as they are unable to counteract dialysis induced volume depletion^[66,77]. Chesterton *et al.*^[31], reviewed the importance of assessment of BRS in CKD patients, especially its relevance in prediction of vasomotor instability during dialysis. The authors inferred from literature that there are demonstrable pathological alterations in CKD, contributing to structural and functional changes in the cardiovascular system that may result in both haemodynamic instability and cardiovascular mortality. Understanding the associations between conventional markers of haemodynamic instability and BRS (as a measure of autonomic function) will allow early and better risk stratification, prevention and management in CKD patients.

EVIDENCE OF IMPAIRED BRS IN CKD

Baroreceptor reflex control, as studied by BRS is reduced in CKD patients and worsens with the disease severity (Table 2). Studies have also compared BRS of patients on different treatment modality of CKD - hemodialysis, peritoneal dialysis and RT with inconsistent results. Although the literature on BRS assessment in CKD is scarce, but most existing studies suggest that dialysis fails to improve BRS in CKD patients while renal transplant undoubtedly improves it. Few studies have also examined the correlation of BRS with vascular compliance and autonomic parameters in-order to understand the pathophysiology of baroreflex dysfunction in CKD patients. Although this still remains to be studied in further details.

CONCEPTUAL MODEL EXPLAINING THE REDUCED BRS IN CKD

We have previously seen in the earlier section (Figure 2) the complete baroreflex arc. Conceptually a defect anywhere in this loop could result in impaired BRS in CKD.

Till now different schools of thoughts have been categorized to summarize the defect in baroreflex function in CKD: (1) Vascular vs Neural debate; (2) Structural vs functional mechanisms.

As a matter of fact, none of these contemplations are full-proof and mutually exclusive. There exists a grey area of overlap of these factors resulting in baroreflex dysfunction.

In the next section, we will discuss the limited evidence available to possibly speculate the pathophysiology of reduction in BRS in CKD patients.

PATHOPHYSIOLOGY OF BAROREFLEX DYSFUNCTION IN CKD

Vascular vs neural

The baroreflex arc is integral to the short-term regulation of BP and is under autonomic regulation. A change in BP results in an alteration in transmural stretch within the baro-sensitive central arteries. This causes activation of the baroreceptors (level 1 in Figure 3) located within the adventitia of arterial wall. Modified firing from these receptors is transmitted *via* the afferent nerves (level 2 in Figure 3) to the central autonomic centre (level 3 in Figure 3). Sympathetic and parasympathetic systems (level 4 in Figure 3) influencing vessels and heart (level 5 in Figure 3) constitute the efferent response. Thus, a change in BP results in a corresponding change in the RR interval and vessel tone, restoring BP to normal limits.

BRS is well recognized as a composite marker of the overall integrity of the baroreflex arc^[31]. BRS is therefore determined by the mechanical properties of the arterial wall which constitutes the vascular component, and the parasympathetic and sympathetic nervous system

forming the neural component.

Chesterton *et al.*^[31] found that BRS is impaired in CKD which explains the development of intra-dialytic hypotension (IDH) in these patients. IDH is associated with increased mortality in hemodialysis (HD) patients. Additionally, they investigated the link between vascular calcification (measure of arterial structure), arterial stiffness (measure of arterial function) and BRS in chronic HD patients and concluded that there is a positive association between vascular calcification and BRS. Thus the impaired BRS observed in CKD patients could be due to the excessive vascular calcification observed in them.

In concordance Kaur *et al.*^[34] studied the reversibility of arterial stiffness indices along with BRS before, at 3 mo and 6 mo after RT in-order to understand the temporal connection between these parameters. They reported the normalization of BRS in ESRD patients by 6 mo which followed the early improvement in arterial stiffness.

On similar lines, Boutouyrie *et al.*^[83] also theoretically categorized the baroreflex loop into vascular compartment which includes the wall stretch component (receptor level) and neural comprising of afferent, centre and efferent arc of baroreflex. Notably, baroreceptors embedded in the adventitia of central arteries are sensitive only to vessel wall stretch and not directly to intravascular pressure. Pressure changes inside the vascular lumen need to get translated as vessel wall stretch to get sensed by baroreceptors. This pressure to stretch conversion is dependent on arterial compliance and thus, stiffness of large arteries become a crucial determinant of the vascular component of baroreflex^[74,84]. CKD is associated with both vascular remodelling and autonomic dysfunction. The authors commented on a previous study^[34] and discussed that questions still remain regarding how transplantation improves baroreflex - is it through amelioration of arterial properties or neural components or/and a relative contribution of both.

This puzzle remains unresolved till date. Most available data is suggestive of a probable defect at level 1 that is the sensing by baroreceptors itself. Although there exists data regarding dysfunction at other sites also in human and animal studies - level 2 - afferents^[85,86], level 3 - centre^[86,87], level 4 - efferents^[85,88,89] and level 5 - end organ^[90-92] in CKD.

By studying in detail the large artery and neural parts components of baroreflex arc, studies in future may help in understanding this concept further.

Structural vs functional modulation of the arterial baroreflex

Large artery structural changes are considered to be the predominant mechanism responsible for decreased BRS^[74]. There is an emerging concept of the role of "functional mechanisms" responsible for altered baroreflex function which could be either at the level of peripheral sensory endings and/or at the central nervous system.

Table 2 Baroreflex sensitivity in chronic kidney disease

Ref.	Number of patients Study design	Method of BRS assessment	Results
Pickering <i>et al</i> ^[65]	32 patients on HD serially studied	Intra-venous bolus of phenylephrine	BRS was found to be low HD improved reflex sensitivity over the long term, but did not have any consistent immediate effect
Lazarus <i>et al</i> ^[64]	13 patients on HD and 5 controls Cross-sectional	Intra-venous angiotensin and inhaled amyl nitrite	BRS lower in patients than controls for both pressor and depressor stimuli
Tomiyama <i>et al</i> ^[78]	22 non-dialysed patients and controls	Intra-venous bolus of phenylephrine and inhaled amyl nitrite	Lower BRS in patients as compared to controls
Agarwal <i>et al</i> ^[62]	Cross-sectional 25 non-dialyzed patients and 8 controls	Intra-venous bolus of phenylephrine	Lower BRS in patients 8 patients restudied after HD, BRS lower in hypotension-prone <i>vs</i> normotensive group 12 patients restudied after RT, BRS improved
	8 patients reassessed after 6.6 +/- 1.0 wk of hemodialysis 12 patients were restudied 24 +/- 4.0 wk after renal transplantation		
Gerhardt <i>et al</i> ^[67]	20 patients of HD, RT and controls each Cross-sectional	Sequence analysis	Reduced BRS in CKD <i>vs</i> Controls Similar BRS in RT and controls
Gao <i>et al</i> ^[79]	17 ESRD patients and 29 controls Cross-sectional	Sequence analysis	BRS was 62% lower in ESRD than controls
Johansson <i>et al</i> ^[80]	216 hypertensive CKD patients with 43 age-matched controls	Spontaneous method	BRS was reduced by 51% in CKD patients as compared with controls Greater reductions in BRS noted in diabetic <i>vs</i> non-diabetic patients
Chan <i>et al</i> ^[32]	10 hypertensive ESRD patients receiving conventional hemodialysis were studied before and 2 mo after conversion to nocturnal hemodialysis Assessed BRS along with total arterial compliance	Spontaneous method	Improvement in BRS by nocturnal HD as compared to conventional HD Increases in BRS correlated with increases in total arterial compliance
Bavanandan <i>et al</i> ^[81]	105 non-dialysis CKD patients Baseline and follow-up of 42 mo Studied relationship with increasing degrees of uremia Recorded primary (death, dialysis, transplantation) and secondary (fatal and nonfatal cardiovascular events) outcome measures	Spontaneous method	Nondialysis dependent CKD patients have impaired BRS BRS is related to decreasing GFR A trend towards poorer prognosis in patients with impaired BRS
Studinger <i>et al</i> ^[33]	Juvenile study group with 14 HD patients, 14 RT and 14 controls BRS with HRV and carotid artery stiffness	Pharmacological and spontaneous method	BRS was markedly reduced in HD as compared to controls Carotid artery stiffness was higher in HD than controls and was inversely related to BRS HRV was also compromised in HD, and was directly related to BRS No significant differences in any of these variables between RT and controls Decreased baroreflex function in juvenile HD is partly due to loss of carotid artery elasticity and partly due to impaired heart rate variability. Renal transplantation may partly prevent impairment or improve compromised baroreflex function in young patients with ESRD
Chesterton <i>et al</i> ^[31]	40 HD patients Assessed BRS with arterial calcification and arterial stiffness indices	Spontaneous method	Reduced BRS in HD patients Reduced BRS is associated with increased vascular calcification and arterial stiffness
Lacy <i>et al</i> ^[82]	55 non-dialysis non-diabetic CKD patients BRS relationship with arterial stiffness and GFR	Spectral method	BRS reduced as renal disease severity increases Reduced GFR was correlated with increased PWV and decreased cardiac BRS
Rubinger <i>et al</i> ^[35]	52 HD, 44 RT and 41 controls 16 patients before and after transplant BRS with HRV and BPV	Spontaneous method	Non-dialysis non-diabetic CKD patients with decreasing GFR have reduced cardiac BRS and increased large artery stiffness In HD patients, BPV was increased, while HRV and BRS were markedly decreased as compared to controls RT was associated with normalization of BPV at short term (\leq 1 yr) and long term and with improvement of HRV at a long-term ($>$ 1 yr) follow-up. After RT baroreceptor indices were significantly increased and returned to values similar to those of the control

Chesterton <i>et al</i> ^[77]	34 chronic HD Cross-sectional Relation with intra-dialytic hypotension	Spontaneous method	Impaired BRS predicts intra-dialytic hypotension
Kaur <i>et al</i> ^[34]	23 ESRD patients studied prospectively before and at 3 and 6 mo after RT BRS with central arterial stiffness and HRV and BPV	Spontaneous method	RT normalizes BRS in ESRD patients by 6 mo which follows the improvement in the central arterial stiffness

HD: Hemodialysis; RT: Renal transplantation; CKD: Chronic kidney disease; ESRD: End stage renal disease; GFR: Glomerular filtration rate; HRV: Heart rate variability; BPV: Blood pressure variability; BRS: Baroreflex sensitivity.

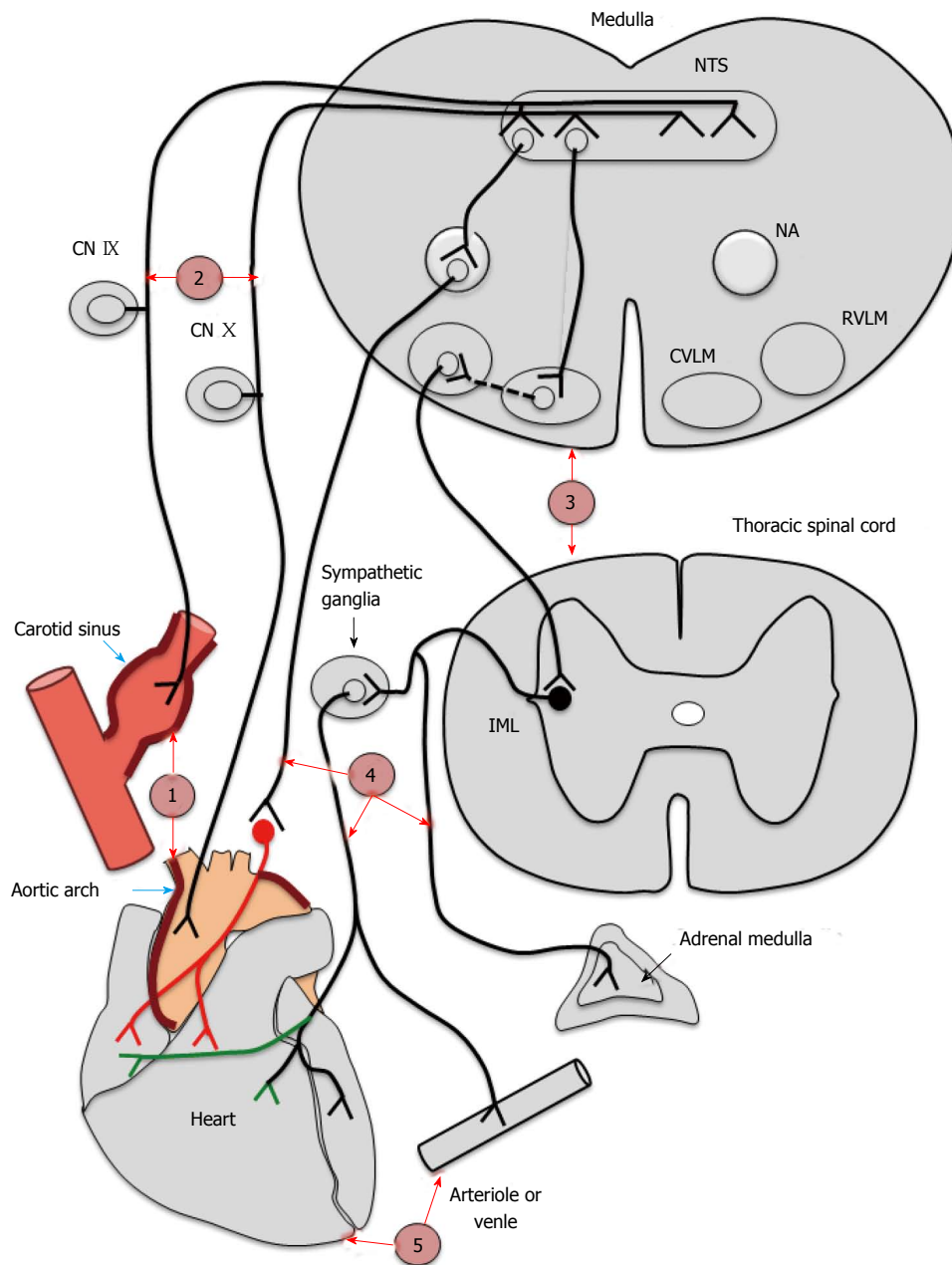


Figure 3 Probable levels of defect in baroreflex arc in chronic kidney disease. Depicts the different probable levels of defect in chronic kidney disease. Level 1 represents the baroreceptors affected by calcification of central arteries. Level 2, 3, 4 and 5 represents afferents (IX and X nerves), centres, efferents and endorgans (heart and vessels) respectively. CN IX and CN X: Cranial nerve IX and X; NTS: Nucleus tractus solitarius; NA: Nucleus ambiguus; CVLM: Caudal ventrolateral medulla; RVLM: Rostral ventrolateral medulla; IML: Intermediolateral gray column.

Structure of the central arteries determines the deformation and thus the strain of baroreceptor endings with changes in blood pressure^[93,94]. That is the reason

for structural changes in the large arteries and increased arterial stiffness being considered the cardinal mechanism responsible for the reduced BRS and resetting of

baroreceptors in hypertension, atherosclerosis, and aging.

The current concept focuses on the functional mechanisms and thus the postulate that baroreceptor activity is not merely a manifestation of associated vascular strain.

Studies have identified various mechanisms involved in the modulation of the baroreflex arc. These are referred to as functional factors to differentiate them from structural changes. Based on their site of action, functional factors are categorized into two: (1) peripheral sensory mechanisms involving baroreceptors and or sensory afferents; and (2) central mechanisms involving the neural areas coupling the afferent sensory stimuli to efferent autonomic responses^[95].

Chapleau *et al*^[96] studied the role of functional mechanism in baroreflex alteration in hypertensives and aged people. They examined on both cultured baroreceptor nodose neurons and isolated carotid sinus preparation of dogs and rabbits.

In their study^[96], they found that peripheral sensory mechanisms include: (1) Lack of endogenous PGI₂ and increase in free radicals and platelet aggregation which result in deranged baroreflex function in chronic hypertension and atherosclerosis; (2) Stretch activated channel and transient outward K current which are responsible for mechano-electrical transduction and adaptation of baroreceptors respectively; and (3) Na-K pump inhibition, which occurs with fall in arterial pressure and leads to prompt (within minutes) reversal of chronic baroreceptor resetting in chronic hypertensive rabbits. The rapidity of response rules out structural change and could be due to functional change.

In their study^[96], they have also commented on central mechanisms which include: (1) Loss of inhibition of sympathetic system and inefficient coupling of afferent stimuli to efferent response which could be attributed to reduced central arterial compliance and rapid frequency of baroreceptor discharge. It has been seen that 3 and a low frequency (< 3 Hz) of baroreceptor discharges sustain the reflex inhibition of sympathetic system; and (2) Defect in neural centres mediating the baroreflex arc. Authors have suggested that this may be the chief cause of the reduction in baroreflex functioning with aging.

Notionally, chronic kidney patients might have a similar structural and functional defect and functional changes may precede the structural changes unlike the present-day postulation but this concept has not been studied in CKD patients yet.

CONCLUSION

CKD patients have high cardiovascular mortality and morbidity. Baroreceptor function assessment is an independent predictor of cardiovascular risk. There are different methodological techniques and determinants of BRS. The underlying patho-physiology of baroreflex dysfunction is still unclear but probable defect seems

to be central arterial stiffness in CKD patients resulting in dampened firing by baroreceptors (receptor level defect).

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Secondary amyloidosis in autoinflammatory diseases and the role of inflammation in renal damage

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Abstract

The release of proinflammatory cytokines during inflammation represents an attempt to respond to injury, but it may produce detrimental effects. The inflammasome is a

large, multiprotein complex that drives proinflammatory cytokine production in response to infection and tissue injury; the best-characterized inflammasome is the nod-like receptor protein-3 (NLRP3). Once activated, inflammasome leads to the active form of caspase-1, the enzyme required for the maturation of interleukin-1beta. Additional mechanisms bringing to renal inflammatory, systemic diseases and fibrotic processes were recently reported, *via* the activation of the inflammasome that consists of NLRP3, apoptosis associated speck-like protein and caspase-1. Several manuscripts seem to identify NLRP3 inflammasome as a possible therapeutic target in the treatment of progressive chronic kidney disease. Serum amyloid A (SAA), as acute-phase protein with also proinflammatory properties, has been shown to induce the secretion of cathepsin B and inflammasome components from human macrophages. SAA is a well recognised potent activator of the NLRP3. Here we will address our description on the involvement of the kidney in autoinflammatory diseases driven mainly by secondary, or reactive, AA amyloidosis with a particular attention on novel therapeutic approach which has to be addressed in suppressing underlying inflammatory disease and reducing the SAA concentration.

Key words: Inflammation; Autoinflammatory disease; Chronic kidney disease; Interleukin-1; Dialysis; Caspase; Proteinuria; Amyloidosis; Nod-like receptor protein-3

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Core tip: Inflammation may also negatively produce elevation of proinflammatory cytokines. Recently, attention was addressed to the formation of the intracellular inflammasome nod-like receptor protein-3 (NLRP-3) activating caspase-1, the enzyme required for the maturation of interleukin-1. IL1, in turn, regulate serum amyloid A, a major acute-phase with also proinflammatory properties. An interesting new scenario on the pathogenesis of renal diseases (namely ANCA-

associated glomerulonephritis vasculitis, urate-crystal nephropathy, contrast nephropathy, acute kidney injury, reactive systemic amyloidosis) and reactive systemic amyloidosis was opened, and NLRP3 inflammasome was recently identified as a possible therapeutic target in the treatment of chronic kidney disease.

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INTRODUCTION

Inflammation is a protective process, an attempt of the organism to respond to the harmful stimuli and at the same time to initiate the healing process for the tissue. Although the release of proinflammatory cytokines may have acute beneficial effects, chronic systemic elevation is likely to produce detrimental effects^[1].

Inflammation is central to the pathogenesis of many renal diseases: The innate immune system, a first line defense against pathogens, is usually involved in the initiation and propagation of inflammation and moreover, chronic inflammation may contribute to progression of acute or chronic kidney disease (CKD).

NLRP3 mediated inflammation

Recently, several authors^[2,3] seem to indicate additional mechanisms that may orchestrate renal inflammatory and fibrotic processes by the formation and activation of the intracellular inflammasome that consists of nod-like receptor protein-3 (NLRP-3), apoptosis associated speck-like protein (ASC) and caspase-1.

In the last few years several authors^[2-9] underlined the importance of the NLRP3 inflammasome activation, the currently most fully characterized inflammasome, as an important player in renal injury. An interesting new scenario on the pathogenesis of renal diseases beyond the acquired knowledge in the rheumatologic field was opened, and NLRP3 inflammasome was recently identified as a possible therapeutic target in the treatment of progressive CKD.

Inflammasome: The inflammasome is a large, multi-protein complex that drives proinflammatory cytokine production in response to infection and tissue injury.

The best-characterized inflammasome is the NLRP3 inflammasome. On assembly of the NLRP3 inflammasome, post-translational processing and secretion of pro-inflammatory cytokines IL-1 β and IL-18 occurs; in addition, cell death may be mediated *via* caspase-1^[10].

Interleukin-1 (IL-1), previously known as endogenous pyrogen, osteoclast activating factor, catabolin, hemopoietin-1, lymphocyte activating factor, or epidermal-derived thymocyte activating factor, is produced as an

inactive precursor form upon cell activation. Its release requires the activation of different molecules gathered under the name of "inflammasome".

The activation of inflammasome leads to the active form of caspase-1, the enzyme required for the maturation of IL-1. The release of IL-1 requires the activation of the cell by ATP through its P2X7 receptor that involvement of K⁺ and Ca²⁺ channels and the action of a phosphatidylcholine-specific phospholipase. Necrotic cells produced by pressure disruption, but also hypoxic injury, uric acid crystals, bacterial toxins^[11] or complement-mediated damage were capable of activating the NLRP3 inflammasome, triggered in part through ATP produced by mitochondria released by damaged cells (Table 1).

Some authors^[12] indicate that the activation of the NLRP3 inflammasome requires two separate signals (Figure 1). The first signal, which can derive from Toll-like receptors, Tumor Necrosis Factor Receptors or IL-1R signaling, needs to activate nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B) for the transcription and translation of the immature pro-forms of IL-1 β and IL-18. As a second step, enzymatic cleavage is needed to secrete these pro-inflammatory ILs into the extracellular space.

The non-immune renal parenchymal cells do not seem to release IL-1 β , as they do not express pro-IL-1 β upon NF- κ B activation^[8], however, several reports document the expression and release of IL-18 from tubular epithelial cells (TECs)^[13-15]. This would seem to indicate that the NLRP3 inflammasome and caspase-1 axis may also be in renal non-immune cells. Moreover, Zhang *et al.*^[16] using a confocal microscopy, documented NLRP3 and ASC to be expressed by glomerular podocytes.

Intrinsic renal cells express components of the inflammasome pathway: This is mostly prominent in TECs and, to a lower degree, in glomeruli. Several primary renal diseases and systemic diseases affecting the kidneys are associated with NLRP3 inflammasome/IL-1 β /IL-18 axis activation. Most of the disorders studied have been acute inflammatory diseases: The disease spectrum includes ureteric obstruction, ischaemia reperfusion injury, glomerulonephritis, sepsis, hypoxia, glycerol-induced renal failure, and crystal nephropathy^[17].

The German group from Munich recently described^[7] the role of the NLRP3 inflammasome in oxalate nephropathy and found that calcium oxalate crystals kill TECs, which leads to the release of ATP and potentially other NLRP3-agonistic DAMPs that trigger IL-1 β secretion by renal dendritic cells.

In addition, renal dendritic cells ingest oxalate crystals by phagocytosis and subsequent lysosomal leakage activates NLRP3. Acute oxalate nephropathy was significantly attenuated in NLRP3-, ASC- and caspase-1-deficient mice. Finally, acute oxalate nephropathy had been shown to be prevented by therapeutic IL-1 blockade with anakinra, a IL-1 receptor antagonist approved by the United States Food and Drug Administration for the treatment of rheumatoid arthritis. The results of this study suggest a potentially similar pathogenic role

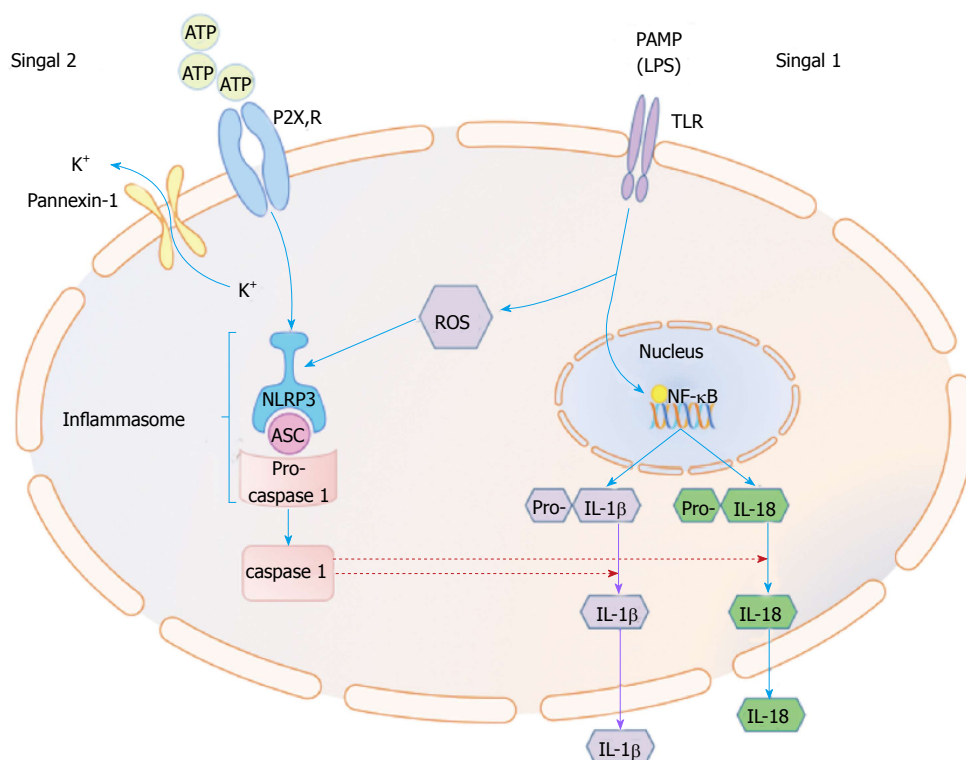


Figure 1 Model of nod-like receptor protein-3 inflammasome activation and the role of the nod-like receptor protein-3 inflammasome in the two-step activation of interleukin-1 β and interleukin-18^[10,12]. Activation of the NLRP3 inflammasome requires two signals. Signal 1: Activation of TLRs, IL-1Rs and TNFRs induces the transcription and translation of NF- κ B to produce pro-forms of IL-1 β and IL-18; Signal 2: Enzymatic cleavage by (caspase-11-driven) caspase-1 to secrete mature cytokines, IL-1 β and IL-18. ROS: Reactive oxygen species; TLR: Toll-like receptor.

of the NLRP3 inflammasome in other crystal-related nephropathies such as cast nephropathy, contrast nephropathy, acute kidney injury (AKI) in rhabdomyolysis or urate nephropathy^[17].

The role of the NLRP3 inflammasome arthritis in urate crystal-induced is well described^[18] and the block of IL-1 may be considered a good therapeutic option in patients with gouty arthritis and renal failure^[19].

Moreover, other authors reported that the upcoming data on the NLRP3 inflammasome support the evolving danger signaling concept of renal inflammation^[20]. More recently Schreiber *et al.*^[21] described their experience in antineutrophil cytoplasmic antibodies (ANCA)-activated phagocytes that cause vasculitis and necrotizing crescentic glomerulonephritis (NCGN). The authors supposed that ANCA-induced phagocyte NADPH oxidase generated tissue-damaging reactive oxygen species that restrains inflammation, downregulated caspase-1, thereby keeping the inflammasome in check, reducing IL-1 generation and limiting ANCA-induced inflammation. The authors concluded that IL-1 receptor blockade by anakinra might provide a promising strategy in NCGN. More than 25 years ago, even in patients on hemodialysis, it was shown that the involvement of monocyte activation brings to the release of IL-1 and related cytokines, as already reported in 1988 by Dinarello^[22].

Mulay *et al.*^[7] experimentally showed in mice that renal CaOx crystal deposition was associated with diffuse

neutrophil infiltrates and tubular necrosis mainly at the inner stripe of the outer medulla, as demonstrated by the disintegration of TECs and granular casts in tubular lumen. The structural alterations of oxalate nephropathy were associated with renal failure^[4]. Clodronate liposome was used in WT mice or diphtheria toxin in CD11c DTRg mice to demonstrate that CaOx-induced intrarenal IL-1 secretion originated from the intrarenal network of interstitial mononuclear phagocytes.

On hypothesizing that therapeutic blockade of IL-1 might be able to interfere with this pathomechanism and protect against renal failure, the authors^[2] used anakinra: Intraperitoneal injection of anakinra dose-dependently reduced tubular injury and neutrophil recruitment and improved renal excretory function during oxalate nephropathy in mice. The authors concluded that IL-1 mediated inflammation and tissue damage in kidney injury induced by CaOx crystals and thus IL-1 blockade protected from renal failure in oxalate nephropathy in mice.

Both experimental and human studies show a detrimental role for NLRP3 in the development of acute and chronic tubule-interstitial disease^[6]. To confirm this Duewell *et al.*^[23], using a novel microscopic technique, a combination of laser reflection and fluorescence confocal microscopy to identify in mice crystalline materials and immune cells, recently reported that minute cholesterol crystals were present in early diet-induced atherosclerotic lesions and that their appearance coincided with

Table 1 Pathogen associated molecular pattern and damage associated molecular pattern that trigger nod-like receptor protein-3 activation^[12]

Type	Molecule/molecular pattern
PAMP	Leptospiral interrogans/glycolipoprotein Influenza Streptococcus pyogenes/streptolysin O Staphylococcus aureus/alpha hemolysin
DAMP	ATP Nigericin Histones U1snRNP ribonucleoprotein dsDNA/nucleosomes MSU crystals Uromodulin Biglycan Silica Alum Calcium oxalate Asbestos Amyloid-β Hemazoin Hyaluronan

PAMP: Pathogen associated molecular pattern; DAMP: Damage associated molecular pattern; ROS: Reactive oxygen species/oxidative stress; PAMP: Pathogen-associated molecular pattern; DAMP: Damage-associated molecular pattern; ATP: Adenosine tri phosphate; MSU: Mono sodium urate.

the first appearance of inflammatory cells.

To test whether cholesterol crystals could activate the release of IL-1 β , the authors incubated lipopolysaccharides-primed human peripheral blood mononuclear cells with cholesterol crystals: Cholesterol crystals induced a robust, dose-responsive release of cleaved IL-1 β in a caspase-1 dependent manner. The authors also demonstrated that cholesterol crystals also activated the NLRP3 inflammasome in phagocytes *in vitro* in a process that involved phago-lysosomal damage. Crystalline cholesterol acts as an endogenous danger signal and its deposition in arteries or elsewhere was an early cause rather than a late consequence of inflammation.

Most importantly, mice whose bone marrow-derived cells lacked NLRP3 inflammasome components, or IL-1 cytokines, were markedly resistant to developing atherosclerosis. The lesional area in the aorta of these mice was reduced on average by 69%, compared to chimeric LDLR-deficient mice that had wild-type bone marrow.

Amyloidosis: Amyloidosis is a disorder of protein folding in which normally whole or fragments of normally soluble proteins are deposited as abnormal, insoluble fibrils that disrupt tissue structure, so causing disease. In systemic amyloidosis the deposits may be present in the parenchyma of the viscera and tissues, causing progressive organ dysfunction leading patients to death. Systemic amyloidosis, fatal within 6 mo of diagnosis in up to 20% of patients, causes about one per thousand deaths in developed countries and remains an important

Table 2 Over 30 proteins capable of amyloid formation have been identified

Immunoglobulin light chains in primary systemic amyloidosis
Ig heavy chain
Beta2-microglobulin in dialysis-associated arthropathy
Amyloid beta protein in alzheimer disease and down syndrome
Hereditary forms (including transthyretin, apolipoprotein A- I and A- II, gelsolin, lysozyme, fibrinogen a-alpha chain
Amyloid a in secondary amyloidosis

unmet medical need. There are about 30 different types of amyloid in humans, characterized by the particular specific protein that forms the fibrils^[24] (Figure 2).

The core structure of all amyloid fibrils consists of antiparallel β -pleated sheets arranged with their long axes perpendicular to the long axis of the fibril. This structure specifically binds the histochemical dye, Congo-red, from alkaline alcoholic solutions, in an ordered molecular array which gives pathognomonic red-green birefringence when viewed in strong cross-polarized light. This is the gold standard for histological diagnosis of amyloid^[24] (Table 2).

There are therefore both acquired and hereditary forms of amyloidosis. The most common form of systemic amyloidosis is the AL type. The international nomenclature comprises A for amyloidosis and the second and other letters identify the amyloid fibril protein, in this case L for monoclonal immunoglobulin light chains^[24].

AL amyloidosis, formerly known as primary amyloidosis, is thus a complication of monoclonal gammopathy of any type ranging from myeloma through monoclonal gammopathy of uncertain significance, to the whole variety of B/plasma cell dyscrasias. It accounts for about 60% of all cases.

AA amyloidosis, formerly known as secondary or reactive systemic amyloidosis, is a complication of chronic inflammatory and infective diseases in which there is a sustained acute-phase response with overproduction of serum amyloid A (SAA) protein, a very sensitive and dynamic major acute-phase protein. Although becoming rare in the developed world due to greatly improved treatments for inflammatory arthritides, Crohn's disease, chronic infection, *etc.* AA amyloidosis is still a fairly common disease in medicine department and nephrologist's counseling may be required for the detection of proteinuria or renal failure^[25].

In the past ten years, thanks to more aggressive treatment schedules and to the increasing availability of anti-TNF treatments, some authors^[26,27] report that the incidence of AA amyloidosis in chronic arthritides has slowly decreased.

This has led to a relative increase in the rate of other conditions that are well-recognized to significantly associate with AA, such as Crohn's disease, hereditary periodic fevers, malignancies, systemic vasculitides and diseases predisposing to recurrent infections, including

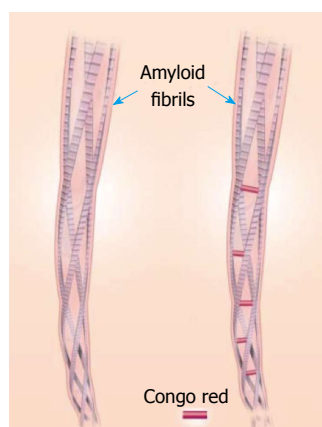


Figure 2 Structural features of amyloid^[25].

cystic fibrosis, bronchiectasis, epidermolysis bullosa, cyclic neutropenia, acquired or inherited immunodeficiencies, injection-drug use and acne conglobata.

SAA is a major acute-phase protein present in serum but also shown to possess proinflammatory properties, as meaning that it can induce the release of cytokines from different cell types, including THP-1 monocytes, human neutrophils, and mast cells.

SAA is mainly produced in the liver under the regulation of IL-1, IL-6, and TNF- α , but its expression has also been demonstrated in other cell types, including macrophages, endothelial cells, and smooth muscle cells.

Moreover, SAA has been shown to induce the secretion of cathepsin B and inflammasome components from human macrophages. As processing of SAA by cathepsin B may result in production of amyloidogenic SAA fragments: Experimentally, SAA has demonstrated to induce a strong expression of IL1 β and TNF α in human macrophages^[28].

SAA mediates its effect through activation of NLRP3 inflammasome: SAA is a potent activator of the NLRP3 inflammasome *via* a cathepsin B- and P2X7-dependent manner and is the first physiological proinflammatory mediator that can provide signals needed for expression of pro-IL-1 (as shown in Figure 1) and activation of the inflammasome cascade, resulting in activation of caspase-1 and secretion of mature IL-1 β so resulting in formation of amyloidogenic fragments^[28]. The conversion of the circulating soluble protein SAA into stable, highly ordered, amyloid fibrils that accumulate extracellularly causing organ damage is a multi-step process.

As an acute phase reactant secreted by the liver under the transcriptional control of IL-1 and IL-6, SAA increases up to 1000 fold following an inflammatory stimulation. If such stimuli persist, as occurs in several chronic diseases, SAA concentration may reach a critical threshold over which it becomes prone to aggregation. Moreover, the estimated ten years' survival was reported to be much higher in the patients with lower SAA levels, below 10 mg/dL^[29].

A β -2m amyloidosis, so-called DIALYSIS-RELATED AMYLOIDOSIS, is a serious complication of long-term dialysis for end-stage renal failure in which β 2-microglobulin, normally catabolized by the kidneys, is not adequately cleared and accumulates in the plasma, rising in concentration from its normal value of 1-2 mg/L to up to 70 mg/L^[30].

All amyloid deposits have the feature to be largely ignored by the usually very efficient physiological mechanisms by which abnormal protein debris is cleared from the interstitial space in the tissues. Dead cells, effete matrix and structural proteins, blood cells and plasma proteins extravasated in injury, are normally rapidly cleared with no local or systemic clinical consequences. In contrast, although macrophages and giant cells are occasionally seen, especially around local rather than deposited as amyloid fibrils in and around bones and joints, causing pain, bone cysts and pathological fractures.

Here we will address our description on the involvement of the kidney in autoinflammatory disease driven mainly by secondary, or reactive, AA amyloidosis.

AA AMYLOIDOSIS

A clear example of renal involvement in autoinflammatory disease with amyloid A deposition may be considered the Muckle-Wells (MWS) disease associated with AA-amyloidosis. MWS is inherited as an autosomal dominant condition, meaning each child of a sufferer has a 50% chance of developing the syndrome.

MWS is a rare genetic autoinflammatory syndrome and the intermediate-severity form of cryopyrin-associated periodic syndrome (CAPS). As with other forms of this syndrome, it presents with recurrent episodes of fever, skin rash, joint pain, abdominal pain and conjunctivitis, but in addition sufferers typically develop a progressive sensorineural deafness and amyloidosis^[9].

The protein affected in MWS is cryopyrin, produced by the NLRP3 gene located on chromosome 1. The gene is expressed in white blood cells (mainly neutrophils) and chondrocytes (cartilage cells). Cryopyrin is an essential component of the inflammasome, an intracellular protein complex involved in the innate immune system. The abnormal inflammasome in MWS allows unrestricted activation of the enzyme caspase-1, which in turn causes overproduction of active IL-1, switching on the inflammatory cascade in an uncontrolled manner^[31].

MWS can have severe consequences due to chronic high levels of inflammation in the body. This can be life-threatening if generalized amyloidosis of the AA type develops, due to long-term buildup of amyloid protein products from the chronic inflammation in MWS. Organ damage results from the extracellular deposition of proteolytic fragments of the acute-phase reactant SAA as amyloid fibrils^[9]. A sustained high concentration of SAA is the prerequisite for developing AA amyloidosis.

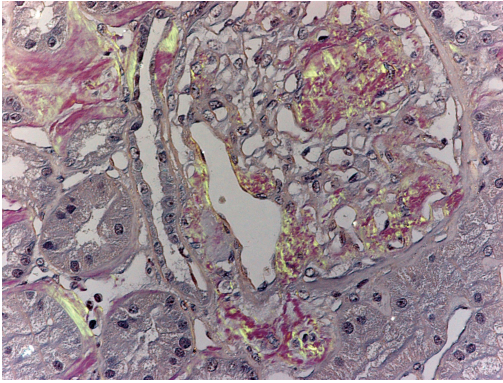


Figure 3 Renal biopsy: Amyloid fibrils bind congo red stain, yielding the pathognomonic apple-green birefringence under cross-polarized light microscopy^[9].

The kidneys, liver and spleen are the main target organs of AA amyloid deposits (Figure 3).

In more than 90% of the patients proteinuria, nephrotic syndrome and/or renal dysfunction dominate the clinical picture at onset^[9,24,26,27]. If not effectively treated, this disease invariably leads to end stage kidney disease^[9] and renal replacement therapy, that are still associated with a poor outcome^[27].

Over 25% of MWS patients have elevated serum amyloid, and at least 25% have amyloidosis. Serum AA testing is essential to follow, along with C-Reactive Protein (C-RP), Erythrocyte Sedimentation Rate (ESR) and other laboratory tests. Amyloidosis is also a risk to some patients affected by different types of CAPS, a group of autoinflammatory disorders characterized by recurrent episodes of systemic inflammation marked by fever, tissue inflammation, particularly of the joints and skin, and other constitutional symptoms, clinically defined by a spectrum of varying severity. Amyloidosis may be associated in familial cold autoinflammatory syndrome and in neonatal-onset multisystem inflammatory disease/chronic infantile neurological cutaneous and articular syndrome, but not so much as in MWS. Generalized amyloidosis is due to a permanent buildup of amyloid in the kidneys, liver and elsewhere, that can be fatal^[32].

Clinical AA amyloidosis is typically preceded by many years of active inflammation before presenting, most commonly with renal involvement^[33].

In AA amyloidosis renal dysfunction is reported to be the predominant disease manifestation. Mortality, amyloid burden, and renal prognosis all significantly correlated with the SAA concentration during follow-up. The risk of death was reported to be 17.7 times as high among patients with highest SAA concentrations. In the previously reported^[25] largest study on AA amyloidosis involving 374 patients, the most frequent underlying disorder was inflammatory arthritis and only rare causes of AA amyloidosis included vasculitis, sickle cell anemia, malignant disease, epidermolysis bullosa, and cyclic neutropenia. Renal involvement was reported to be frequent: In 97% of patients, more than 500 mg

of proteinuria per day were present or the serum creatinine concentration was more than 1.5 mg/dL. The relative risk of progression to end-stage renal failure was also increased among patients whose renal function was relatively worse at baseline, with an increase by a factor of 5 for each doubling of the baseline serum creatinine concentration ($P < 0.001$).

Fortunately, the cardiac involvement is not so frequent in AA amyloidosis and it is reported to be present in only 1 patient, and findings consistent with cardiac infiltration were present in only 2 among 224 patients who underwent echocardiography^[34].

A worse renal outcome in patients with chronic sepsis or Crohn's disease was reported^[28], possibly related to the high frequency of surgical intervention and administration of immunosuppressive drugs, probably due to greater severity of disease associated or not at increased risk of infection.

Therapy

Further studies need to elucidate whether persistent inflammation serves as a catalyst by sensing and converting the endothelium into a proinflammatory surface that makes the vasculature more vulnerable to the effects of other circulating risk factors. Such a scenario is supported by the strong documented association between inflammatory markers and endothelial dysfunction in patients with CKD.

Similarly, effective anti-inflammatory treatment, or whatever is needed to control the acute-phase response and maintain circulating SAA serum levels in the normal range, is life saving in AA amyloidosis^[9,25,27]. Rigorous compliance with colchicine therapy for Familial Mediterranean Fever (FMF) prevents and ameliorates AA amyloidosis even in patients who do not experience complete relief of symptoms. The key is to control SAA production, closely monitoring SAA serum levels in all patients with AA amyloidosis, and tailoring their treatment to keep these as low as possible. However, many patients are already in severe or end-stage organ failure when diagnosed with amyloidosis and new approaches are desperately needed to save them.

Treatment of AA amyloidosis has to be addressed in suppressing underlying inflammatory disease and reducing the SAA concentration as much as possible. If not effectively treated, this disease invariably leads to end stage kidney disease and renal replacement therapy, that are still associated with high mortality rate^[25].

In a unpublished experience we observed in a female patient aged 40, affected by Chron disease with nephrotic proteinuria of 14 g/daily and CKD stage 3 secondary to renal AA amyloidosis histologically proven, the control of the baseline chronic bowel inflammatory disease with the monoclonal antibody adalimumab, a TNF-alfa inhibitor, significantly reduced the proteinuria levels up to 6 g/daily, while still remaining in the nephrotic range. The TNF-inhibitor therapy also reduced SAA levels from more than 4 mg/dL (normal values <

0.5) up to quite normal values (0.61 mg/dL).

Among AA amyloidosis therapy, some years ago interest was pointed on eprodisate, structurally similar to heparin sulfate, a glycosaminoglycan that is known to promote fibril assembly, inducing amyloid formation. Eprodisate, negatively charged, sulfonated molecule that is structurally similar to heparin sulfate and works by competitively inhibiting the interaction between SAA and glycosaminoglycans.

A RCT was conducted^[35] enrolling 180 patients with AA amyloidosis-associated nephropathy; patients were treated with eprodisate or placebo for 24 mo: The authors reported that the treatment was associated with a 42% reduction in the risk of worsening renal disease (as measured by creatinine clearance) or death (CI: 0.37-0.93; $P = 0.02$), compared with placebo. Surprisingly, there was no significant difference in terms of the overall changes in proteinuria: A second phase III trial is now ongoing.

Higher levels of aspecific laboratory inflammatory markers such as C-RP and sTNF are independently associated with faster rates of kidney function loss in CKD^[36]. Pravastatin, a HMG-CoA reductase inhibitor, was reported to prevent loss of kidney function to a greater extent in CKD individuals with coronary artery disease (CAD) with greater evidence of inflammation, although this was of borderline significance. These data suggest that inflammation may mediate the loss of kidney function among subjects with CKD and concomitant CAD^[37].

Some years ago, other authors experimentally found that inhibition of the isoprenoid pathway by another statin, lovastatin, resulted in a dose-dependent reduction of amyloid formed in mouse recombinant SAA produced in *Escherichia coli*, hypothesizing the isoprenoid metabolism as a potential target for prevention and treatment of AA amyloidosis^[38].

More recently, Luo *et al.*^[39] studied the effects of another statin, rosuvastatin (RSV), and observed that, compared with controls, diabetic Sprague-Dawley rats showed severe metabolic disorder, cardiac dysfunction, fibrosis, disorganized ultrastructure, and excessive activation NLRP3 inflammasome, ASC, IL-1 β and mitogen-activated protein kinases. The NLRP3 inflammasome was found activated in response to high levels of glucose. RSV was added and continued for 8 wk. The effect and underlying mechanisms of action of RSV in diabetic cardiomyopathy (DCM) and whether NLRP3 was a target for RSV in DCM, was studied. The authors concluded that, compared with diabetics rats alone, RSV experimentally ameliorated the overexpression of NLRP3 inflammasome and silencing NLRP3, ameliorated cardiac remodeling and dysfunction, so identifying RSV as a significant potential therapy *via* inhibition of NLRP3 inflammasome.

Due to the strong association between proinflammatory cytokines and complications common in ESRD, such as vascular calcification and wasting, the potential role of both general and targeted anticytokine

treatment strategies in ESRD patients needs further evaluation^[40]. Inflammation has to be considered an important target for pathogenetic interventions both in AKI and in progression of CKD, as recently suggested^[41].

Therapeutic interventions that suppress inflammation and oxidative stress may address both short-term (dynamic) and long-term (structural) contributors to a decline in the GFR in patients with CKD and could possibly stabilize or even improve kidney function^[9,42].

However, despite major technologic improvements in dialysis techniques, a lot of haemodialysis and peritoneal dialysis patients show serological evidence of an activated inflammatory response, as clearly indicated by increased circulating levels of non-specific markers of inflammation and proinflammatory cytokines such as IL-6.

Dialysis treatment save the lives of patients with ESRD but it does not cure the burden of clinical consequences related to uremic state, *i.e.*, the marked risk for atherosclerotic cardiovascular disease and inflammation. Renal transplantation (TPX) can be considered in selected patients progressing to ESRD, but unfortunately, it is a choice not offered to all ESRD patients due to the low number of transplants performed in some countries.

Novel treatments to control inflammation processes, and also to prevent progression of renal damage, are under development and anti-cytokine agents are becoming the mainstay of therapy to prevent and treat AA, including patients with FMF that do not respond or do not tolerate adequate colchicine dosages and targeting key molecular events in the fibrillogenesis process^[43]; also the role of other drugs are in progress^[44].

Unfortunately, control of fibril-protein production is not possible in some forms of amyloidosis and in others it is often slow. There is no therapy that directly targets amyloid deposits for enhanced clearance. However, all amyloid deposits contain the normal, non-fibrillar plasma glycoprotein, serum amyloid P component (SAP).

Other authors^[45] showed that administration of anti-human-SAP antibodies to mice with amyloid deposits containing human SAP triggers a potent, complement-dependent, macrophage-derived giant cell reaction that swiftly removes massive visceral amyloid deposits without adverse effects. Interestingly, the authors found that a combination of a drug that depletes circulating SAP and an antibody that targets residual SAP within the deposits results in clearance of amyloid deposits. A humanized version of the anti-SAP antibody has been developed with a view to clinical evaluation of this dual approach, hypothesizing this combined therapy to eliminate amyloid deposits.

IL-1 blockade

Clinical observations to date suggest that although IL-1 plays a key role in activation of the innate immune system, blockade of this cytokine appears to have few adverse effects. Anti-IL-1 therapy appears to

increase the risk of infection only marginally, and there is no clear evidence for increased risk of malignancy, despite lymphoma and other types of cancer have been reported in children treated with TNF blockers, often when along with certain other drugs (such as azathioprine or 6-mercaptopurine). Safety block of IL-1 after 12 mo after renal TPX was reported^[46] also in a renal transplanted patients affected by MWS with systemic amyloidosis treated with triple immunosuppressive drug regimen and at the same time canakinumab: No flares of MWS was observed during this period.

In our experience we did not observe increased hospitalization rate due to infections or malignancy in two patients affected by Muckle Wells syndrome treated with IL-1 blockers who had been followed for over three years^[9].

CONCLUSION

The release of proinflammatory cytokines during inflammation represents an attempt to respond to injury, but it may produce detrimental effects. The best-characterized inflammasome is the NLRP3 that, once activated, leads to the active form of caspase-1, the enzyme required for the maturation of IL-1 β . SAA, as acute-phase protein with also proinflammatory properties, is a well recognized potent activator of the NLRP3. Additional mechanisms bringing to renal inflammatory, systemic diseases and fibrotic processes, resulting in kidney insufficiency were recently reported, *via* the activation of the inflammasome.

Currently, treatment options in amyloidosis rely on reducing the supply of the precursor protein and thus depend absolutely upon accurate typing of the amyloid. Intercalating agents able to induce physical disruption of the fibrillar structure of the native fibrils, once mature fibrils have been deposited, are under study in some types of non-AA amyloidosis, hence producing an intermediate: It so resulting to be more readily available for enzymatic degradation.

The administration of anti-human SAP antibodies^[47] to mice with amyloid deposits containing human SAP triggers a potent, complement-dependent, reaction that swiftly removes massive visceral amyloid deposits without adverse effects. These promising results achieved in mouse models based on intermediary metabolism may not be extended to humans, so specific trials are needed to test this hypothesis also in humans.

The role of statins is a new aspect targeted towards NLRP3 and not only in ameliorating dyslipidemic profile: Treatment with statins may represent a promising further test for this well-known class of drugs beyond the CV risk reduction, mediated by the reduction of lipidic profile^[39].

Recently, great interest is growing on the role of NLRP3 inflammasome that incorporates several signals of tissue injury, infectious or non-infectious, and

consequently brings, *via* the activation of caspase-1, to the secretion of the pro-inflammatory cytokines IL-1 β and IL-18. Block of the IL-1 system seems to be a fascinating option to counteract caspase activation and reducing IL-1 levels and consequently also SAA levels, that appear to be dramatically reduced within normal values even in patients with border line levels up to thousands of times. The mainway is to control the primary cause of inflammation and IL-1 blockers have demonstrated in rheumatologic field to be really effective and safe, even when associated to important immunosuppressant therapy, such as in kidney transplant patients. Moreover, a possible targeted intervention of IL-1 receptor blockade, even in active vasculitis, was recently suggested^[22].

Several question points remain open, such as whether it is right to consider IL-1 block as target for treating CKD. And also if it is really the NLRP3 inflammasome a gauge of kidney injury damage or if we can specifically target the NLRP3 inflammasome for therapeutic intervention, as recently postulated by other authors^[12].

The direct involvement of NLRP3 in kidney disease has not been demonstrated yet, despite recently several manuscripts address a reasonable suspicion about it. Moreover, a deeper knowledge on the role of NLRP3 inflammasome and of reactive AA amyloidosis in renal diseases is requested. Whether blocking IL-1 is really effective in delaying the progression of renal damage has yet to be demonstrated by large trials, despite, at the moment, the high costs severely limit the use of such drugs.

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Role of calcium in polycystic kidney disease: From signaling to pathology

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Abstract

Autosomal dominant polycystic kidney disease (ADPKD) is the most common inherited monogenic kidney disease. Characterized by the development and growth of cysts that cause progressive kidney enlargement, it ultimately leads to end-stage renal disease. Approximately 85% of ADPKD cases are caused by mutations in the *PKD1* gene, while mutations in the *PKD2* gene account for the remaining 15% of cases. The *PKD1* gene encodes for polycystin-1 (PC1), a large multi-functional membrane receptor protein able to regulate ion channel complexes, whereas polycystin-2 (PC2), encoded by the *PKD2* gene, is an integral membrane protein that functions as a calcium-permeable cation channel, located mainly in the endoplasmic reticulum (ER). In the primary cilia of the epithelial cells, PC1 interacts with PC2 to form a polycystin complex that acts as a mechanosensor, regulating signaling pathways involved in the differentiation of kidney tubular epithelial cells. Despite progress in understanding the function of these proteins, the molecular mechanisms associated with the pathogenesis of ADPKD remain unclear. In this review we discuss how an imbalance between functional PC1 and PC2 proteins may disrupt calcium channel activities in the cilium, plasma membrane and ER, thereby altering intracellular calcium signaling and leading to the aberrant cell proliferation and apoptosis associated with the development and growth of renal cysts. Research in this field could lead to the discovery of new molecules able to rebalance intracellular calcium, thereby normalizing cell proliferation and reducing kidney cyst progression.

Key words: Autosomal dominant polycystic kidney disease; Calcium signaling; cAMP; Cell growth; Non-capacitative calcium entry

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Core tip: In the present article, we discuss: (1) the regulation of calcium signaling in the primary cilia of autosomal dominant polycystic kidney disease (ADPKD) cells and the downstream processes that lead to cystogenesis; (2) how calcium impairment promotes cell proliferation by activating different signaling pathways; (3) the activity of non-capacitative calcium entry channels, which in PKD1-silenced cells stimulates cell growth by Ca^{2+} oscillations and nuclear factor of activated T-cells activation, highlighting new findings showing the role of polycystin-2 in calcium oscillations; (4) the impairment of intracellular calcium signaling associated with apoptosis; and (5) the use of calcium channel blockers and calcium modulators in the treatment of ADPKD.

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INTRODUCTION

Autosomal dominant polycystic kidney disease (ADPKD) is the most common inherited pathology of the kidneys, having an incidence of 1:500-1:1000 individuals. It accounts for roughly 10% of cases of end-stage renal disease^[1,2], which results from the progressive bilateral development and expansion of fluid-filled cysts arising from the de-differentiation of renal tubule epithelial cells^[1]. ADPKD is caused by the mutation of two genes: *PKD1*, which accounts for 85% of cases, and *PKD2*, associated with the remaining 15% of cases^[1]. In ADPKD, the focal cyst development in the kidneys seems to be associated with a somatic second hit brought on by either loss of heterozygosity or other mutations in renal cyst lining epithelial cells^[3,4]. *PKD1* and *PKD2* genes encode for polycystin-1 (PC1) and polycystin-2 (PC2) proteins, respectively^[1,5]. PC1 is an integral membrane receptor with a large extracellular region consisting of a variety of domains involved in cell-cell and cell-matrix interactions. It also bears 11 transmembrane domains, and a short cytoplasmic segment containing motifs involved in signal transduction^[1]. PC2, on the other hand, is an integral transmembrane protein mainly localized to the endoplasmic reticulum (ER); it is anchored to cell membranes by six transmembrane regions, and has two cytoplasmic N- and C-terminal tails. PC2 functions as a nonselective cation channel that transports calcium, and shows significant homology with transient receptor potential (TRP) channels^[5-8]. Indeed, the polycystins and their homologous proteins are considered a new subfamily of TRP channels, and accordingly known as TRP polycystic proteins^[1].

An interaction between PC1 and PC2 forms the so-

called polycystin complex. This is mainly confined to the primary cilia of kidney epithelial cells, where it acts as a flow sensor, triggering intracellular calcium release *via* the activation of the PC2 channel in response to fluid-flow changes. Disruption of this complex impairs intracellular calcium influx, and leads to the development and expansion of kidney cysts^[1,9,10].

Polycystins are able to regulate calcium channel activity not only in the cilia, but also in other cellular compartments, including the plasma membrane and ER. Indeed, PC1 and PC2 co-assembly has been seen to generate a cation-permeable current through the plasma membrane^[11], and PC1 and PC2 are known to regulate intracellular calcium release in the ER through their interaction with the inositol 1,4,5-trisphosphate receptor (IP₃R)^[12-14]. In this context, PC2 enhances calcium release from the ER by stimulating the activity of the IP₃ receptor, while PC1 inhibits this process by reducing PC2-IP₃R interaction *via* a mechanism involving the stromal interaction molecule-1 (STIM1) and the PI3K/Akt pathway^[12,15]. PC1 can also regulate other types of calcium channels, including non-capacitative calcium entry (NCCE) channels, which are able to generate intracellular calcium oscillations^[16]. PC2, on the other hand, regulates intracellular calcium release by either interacting with the calcium channels TRPC1 and TRPV4 on the plasma membrane and in primary cilia, and/or through an association with ryanodine and IP₃ receptors in the ER^[8,17-19]. Moreover, PC2 appears to be able to generate a non-specific voltage-dependent cation current in native HEK293 kidney cells. This current is strongly associated with PC2 activity, and is completely abolished by the depletion of PC2 protein^[20].

Taken together these findings suggest that PC1 and PC2 may affect calcium influx from different cellular compartments, including cilium boundaries (cilioplasm), plasma membrane and ER. Dysregulation of calcium signaling due to loss of polycystin function causes the aberrant activation of different pathways associated with abnormal cell proliferation and fluid secretion, thereby leading to the development and expansion of kidney cysts. However, the cascade of events that occur between polycystin dysfunction and kidney cyst formation in ADPKD is not yet fully understood.

In this review, we discuss the impact of polycystin loss of function on calcium signaling, which may alter different pathways associated with the cell growth and apoptosis that are a typical hallmark of ADPKD. Moreover, we report the potential effects of calcium dysregulation on kidney cyst formation and progression. Finally, we also discuss the state of the art in calcium channel modulators, able to restore normal calcium release and therefore appealing targets for ADPKD treatment.

ROLE OF THE POLYCYSTIN COMPLEX IN PRIMARY RENAL CILIA

It is well known that PC1 and PC2 co-localize in the

primary cilia of kidney epithelial cells, performing a mechano-sensor function by transducing calcium signals in response to changes in tubular fluid flow. Loss or dysfunction of either PC1 or PC2 causes the inability of cells to sense mechanical stimuli due to bending of the cilia, which leads to abnormal cell morphology and polarity, and thereby contributes to renal cyst formation^[10,21,22]. The calcium signaling triggered by fluid shear stress initiates in the primary cilia through PC2-dependent calcium release. This is initially confined to the cilioplasm, but, through the ryanodine receptor, the same calcium signal subsequently activates a cytosolic calcium response that induces calcium influx from intracellular stores^[23].

PC2, as mentioned above, is also able to interact with the transient potential receptor channels TRPC1 and TRPV4. PC2 and TRPC1 assemble to form a heteromultimeric channel, not associated with PC1, which is activated in response to G-protein-coupled receptor stimulation, and shows a pattern of single-channel conductance distinct from that of the individual PC2 and TRPC1 channels^[24]. Direct or indirect activation of the PC2/TRPC1 complex, either by cilium bending or through the activation of plasma membrane GPCRs, may affect the mechano-transduction of cilium-associated calcium signals^[24]. PC2 can also form a heteromeric channel complex with TRPV4. This complex displays molecular mechano-sensor properties, being able to generate flow-induced calcium influx, which seems to be abolished by the depletion of TRPV4 channel in renal epithelial cells^[18]. It is also plausible that polycystins cooperate with other proteins located in the cilium, such as cystin, polaris, inversin, and kinesin-II, as defects in these proteins may lead to the formation of kidney cysts^[21,25].

In the primary cilia, PC1/PC2, PC2/TRPC1 and PC2/TRPV4 complexes regulate the calcium signaling activated by cilium deflection due to changes in fluid flow. Surprisingly, however, depletion of TRPC1 and TRPV4 is not associated with cyst formation, despite it altering ciliary calcium signaling. Hence, the impairment of ciliary calcium signaling alone is not sufficient to trigger kidney cyst development, a process which, instead, seems to be closely linked to the activity/function of PC1 and PC2 proteins. In fact, a recent study has shown that ablation of the cilia in both PC1- and PC2-deficient cells reduces cyst growth, suggesting that the loss of cilia may cause milder cyst progression than in the cilia-equipped ADPKD cells^[26]. Moreover, cilia-dependent cyst growth is not associated with the activation of the mitogen-activated protein kinase/extracellular signal-regulated kinase, mammalian target of rapamycin (mTOR) or cyclic adenosinemonophosphate (cAMP) pathways^[26]. As a whole, these findings suggest that the polycystins govern ciliary signaling by an unknown mechanism when the normal kidney epithelial cell phenotype is maintained. In ADPKD, the inactivation of polycystins alters cilia-dependent signaling, thereby promoting the formation of the characteristic kidney cysts.

CALCIUM SIGNALING AND CELL PROLIFERATION IN ADPKD CELLS

ADPKD is strongly associated with the altered cell proliferation of cystic kidney epithelial cells that represents a typical hallmark of the disease. The disruption of calcium signaling associated with PC1 and PC2 deficiency could be the primary event behind the increased cell growth seen in ADPKD. Indeed, we do know that calcium restriction in ADPKD cells causes cAMP-dependent activation of the B-Raf/mitogen-activated protein kinase kinase (MEK)/extracellular-signal-regulated kinases (ERK) pathway, which results in increased cell growth^[27]. Moreover, this reduction in intracellular calcium levels also inhibits the activity of AKT kinase, a negative regulator of B-Raf^[27]. In cystic cells, normal growth can be restored by increasing their cytosolic calcium concentration, which increases AKT activity and inhibits cAMP-dependent B-Raf/ERK activation^[28].

Low intracellular calcium levels may also stimulate the activity of the ciliary calcium-sensitive adenylyl cyclases AC5 and AC6, as well as the plasma-membrane-anchored AC6, leading to the elevation of cAMP^[29,30]. Therefore, loss of polycystin function may promote the activity of AC5/6 by reducing intracellular Ca^{2+} release from the cilia, ER and plasma membrane^[29,30] (Figure 1). Indeed, it has recently been reported that the double knockout of *PKD1* and *AC6* genes decreases cystogenesis, improves renal function and increases survival in a mouse model of ADPKD. These improvements in renal function occur through a reduction in cAMP levels and inhibition of the B-Raf/MEK/ERK pathway, suggesting that AC6 could be a key mediator of cyst formation in ADPKD^[31]. In addition, cAMP elevation may activate the cAMP-response element-binding protein, which promotes cell proliferation in an epidermal growth factor receptor (EGFR)-activation-dependent manner by stimulating expression of the EGF-like peptide amphiregulin^[32]. EGFR signaling is dependent upon a mechanism involving the sequential activation of Ras, Raf-1, MEK and ERK. It can converge on the same pathway activated by cAMP, thereby leading to activation of the ERK kinases that promote cell proliferation in ADPKD cells^[33] (Figure 1). Furthermore, the abnormal activity of mTOR kinase has been observed to contribute to increased cell proliferation and cyst formation in ADPKD cyst-lining epithelial cells. In normal kidney epithelial cells mTOR activity is inhibited by PC1, which interacts with TSC1/TSC2, an inhibitory complex of mTOR, preventing its inactivation. Conversely, in ADPKD cells polycystin dysfunction promotes mTOR activation by inhibition of the TSC1/TSC2 complex, through a mechanism involving the cAMP-dependent B-Raf/ERK pathway^[34,35] (Figure 1).

The expression of full-length PC1 has been shown to inhibit intracellular calcium release in response to ATP in Madin-Darby canine kidney (MDCK) cells, in a mechanism that involves the interaction of STIM1 with

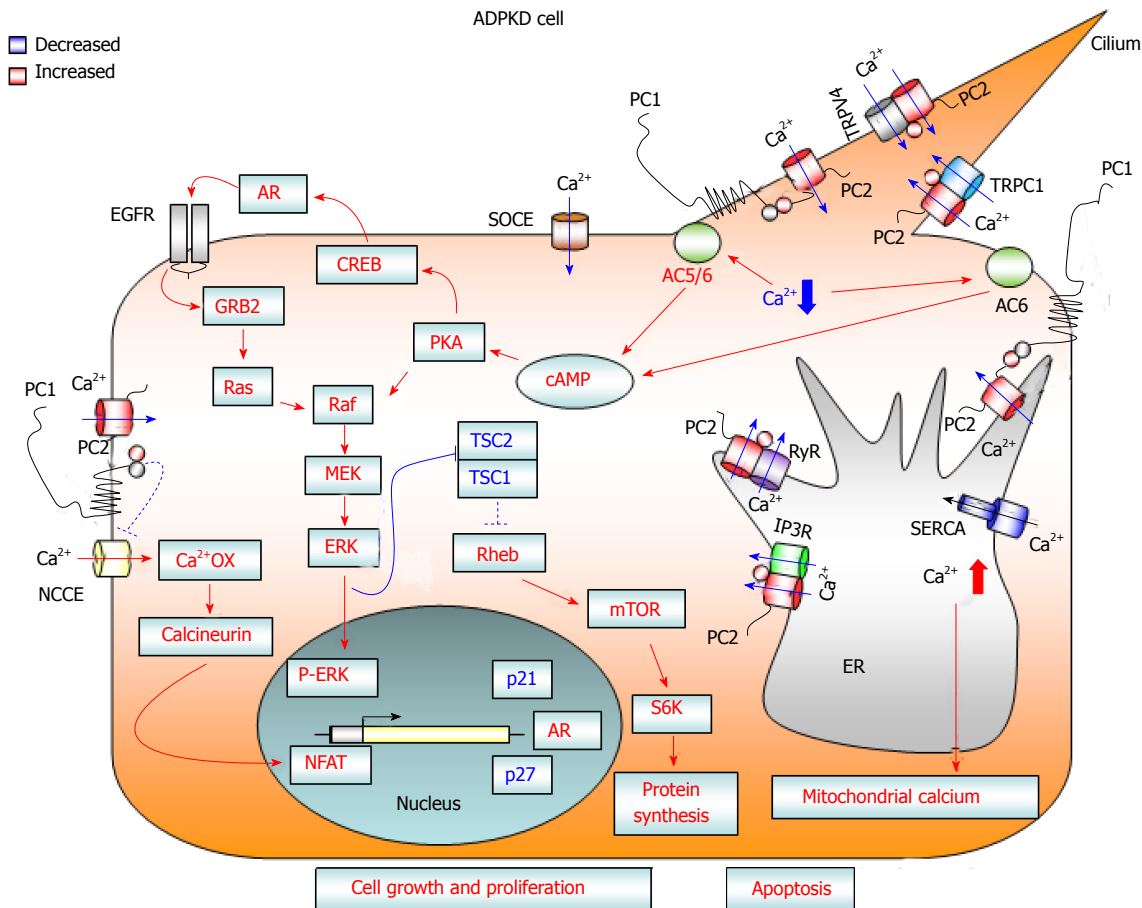


Figure 1 Diagram showing calcium-dependent dysregulated signaling pathways that promote cell proliferation and apoptosis in autosomal dominant polycystic kidney disease cells. Loss of PC1 and/or PC2 function causes a reduction in cytosolic calcium influx from three different cellular compartments: (1) the primary cilium after mechanical stimuli; (2) the endoplasmic reticulum, in an IP₃R- and RyR-dependent manner; and (3) the plasma membrane, through a reduction in SOCE channel activity. The reduced concentration of cytosolic calcium may activate Ca²⁺ sensitive adenylyl cyclases 5 and 6, leading to a rise in cAMP. Increased levels of cAMP cause the activation of B-Raf/MEK/ERK and CREB/AR/EGFR pathways, as well as stimulating mTOR signaling, through the active form of ERK kinases that inactivate the TSC1/TSC2 complex. Moreover, deficiency of PC1 and/or PC2 enhances the activity of NCCE channels, which, by increasing calcium oscillation frequency, results in the activation of the transcription factor NFAT. The abnormal activation of these signaling pathways promotes cell proliferation and kidney cyst formation. In addition, the reduction in Ca²⁺ influx from the ER to the cytosol caused by a deficiency in PC2 channel activity brings about an imbalance in ER calcium concentration, resulting in ER Ca²⁺ overload. The increased ER calcium concentration sensitizes kidney cystic cells to apoptotic stimuli by abnormal ER calcium release, which may induce mitochondrial damage and thereby lead to cytochrome C release and activation of apoptosis. AC 5/6: Adenylyl cyclase 5/6; AR: Amphiregulin; Ca²⁺ OX: Calcium oscillations; cAMP: Cyclic adenosine monophosphate; CREB: cAMP response element binding transcription factor; EGFR: Epidermal growth factor receptor; ER: Endoplasmic reticulum; ERK: Extracellular-signal-regulated kinases; GRB2: Growth factor receptor-bound protein 2; IP₃R: Inositol 1,4,5-trisphosphate receptor; MEK: Mitogen-activated protein kinase kinase; mTOR: Mammalian target of rapamycin; NCCE: Non-capacitative calcium channel entry; NFAT: Nuclear factor of activated T-cells; PKA: Protein kinase A; PC1: Polycystin-1; PC2: Polycystin-2; S6K: Ribosomal S6 kinase; Raf: Rapidly accelerated fibrosarcoma kinase; Ras: Rat sarcoma viral oncogene homolog family; Rheb: Ras homolog enriched in brain; RyR: Ryanodine receptor; SERCA: Sarcoplasmic endoplasmic reticulum calcium ATPase; SOCE: Store-operated calcium channel entry; TRPC1: Transient receptor potential channel 1; TRPV4: Transient receptor potential cation channel subfamily V member 4; TSC: Tuberous sclerosis complex.

IP₃R, and reduces the association between PC2 and the IP₃ receptor^[15]. Moreover, PC1 seems able to regulate intracellular calcium release and PC2-IP₃R-STIM1 interaction through the PI3K/Akt signaling pathway^[15]. The exogenous expression of the C-terminal fragment of PC1 (PC1-Cter) could function as a dominant negative effector, causing an increased intracellular calcium release in response to ATP treatment, as seen in HEK-293 cells^[36]. Furthermore, PC1-Cter-expressing cells not only exhibit increased levels of basal calcium, but also show enhanced cell proliferation, which is associated with the activation of ERK kinases^[37]. Consistently, the transfection of HEK-293 cells with the C-terminal tail of PC1 has been observed to cause an

increase in both basal and intracellular calcium release, leading to the activation of the nuclear factor of activated T-cells (NFAT)^[38]. Moreover, NFAT activation, associated with increased cell proliferation in HEK-293 cells, is also observed after the downregulation of PC1 by RNA interference^[16]. NFAT activation occurs through a rise in the frequency of intracellular calcium oscillations, caused by the increased activity of NCCE channels^[16] (Figure 1). In HEK293 cells, these calcium oscillations can be increased by either reduced or undetectable levels of PC1, but are only induced by the absence of PC2 (Figure 2A). Normal calcium oscillations can be restored in PKD1-deficient cells *via* the reintroduction of mouse wild-type PC1, and in PKD2 knockout cells *via* the

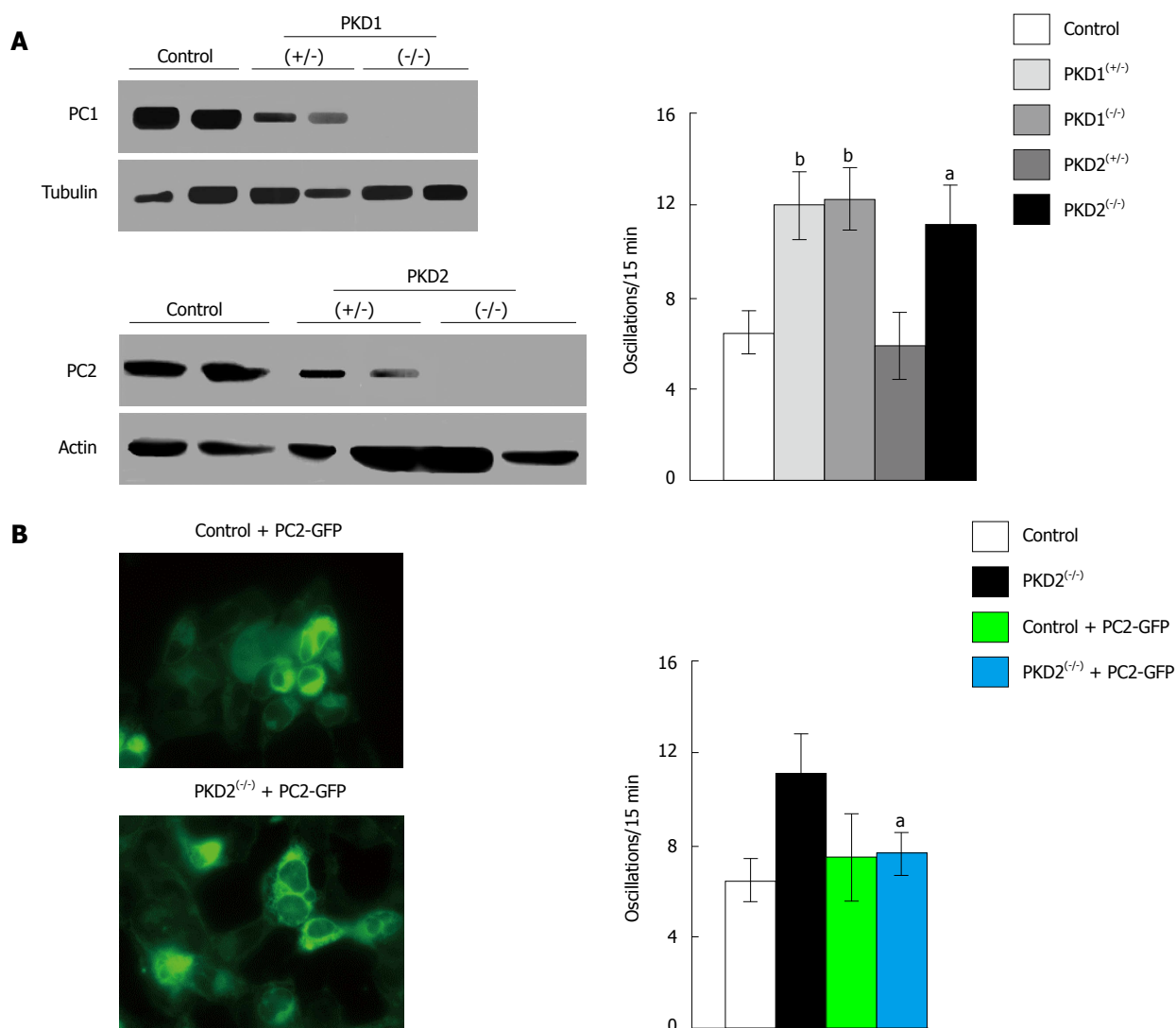


Figure 2 Downregulation of *PKD1* and *PKD2* genes increases fetal bovine serum-induced calcium oscillations in HEK293 cells. A: The stable transfection of HEK293 cells with plasmids containing specific anti-*PKD1* and anti-*PKD2* sequences causes a partial (+/-) or complete (-/-) downregulation of PC1 and PC2 expression compared with HEK293 cells stably transfected with scramble sequences (control). *PKD1* and *PKD2* gene silencing was evaluated by Western blotting using anti-PC1 and anti-PC2 antibodies. Calcium oscillations were increased in both partially (+/-) and fully (-/-) cells silenced for the *PKD1* gene, as well as in fully (-/-) *PKD2*-silenced cells, as compared with scramble-treated cells (control). The number of oscillations/15 min were: 12 ± 1.5 in *PKD1*^(+/-) cells, 12.2 ± 1.42 in *PKD1*^(-/-) cells and 11.13 ± 1.79 in *PKD2*^(-/-) cells, vs 6.39 ± 1.09 in control cells (^b $P < 0.01$; ^a $P < 0.05$); B: The expression of full-length exogenous PC2 fused with GFP in *PKD2*^(-/-) cells restores normal calcium oscillations (11.13 ± 1.79 oscillations/15 min in *PKD2*^(-/-) cells vs 7.72 ± 1.07 in *PKD2*^(-/-) cells transiently transfected with *PKD2*-GFP cDNA; ^a $P < 0.05$). Western blotting, oscillation recording and cell imaging were performed as previously reported^[16]. Data, obtained from three different experiments analyzing at least 45 cells for every HEK293 clone, are represented as mean \pm standard deviation. Analysis of data was performed using Student's *t* test, and differences were considered significant at a value of $P < 0.05$. PKD: Polycystic kidney disease; HEK293: Human embryonic kidney cells; GFP: Green fluorescent protein; PC: Polycystin.

transfection of full-length PC2 (reference^[16] and Figure 2B). These findings suggest that PC1 could negatively regulate NCCE channels in a mechanism involving PC2 expression.

Taken as a whole, the evidence above suggests that the increased cell proliferation, fluid secretion and kidney cyst development seen in ADPKD may arise due to either the loss of polycystin complex function or an imbalance in the PC1/PC2 ratio causing intracellular calcium changes that trigger the B-Raf/MEK/ERK signaling cascade, as well as mTOR and NFAT pathways (Figure 1). However, the different effects on intracellular calcium concentration and downstream events observed

with PC1 fragments and full-length PC1 expression suggest that further investigations are needed to clarify the function of polycystins in the regulation of calcium signaling.

CALCIUM SIGNALING AND APOPTOSIS IN ADPKD CELLS

In ADPKD, cyst formation and expansion rely on multiple mechanisms, including apoptosis, whose levels are higher in kidney cells from patients with ADPKD with respect to healthy individuals^[39]. As apoptosis is one of the multiple cellular processes regulated by calcium

signaling, this increase in apoptosis may be associated with abnormal intracellular calcium influx. Indeed, it has been demonstrated that cell sensitivity to apoptotic stimuli can be enhanced by calcium accumulation in the ER of renal epithelial cells deprived of functional PC2^[40]. Conversely, expression of the PC2 protein, which functions as a calcium channel, inhibits apoptosis by lowering ER calcium levels^[40]. Therefore, polycystin dysfunction appears to bring about an imbalance in ER calcium concentration through a reduction in the activity of the PC2 channel, causing calcium overload in the ER. This increase in ER calcium concentration, and its subsequent release, sensitizes cystic kidney cells to apoptotic stimuli. The excess calcium released from the ER is absorbed by the mitochondria, potentially causing damage that may lead to the release of cytochrome C, which activates the programmed cell death (Figure 1). In light of these findings, it seems that PC2 may function as an anti-apoptotic calcium channel in kidney epithelial cells^[40]. Likewise, programmed cell death in kidney cells may be also regulated by PC1. In fact, apoptosis is prevented in MDCK cells, through the activation of the phosphatidylinositol 3-kinase/Akt signaling pathway, by the expression of full-length PC1^[41,42].

CALCIUM CHANNELS AS A TARGET FOR ADPKD THERAPY

Drugs able to inhibit mTOR and cAMP-related pathways have already completed clinical trials. In particular, the use of the vasopressin V2 receptor Tolvaptan has led to significant improvements in renal function^[43], although treatment with mTOR signaling pathway inhibitors did not yield satisfactory results^[44]. Investigation into the calcium modulator molecules as an alternative treatment for ADPKD has also begun. To this end, significant results have already been achieved in preclinical trials of triptolide, an active diterpene that induces intracellular calcium release through a PC2-dependent mechanism. Treatment with this molecule improved renal function in a mouse model for ADPKD, inhibiting cyst expansion by restoring normal calcium signaling and cell proliferation^[45,46]. Conversely, retrospective studies have shown that treating ADPKD patients with calcium channel blockers provokes a worsening of renal function, as compared to untreated patients, by reducing the glomerular filtration rate^[47]. However, treatment of PKD2(-/WS25) ADPKD mice with R-568, a type-2 calcimimetic molecule that triggers the activation of calcium-sensing receptors, showed no detectable effect on cystogenesis^[48]. Nonetheless, despite the unsatisfactory results yielded by current therapeutic interventions relying on calcium channel modulators, it is worthwhile continuing this line of research, as further studies into other calcium regulators may lead to the discovery of more efficient drugs.

CONCLUSION

Polycystin complex, formed by the interaction between

PC1 and PC2, may function as a calcium-permeable receptor-channel complex able to regulate intracellular calcium signaling. As both PC1 and PC2 are mutated in ADPKD, and in light of their effects on cell proliferation and apoptosis, considered typical hallmarks of ADPKD, it is highly plausible that such mutations play a central role in the disease. In complex or alone, PC1 and PC2 can both act in different cellular compartments, including the plasma membrane, endoplasmic reticulum and primary cilium, but the downstream effects of their dysfunction in ADPKD have still not been clarified. Nevertheless, it is known that functional loss of either PC1 or PC2 causes calcium signaling disruption, which is considered a primary event for kidney cyst formation in ADPKD. Although intracellular Ca²⁺ alteration abnormally activates several pathways that stimulate cell proliferation in ADPKD cystic cells, including cAMP-dependent B-Raf/MEK/ERK signaling and mTOR, EGFR and NFAT pathways, these are not activated in the cyst formation process associated with ciliary signaling impairment. Further investigation is therefore required to clarify the function of polycystins, and in turn identify new targets for ADPKD treatment.

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Percutaneous nephrolithotomy in pediatric age group: Assessment of effectiveness and complications

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Abstract

Management of kidney stone disease in pediatric

population is a challenging condition in urology practice. While the incidence of kidney stone is increasing in those group, technological innovations have contributed to the development of minimally invasive treatment of urinary stone disease such as mini-percutaneous nephrolithotomy (mini-PCNL), micro-PCNL, ultra mini-PCNL. In this review we tried to evaluate the effect of new treatment techniques on pediatric kidney stones.

Key words: Percutaneous nephrolithotomy; Pediatric; Kidney stone; Urolithiasis

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Core tip: In this article, minimally invasive treatment options of pediatric kidney stone disease are examined. Also, the effectiveness and complication rates of these techniques were reviewed in the light of recent publications.

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INTRODUCTION

The incidence of kidney stones in pediatric population is increasing and is reported that 50 cases per 100000 children^[1]. The majority of kidney stones contain calcium. Most consist of calcium-oxalate but to a lesser extent calcium phosphate. Much less commonly kidney stones consist of urate, cysteine or struvite. Unlike adults, urinary stone disease in pediatric population is associated with genetic, metabolic and anatomical causes. Children with urolithiasis are considered high

risk for recurrent stone formation, and it is crucial for children to receive a treatment method that will provide them stone free^[2].

Most pediatric urinary stones can be managed effectively by minimally invasive treatment modalities such as extracorporeal shock wave lithotripsy (SWL), percutaneous nephrolithotomy (PCNL), retrograde intrarenal surgery (RIRS)^[3]. However, PCNL can have a significant role in cases involving large and/or SWL resistant stones. According to the European Association of Urology guidelines, PCNL is recommended as primary treatment option for large renal stones (> 20 mm) and also for > 10 mm stones of the lower renal pole^[4].

The surgical management of pediatric kidney stones with PCNL has been developed due to improvement of endourologic devices and acquired experiences. Standard PCNL required 24-30 F nephrostomy sheath for renal access. But this method is associated with complications such as hemoglobin drop, blood transfusion, damage of renal parenchyma, and postoperative analgesic requirement. In order to decrease morbidity associated with PCNL in pediatric patients small size instruments have been used. Thus, PCNL is performed with small size endoscopes *via* smaller percutaneous tract in diameters ranging from 11 F to 20 F and this was named as Miniperc or Mini-PCNL^[5]. Recently, Micro-PCNL or microperc has been described as another minimally invasive PCNL technique that is performed through a 4.8 F all-seeing needle^[6].

The literature was reviewed for success and complication rates regarding recent PCNL techniques in pediatric age group.

MINI-PCNL: SURGICAL TECHNIQUE, SUCCESS AND COMPLICATION RATES

The first pediatric PCNL was described using a 15 F peel-away sheath and 10 F pediatric cystoscope by Helal *et al.*^[7] in 1977. Yet, this technique was developed using an 11 F access sheath by Jackman *et al.*^[8] in pediatric patients. Since then, the new form of PCNL has become a treatment option for adults as well^[9,10]. The first 12 F nephroscope was presented to perform mini-PCNL in 2001^[9]. The new device consisted of 15 F and 18 F sheaths, a system of continuous low pressure irrigation, and a 6 F working channel. In time, this technique has developed and also accumulated in the pediatric patients for the treatment of renal stones regardless of the size of the stone. There is no common consensus as to exact size that is used for mini-PCNL, but usually access sheaths below 20 F is accepted^[11].

Mini-PCNL is performed under general anesthesia. After introduction of anesthesia with the patient in the lithotomy position, retrograde ureteral catheterization is performed with 3-5 F ureteral catheter to fill the collecting system during percutaneous access. Then, the patient is repositioned in the prone position with a 30°-45° upward tilt of the affected site. Adequate

padding of the pressure points should be done to prevent pressure induced injuries and neuropraxias^[12,13]. Prone position is the most preferred technique but it has been reported that supine position *vs* prone position has equal safety and effectiveness^[14]. Percutaneous renal access is achieved under the fluoroscopic and/or ultrasonic guidance. A lower pole posterior calyx access is preferred, but site of renal puncture may vary depending on localization and burden of stone and renal anatomy. Puncture tract dilatation is performed with dilators, followed by placement of the sheath. According to the endoscopic equipment used in mini-PCNL different sheath size has been reported in literature. Although most preferred one is 16 F sheath, 15 F, 16 F, 18 F or 20 F sheaths have been used. Also, the most common endoscopes used are 9 F, 5 F ureteroscope, 12 F and 15 F mini-nephroscopes^[15,16]. According to the localization of the stone 7 F, 9 F and 14 F flexible ureteroscopes can be used. Stone disintegration is usually performed with laser and/or pneumatic lithotripsy that vary according to the surgeon preference^[17].

PCNL is a challenging procedure in pediatric population because of the small kidney and the low tolerance to blood loss. The use of the mini-PCNL technique is becoming increasingly popular in the treatment of kidney stones in pediatric patients.

In the first publications, standard PCNL technique was performed for the treatment of kidney stone in children and stone-free rate (SFR) has been reported to be 47%-98%^[18,19]. Adult instruments were used with minimal complications. Badway *et al.*^[19] reported their results of 60 children using a 26 F and 28 F Amplatz sheath. SFR was reported approximately 84% with PCNL monotherapy, with only one procedure being abandoned due to intraoperative bleeding. Samad *et al.*^[18] performed 188 PCNLs using a 17 F or 26 F nephroscope in children aged 6-16 years. SFR was reported 47% after PCNL monotherapy and transfusion rate was 3%. Bilen *et al.*^[20] compared the use of 26 F, 20 F and 14 F Mini-PCNL. The mean patient age of the children in each group was 13.2 years, 5.9 years and 6.3 years, respectively. The stone burden, previous surgery and the mean haemoglobin drop postoperatively did not change between the groups; however, the blood transfusion rate was higher in the 26 F and 20 F Amplatz sheath groups. The SFR was highest in the Mini-PCNL group, at 90%, compared to 69.5% in the 26 F and 80% in the 20 F group.

There is no consensus on definition of SFR. It is usually considered as stone fragments smaller than 3 or 4 mm. But untreated residual fragments can cause a stone related events. Due to the fact that pediatric patients have a risk for stone recurrence. It is important to achieve complete stone clearance by selected treatment methods in the treatment of kidney stones in pediatrics^[21].

Wang *et al.*^[22] reported their results of 247 renal units with calculi in 234 patients who underwent mini-PCNL aged under 3 years. All procedure were performed by

Table 1 Mini- percutaneous nephrolitotomy

Ref.	Year	Renal unit	Mean age	Stone size (mean)	Tract	Mean operative time (min)	Initial SFR %	Complications (% , overall)
Ozden <i>et al</i> ^[24]	2010	100	9.5 yr	507.5 mm ²	20.8 F (mean)	79.1	85	25
Zeng <i>et al</i> ^[25]	2012	20	20.6 mo	2.2 cm	14-16 F	77.5	95	NR
Resorlu <i>et al</i> ^[26]	2012	106	9.6 yr	23.7 mm	12-22 F	76.3	85.8	17
Yan <i>et al</i> ^[27]	2012	27	42.6 mo	1.85 cm	14-16 F	86.5	85.2	15
Wah <i>et al</i> ^[28]	2013	23	4.76 yr	3.44 cm ²	16 F	109.4	83.6	14
Onal <i>et al</i> ^[29]	2013	1205	8.8 yr	4.09 cm ²	Cutoff size 20 F	93.5	81.6	27.7
Elderwy <i>et al</i> ^[30]	2014	47	8 (median) yr	2.3 cm (median)	20-24 F	90	91.4	10.6
Desoky <i>et al</i> ^[31]	2015	22	9.5 yr	2.4 cm	20 F	65.1	90.9	36.3
Brodie <i>et al</i> ^[15]	2015	46	7.3 yr	NM	16 F	NR	76	NR

NR: Non reported; NM: Not measured; SFR: Stone-free rate.

Table 2 Modified clavien classification

Grade I	Any deviation from the normal postoperative course without the need for treatment
Grade II	Requiring pharmacological treatment with drugs Blood transfusions and total parenteral nutrition are also included
Grade III	Requiring surgical, endoscopic or radiological intervention
Grade III a	Intervention not under general anesthesia
Grade III b	Intervention under general anesthesia
Grade IV	Life-threatening complication requiring IC/ICU management
Grade IV a	Single organ dysfunction (including dialysis)
Grade IV b	Multiorgan dysfunction
Grade V	Death of a patient

ICU: Intensive care unit.

single tract, including 245 14 F tracts, 1 16 F tract and 1 12 F tract, respectively. 191 cases had stone burden 1-2 cm² and 30 cases stone burden > 2 cm², 26 cases < 1 cm². Mean operating time was 32.5 min (range 21-62 min). Complete stone free rate has been reported as 240 renal unit (97.2%). In another mini-PCNL study SFR rates has been reported as 90.8% in stone burden < 20 mm, but 76.3% in stone burden > 20 mm^[23]. In Table 1, there is an overview of the recent published data of mini-PCNL.

Due to the minimally invasive nature of mini-PCNL in the case of providing complete stone clearance and a clear nephrostomy tract makes the procedure in tubeless manner. Bilen *et al*^[32] evaluated result of tubeless (ureteral catheter but no nephrostomy drainage tube) vs conventional mini-PCNL (nephrostomy drainage tube) in infants and preschool children. In this study with 28 renal unit in 26 patients, the tubeless mini-PCNL group had significantly shorter surgery and fluoroscopy times. Complications rates were higher and duration of hospitalization were longer in the nephrostomy group. Stone-free rates were reported as 91.6% and 78.5% in tubeless and nephrostomy group, respectively.

The aim of the minimally invasive PCNL is to reduce complications such as blood loss, intraoperative -postoperative pain and hospital stay. On the other hand it is believed that a small calibre tract is less injurious to nephrons. But many authors have reported that 24-26 F dilataion does not cause significant morbidity in children, it has been reported that there is no advantage in using a small access based on renal scaring alone^[33].

The caliber and number of tracts are associated with intraoperative hemorrhage during PCNL in children^[34]. Complication rates have significantly reduced with the development of the smallest and least traumatic endoscopic appliances. Moreover, it is reported that there is a significant correlation of intraoperative bleeding with duration of surgery, stone burden and sheath size^[35]. In addition that it is stated that operative time, sheath size, mid calyceal puncture and partial staghorn formation are independent predictors of complications^[29].

It is important that using a common definition in the expression of complication to determine the risk factors for complications. Recently, the modified Clavien system for classifying surgical complications has been used for this purpose^[36]. But complications are not always reported according to this system in recent publications (Table 2). Modified Clavien Classification has been shown.

The first time, Ozden *et al*^[24] indicated perioperative complications of PCNL in pediatric patients using the modified Clavien grading system. Transient fever (grade I) is one of the most frequent complication. But it is not always microbial in origin^[37]. It is determined that transient fever rate is 31% in 188 PCNLs. However, postoperative infection is reported in approximately 6% of pediatric patients^[20,38].

Bleeding is a serious complication during intraoperative and postoperative period in pediatric patients which is associated with sheath size, stone burden, number of tracts and operative time. Hemoglobin drop requiring transfusion (grade II) is reported in 0.4%-24% of patients^[39,40]. In another study higher hemoglobin drop

Table 3 Complication rates of mini- percutaneous nephrolithotomy according to modified clavien classification

Ref.	Year	Renal unit	Overall complication rate (%)	Grade I - II (%)	Grade III (%)	Grade IV-V (%)
Ozden <i>et al</i> ^[24]	2010	100	25	21	4	-
Resorlu <i>et al</i> ^[26]	2012	106	17	17	-	-
Yan <i>et al</i> ^[27]	2012	27	15	15	-	-
Wah <i>et al</i> ^[28]	2013	23	14	13.6	0.4	-
Onal <i>et al</i> ^[29]	2013	1205	27.7	23.04	3.46	1.2
Pan <i>et al</i> ^[42]	2013	59	11.9	11.9	-	-
Elderwy <i>et al</i> ^[30]	2014	47	10.6	8.5	2.1	-
Desoky <i>et al</i> ^[31]	2015	22	36.3	22.7	13.6	-

has been determined in pediatric patients performed PCNL when size of the tract dilatation exceeded 22 F^[34].

There is a debate on the classification of grade III complication, is that auxiliary procedures such as RIRS, SWL and second look PCNL. It is recommended to consider them as part of treatment strategy. However, such as hydrothorax requiring chest tube or urine leakage requiring urinary diversion can be classified as Clavien grade III complication^[24]. It is said that grade III, IV, V complications should be quite rare and more likely associated with surgical techniques and experience^[41]. Complication rates have been shown in literature in Table 3.

ULTRA-MINI PCNL: SURGICAL TECHNIQUE AND NEW REPORTS

In 2013, the new PCNL technique was described by Desai *et al*^[34] using of a novel 6 F mini nephroscope through an 11-13 F metal sheath to perform holmium: YAG laser lithotripsy. The new procedure was performed in 36 patients with a mean stone size 14.9 mm. Two patient were preschool children. It was reported that mean operative time, stone free rate at postoperative 1st day and 1st month were 59.8%, 88.9%, and 97.2%, respectively. Complication rate were reported as 16.% in 6 patients, according to Clavien classification, including 2 sepsis, 1 urinary extravasation, and 3 fever. The authors determined that there was no needed blood transfusion^[43]. In another study results of 62 patients were reported using a 3.5 F nephroscope. Nephrostomy tract was dilatated up to 13 F. Only four of the 62 patients were children. Mean stone size was 16.8 mm, stone free rate at the 1st month was reported approximately 87%^[44]. There is no sufficient data available to compare this new technique with other methods which use for the treatment of pediatric urinary stones. The new technique's effectiveness and safety remain to be seen in larger prospective studies in pediatric patients.

MICRO-PCNL: SURGICAL TECHNIQUES

Recently, Micro-PCNL or microperc has been described as another minimally invasive PCNL technique that is performed through a 4.85 F all-seeing needle. A three-way connector is attached to the latter, which admits a

saline irrigation tube, 0.9 or a 0.6 mm-diameter micro-optic, a 272 µm laser fiber. The outer diameter of this modified needle is 1.6 mm (4.85 F). The first time this new technique were used in 15 adults. Mean stone size, operation time was 30.4 mm, 101.4 min, respectively. Postoperative complete stone clearance achieved in 11 patients^[45]. Since then this method has adopted to the treatment of pediatric kidney stones. In a study, 24 infant treated with micro-PCNL. The mean age, stone size, operation time were 15.8 mo, 13.5 mm, 53.7 min, respectively. There is no major complication and hemoglobin drop requiring blood transfusion reported^[46]. More experience and more knowledge is needed for the effectiveness of this method.

CONCLUSION

Technological innovations have contributed to the development of minimally invasive treatment of urinary stone disease. It can be said that to increase the efficacy and reduce complications is the main objective of physicians. In this manner new treatment methods which use for minimally invasive management of kidney stones in pediatric population has offered various treatment alternatives to the surgeons. However, level of experience and new publications can contribute us to provide complete stone clearance and to reduce complication rates.

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Central blood pressure and chronic kidney disease

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Abstract

In this review, we focused on the relationship between

central blood pressure and chronic kidney diseases (CKD). Wave reflection is a major mechanism that determines central blood pressure in patients with CKD. Recent medical technology advances have enabled non-invasive central blood pressure measurements. Clinical trials have demonstrated that compared with brachial blood pressure, central blood pressure is a stronger risk factor for cardiovascular (CV) and renal diseases. CKD is characterized by a diminished renal autoregulatory ability, an augmented direct transmission of systemic blood pressure to glomeruli, and an increase in proteinuria. Any elevation in central blood pressure accelerates CKD progression. In the kidney, interstitial inflammation induces oxidative stress to handle proteinuria. Oxidative stress facilitates atherogenesis, increases arterial stiffness and central blood pressure, and worsens the CV prognosis in patients with CKD. A vicious cycle exists between CKD and central blood pressure. To stop this cycle, vasodilator antihypertensive drugs and statins can reduce central blood pressure and oxidative stress. Even in early-stage CKD, mineral and bone disorders (MBD) may develop. MBD promotes oxidative stress, arteriosclerosis, and elevated central blood pressure in patients with CKD. Early intervention or prevention seems necessary to maintain vascular health in patients with CKD.

Key words: Atherosclerosis; Mineral and bone disorder; Oxidative stress; Proteinuria; Renal autoregulation

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Core tip: Wave reflection is a major mechanism that determines central blood pressure in chronic kidney disease (CKD). Diminished renal autoregulatory ability characterizes CKD, allowing an increase in proteinuria. Thus, any elevations of central blood pressure accelerate the progression of CKD. The kidney produces oxidative stress compounds due to proteinuria handling and secondary interstitial inflammation. Oxidative stress facilitates atherogenesis, increases arterial stiffness and central blood pressure. Furthermore, even in early stages of CKD, mineral and bone disorder (MBD) is

developed. CKD-MBD facilitates to induce oxidative stress and elevation of central blood pressure. To keep vascular health in CKD, early intervention or prevention seems mandatory.

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INTRODUCTION

Blood pressure is the product of the cardiac output and total peripheral vascular resistance. In turn, cardiac output is the product of the stroke volume and heart rate. The diastolic and mean blood pressures remain similar along the systemic arterial tree^[1]. Therefore, the aortic and brachial mean blood pressures are comparable^[1]. However, systolic blood pressure differs significantly between the central and peripheral arteries, even within a single cardiac beat. Specifically, the central systolic blood pressure is lower than the brachial systolic blood pressure, which itself is lower than the systolic blood pressure at the dorsal foot artery. At any given site within the arterial tree, the systolic blood pressure increases as the distance from the heart increases^[2].

CENTRAL HEMODYNAMIC MECHANISMS

How does a single heart stroke cause variations in blood pressure from the aorta to the peripheral arteries? Two mechanisms have been proposed (Figure 1): Wave reflection and amplification^[2]. Wave reflection occurs at all levels of the arterial tree^[3]. Reflection occurs in areas where the arterial caliber is decreasing, or at areas where a single artery divides into two or three branches. Each wave reflection causes a backward wave in arterial system. If all backward waves could be integrated, the single wave would ascend approximately from the aortic bifurcation. Backward and forward waves yield summation effects, resulting in augmented systolic blood pressure. As shown in Figure 2, the augmentation index (AI) is defined as the augmented pressure/forwarding pulse pressure. The summation effects are affected by many factors^[4], including the degree of wave reflection, heart rate, height, and pulse wave velocity (PWV). The reflection magnitude is modulated by the stroke volume and arterial stiffness. A greater stroke volume enlarges the reflection. Increased arterial stiffness, such as that in elderly individuals, also increases reflection. A short height and fast PWV also allow the backward wave to reach the ascending aorta during systole, resulting in a very high central systolic blood pressure and significant stress on the left ventricle^[5,6]. Normally, the backward wave arrives at the ascending aorta in diastole, faci-

tating coronary perfusion. A slower heart rate lengthens the ejection period and allows the backward wave to reach the ascending aorta at late systole. Thus, the early arrival of a large reflection wave at the ascending aorta increases both the central systolic blood pressure and cardiovascular (CV) risk.

Pressure amplification is a physiological phenomenon that is evident in young people with supple, flexible, elastic arteries^[2]. In such individuals, the arterial wall flexes like a whip with each heart stroke. We will attempt the difficult process of explaining pressure amplification without a mathematical analysis. During the systolic phase, the pulse wave arrives at the aorta and proceeds at the speed of the PWV. The PWV speed is well known to increase along with systolic blood pressure. One can divide the forwarding pulse into three parts: Initial, middle, and last. In the initial part, blood travels from the heart to the aorta, which is very soft and becomes distended. Accordingly, the PWV is slow in the initial part and functionally increases the aortic root stiffness with a small increase in blood pressure. During the middle part, the forwarding pulse emerges from the heart. As the aortic root stiffness is higher in the middle part than in the initial part, the PWV also increases. Consequently, some of the middle part catches up with the initial part, leading to a moderate amplification of systolic pressure. Finally, the last part enters the aorta at the fastest PWV, further amplifying the systolic blood pressure. Closure of the aortic valve ends this escalation of systolic pressure. Importantly, this pressure amplification continues along the length of the aorta as the pulse wave travels. Thus, in young people, the central systolic blood pressure remains low, compared with the brachial systolic blood pressure^[7].

Indeed, isolated systolic hypertension is a CV risk in elderly patients, not in young subjects^[7]. The above description may explain why pressure amplification is a major cause of isolated systolic hypertension in young individuals, whereas wave reflection causes central systolic blood pressure elevation in the elderly. The Framingham Study focused attention toward pulse pressure as the best measure of CV risk, at least in older subjects^[7]. Since pulse pressure is a surrogate measure of arterial stiffness, such data indicate that arterial stiffness is a key determinant of CV risk in older subjects. Although there is a debate, the data from Framingham study suggest that diastolic pressure remains the best predictor of coronary heart disease risk in younger subjects. As chronic kidney disease (CKD) is rather common in elderly populations whose artery is stiff due to the remodeling^[2,5,6], wave reflection, rather than pressure amplification, determines the central blood pressure in this patient population.

CENTRAL BLOOD PRESSURE MEASUREMENT METHODS

The central systolic blood pressure places a direct

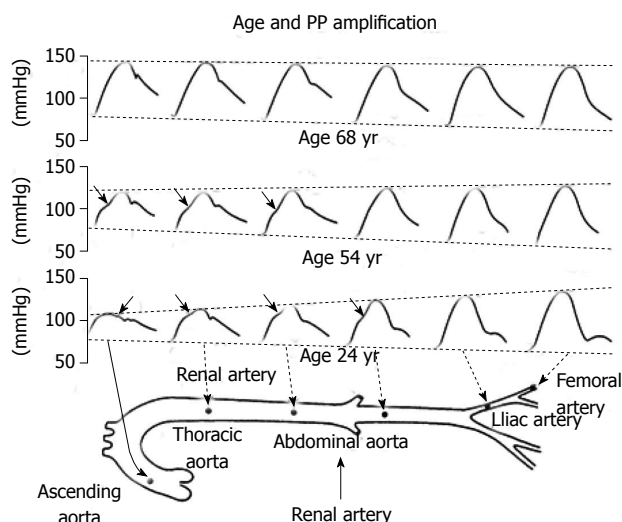


Figure 1 Representative pulse waveforms along the aorta in young, middle-aged and elderly persons. In younger subjects (age: 24 yr), the rate of propagation is relatively low in arterial vessels, which become progressively narrower and less distensible. Because of the summation of the forward and the backward wave at each point of the arterial tree, peak systolic blood pressure increases markedly from central to peripheral arteries, while end-diastolic blood pressure tends to be reduced and mean arterial pressure remains unchanged. In older subjects (age: 68 yr), because of the more rapid propagation of pressure wave with resulting changes in wave reflections, the amplification of PP disappears, making that central and peripheral BP become identical. At 54 yr of age, the situation is intermediate between younger and older subjects^[2].

burden on the left ventricle and is a better predictor of CV prognosis than the brachial blood pressure. Central blood pressure correlates better with real blood pressure for heart and great vessels than brachial blood pressure. A lower central blood pressure is associated with a better CV outcome, regardless of brachial blood pressure. Until recently, intravascular catheterization was only the method to measure central blood pressure efficiently. This method is direct and accurate, and therefore remains the gold standard for central blood pressure assessment. However, it is so invasive that only selected patients can undergo such an evaluation. Recent progress in medical technologies has enabled non-invasive assessments of central blood pressure.

Currently, two devices that provide consistent central blood pressure readings are available on the market^[8]. First, Karamanoglu *et al.*^[9] performed invasive simultaneous measurements of both the brachial and aortic pulse waveforms and used a Fourier analysis to generate a generalized transfer function. This transfer function allows the estimation of an aortic waveform from a brachial waveform. The transfer function was later used to develop a device that uses a tonometer to access the radial pulse waveform and estimate an aortic pulse waveform (Figure 3). This device is able to calculate the aortic blood pressure through calibration with indirect brachial blood pressure measurements obtained *via* the cuff method. Second, Takazawa *et al.*^[10] independently developed a new device to access the central blood pressure. The authors invasively measured the aortic blood pressure during cardiac catheterization,

while simultaneously indirectly measuring both the radial pulse waveform and brachial blood pressure. They found that the second peak of radial pulse waveform correlated with the aortic waveform peak, thus enabling an indirect estimation of the aortic systolic blood pressure without using the generalized transfer function (Figure 4).

Notably, cuff measurements of brachial blood pressure *via* oscillometric methods have such large errors that invasive measurements of the brachial blood pressure are approximately 10 mmHg higher than non-invasive measurements^[8]. Both devices have been described as calibrating the central blood pressure through the indirect measurement of brachial blood pressure. Thus, invasive measurement yields central blood pressure values approximately 10 mmHg higher than device-assisted indirect central blood pressure values. Although we are very familiar with the indirect measurement of brachial blood pressure, great cautions are required when discussing the accuracy of the method to assess the exact blood pressure.

INCREASED CENTRAL BLOOD PRESSURE IS AN IMPORTANT CV RISK

Recent clinical studies have shown that the increase in central blood pressure is a stronger CV risk than the brachial blood pressure. Williams *et al.*^[11] divided a cohort of enrolled hypertensive Anglo-Saxon and Scandinavian patients into two groups: Those treated with calcium channel blocker-based medications, and treated with beta-blocker-based regimens. During the follow-up period, both groups exhibited similar brachial blood pressure control. However, fewer CV events occurred in patients receiving calcium channel blocker-based therapy. Importantly, the central blood pressure was significantly lower in those treated with calcium channel blockers than in those treated with beta-blockers (Figure 5). The authors also demonstrated that central blood pressure contributed to the number of total CV events and the development of renal impairment, suggesting that a correct central blood pressure measurement is a more accurate parameter than brachial blood pressure in preventing CV and renal events. Roman *et al.*^[12] performed a population-based longitudinal study of prevalent and incident CV disease in 3502 American Indians; 319 of these subjects suffered fatal and non-fatal CV events during a 5-year follow-up. The authors concluded that the measurement of central blood pressure more strongly predicts CV events than does brachial blood pressure. However, Chirinos *et al.*^[13] enrolled 2606 patients with CKD patients and observed the incidence of hospitalization for new-onset heart failure over a 3.5-year period. These authors concluded that a fast aortic PWV, but not a high central blood pressure, predicted heart failure. It is difficult to distinguish heart failure from fluid retention in patients with CKD partly due to vascular remodeling including calcification. In contrast, our previous study indicated

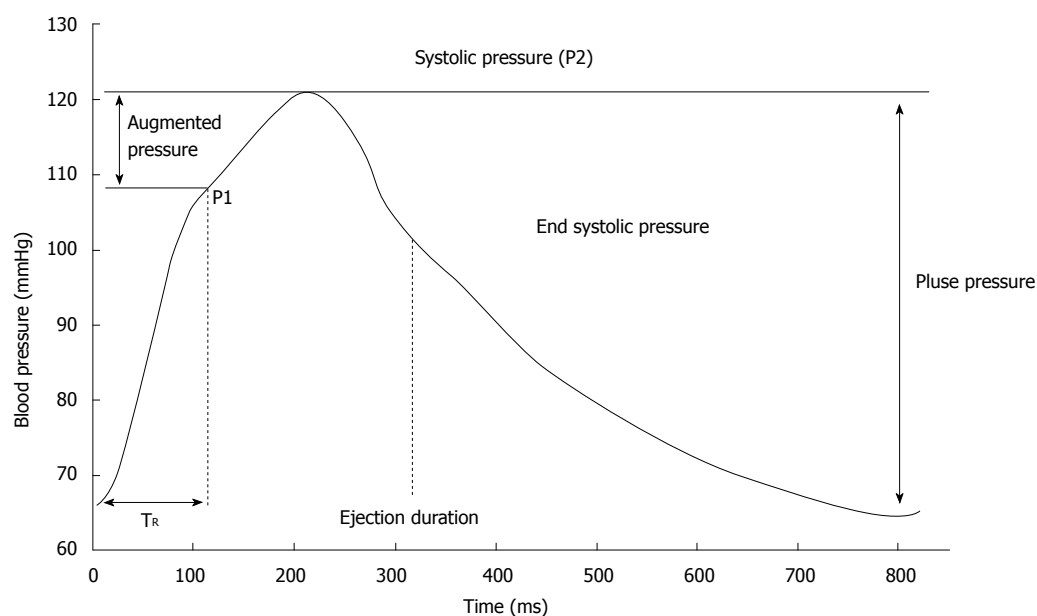


Figure 2 Example central pressure waveform. TR indicates timing of the reflected pressure wave; P1 and P2 represent the first and second systolic peaks, respectively^[3]. Augmentation index is defined as augmented pressure/forwarding pulse pressure (P1).

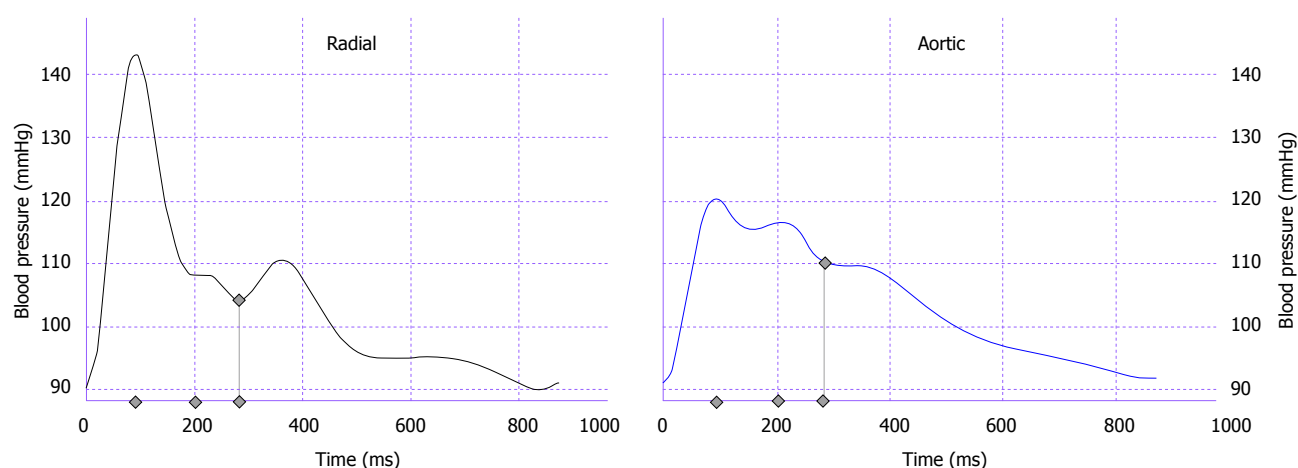


Figure 3 Estimation of central pulse waveform. Radial tonometry detects radial pulse waveform with high systolic blood pressure over 140 mmHg (left panel). Using this radial waveform, generalized transfer function calculates aortic pulse waveform (right panel). Please note that aortic systolic blood pressure is 120 mmHg (Available from: URL: <http://hogimed.fr/?q=sphyg%20p1>).

that AI predicted CV events in hemodialysis patients^[14]. Collectively, these data suggest that the blood pressure in the ascending aorta is a significant CV risk for the development of atherosclerotic CV diseases.

What about abdominal aortic blood pressure, to which the kidney is exposed? The backward wave travels for a shorter distance and meets the forwarding wave sooner in the aorta at the renal artery level, compared to the ascending aorta. Thus, the backward wave augments the forwarding wave in mid-systole, leading to greater summation effects^[5]. The pressure amplification at this level is also greater than in the ascending aorta because the forwarding wave travels a longer distance. Collectively, the renal arterial pressure should fall between the aortic root and brachial blood pressures. Indeed, Hope *et al.*^[15] examined blood pressure profiles

along the aorta during cardiac catheterization in patients with an average age of 65 years, and reported that the aortic systolic pressure was approximately 10 mmHg higher at the level of the kidney than in the ascending aorta.

CENTRAL BLOOD PRESSURE AS A CAUSE OF CKD

When exposed to high blood pressure, the arteries and arterioles constrict and increase their vascular resistance to buffer the direct transmission of systemic pressure to capillary beds in the terminal organs, in a process called autoregulatory or myogenic vasoconstriction^[16]. In addition to myogenic constriction, tubuloglomerular feedback (TGF) affects renal autoregulation^[17]. TGF

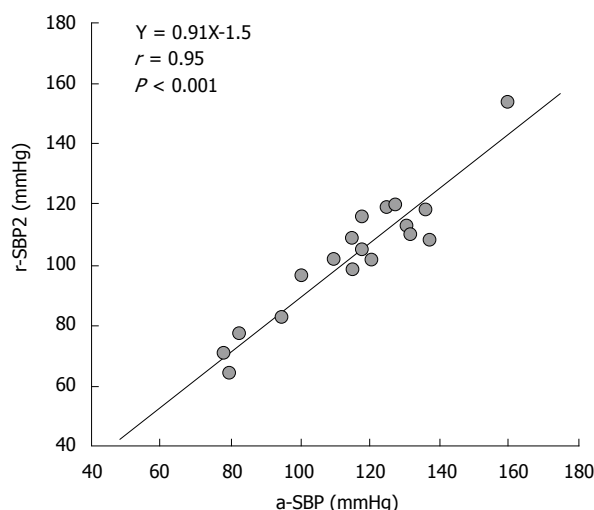


Figure 4 Relationship between radial second peak of systolic blood pressure and aortic systolic blood pressure. There is a strong positive relation between two^[10]. r-SBP2: Radial second peak of systolic blood pressure; a-SBP: Aortic systolic blood pressure.

is the mechanism specific for the kidney to maintain glomerular filtration rate constant. Elevations of blood pressure temporally increase both glomerular capillary pressure and filtration rate. This elicits an increase in tubular flow that reaches macula densa. Then, the reabsorption by macula densa is increased. Macula densa cells release the mediator to constrict afferent arteriole, thereby returning both glomerular capillary pressure and filtration rate to the baseline. Many studies have repeatedly demonstrated that the dysregulation of these autoregulatory responses in CKD^[18]. Nephritic patients commonly exhibit mesangial changes, which damage the effectiveness of TGF^[19]. In nephrosclerosis, afferent arteriolar changes such as hyalinosis (benign nephrosclerosis) and fibrinoid necrosis (malignant nephrosclerosis) preclude the normal autoregulatory behavior of the afferent arteriole^[20]. In diabetes, hyperglycemia facilitates the re-uptake of NaCl through sodium-glucose co-transporters at the proximal tubules. NaCl delivery to the macula densa is reduced in diabetes, thereby reducing the TGF^[21]. CKD is characterized by the diminished TGF, activating renin-angiotensin system, causing secondary hyperaldosteronism, volume expansion and hypertension. Consequently, in patients with CKD, systemic blood pressure is transmitted rather directly to the glomeruli, partly because of inadequate renal autoregulatory adjustments. Thus, glomerular hyperfiltration and hypertension are commonly observed in this patient population.

Proteinuria is a clinical marker of glomerular hypertension^[21]. We performed clinical studies to determine the role of central hemodynamics in CKD progression. As the aortic blood pressures at the renal artery and aortic root differ, we focused on central hemodynamic parameters such as AI and the time for reflection (TR), rather than the central blood pressure itself. TR indicates the time required for the reflection pressure to arrive

at the ascending aorta (Figure 2). As discussed above, an inappropriate activation of renin angiotensin system is common in CKD. Our previous data indicated that AI correlated positively with proteinuria in 99 non-diabetic patients with CKD^[5]. Among 44 patients with angiotensin inhibition, a higher basal AI led to a greater annual decrease in creatinine clearance (Figure 6), suggesting that in addition to angiotensin, AI is a risk factor for the progression of non-diabetic CKDs. We further performed an observational study of 42 non-diabetic patients with CKD^[22]. A multivariate regression analysis revealed a correlation between annual increases in serum creatinine and the TR, suggesting that the TR predicts the progression of renal dysfunction in patients with CKD. Finally, we performed a randomized controlled trial of 59 hypertensive CKD patients to assess the long-term effects of calcium antagonists on AI^[23]. All patients received an angiotensin receptor blocker and amlodipine or azelnidipine. Compared to amlodipine, azelnidipine reduced proteinuria and AI to a greater extent. Consequently, these data support the notion that any reductions in the abdominal aortic blood pressure would decrease proteinuria, thus slowing the progression of CKD.

Recent findings indicate an increase in blood pressure variability causes kidney damage^[24]. Blood pressure exhibits beat-to-beat, day-to-day, and visit-to-visit variations. Renal autoregulatory adjustments occur with some delay following changes in blood pressure^[16,17]. The myogenic mechanism requires a few second to initiate, and the TGF requires a slightly longer time to complete its final adjustment. If the blood pressure suddenly decreases, the low blood pressure must perfuse the kidney, which exhibits high vascular resistance due to the remaining autoregulatory vasoconstriction; this situation presumably leads to renal ischemia. If the blood pressure increases abruptly, this high systemic blood pressure is transmitted to the glomeruli rather directly before an adequate autoregulatory increase in renal vascular resistance can occur. Thus, marked blood pressure variability may induce ischemia-reperfusion type renal damage^[25]. Of interest, a high aortic pulse pressure was found to correlate with an increase in beat-to-beat blood pressure variability^[26]. Collectively, central hemodynamic abnormality hastens the progression of CKD presumably by causing renal ischemia in addition to glomerular hypertension.

INCREASED CENTRAL BLOOD PRESSURE AS A CONSEQUENCE OF CKD

The glomeruli continuously leak proteins into the ultrafiltrate^[27]. These filtered proteins are largely taken up by proximal tubular cells and handled in one of two ways. Under physiological conditions, the glomerulus leaks a small amount of proteins that are absorbed by proximal tubular cells and subjected to acid hydrolysis. Under pathological conditions, such as proteinuric CKD, each

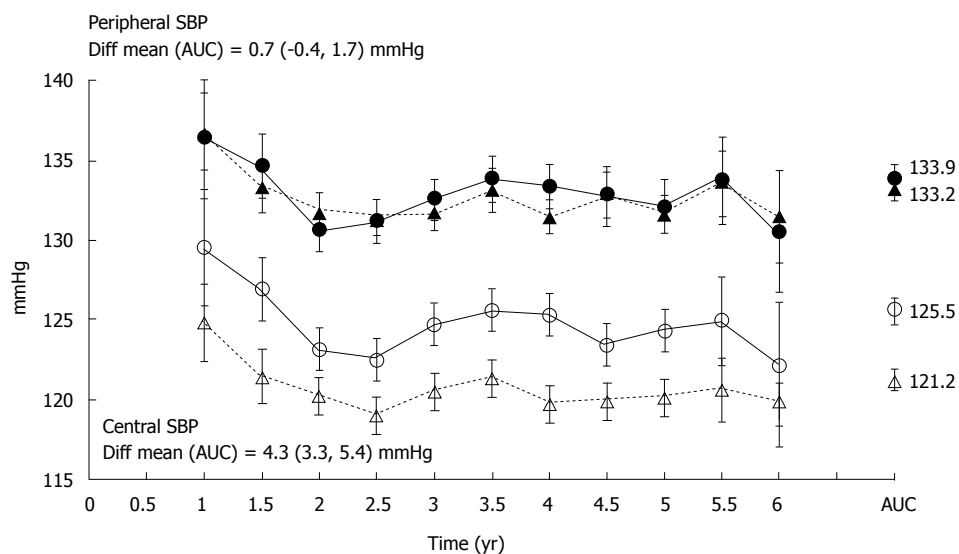


Figure 5 Principal results of ASCOT-CAFÉ study. Brachial blood pressure was similar between the patients treated with beta-blocker-based medication (close circles) and calcium channel blocker-based treatment (closed triangles). However, central blood pressure was higher in the former (open circles) than the latter group (open triangles)^[11]. SBP: Systolic blood pressure.

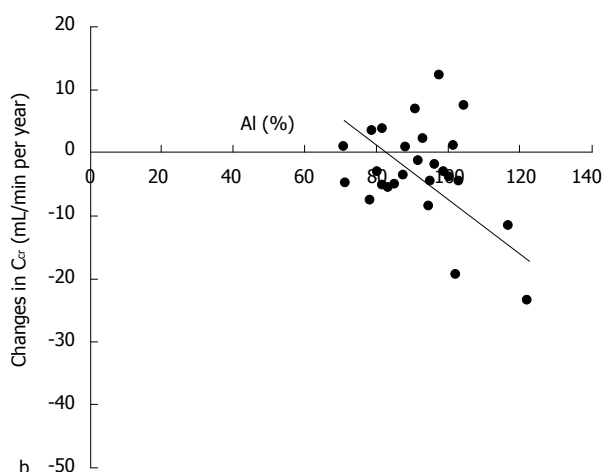


Figure 6 Relationship between annual changes in creatinine clearance and augmentation index. There is an inverse relation between two^[6].

glomerulus leaks a large amount of proteins. Because the capacity of the proximal tubules to hydrolyze proteins is limited, oxidative degradation begins to break down the absorbed proteins, thus triggering an atherogenic chain reaction. Reactive oxidative species (ROS) diffuse into the peritubular capillary and oxidize circulating molecules such as low-density lipoprotein cholesterol (LDL-C). Oxidized LDL-C consequently induces inflammation in the arterial wall to initiate an atheroma^[28]. Subsequently, these atheromas become focal points for *de novo* oxidative stress and promote progression to systemic atherosclerosis. In addition to generating oxidative stress, the proximal tubular cells secrete various chemokines that recruit inflammatory cells into the renal interstitium. In turn, interstitial inflammation accelerates oxidative stress. Non-proteinuric CKDs, such as hydronephrosis, cystic kidney disease, and ischemia-reperfusion, also increase

oxidative stress^[25]. Cystic expansion or increased intra-tubular pressure causes tubular cell damage and can induce apoptosis or necrosis. In a manner similar to that observed in proteinuric CKDs, inflammatory cells accumulate in the renal interstitium to remove the debris and replace it with fibrotic material. Epithelial-mesenchymal transition may contribute to fibrosis process. Interstitial inflammation also triggers an atherogenic chain reaction in non-proteinuric CKDs.

Atherosclerosis is characterized by arterial stiffness, for which PWV is a good index. The carotid-femoral (cf) PWV has been used in many studies. We previously conducted an observational study of 102 hypertensive patients with CKD^[29]. These patients were divided into two groups according to the use or non-use of an angiotensin converting enzyme inhibitor or angiotensin receptor blocker and observed for 4 years. The heart-femoral (hf) PWV was measured repeatedly. Compared with cfPWV, which measures arterial stiffness below the aortic arch, hfPWV assesses arterial stiffness across the total aorta and iliac artery. Brachial blood pressure was similarly controlled in both groups. However, although gradual hfPWV elevation was observed in the group without angiotensin inhibition, this value remained unchanged in the patients under angiotensin inhibition. As expected from the lack of a progressive increase in PWV^[30], angiotensin inhibition reduced both CV and renal deaths. In addition, PWV correlated positively with AI in patients with CKD^[22]. In other words, a rapid PWV indicates a high AI and central blood pressure. Maintenance of the central blood pressure within a normal range is a mandatory step in maintaining renal blood flow and glomerular filtration without inducing oxidative stress. It would be advantageous to slow the progression of renal dysfunction in CKD. From these results, we propose a working hypothesis in which a vicious cycle exists between CKD and increased central

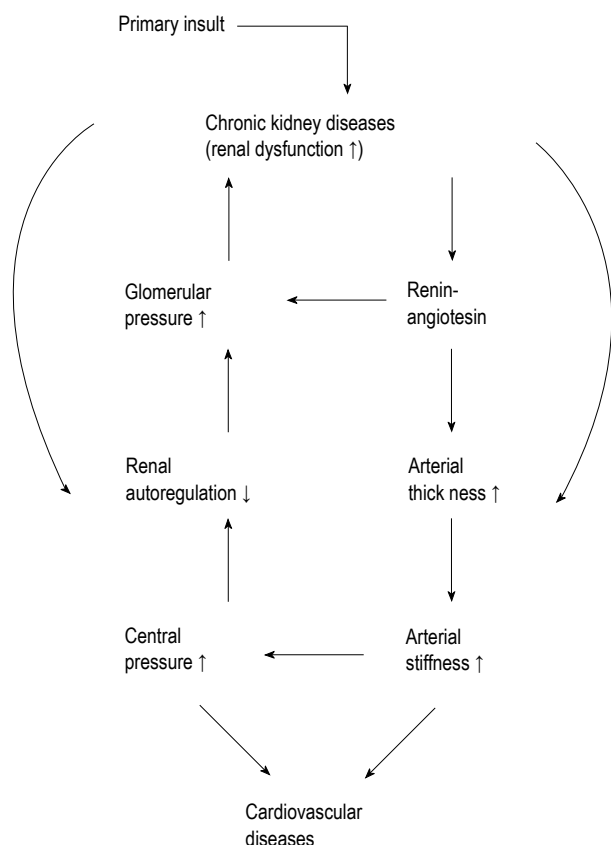


Figure 7 Working hypothesis underlying cardiovascular disease and chronic kidney disease. Lowering central blood pressure could cut vicious cycle between CVD and CKD^[29]. CKD: Chronic kidney disease; CVD: Cardiovascular disease.

blood pressure (Figure 7).

Mineral and bone disorders (MBDs) also underlie the development of CV diseases in CKD^[31]. Notably, MBDs are initiated at an early stage of CKD. For example, renal *klotho* expression is already reduced at CKD stage 2. This situation induces an increase in FGF23 expression. In CKD stage 3, a decreased calcitriol level and secondary hyperparathyroidism are common observations. In CKD stage 4-5, hyperphosphatemia and hypocalcemia become evident. FGF23 increases ROS production in vascular smooth muscle cells and induces cardiac hypertrophy^[32,33]. In vascular smooth muscle cells, the excessive uptake of phosphate through Pit1 induces the expression of osteocyte-specific genes, thus changing the cellular phenotype from vascular to bone^[34]. Arterial calcification is a significant CV risk, especially in patients with advanced CKD^[35]. Aortic root calcification is common in this population. Lam *et al.*^[36] demonstrated that aortic root remodeling is a significant CV risk. We performed a cross-sectional study to characterize the central hemodynamics in 1392 CKD patients. As shown in Figure 8, the AI was lower in stage 5 than in stage 1^[37]. Because of the marked increase in aortic root stiffness, the forwarding wave cannot adequately stretch the aortic root; subsequently, the forwarding pressure increases to the extent that the reflection pressure contributes slightly to the peak aortic

pressure, thus reducing the AI. Collectively, our results provide functional evidence that aortic root stiffness is markedly increased in stage 5 CKD, which would account for the high CV risk faced by advanced CKD patients. In addition, our data indicated that diastolic blood pressures were lower in CKD stages 3-5 were lower than stage 1. Under physiological conditions, aorta stores approximately half of the stroke volume during systole. The pooled blood keeps the organ well perfused during diastole. Increased aortic stiffness not only decreases this storage capacity and thus reduces coronary perfusion, but also elicits central high blood pressure and resultant left ventricular hypertrophy^[38,39]. Thus, increased aortic stiffness exacerbates myocardial ischemia, worsening CV prognosis.

POSSIBLE THERAPIES FOR CENTRAL HIGH BLOOD PRESSURE IN CKD

Most patients with CKD manifest hypertension, and the selection of antihypertensive agents might determine their central blood pressure^[40]. Vasodilating antihypertensive agents, including calcium channel blockers, angiotensin receptor blockers, converting enzyme inhibitors, and alpha-adrenergic blockers, preferentially reduce the central blood pressure rather than the brachial blood pressure (Figure 9). Aliskiren was not available in the market when this study was performed. In contrast, non-vasodilating antihypertensive medications, such as diuretics and beta-adrenergic blockers, similarly reduce the central and brachial blood pressures. Thus, the administration of vasodilator antihypertensive agents to hypertensive patients with CKD more efficiently lowers the central blood pressure, compared with non-vasodilator antihypertensive medications, thereby ameliorating proteinuria and preventing the development of atherosclerotic CV diseases. In this regard, patients whose blood pressure had not reached goal values, despite treatment with an angiotensin receptor blocker, were evaluated in a retrospective study^[41]. Patients treated with additional calcium channel blockers or additional diuretics were compared. Both calcium channel blocker and diuretic treatment considerably reduced the brachial blood pressure. However, although both agents reduced the AI, calcium channel blockers yielded greater improvements in this parameter. Compared with those using diuretics, patients using calcium channel blockers exhibited a greater decrease in protein excretion. Interestingly, decreases in proteinuria correlated with reductions in AI. Similarly, Bakris *et al.*^[42] demonstrated that combined treatment with converting enzyme inhibitors and calcium channel blockers provided better renal protection than combined treatment with both converting enzyme inhibitors and diuretics. Although angiotensin receptor blockers, converting enzyme inhibitors, and direct renin inhibitors have been established as first-line antihypertensive drugs for proteinuric patients with CKD^[43], calcium channel

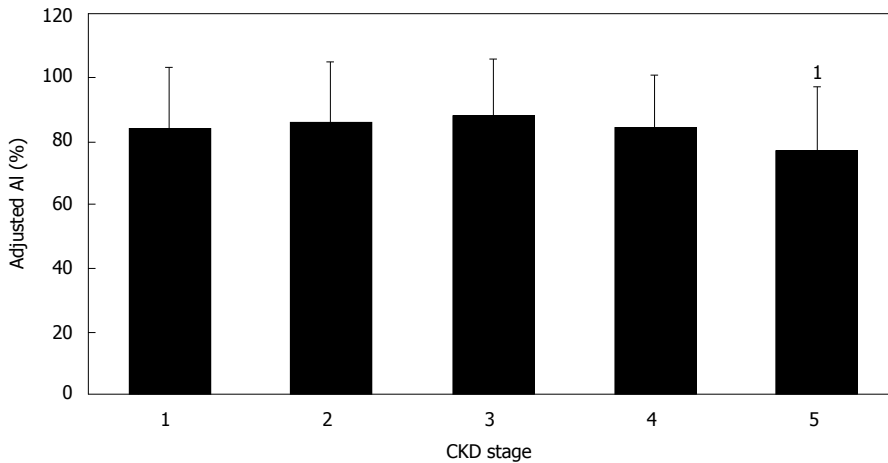


Figure 8 Comparison of augmentation index among all chronic kidney disease stages. AI was adjusted with confounding factors including age, blood pressure, pulse rate, and vasodilator antihypertensive drugs. ¹Indicated significant difference from stage 1^[37]. CKD: Chronic kidney disease.

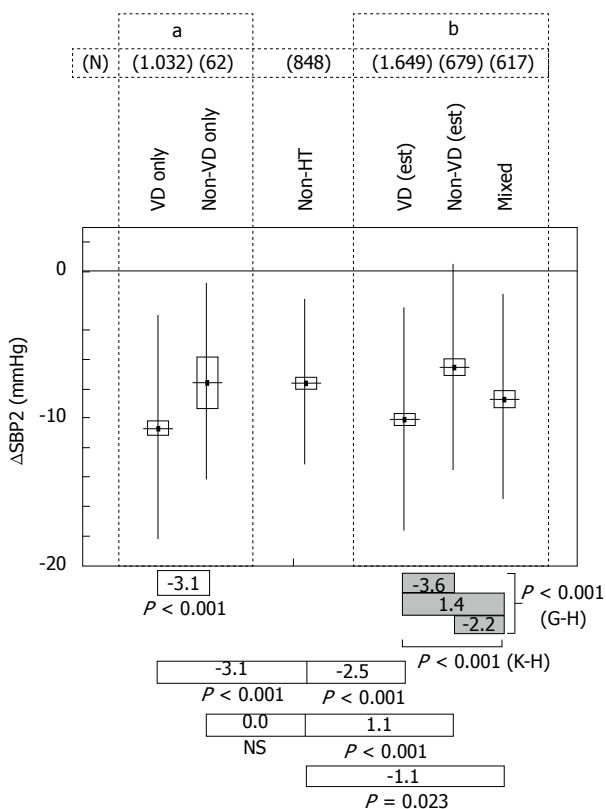


Figure 9 Disparate effects of antihypertensive drugs on central blood pressure. Differences between brachial systolic blood pressure and central systolic blood pressure (Δ SBP2) were compared between vasodilator (VD) and non-vasodilator (non-VD) antihypertensive medications. Δ SBP2 was adjusted by age, gender, height, BMI, diastolic blood pressure and use of nitrate. The comparison between actual VD and non-VD only regimen was shown in left panel (A). Right panel (B) depicted comparisons among VD(est), non-VD(est) and Mixed combination of VD and non-VD. "(est)" indicated including data derived from mixed combination, for which the effects of VD and non-VD alone on Δ SBP2 were estimated. Non-hypertensive population (non-HT) was used as physiological reference for Δ SBP2. VD antihypertensive drugs included angiotensin receptor blocker, calcium channel blocker, converting enzyme inhibitor and alpha-blocker. Non-VD group includes beta-blocker and diuretics. Mann-Whitney U test was used to compare the means, unless otherwise specified. K-W and G-H described Kruskal-Wallis and Games-Howell multiple comparison test^[40].

blockers appears more suitable than diuretics for second-line antihypertensive treatment. In addition, calcium channel blockers flatten intra-individual variations in blood pressure^[44]. Therefore, calcium channel blockers appear to retard the progression of hypertensive non-proteinuric CKD by preventing additional ischemia-reperfusion renal damage.

Endothelial cells secrete nitric oxide in response to shear stress, thus relaxing the arteries^[45]. Alternatively, oxidative stress reduces the bioavailability of nitric oxide, which elicits vasoconstriction and arterial remodeling^[46]. Clinically, flow-mediated vasodilation (FMD) can be used to assess endothelial function^[47]. Blood flow stimulates the endothelium to release vasodilators such as nitric oxide, and the effects of the vasodilators can be assessed by monitoring arterial diameter with ultrasound device. We enrolled 36 CKD patients with dyslipidemia to evaluate the effects of statin on FMD^[27]. Although FMD was reduced in patients with CKD, this parameter correlated inversely with the magnitude of proteinuria. Furthermore, atorvastatin treatment improved both FMD, as well as LDL-C levels. In addition, our previous data suggest that combined treatment with statins and angiotensin inhibitors attenuated the progressive increases in PWV observed in hemodialysis patients^[48]. Statins exert pleiotrophic actions, including immunomodulation, anti-inflammation, and oxidative stress reduction^[49]. Statins also inhibit both podocyte injury and protein re-uptake by proximal tubules (Figure 10)^[27]. As discussed, oxidative stress is a mediator of atherosclerosis development in CKD. In patients with CKD, the judicious use of statins might help to end the vicious cycle between the progression of renal dysfunction and central high blood pressure.

A recent study demonstrated that when compared with calcium carbonate treatment, sevelamer hydrochloride treatment for the control of hyperphosphatemia slowed coronary artery calcification and suppressed advanced glycation end products (AGEs) in hemodialysis patients^[50]. Similarly, our previous data indicated

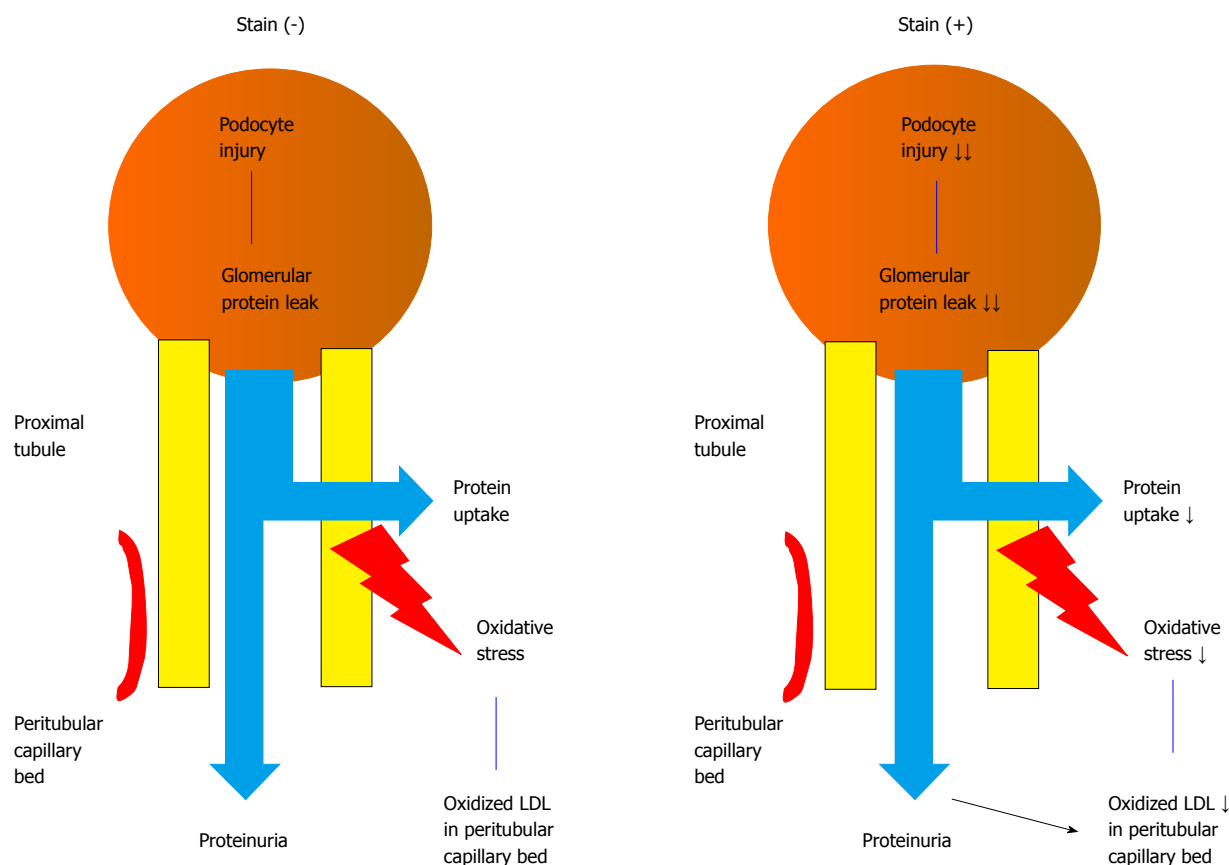


Figure 10 Multiple actions of statin on chronic kidney diseases: Statin decreases proximal tubular uptake of protein leaked from glomeruli, reducing oxidative stress. Statin also improve podocyte injury, reducing glomerular protein leak^[27]. LDL: Low-density lipoprotein.

that switching from calcium carbonate to sevelamer hydrochloride reduced LDL-C levels and attenuated progressive increases in PWV in hemodialysis patients^[51]. Another vicious cycle appears to link oxidative stress and AGEs^[52]. AGEs induce ROS in vascular cells, leading to ongoing AGE formation and atherogenesis. Therefore, a blockade of ROS or AGE formation might interrupt this vicious cycle. In contrast to the inverse association between 25-hydroxyvitamin D and hypertension risk, 1,25-dihydroxyvitamin D was positively associated with risk of hypertension^[53]. Thus, careful supplementation of vitamin D is mandatory for CKD patients. These observations suggest that an appropriate treatment for hyperphosphatemia would cut this vicious cycle and arrest further increases in arterial stiffness (especially aortic root stiffness) and central hemodynamic deteriorations in patients with stage 5 CKD.

CONCLUSION

It is not possible to determine the exact central blood pressure from brachial blood pressure. However, central blood pressure is a stronger predictor of CV and renal diseases, compared with brachial blood pressure, and should therefore be used to guide antihypertensive therapy. In CKD patients, the arteries, including the aorta, become stiff even at early stages of disease^[54]. As the proportions of elderly citizens are increasing within

populations, the prevalence of CKD might also increase. For these patients, central blood pressure measurements and subsequent therapeutic interventions could improve their renal and CV prognoses. However, even Western medicine remains far from meeting this goal. We hope that this review will enlighten all individuals with an interest in medical care, including medical staff members, nephrologists and cardiologists, to the details of this issue.

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Retrospective Study

Incidence and prevalence of hepatitis B and hepatitis C viruses in hemodialysis patients in Lebanon

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Institutional review board statement: This study is retrospective, involving anonymous clinical data without affecting the patient's rights and welfare. These data were obtained directly from the ministry of public health registries in collaboration with its general director. No IRB approval was required.

Informed consent statement: We performed a retrospective study using anonymous patients data collected through the ministry of public health which routinely compiles all HBsAg and HCV serology results from the affiliated HD centers across Lebanon on a monthly basis. Since then, no informed consent was required.

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Abstract

AIM: To determine the incidence and the prevalence of hepatitis B and C viral infections in patients on hemodialysis (HD) across Lebanon.

METHODS: We reviewed the data registry at the Lebanese Ministry of Public Health where records of monthly hepatitis B surface antigen (HBsAg) and hepatitis C virus (HCV) serology are reported from 60 affiliated HD centers across Lebanon. All patients who were on HD or who started HD between October 2010 and July 2012 were included in the study. Patients from seven HD centers were excluded due to inadequate and incomplete results reporting. During the selected

period, HBsAg and HCV serology were available for 3769 patients from 53 HD centers distributed at all Lebanese governorates. The prevalence was calculated by dividing the number of patients with positive HBsAg or HCV serology to the total number of patients. The Incidence was calculated by dividing the number of newly acquired infection to number of patients-years (p-y). Incidence rates at different governorates were compared to each other using two tailed *Z* test and a *P* value of < 0.05 was considered significant.

RESULTS: Sixty out of 3769 HD patients were found to have positive HBS Ag and 177 out of 3769 were positive for HCV Antibodies. The prevalence of hepatitis B virus (HBV) and HCV in HD patients across Lebanon was 1.6%, and 4.7%, respectively. The comparison of prevalence according to geographic distribution could not be done accurately due to the frequent shift of patients between dialysis centers at different governorates. The incidence rate was 0.27 per 100 p-y for HBV and 0.37 per 100 p-y for HCV. There was no significant difference concerning the incidence of HBV between HD centers at different governorates (all *P* values > 0.1), but this difference was highly significant concerning the incidence rates of HCV which occurred predominantly in the southern centers (1.47 per 100 p-y) with a *P* value of 0.00068 and 0.00374 when compared to Mount Lebanon (0.21 per 100 p-y) and the Northern centers (0.19 per 100 p-y), respectively.

CONCLUSION: The incidence rate of HBV and HCV is very low in the Lebanese HD centers and their prevalence is decreasing over the last two decades.

Key words: Hemodialysis; Prevalence; Hepatitis C virus; Incidence; Hepatitis B virus; Lebanon

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Core tip: This is the largest and most statistically significant study addressing the prevalence and the incidence of hepatitis B virus (HBV) and hepatitis C virus (HCV) in 3769 patients on hemodialysis (HD) through 88% of all HD centers across Lebanon over a period of 22 mo. The prevalence of HBV and HCV in the studied population was 1.6%, and 4.7%, respectively. The incidence rate was 0.27 per 100 p-y for HBV, and 0.37 per 100 p-y for HCV. These values are amongst the lowest rates reported in other countries, which is most probably related to good adherence to infection control standards in the Lebanese HD centers.

Abou Rached A, El Khoury L, El Imad T, Geara AS, Jreijiry J, Ammar W. Incidence and prevalence of hepatitis B and hepatitis C viruses in hemodialysis patients in Lebanon. *World J Nephrol* 2016; 5(1): 101-107 Available from: URL: <http://www.wjgnet.com/2220-6124/full/v5/i1/101.htm> DOI: <http://dx.doi.org/10.5527/wjn.v5.i1.101>

INTRODUCTION

The susceptibility to acquire viral hepatitis during hemodialysis (HD) has several potential underlying reasons related to both the patient and the HD procedure. First, although the rate of blood products transfusions has decreased since the introduction of erythropoietin stimulating agents, HD dependent patients still subjects of recurrent transfusions. Second, HD machines and membranes are shared between different patients which increases the risk of direct blood cross contamination within one HD unit. Vaccination does not offer the same level of protection against HBV transmission in HD patients as in the general population and finally and once exposed to HBV or HCV, End stage renal disease (ESRD) patients are more prone to become chronic carriers compared to the general population^[1]. Acquiring an HBV and/or HCV infection has long-term impact on morbidity and mortality of HD patients. It has been suggested that HCV seropositivity is associated with all-cause as well as cardiovascular mortality in HD patients^[2]. In addition, HBV and/or HCV infection changes the clinical course and the prognosis after kidney transplantation. In Lebanon, HBV and HCV prevalence in HD patients has not been widely studied previously. The available studies were limited to few dialysis centers and date back to the late 1990's^[3-5]. Reassessing the extent of the problem for both viruses establishes the infection control protocols and the general means to prevent transmission of hepatitis infection in ESRD patients on HD.

The main objective of this study is to determine the incidence and the prevalence of HBV and HCV infections in ESRD patients on HD across Lebanon. The current common practice is that ESRD patients on HD should be screened for HBV and HCV infection before the initiation of HD and monitored monthly thereafter. This serology is reported to the ministry of public health (MOPH) on a monthly basis. Our goal is that by establishing the annual incidence of HBV and HCV infection in HD patients in the different centers in Lebanon, we will be able to document how extensive is the viral hepatitis in the Lebanese HD centers. In addition, a secondary objective is to compare the incidence and the prevalence between the different Lebanese regions in order to localize a potentially high risk center.

MATERIALS AND METHODS

Each dialysis center in Lebanon is required to report monthly serology for both HBV (HBsAg) and HCV (HCV antibody) for all its HD patients.

We reviewed the MOPH registry, which compiles all Lebanese dialysis centers data, for the period extending from October 2010 to July 2012. Using this data; we conducted an assessment of the prevalence and incidence of HBV and HCV in the HD population in Lebanon.

The study population included all the patients' who

Table 1 Distribution of hemodialysis centers across Lebanon

Governorates	No. of centers present	No. of centers with available data	No. of patients studied during this period
Beirut	6	6	559
Mount Lebanon	27	24	1632
Bekaa	7	5	394
South	6	5	339
North	11	11	757
Nabatieh	3	2	88
Total	60	53	3769

Table 2 Calculated total patient months for hepatitis B

Governorates	Total number of patients	Total patient-month	Patients with positive HBsAg	Newly acquired hepatitis B
Beirut	559	7232	8	2
Mount Lebanon	1632	23955	19	3
Bekaa	394	4026	9	2
South	339	4379	5	1
North	757	13014	18	4
Nabatieh	88	1120	1	0
Total	3769	53726	60	12

HBsAg: Hepatitis B surface antigen.

underwent HD during the period extending from October 2010 to July 2012 (*i.e.*, both already established ESRD and newly diagnosed ESRD initiated on HD). We only excluded dialysis units and patients who had incomplete, or did not report data for part or for the whole studied period (7 HD centers from a total of 60 HD centers).

Incidence analysis

Since we had monthly serology and the patients were starting or stopping dialysis at different date during the studied period, we calculated the incidence using a patient-month (p-m) unit. Each HD patient was represented in the incidence analysis by the total number of months spent undergoing HD (*i.e.*, if a patient was on dialysis only for 10 mo he will be counted in the analysis as 10 p-m).

In the incidence analysis, we excluded all the patients with positive serology at the start of the studied period. For the patients who did not seroconvert during the period they were receiving HD, we counted the total months during which the patient was on HD. For patients who eventually acquired hepatitis viral infection, we only counted the total months before the acquiring infection (the period during which the patient was at risk of acquiring an infection).

Incidence (per patient-month) = (Total number of acquired infection)/(Total patient|month)

At the end of the incidence calculation, we converted the unit p-m to patient-year (p-y) by dividing the final number by 12.

Incidence (per patient-year) = [(Total number of acquired infection)/(Total patient|month)]/12

We did the statistical analysis separately for HBV and HCV.

To compare the incidence between the different governorates and since the population is independent, we used a Z-test to compare head to head the incidence of acquiring hepatitis viral infection between the different Governorates. We used a $P < 0.05$ with a two-tailed Z-test to reject the null hypothesis with a 95 percent certainty. Since our sample size was large, our analysis did fulfill the requirement of a study power more than 80%.

Prevalence analysis

We divided the total number of patient with positive

serology for HBV and HCV separately by the total number of patients studied in this period of time.

Prevalence = (Total number of patients with positive serology)/(Total number of patients)

There are a total of 60 HD centers in Lebanon, fifty three (88.3%) had a complete data reporting and were included in the analysis. To evaluate the geographic differences in viral hepatitis in Lebanon, each dialysis unit was allocated to a Governorate based on its geographic location. Table 1 layout the geographic distribution of the HD units across Lebanon. The centers with missing data and not included in the analysis were 3 of 27 (11%) of the Governorate of Mount Lebanon (Haroun Hospital; Siblin Governmental Hospital and Serhal Hospital), 2 of 7 (28%) in the Bekaa (Hermel Governmental Hospital, Hraoui Governmental Hospital), 1 of 6 (16%) in South (Hammoud Hospital) and 1 of 3 (33%) in Nabatieh (Nabatieh Governmental Hospital).

RESULTS

Sixty out of 3769 HD patients studied during a 22-mo period from October 2010 to July 2012 were found to have positive HBsAg and 177 out of 3769 were positive for HCV Antibodies during anytime for this period. The Prevalence of HBV and HCV in HD patients across Lebanon was 1.6% and 4.7%, respectively.

The prevalence of HBV in HD units by governorate was distributed as follows: 1.43% for Beirut, 1.16% for Mount Lebanon, 2.28% for Bekaa, 1.47% for the South, 2.37% for the North and 1.13% for Nabatieh. The prevalence of HCV by Governorate was distributed as follows: 3.57% for Beirut, 4.47% for Mount Lebanon, 5.58% for Bekaa, 7.07% for the South, 5.01% for the North and 0% for Nabatieh. We did not analyze the difference in the prevalence between each Governorate since it is difficult to interpret such results due to the frequent shift of patient between dialysis units (*i.e.*, some patients switch HD center between seasons and relocate to Mount Lebanon or Beirut during the winter season). The geographical distribution is detailed in Tables 2 and 3.

The incidence of HBV in HD centers in Lebanon was 0.27 per 100 p-y, while for HCV it was 0.37 per 100 p-y (Table 4). No newly acquired infection for both HBV and

Table 3 Total patient-months distribution for hepatitis C

Governorates	Total number of patients	Total patient-month	Patients with positive HCV Abs	Newly acquired hepatitis C
Beirut	559	7055	20	3
Mount Lebanon	1632	22970	73	4
Bekaa	394	3797	22	2
South	339	4078	24	5
North	757	12597	38	2
Nabatieh	88	1142	0	0
Total	3769	51639	177	16

HCV: Hepatitis C virus.

HCV were observed in Nabatieh.

While comparing the incidence of HBV in HD units between different governorates in Lebanon, no statistically significant difference was found (with a *P*-value always higher than 0.05) (Table 5). In Contrast, a statistically significant difference was found between the incidence of HCV in the South (1.47 per 100 p-y) compared to Mount Lebanon (0.21 per 100 p-y) and the North (0.19 per 100 p-y) showing a higher incidence in the South with a *P*-value of 0.00068 and 0.00374 respectively (Tables 5 and 6 for a list of the different calculated *P*-values).

DISCUSSION

It is well known that HD patients are at high risk for HCV and HBV infections. In Lebanon three small studies were done concerning the prevalence of HCV in HD patients. Naman *et al*^[4] reported in 1996 that the prevalence of HCV among HD in Lebanon was 27% with a high variety between the 5 centers studied (10%-39%), Abdelnour *et al*^[3] reported in 1997 in various hospitals a prevalence of 16%, Abourached *et al*^[5] reported in 2006, a prevalence of 13% (2.2%-38%) in 17 HD centers in Lebanon.

In this epidemiologic study covering more than 88% of the HD centers in Lebanon, the prevalence of anti-HCV antibodies in ESRD patients undergoing HD was 4.7%, showing a decrease in the prevalence of HCV among HD patients in Lebanon over the last two decades. The lowest prevalence was in Beirut (3.5%) and the highest in the South (7%). We observed a high variability among the 53 different centers studied, ranging from as low as 0% to as high as 20%.

The reduction in HCV prevalence in HD patients is a common trend across several countries and it was mainly related to the reduction in the number of transfusions in HD patients and the improvement of the laboratory screening techniques for detection of anti-HCV antibodies in blood donors. The prevalence of HCV infection in patients on HD is highly variable but clearly much higher than in the general population of the respective countries. In phase one of the Dialysis Outcomes and Practice Patterns Study (DOPPS), a prospective observational study of adult HD patients

Table 4 Incidence of hepatitis B and hepatitis C among hemodialysis centers in Lebanon

Governorates	Incidence of HBV (per 100 p-y)	Incidence of HCV (per 100 p-y)
Beirut	0.33	0.51
Mount Lebanon	0.15	0.21
Bekaa	0.59	0.63
South	0.27	1.47
North	0.37	0.19
Nabatieh	0	0
Across Lebanon	0.27	0.37

HCV: Hepatitis C virus; HBV: Hepatitis B virus.

randomly selected from 308 representative dialysis facilities in France, Germany, Italy, Japan, Spain, the United Kingdom, and United States, an overall HCV prevalence of 13% was found in 8615 patients^[6].

Globally the prevalence of HCV among patients undergoing HD varies from as low as 6.1% in Germany in 2002^[7] to as high as 76% in Casablanca in 2005^[8]. In general, North Africa and the Middle East were cited as high prevalence areas, both in the general population and in HD patients, by the WHO in 1999^[9]. Previous studies from the region have reported a prevalence of anti-HCV antibodies in HD patients of 50% in Saudi Arabia in 2000^[10], 19.1% in Tunisia in 1994^[11], 20.2% in Turkey in 2006^[12] and 34.6% in Jordan in 2007^[13].

Concerning the prevalence of HBV, Abourached *et al*^[5] reported on 2007 a prevalence of 2.62% (0%-6.5%) of HBsAg in 17 HD centers in Lebanon, our study showed a decrease of the prevalence to 1.6%, ranging from 1.4% in Nabatieh and Bekaa, to 2.4% in the North. This prevalence is slightly elevated than that reported in different study In Lebanon concerning the general population^[14]. We observed a high variability among the 53 different centers, ranging from as low as 0% to as high as 15%. This observed prevalence is lower than that reported in the 2008 by the Saudi Centre for Organ Transplantation (SCOT) report, where HBV seropositivity was 4.6% in the Saudi HD population while among Jordanian HD patients it was 5.9%^[15]. It is also lower than that reported in HD patients in other regions including Europe (4.1%), Japan (2.2%) and the United States (2.4%) during the period extending from 1996 to 2002^[16]. A study sample from the DOPPS, which included 8615 adult HD patients from 308 dialysis facilities in Western Europe and the United States, reported prevalence rates for HBV infection ranging from 0% to 6.6%^[17]. Studies from less developed countries estimated that the proportion of HBsAg carriers in the HD population varies from 2% to 20%^[18,19].

Prospective follow up of seronegative HD patients enabled us to observe 12 newly acquired infection for HBV and 16 newly acquired infection for HCV during a twenty two months period. We observed a 0.37 per 100 p-y incidence of new HCV infections during the 22-mo observation period. The reported incidence of new HCV infections varies considerably between countries. A rate

Table 5 *P*-values for hepatitis B

HBV	Beirut	Mount Lebanon	Bekaa	South	North	Nabatieh
Beirut		<i>P</i> = 0.3759 NS	<i>P</i> = 0.5562 NS	<i>P</i> = 0.8702 NS	<i>P</i> = 0.8948 NS	<i>P</i> = 0.579
Mount Lebanon			<i>P</i> = 0.1059 NS	<i>P</i> = 0.6073 NS	<i>P</i> = 0.2208 NS	<i>P</i> = 0.7086 NS
Bekaa				<i>P</i> = 0.5038 NS	<i>P</i> = 0.5709 NS	<i>P</i> = 0.4578 NS
South					<i>P</i> = 0.778 NS	<i>P</i> = 0.6159 NS
North						<i>P</i> = 0.5547 NS
Nabatieh						

NS: Non-significant; HBV: Hepatitis B virus.

Table 6 *P*-values for hepatitis C

HCV	Beirut	Mount Lebanon	Bekaa	South	North	Nabatieh
Beirut		<i>P</i> = 0.229 NS	<i>P</i> = 0.8164 NS	<i>P</i> = 0.1274 NS	<i>P</i> = 0.2598 NS	<i>P</i> = 0.4854 NS
Mount Lebanon			<i>P</i> = 0.1822 NS	<i>P</i> = 0.00068	<i>P</i> = 0.9079 NS	<i>P</i> = 0.6548 NS
Bekaa				<i>P</i> = 0.2951 NS	<i>P</i> = 0.2036 NS	<i>P</i> = 0.438 NS
South					<i>P</i> = 0.00374	<i>P</i> = 0.2346 NS
North						<i>P</i> = 0.6707 NS
Nabatieh						

NS: Nonsignificant; HCV: Hepatitis C virus.

as low as 0.4% was observed in France from 1997 to 2000^[20] but higher rates have been reported from the Mediterranean region. According to the 2008 SCOT report, the annual rate of HCV sero-conversion in Saudi HD patients was 7%-9% while in Jordan it was 2.6%^[14]. The incidence rate of 0.27 per 100 p-y for HBsAg is slightly less than that reported in Europe, Japan and the United States (0.4-1.8 per 100 p-y)^[17]. While comparing the incidence of HBV infection among the different Governorates, no statistically significant difference was found. In contrast, a statistically significant difference in the incidence of HCV infection was found between the South and both Mount Lebanon and the North. This interestingly high incidence of HCV infection of 1.47 per 100 p-y found in the South need to be assessed further to be able to find a potential reason for this higher incidence.

In this study we collected data from 53 centers distributed across all the six Governorates of Lebanon from the total 60 centers reporting monthly serology to the MOPH, giving us a total of 3769 patients studied over a 22-mo period. This significantly increased the statistical power and the validity of the results.

In general, the prevalence and incidence of HBV and HCV infections in HD patients are directly related to the prevalence of these infections in the general population, the quality of healthcare services in a community and

the standards of infection control practices in HD units. In Lebanon, patients on maintenance HD were found to have a higher prevalence of HCV infection of 4.7% when compared to the general population since, according to available data, anti-HCV prevalence rate of 0.2% to 0.4% of the general population in Lebanon^[21], but we noted a significant decrease of this prevalence in this group of patients during the last 20 years. Also the prevalence of HBV was slightly higher than the general population 2.62% vs 2.2%.

This higher prevalence of HBV and HCV infection in HD compared to the general population has been confirmed by several reports from different countries^[22].

The incidence of HBV and HCV in HD centers in Lebanon is very low compared to others countries in the region, this can be due to the good applications of the standards of infection control practices in HD centers and the strict surveillance by the MOPH.

A notable result of this study was the significantly higher incidence of HCV found mainly in the South that may be due to variation in the degree of implementation of the universal precautions to prevent nosocomial transmission. In order to evaluate the reason for the variation between the different HD units, a more detailed evaluation of each dialysis patient should be done especially in the units with the lowest and the highest incidence for HBV or HCV. Such studies would assist in

guiding interventions aiming to reduce the occurrence of these infections and thus reduce the morbidity and mortality of the HD population in Lebanon.

Finally this study demonstrated a reduce in the prevalence of HBV and HCV infections in HD centers during the last 2 decades and a low incidence rate due to the good applications of the standards of infection controls practice.

COMMENTS

Background

End stage renal disease (ESRD) patients on hemodialysis (HD) are particularly at higher risk for acquiring hepatitis C virus (HCV) and hepatitis B virus (HBV) than the general population, due to the sharing of contaminated machines within the same center and the higher rates of blood transfusions. Such infections have a negative impact on the clinical course of ESRD causing higher rates of morbidity and mortality. Since then, it is essential to determine the prevalence and the incidence of HBV and HCV infections in HD patients in each country, then to decide accordingly about further interventions to control such infections.

Research frontiers

The prevalence of HCV and HBV in patients on HD in Lebanon was only addressed by small studies including few HD centers. This study will determine the prevalence and incidence of HCV and HBV in a significantly larger population including 3769 HD patients through 88% of all HD centers across Lebanon between October 2010 and July 2012.

Innovations and breakthroughs

Previous studies done on smaller sample sizes limited to some Lebanese HD centers reported a prevalence of 2.6% and 13%-27%, for HBV and HCV, respectively. No reports are yet available about their incidence rate. Although accurate comparison could not be done due to the different sample sizes, this study showed a reduction in HBV and HCV prevalence through HD patients in Lebanon (1.6% and 4.7%, respectively). On the other hand, their calculated prevalence and incidence rates were found to be among the lowest values reported in other countries, either in the Middle East, or in Europe and United States. Another notable result of this study was the significantly higher incidence rate of HCV in the Southern Lebanese HD centers.

Applications

This study reflects an appropriate adherence to standards of infection control in the Lebanese HD centers limiting the spread of HBV and HCV between HD patients. However, it emphasizes the need for further investigations to reestablish those standards in some centers having significantly higher incidence rate of HCV, located mainly in the South of Lebanon.

Peer-review

In this manuscript, the authors report on the prevalence and incidence of hepatitis B and hepatitis C among HD patients in Lebanon. This paper is clinically interesting.

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Retrospective Study

Renal and perinephric abscesses in West China Hospital: 10-year retrospective-descriptive study

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Institutional review board statement: This study was reviewed and approved by West China Hospital Sichuan University Clinical Trials and Biomedical Ethics Committee.

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Abstract

AIM: To elucidate the clinical, radiological and laboratory profiles of renal abscess (RA) and perinephric abscess (PNA), along with related treatment and outcome.

METHODS: Ninety-eight patients diagnosed with RA or PNA using the primary discharge diagnoses identified from the International Statistical Classification of Diseases and Related Health Problems Tenth Edition (ICD-10) codes (RA: N15.101, PNA: N15.102) between September 2004 and December 2014 in West China Hospital were selected. Medical records including patients' characteristics, symptoms and signs, high-risk factors, radiological features, causative microorganisms and antibiotic-resistance profiles, treatment approaches, and clinical outcomes were collected and analyzed.

RESULTS: The mean age of the patients was 46.49 years with a male to female ratio of 41:57. Lumbar pain (76.5%) and fever (53.1%) were the most common symptoms. Other symptoms and signs included chills (28.6%), anorexia and vomiting (25.5%), lethargy (10.2%), abdominal pain (11.2%), flank mass (12.2%), flank fistula (2.0%), gross hematuria (7.1%), frequency (14.3%), dysuria (9.2%), pyuria (5.1%) and weight loss (1.0%). Painful percussion of the costovertebral angle (87.8%) was the most common physical finding. The main predisposing factors were lithiasis (48.0%), diabetes mellitus (33.7%) followed by history of urological surgery (16.3%), urinary tract infections (14.3%), renal function impairment (13.3%), liver cirrhosis (2.0%), neurogenic bladder (1.0%), renal cyst (1.0%), hydronephrosis (1.0%), chronic hepatitis B (1.0%), post-discectomy (1.0%) and post-colectomy (1.0%). Ultrasound (US) and computed tomography were the most valuable diagnostic tools and US was recommended as the initial diagnostic imaging choice. *Escherichia coli* (51.4%), *Staphylococcus aureus* (10.0%) and *Klebsiella pneumoniae* (8.6%) were the main causative microorganisms. Intravenous antibiotic

therapy was necessary while intervention including surgical and nonsurgical approaches were reserved for larger abscesses, multiple abscesses, PNAs and non-responders.

CONCLUSION: Heightened alertness, prompt diagnosis, and especially proper antibiotics in conjunction with interventional approaches allow a promising clinical outcome of renal and perinephric abscesses.

Key words: Renal abscess; Causative pathogens; Perinephric abscess; Diagnosis; Antibiotic resistance; Interventional treatment; Conservative treatment

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Core tip: Renal and perinephric abscesses are uncommon but potentially lethal infectious diseases and the case-fatality rates most frequently cited in previous studies are still high. However, the previous case-fatality rates need to be updated, since prompt diagnosis and appropriate therapeutic strategies have contributed to lower mortality. This article reports the characteristics of patients identified with renal or perinephric abscesses and shares the management experience and outcome in West China Hospital during the last decade.

Liu XQ, Wang CC, Liu YB, Liu K. Renal and perinephric abscesses in West China Hospital: 10-year retrospective-descriptive study. *World J Nephrol* 2016; 5(1): 108-114 Available from: URL: <http://www.wjgnet.com/2220-6124/full/v5/i1/108.htm> DOI: <http://dx.doi.org/10.5527/wjn.v5.i1.108>

INTRODUCTION

Renal abscess (RA) is defined as encapsulated pus confined to the renal parenchyma and is further divided into renal cortical or corticomedullary abscess^[1]. Perinephric abscess (PNA) is a collection of suppurative material located between Gerota's fascia and the renal capsule^[2]. Complications of urinary tract infections (UTIs) and hematogenous seeding from primary infected sites are the common source of infection^[2,3]. Additionally, rupture of renal cortical abscess or renal carbuncle can result in the formation of PNA^[2].

As a result of its anatomical location and potential to spread, RA is potentially lethal and the prognosis can be poor, especially in immunosuppressed and cachectic patients^[1,4]. PNA originates from hematogenous dissemination, and often has an acute presentation with pain and high spiking temperatures^[2], while in most cases, PNA is notoriously silent clinically^[2,3], thereby the diagnosis can be challenging^[2]. It is reported that only 35%-38% of patients with PNA are correctly diagnosed at the time of admission^[5,6]. The mortality rates of RA and PNA in recent series are reported to range from 1% to 14%^[3,7-12], while complicated abscess may carry

a higher mortality^[1]. Due to the above situation, a retrospective-descriptive study was conducted with 98 relevant cases identified with RA and PNA between September 2004 and December 2014 in West China Hospital, in an attempt to recognize the disease and describe our experience with it over the past 10 years.

MATERIALS AND METHODS

The data presented in this study were obtained from medical records of patients selected using the primary discharge diagnoses identified from the International Statistical Classification of Diseases and Related Health Problems Tenth Edition (ICD-10) codes (RA: N15.101, PNA: N15.102) during the last decade in our hospital. Suspected patients were diagnosed based on both clinical and radiological criteria. Abscesses ≤ 3 cm were defined as small, medium 3-5 cm, and > 5 cm large. The rule that culture findings guide selection of an antibiotic regimen was firmly followed, while before the culture results were obtained, initial empirical antibiotics were provided once a clinical diagnosis of RA and PNA was made. Antibiotics typically included piperacillin plus amikacin and metronidazole, piperacillin/tazobactam or third-generation cephalosporin plus metronidazole, or quinolones plus metronidazole. When patients had a severe condition such as sepsis or were prone to infection with extended-spectrum β -lactamase (ESBL)-producing organisms, carbapenem antibiotics (*e.g.*, imipenem/cilastatin) were also included in prescription.

Treatment modes were subdivided into two groups: Conservative treatment and interventional treatment. The latter comprised five categories: Antibiotics plus percutaneous drainage; antibiotics plus double J tube insertion; antibiotics plus nephrostomy; antibiotics plus surgical drainage; and antibiotics plus nephrectomy. Since improvement in clinical manifestations usually precedes that in radiological imaging findings, patients were mainly assessed by their clinical improvement. The clinical outcome was classified as cure, clinical improvement (mainly including remission or disappearance of initial symptoms, shrinkage of the abscess cavity upon imaging, recovery of white blood cell and neutrophil counts, and negative results for blood and urine culture), or death.

Biostatistics

The statistical methods of this study were reviewed by Liang Huang from Center of Infectious Diseases, West China Hospital of Sichuan University, Chengdu, Sichuan Province, China.

RESULTS

Patient characteristics

Among the 98 patients, there were 41 (41.8%) men and 57 (58.2%) women. The age ranged from 18 to 75 years with a mean of 46.49 ± 15.07 years. RA was observed in 68 (69.4%) patients and PNA in 30 (30.6%).

Table 1 Characteristics of patients with renal or perinephric abscesses *n* (%)

Variables	Value
	Total (<i>n</i> = 98)
Lumbar pain	75 (76.5)
Fever	52 (53.1)
Chills	28 (28.6)
Anorexia and vomiting	25 (25.5)
Lethargy	10 (10.2)
Abdominal pain	11 (11.2)
Flank mass	12 (12.2)
Flank fistula	2 (2.0)
Gross hematuria	7 (7.1)
Frequency	14 (14.3)
Dysuria	9 (9.2)
Pyuria	5 (5.1)
Loss of weight	1 (1.0)
Painful percussion of the CVA	86 (87.8)

CVA: Costovertebral angle.

Fifteen patients (15.3%) had no identifiable systemic or urological disorder that might have been involved in abscess formation, whereas for other patients, the spectrum of predisposing factors remained consistent with conventional predisposing factors: Diabetes mellitus (*n* = 33, 33.7%); lithiasis (*n* = 47, 48.0%) which included renal calculi (*n* = 32, 32.7%), ureteric calculi (*n* = 5, 5.1%), renal and ureteric calculi (*n* = 10, 10.2%); history of urological surgery (*n* = 16, 16.3%); UTIs (*n* = 14, 14.3%); renal function impairment (*n* = 13, 13.3%); liver cirrhosis (*n* = 2, 2.0%); neurogenic bladder (*n* = 1, 1.0%); and other diseases (*n* = 5, 5.1%) including one each with renal cyst, hydronephrosis, and chronic hepatitis B, post-discectomy and post-colectomy.

The most common initial symptoms were lumbar pain (*n* = 75, 76.5%) and fever (*n* = 52, 53.1%). Each grade of fever was observed: 38 °C–39 °C in approximately 31.6%, 39.1 °C–41 °C in approximately 20.4%, and absent or low-grade fever in 48.0%. Painful percussion of the costovertebral angle (*n* = 86, 87.8%) was the most common physical finding (Table 1). Patients with RA in this study were more inclined to experience lethargy than PNA (*P* < 0.05) and there was no statistical significance in other symptoms when compared RA with PNA (*P* > 0.05).

Laboratory data and abscess characteristics

There was no significant difference between RA and PNA in white blood cell count (*W* = 996.5, *P* > 0.05), neutrophil count (*W* = 947, *P* > 0.05), hemoglobin (*W* = 0.9773, *P* > 0.05), blood urea nitrogen (*W* = 992, *P* > 0.05) and serum creatinine (*W* = 1038, *P* > 0.05). Hematuria and leukocyturia were most common findings in urine test (Table 2). Of the 98 patients, 77 (78.6%) patients had a solitary abscess and 10 (10.2%) had multiple abscesses. The right side (55.1%) remained the predominant anatomical site and bilateral abscesses were found in two (2.0%) cases. The average size of

Table 2 Blood and urine analysis

Variables	Value	
	RA	PNA
Blood analysis	64	30
WBC (10 ⁹ /L)	10.82 (range: 2.42–29.95)	12.40 (range: 2.68–25.45)
NEUT (%)	81.00 (range: 48.30–96.00)	79.00 (range: 49.30–94.70)
HGB (g/L)	105.75 ± 22.52	101.66 ± 20.13
BUN (mmol/L)	5.26 (range: 1.10–20.30)	5.10 (range: 2.80–23.18)
Serum creatinine (umol/L)	82.00 (range: 25.10–346.00)	86.60 (range: 49.00–560.0)
Urine analysis	64	30
No finding (%)	13 (13.3)	3 (3.1)
Hematuria (%)	47 (48.0)	23 (23.5)
Pyuria (%)	16 (16.3)	8 (8.2)
Proteinuria (%)	32 (32.7)	16 (16.3)
Leukocyturia (%)	41 (41.8)	23 (23.5)

RA: Renal abscess; PNA: Perinephric abscess; WBC: White blood cell; NEUT: Neutrophil count; HGB: Hemoglobin; BUN: Blood urea nitrogen.

RA and PNA was 6.25 (range: 0.50–17.00) cm and 8.35 (range: 4.50–20.00) cm, respectively. The average size of abscess was 4.00 (range: 1.80–10.50) cm in the conservative group, and 7.65 (range: 0.50–20.00) cm in the interventional group (Table 3).

Microbiological data

The results of blood, abscess and urine culture were available for 92, 54 and 91 patients, respectively. Blood and urine cultures were positive in 13 (14.1%) and 23 (25.3%) patients, respectively, and pathogenic organisms were isolated from pus in 33 (61.1%) cases. Of all the positive cultures (*n* = 69), the most frequently isolated pathogen was *Escherichia coli* (*n* = 35, 50.7%) followed by *Staphylococcus aureus* (*S. aureus*) (*n* = 7, 10.1%), *Klebsiella pneumoniae* (*K. pneumoniae*) (*n* = 6, 8.7%), *Pseudomonas aeruginosa* (*n* = 3, 4.3%), *Candida spp.* (*n* = 7, 10.1%), *Enterobacteriaceae* (*n* = 6, 8.7%), *Enterococcus faecium* (*n* = 2, 2.9%), *Enterococcus faecalis* (*n* = 1, 1.4%) and *Aspergillus spp.* (*n* = 2, 2.9%). *E. coli* was more frequently found in patients with RA than those with PNA ($\chi^2 = 6.832$, *P* < 0.01), while there was no significant difference in the distribution of *K. pneumoniae* (*P* > 0.05), *S. aureus* (*P* > 0.05) and *Candida spp.* (*P* > 0.05) (Table 4). We detected ESBL in the isolated strains of *E. coli* in 12 (17.4%) cases and *K. pneumoniae* in one (1.4%) case. We analyzed the antibiotic resistance of *E. coli*, *S. aureus* and *K. pneumoniae* isolated from blood, abscess and urine culture (Table 5).

Imaging studies

Imaging results were available for 97 patients. Ultrasound (US) was applied in 80 cases and alone in 31 (31.6%) cases. Computed tomography (CT) was performed in 63 cases and alone in 16 (16.3%) cases. Magnetic resonance imaging (MRI) was applied in three cases and alone in one (1.0%) case. The imaging results are shown in Table 6.

Table 3 Treatment and outcome

Variables	Abscess size (cm)	Hospital stay (d)	No. of Patients (RA/PNA)	Cure (RA/PNA)	Clinical improvement (RA/PNA)	Death (RA/PNA)
Ab	4.00 (range: 1.80-10.50)	20.7	23 (19/4)	2 (2/0)	21 (17/4)	0 (0/0)
Intervention	7.65 (range: 0.50-20.00)	15.9	75 (49/26)	54 (38/16)	20 (10/10)	1 (1/0)
Ab + PCD			8	3	4	1
Ab + pigtails			2	1	1	0
Ab + nephrostomy			4	0	4	0
Ab + SD			29	21	8	0
Ab + NC			32	29	3	0
RA	6.25 (range: 0.50-17.00)		68	40	27	1
PNA	8.35 (range: 4.50-20.00)		30	16	14	0

Ab: Antibiotic; PCD: Percutaneous drainage; SD: Surgical drainage; NC: Nephrectomy.

Table 4 Causative microorganisms isolated from blood, abscess and urine culture

Variables	Culture		
	Blood (Total = 92)	Pus (Total = 54)	Urine (Total = 91)
No finding	79	21	68
Escherichia coli	8	17	10
Staphylococcus aureus	2	4	1
Klebsiella pneumoniae	1	3	2
Pseudomonas aeruginosa	1	1	1
Other			
Enterobacteriaceae	1	3	2
Enterococcus faecium	0	0	2
Enterococcus faecalis	0	1	0
Candida	0	2	5
Aspergillus	0	2	0

Table 5 The antibiotic resistance rate of causative pathogens isolated

Variables	Causative pathogens isolated		
	Escherichia coli (n = 35)	Klebsiella pneumoniae (n = 6)	Staphylococcus aureus (n = 7)
Penicillin (%)	85.0	100.0	62.5
Levofloxacin (%)	46.2	0	50.0
Gentamicin (%)	53.3	0	50.0
Amikacin (%)	5.0	0	-
Cefotaxime (%)	61.1	50.0	50.0
Ceftriaxone (%)	63.2	50.0	-
Imipenem/cilastatin (%)	0	0	60.0
Vancomycin (%)	-	-	0
ST (%)	70.0	0	37.5

ST: Trimethoprim-sulfamethoxazole.

Treatment and outcome

The average hospitalization duration was 17 d (range 5-92 d). Of the 98 patients, 23 (23.5%) received conservative treatment and 75 (76.5%) received an interventional procedure. Fifty-seven (58.2%) patients were cured, 40 (40.8%) showed clinical improvement by the time of hospital discharge, and one (1.0%) died of multiple organ dysfunction syndrome. Interventional treatment contributed to a better clinical outcome than conservative treatment ($Z = -3.897$, $P < 0.01$). The outcome tended to be better in patients with RA than in those with PNA irrespective of the therapeutic mode ($Z = -8.027$, $P < 0.01$) (Table 3).

DISCUSSION

RA refers to a collection of purulent material within the kidney^[13]. PNA represents an extensive infection in the perinephric space^[2]. It is reported that approximately 30% of PNAs come from hematogenous dissemination^[2], whereas in most cases, they result from rupture of RA^[12,14]. Previous data show that > 80% of PNAs occur secondary to renal tract calculi with ascending UTIs^[15].

RAs and PNAs are seen in all age groups and those aged 42.3-71.62 years were previously reported to be the dominant population^[10-12,16-19]. A similar result was found in the present study. A slight predominance in

Table 6 Imaging results

Results	Imaging tool		
	US (Total = 80)	CT (Total = 63)	MRI (Total = 3)
Negative (%)	2 (2.0)	1 (1.0)	
Abscess (%)	23 (23.5)	37 (37.8)	1 (1.0)
Hydronephrosis (%)	26 (26.5)	8 (8.2)	
Mass (%)	13 (13.3)	11 (11.2)	2 (2.0)
Echogenic alteration (%)	11 (11.2)		
Cyst (%)	4 (4.1)	4 (4.1)	
Hematoma (%)	1 (1.0)	2 (2.0)	

US: Ultrasound; CT: Computerized tomography; MRI: Magnetic resonance imaging.

women was noted, while in previous studies, the male: Female ratio was reported as 1:3-1:7^[7,18,20], and a female predominance as high as 91.8% was also observed^[16].

Diagnosis of RA or PNA remains challenging because the symptoms can be insidious and obscure^[1,2]. Patients with RA may present with fever, chills, flank or abdominal pain, fatigue, nausea, decreased appetite, weight loss and even persistent hiccups^[7,21,22]. In our study, fever was not always accompanied by chills and the high percentage of absent/low-grade fever might be explained by prior antibiotic therapy. Patients with PNA often present with anorexia, nausea and vomiting, flank

pain, flank mass, signs of sepsis, weight loss, fistula formation and urinary tract complaints^[2,4,15,22]. However, patients with RA were more likely subjected to lethargy in this study.

Consistent with previous studies, lithiasis (48.0%) and diabetes mellitus (33.7%) remained the predominant risk factors in the present study^[3,8,9,12,16,22]. Diabetes mellitus accounts for 33.3%-62.5% of all PNAs^[2,7,23] 43.5%-47% of RAs^[7,16,17] and 28%-50% of RAs and PNAs^[3,7,10,11]. Anatomical malformation of the urinary tract, vesicoureteral reflux and obstructive tumors in renal polycystic disease are other previously described risk factors^[1,18]. There was no significant difference between RA and PNA in white blood cell count, neutrophil count, hemoglobin, blood urea nitrogen and serum creatinine, which suggested that patients with RA and PNA shared similar inflammation reaction level and risk of renal impairment in this study.

US, CT and MRI were necessary to establish reliable preoperative diagnosis. US as the initial and classical imaging modality is utilized to measure renal size, discern focal lesions, and detect the true nature of a fluid-containing mass and obstruction of the collecting system. US is not affected by poor renal function or allergy to contrast material^[24,25]. The accuracy of US in the diagnosis of RA is reported to be 70%-93%^[3,23] with sensitivity and specificity of 78.2% and 88.8%, respectively^[23].

CT has been documented to diagnose RA or PNA with an accuracy of 92%-96.4%^[3,6], with specificity of 88%^[23]. In our study, the accuracy of US and CT was 23.7% and 38.1%, respectively. However, when we combined the imaging results with clinical and laboratory data, the final diagnostic accuracy was 52.0%, and the average duration between admission and diagnosis was 2.16 d. With its convenience, accuracy, availability and low cost, US has made a major contribution to accurate and early diagnosis at our unit.

Since ascending dissemination of UTI has surpassed hematogenous dissemination and become the dominant predisposing factor^[6,18], Gram-negative bacteria, especially *E. coli*, *Proteus* spp. and *K. pneumoniae* have been the most common pathogens in recent years^[3,10,16,18]. In this study, *E. coli* was most frequently isolated from patients with RA than those with PNA. Polymicrobial abscesses have been increasingly frequently observed, ranging from 19.2% to 33.3% in incidence^[7,9]. Two (2.0%) polymicrobial cases were found in the present study. There has been an increasing incidence of abscesses caused by fungi, especially *Candida*, particularly in immunosuppressed patients^[7,12,18], and similar cases were observed in this study.

The selection of antimicrobial therapy ideally should be based on culture findings, however, there is an inevitable delay in obtaining results^[2]. We recommend that empirical broad-spectrum intravenous antibiotics should be initiated for critically ill patients after admission. Once the blood or abscess fluid cultures and bacterial isolation tests are confirmed, targeted antibiotic

regimens should be prescribed accordingly.

There is a consensus that small RAs may resolve with antibiotic treatment alone, and percutaneous or surgical drainage may be suitable for large RAs and PNAs. However there is a continuing argument about the proper treatment of middle-sized abscesses^[3,5,7,16].

Although the option of conservative management of RAs and PNAs seems attractive and feasible and successful cases have been reported^[4,16], those cases were selected and limitations in size, location and number of abscesses were obvious and in Iwamoto's case, the patient had received percutaneous drainage prior to conservative treatment^[4].

In the present study, interventional approaches helped to detect the cause of disease and confirm the diagnosis, and culture of pus/aspirate/debris helped guide selection of an antibiotic regimen. The diagnostic and therapeutic value of percutaneous drainage has been confirmed since early years^[26]. The application of interventional procedures contributed to a lower case-fatality rate and lower risk for intensive care unit (ICU) admission^[6,9]. Patients subjected to interventional treatment achieved a better clinical outcome than those received conservative treatment and the cure rate of interventional treatment was 27 times higher than that of conservative treatment. On the other hand, patients with RA were more likely to achieve a better prognosis than those with PNA irrespective of the therapeutic regimens.

The mean duration of hospitalization in the interventional treatment group was 15.9 d compared with 20.7 d in the conservative treatment group. In 2011-2014, the bed turnover time in the urological department of our hospital was 9.687, 9.623, 8.92 and 8.62 d, respectively. In consideration of the pressure relating to bed turnover time, interventional treatment could be a better alternative mode to meet the social needs.

Several limitations should be noted in the present study. First, the number of patients selected was not large enough, and exclusive reliance on the claims data might have resulted in potential bias. A larger population-based retrospective-descriptive study is needed to extrapolate better and confirm our results. Second, the imaging tools were not manipulated by the same technician, thus there is inevitable error in the imaging results obtained. Finally, the number of causative microorganisms isolated from pus/blood/urine was small. In fact, the isolation rates of ESBLs of *E. coli* and *K. pneumoniae* (excluding ICU) in our hospital in 2013 were 59.8% and 29.7%, respectively. The antibiotic resistance rate of *E. coli* to penicillin, cefotaxime, gentamicin, amikacin, trimethoprim-sulfamethoxazole and imipenem/cilastatin was 88.9%, 60.7%, 44.8%, 3.0%, 56.9% and 0.7%, respectively. The antibiotic resistance rate of *K. pneumoniae* was 78.8%, 26.2%, 16.6%, 4.9%, 25.2% and 1.5%, respectively. The antibiotic resistance rate of *S. aureus* to penicillin, gentamicin, sulfamethoxazole and vancomycin was 94.1%, 29.3%, 20.5% and 0%, respectively.

Since RA and PNA can be lethal^[3,6], to reduce the fatality rate when clinical suspicion is around, we recommend that physicians use US for primary evaluation and proceed to CT for confirmation. For small abscesses, intravenous antibiotics alone seem efficient. Interventional regimens are the first-line treatment for larger RAs, multiple abscesses, PNAs, and non-responders. The mode of therapy for medium-sized abscesses should depend on an individual basis, with due consideration of the clinical scenario and risk factors. However, interventional treatment is more capable of offering a promising clinical outcome and better bed turnover time and social benefits.

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COMMENTS

Background

Renal and perinephric abscesses are severe complications of urinary tract infections. Since their symptoms are insidious, the diagnosis can be challenging. Awareness combined with efficient imaging and laboratory results contribute to timely diagnosis, and appropriate treatments can lead to a good outcome and low mortality.

Research frontiers

Conservative treatment is currently reported to be practical for perinephric abscess (PNA) or larger renal abscess (RA) in certain cases, but in most cases, interventional treatment remains the classical therapy.

Innovations and breakthroughs

This study collected 98 patients diagnosed with RA or PNA in West China Hospital during the past decade. The clinical and laboratory profiles of these patients were described and analyzed. The study revealed the local epidemiological features of RA and PNA, and advocated interventional treatment for PNA and large or medium-sized RA.

Applications

This study sorted the clinical and laboratory data of RA and PNA, aiming to help strengthen the awareness of physicians and share the management experience.

Terminology

Extended-spectrum β -lactamase is an enzyme that can hydrolyze β -lactam antibiotics, including penicillins and cephalosporins. Bacteria that can produce extended-spectrum β -lactamase are resistant to β -lactam antibiotics.

Peer-review

The manuscript presents interesting data regarding the renal and perirenal abscesses for a 10-year period.

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Sex bias in response to hepatitis B vaccination in end-stage renal disease patients: Meta-analysis

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Abstract

AIM: To systematically review the literature for studies investigating the potential effect of gender of dialysis patients on the immunogenicity of hepatitis B virus vaccines.

METHODS: Literature searches were conducted by the MEDLINE and Google Scholar. The key words used included "hepatitis B (HB)", "vaccine", "dialysis", "hemodialysis", "sex", "male" and "female". Data of seroresponse to HB vaccine in clinical trials regarding sex of the recipients have been achieved and analyzed. Finally data from 19 clinical trials have been pooled and analyzed.

RESULTS: Analysis of response to HB vaccination in our dialysis population showed males significantly respond less to hepatitis B vaccination ($P = 0.002$, $Z = 3.08$) with no significant heterogeneity detected [$P = 0.766$; heterogeneity $\chi^2 = 14.30$ (df = 19); $I^2 = 0\%$]. A reanalysis of the pooled data was conducted regarding the dialysis mode to evaluate potential differential impact of sex on HB vaccine response. Hemodialysis was the only subgroup that showed a significant difference regarding dialysis mode in response to HB vaccination regarding sex ($P = 0.042$, $Z = 2.03$).

CONCLUSION: This Meta-analysis showed significant effect for the sex of chronic kidney disease and dialysis patients on the immunogenicity of HB vaccine. This sex discrimination was most prominent among hemodialysis patients.

Key words: Hepatitis B virus vaccination; Hepatitis B virus; Immunogenicity; Dialysis patients; Gender; Sex

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Core tip: This study showed that gender of the dialysis patients is a significant factor affecting serresponse to hepatitis B vaccination (HBV) in the immunocompromised population of hemodialysis population. This gender bias was most significantly prominent when patients were under hemodialysis (*vs* other renal replacement therapies including peritoneal dialysis). The relevance of such a finding is to enable the practitioners to be alerted on the effects of HBV vaccinations in dialysis patients and give them clues to individualize vaccination protocols for patients with specific epidemiological characters.

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INTRODUCTION

Hepatitis B virus (HBV) infection is one of the most widespread chronic viral infections in the world with two billion people infected worldwide, and a matter of substantial amounts of financial and health burden throughout the world^[1]. The significance of HBV infection in dialysis setting is even higher, because of the high rate of infection due to contaminations, transfusions and injections, and also the high rate of associated survival disadvantage^[2]. To tackle this problem in this population, hygienic precautions have been developed whose effectiveness has been very well established^[3]. Nevertheless, despite all the precautions, there are still a relatively large proportion of dialysis patients who develop the infection^[4]. For the same reason, hepatitis B vaccination is an inevitable part of any preventive protocol that has been developed and proposed by health societies for the dialysis setting^[5].

As mentioned, vaccination against HBV infection, though very effective, has not thoroughly eradicated the infection in the dialysis patients^[6]. It has been shown that seroconversion due to HBV vaccination in dialysis patients is not perfect; and systematic reviews have shown that there are a number of factors adversely affecting response rate to HBV vaccination in dialysis patients. Erythropoietin use, diabetes mellitus, dialysis mode, vaccine administration mode, adjuvant use, vaccine type (recombinant *vs* plasma-derived), and the effect of age and nutritional status of dialysis patients on the immunogenicity of HBV vaccine are among them. Considering these factors, in a previous paper we proposed individualization of HBV vaccination in dialysis patients based on the epidemiology of the associated factors in their patient population. In the current paper, we systematically review the existing literature for

studies investigating the potential effect of sex of dialysis patients on the immunogenicity of HBV vaccines in their patient population.

MATERIALS AND METHODS

Search strategy and data acquisition

The literature has been searched through the National Library of Medicine's (MEDLINE) database, and Google Scholar; the latter database has been particularly used to find relevant citations of the trials of interest; as well, specific journals have been searched to identify all the associated evidence. The key words used included "hepatitis B", "vaccine", "dialysis", "hemodialysis", "haemodialysis", "peritoneal dialysis", "gender", "sex", "male" and "female". The search has also been repeated using the reference lists of the associated systematic reviews and meta-analyses. There was no restriction in regard to the time of publication for our searches; and all the studies fulfilling the inclusion criteria were included into the analysis, irrespective of their publication year.

Inclusion and exclusion criteria

We used a number of inclusion criteria for the found studies in this systematic review: (1) they had to be available as full text (wherever the full text was not available, we contacted the corresponding author with a kind requests for the full text papers); and (2) their data is presented in a form that could be used construct a database for meta analysis were considered eligible for inclusion. There was no restriction regarding the type of vaccines employed in the trials and they were included into the meta-analysis if their vaccine was either plasma-derived or recombinant DNA preparations. The administered dosages or follow up times or vaccination routs were also not subjects to any preferable inclusion or exclusion. Studies were excluded if: (1) they reported not data on response to HBV vaccination separately for either gender in term of epidemiology of seroconversion for either gender groups; and (2) trials were published as abstracts with no enough methodology description.

End point

The association of the gender of dialysis patients has been associated with seroresponse to HB vaccine in the included trials. In cases both seroprotection and seroconversion had been reported by the included trials, seroconversion has been used as the end-point.

Source of support

This meta-analysis was not supported by any pharmaceutical company. The source of support in this study is a grant from Baqiyatallah University of Medical Sciences, Tehran, Iran.

Literature review

After excluding studies not fulfilling inclusion criteria, 19 clinical trials^[7-25] have been remained whose demo-

Table 1 Basic demographic data of the included clinical trials

Study ID	First author	Ref.	Year of publish	Country of origin	Participant number	Dialysis mode
1	Abdul N Khan	[7]	1996	United States	97	HD and CAPD
2	Kai Ming Chow	[8]	2010	China	87	CAPD
3	Ismail Hamdi Kara	[9]	2004	Turkey	34	HD
4	Baris Afsar	[10]	2009	Turkey	188	HD
5 (ID) 6 (IM)	Andre F Charest	[11]	2000	Canada	97	HD
7	Yao-Lung Liu	[12]	2005	Taiwan	69	HD and CAPD
8	Nancy M Waite	[13]	1995	Canada	77	HD
9	Salwa Ibrahim	[14]	2006	Egypt	29	HD
10	Shih-Yi Lin	[15]	2012	Taiwan	156	HD and CAPD
11	Dede sit	[16]	2007	Turkey	64	HD
12	Gerald DaRoza	[17]	2003	Canada	165	CKD
13	Jamshid Roozbeh	[18]	2005	Iran	62	HD
14	Khalid Al Saran	[19]	2014	Saudi Arabia	144	HD
15	Kevin S Eardley	[20]	2002	United Kingdom	105	HD
16	Sabahattin Ocak	[21]	2008	Turkey	49	HD
17	EO Morais	[22]	2007	Brazil	70	CKD
18	Sh Taheri	[23]	2005	Iran	125	CKD (32), HD (93)
19	Carol Dacko	[24]	1996	United States	32	CAPD
20	Gerald M Fraser	[25]	1994	United States	59	HD and CAPD

CAPD: Continuous ambulatory peritoneal dialysis; HD: Hemodialysis; ID: Intra-dermal; IM: Intramuscular.

Table 2 Demography of the participants in the studies included in the meta-analysis

Author	Ref.	Age (mean \pm SD)	Gender male (%)	Duration of dialysis (mo)
Abdul N Khan	[7]	47 \pm 14 (CAPD) 51 \pm 18 (HD)	26(55%; CAPD) 26 (52%; HD)	18 \pm 23 (CAPD) 56 \pm 73 (HD)
Kai Ming Chow	[8]	60 \pm 11	51/87 (59)	5.8 (median)
Ismail Hamdi Kara	[9]	44 \pm 15	19 (56)	27 \pm 15
Baris Afsar	[10]	NA (for total)	66 (35)	NA (for total)
Andre F Charest	[11]	52 \pm 2 (ID) 46 \pm 2 (IM)	73 (75)	3.4 \pm 1.0 (ID) 4.8 \pm 2.0 (IM)
Yao-Lung Liu	[12]	52 \pm 16 (CAPD) 61 \pm 11 (HD)	28 (41)	43 \pm 33 (CAPD) 60 \pm 49 (HD)
Nancy M Waite	[13]	NA (for total)	49 (64)	NA (for total)
Salwa Ibrahim	[14]	46 \pm 11	19 (66)	80 \pm 59
Shih-Yi Lin	[15]	NA (for total)	64/156(41)	NA
Dede sit	[16]	NA (for total)	31 (48)	NA (for total)
Gerald DaRoza	[17]	60 \pm 15	106 (46)	NA
Jamshid Roozbeh	[18]	NA (for total)	37/62 (60)	NA
Khalid Al Saran	[19]	51 \pm 15	78/66 (54)	40
Kevin S Eardley	[20]	61 \pm 13	58/47 (55)	18
Sabahattin Ocak	[21]	54 \pm 13	56/30 (65)	30 \pm 18
EO Morais	[22]	54.5 (median)	40 (57)	26
Sh Taheri	[23]	50 \pm 17	77 (62)	NA
Carol Dacko	[24]	NA (for total)	19 (59)	NA (for total)
Gerald M Fraser	[25]	NA (for total)	117 (58)	NA

SD: Standard deviation; CAPD: Continuous ambulatory peritoneal dialysis; HD: Hemodialysis; NA: Not available; ID: Intra-dermal; IM: Intramuscular.

graphic data is summarized in Table 1. Demographic data of the 1709 dialysis patients reported in the 19 published papers included in this meta-analysis is presented in Table 2. Details of the vaccination approaches employed in the studies is summarized in Table 3.

Statistical analysis

The Meta analysis has been performed using a random-effects approach. Test of heterogeneity between the studies has been assessed using the I^2 statistics, which describes the proportion of total variation across studies

that is the result of heterogeneity rather than chance. Statistical heterogeneity was present, defined as $P \leq 0.05$ or $I^2 > 50\%$. All statistical analyses was conducted using "metan" user-written commands. The meta-analysis has been performed using software Stata v.9.0 (Stata corp, TX, United States).

RESULTS

Patient characteristics

Demographic and clinical characteristics of the included

Table 3 Vaccination information details in the included clinical trials

Author	Ref.	Vaccination mode	Vaccine type	Vaccine dose	Schedule (mo)
Abdul N Khan	[7]	IM	Recombinant (Engerix-B)	40 mcg	0, 1, (2), 6
Kai Ming Chow	[8]	IM	Recombinant (Engerix-B)	40 mcg and 80 mcg	0, 1, 6
Ismail Hamdi Kara	[9]	IM	Recombinant (Engerix-B)	40 mcg	0, 1, 2, 6
Baris Afsar	[10]	IM	Recombinant	-	0, 1, 2, 6
Andre F Charest	[11]	ID and IM	Recombinant (Engerix-B)	40 mcg (IM); 5 mcg (ID)	0, 1, 2, 6
Yao-Lung Liu	[12]	IM	Recombinant (Engerix-B)	40 mcg	0, 1, 2, 6
Nancy M Waite	[13]	IM	Recombinant (Engerix-B)	40 mcg	0,1,2,6
Salwa Ibrahim	[14]	IM	Recombinant (Engerix-B)	40 mcg	0, 1, 2, 6
Shih-Yi Lin	[15]	IM	Recombinant (Engerix-B)	40 mcg	0, 1, 2, 6
Dede sit	[16]	IM	Recombinant (Hepavax)	40 mcg	0, 1, 2, 6
Gerald DaRoza	[17]	IM	Recombinant and plasma derived	20, 40 and 80 mcg	0, 1, 6
Jamshid Roozbeh	[18]	IM and ID	Recombinant (Herberbiovac-HB)	40 mcg (IM); 20 mcg (ID)	0, 1, 4
Khalid Al Saran	[19]	IM	Recombinant (Engerix-B)	40 mcg	0, 1, 2, 6
Kevin S Eardley	[20]	IM	Recombinant (Aventis MSD)	40 mcg	0, 1, 2, 12
Sabahattin Ocak	[21]	IM	Recombinant (Euvax-B)	40 mcg	0, 1, 2, 6
EO Morais	[22]	ID	Recombinant (Greencross)	2 × 5 mcg	16 injection within 8 wk
Sh Taheri	[23]	IM	Recombinant (Havana)	40 mcg	0, 1, 6
Carol Dacko	[24]	IM	Recombinant (Engerix)	40 mcg	0, 1, 2, 6
Gerald M Fraser	[25]	NA	Recombinant (Engerix-B)	20 mcg	0, 1, 2, 6

ID: Intra-dermal; IM: Intramuscular.

trials have been summarized in Table 1. All of the included clinical trials were published in English and the date of publication ranged from 1994 to 2014. Eight out of the nineteen studies (42%) were from the Middle East [Turkey (4), Iran (2), Saudi Arabia and Egypt each one study] and the remaining were from Canada (3 studies), United States (3 studies), China and Taiwan (3 studies), and United Kingdom and Brazil (1 study, each). In 10 (52.6%) studies, all patients were under hemodialysis while in two (10.5%) only patients under continuous ambulatory peritoneal dialysis (CAPD) was investigated, in 2 (10.5%) patients were chronic kidney disease (CKD) not on renal replacement therapy, in one study patients were either on maintenance hemodialysis or CKD not on dialysis, and in the remaining 4 (21%) studies, both of the dialysis modes were used.

Mean age of the participants in the included cohorts ranged from 44 to 61 years, mean duration of dialysis also ranged from 3.4 to over 80 mo and gender distribution ranged from 35% to 75% in favor of males (Table 2). In two of the studies intradermal mode of vaccination has been used besides the intramuscular mode, and in one study only intradermal mode of vaccine administration had been used. In only one study, some of the patients received plasma-derived vaccines, while in all others, the vaccine was recombinant productions. In 13 trials with intramuscular administration of the vaccine, 40 mcg had been prescribed in all patients, in one study either 40 or 80 mcg was used, and in one another 20, 40 or 80 mcg were used for vaccination. Intradermal administration of vaccine was used in doses ranging from 5 mcg to 20 mcg in different trials. One study had not declared mode of vaccine administration. Schedule of vaccination in four of the studies was 3 times (with different time intervals) and in the others but one, were a 4-times schedule (0, 1,

2, 6). In the remaining one trial, patients either received a 3 or 4 times vaccine administration schedule.

Summary of outcome

Analysis of response to HB vaccination in our dialysis population showed a significant relation to their gender with females significantly responding a better response to vaccination ($P = 0.002$, $Z = 3.08$; Figure 1). As well no significant heterogeneity has been detected in the analysis of the included studies [$P = 0.766$; heterogeneity $\chi^2 = 14.30$ (df = 19); $I^2 = 0\%$].

Reanalysis regarding dialysis mode

Then, a reanalysis of the pooled data was conducted regarding the dialysis mode to evaluate potential differential impact of gender on HB vaccine response. Hemodialysis was the only subgroup that showed a significant difference regarding dialysis mode in response to HB vaccination regarding gender and in other subgroups, gender was not discriminatory factor in vaccine response (Figure 2; HD group: $P = 0.042$, $Z = 2.03$; CAPD group: $P = 0.136$, $Z = 1.49$; HD/CAPD group: $P = 0.618$, $Z = 0.5$; CKD group: $P = 0.302$, $Z = 1.03$; CKD/HD group: $P = 0.448$, $Z = 0.76$).

Reanalysis regarding vaccination schedule

Again, the data had been reanalyzed regarding potential effect of vaccination schedule between the patient groups on the differential vaccine response regarding gender of the patients. Despite a relatively lower p value achieved for schedule "4 times vaccination", none of the subgroups showed any significant difference (Figure 3; "4 times vaccination" group: $P = 0.055$, $Z = 1.92$; "3 times vaccination" group: $P = 0.088$, $Z = 1.71$; "others" group: $P = 0.393$, $Z = 0.86$).

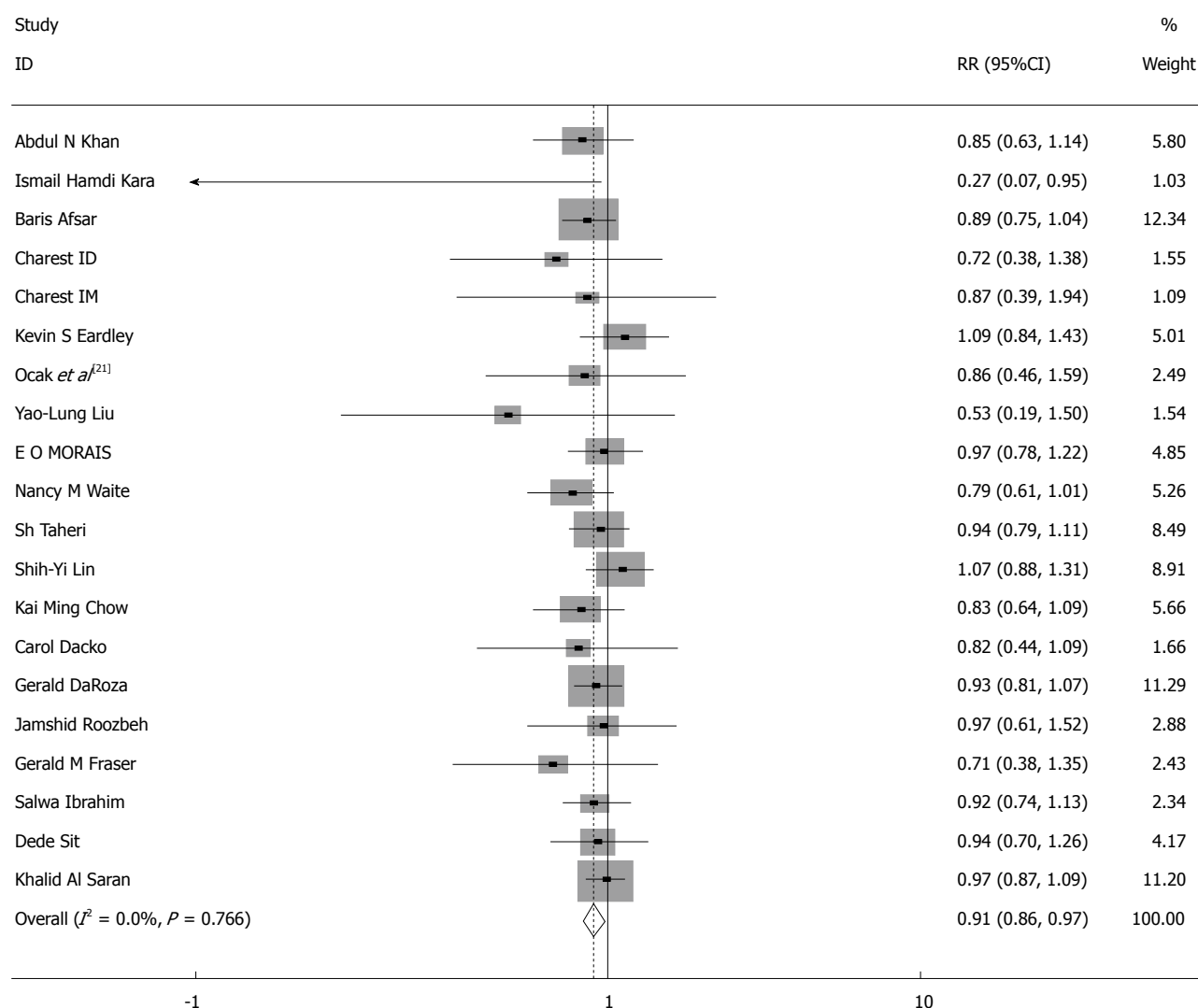


Figure 1 Forest plot: Meta-analysis of the association between gender of the end-stage renal disease patients and seroresponse to hepatitis B vaccination.

Reanalysis regarding vaccine type

The data then had been reanalyzed after removing the only trial in which a plasma-derived vaccine had been used, in order to censor potential effects of vaccine type on the study results. Nonetheless, the findings didn't change significantly ("Recombinant vaccine" group: $P = 0.014$, $Z = 2.47$; "Recombinant or plasma-derived vaccines" group: $P = 0.288$, $Z = 1.06$).

DISCUSSION

In the dialysis setting, HBV vaccination has been confirmed as an essential part of immunization, and guidelines proposed by several experts as well as health organizations almost universally recommended this procedure for this patient population^[5,26,27]. These recommendations are despite the fact that patients with advanced kidney diseases have compromised immune system function, and cannot well respond to any immunization attempt made through vaccination.

The impaired immunogenicity in renal disease

patients has been explained by different mechanisms, most notably impaired cellular immunity system in this population^[28-30]. However, clinical trials have also proposed several other factors having predictive values in this era; but due to the controversial evidence provided by different reports, systematic reviews and meta-analyses have been conducted to pool data of all the published trials to provide a thorough conclusion from the cumulative data. Most of the published systematic reviews on this subject have been performed by Fabrizi *et al*^[31] investigating potential effects of a large number of factors on HBV vaccination in dialysis patients. For example they found no significant effects for using erythropoietin (Epo)^[31] and some other adjuvants^[32] on the immunogenicity of HB vaccination in kidney disease patients; while several other factors significantly associated with seroconversion have also been reported by the same authors that included use of levamisole^[33], granulocyte macrophage-colony stimulating factor^[32] and thymopentin use^[34]. Seroresponse of patients on maintenance hemodialysis vs peritoneal dialysis

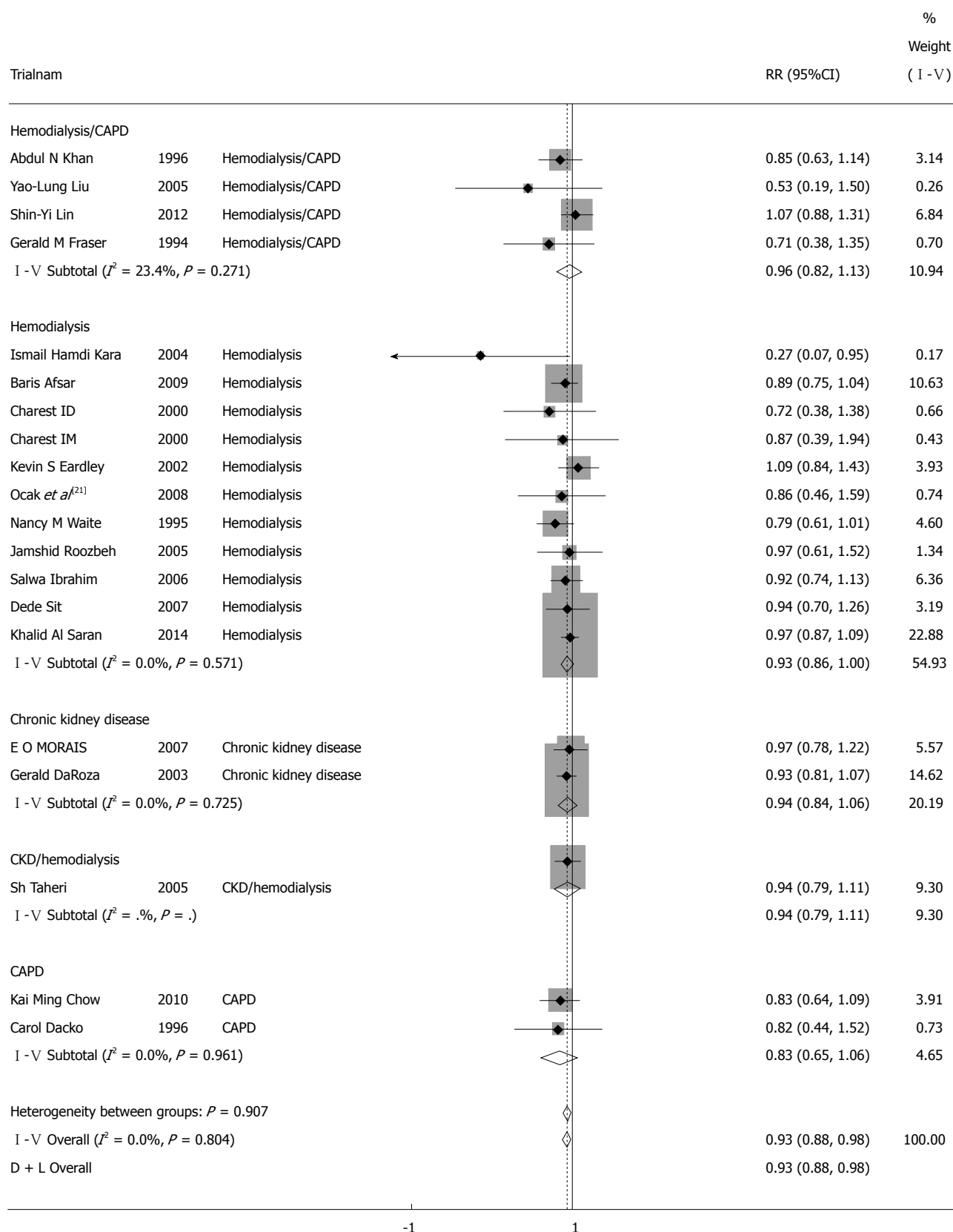


Figure 2 Forest plot: Meta-analysis of the association between gender of the end-stage renal disease patients and seroresponse to hepatitis B vaccination in patients with different therapy modality.

showed no significant difference^[35]; whereas intradermal (vs intramuscular) administration of HB vaccine had

been associated with a significantly higher vaccine response^[36]. Diabetes mellitus^[37] and older age^[38] were

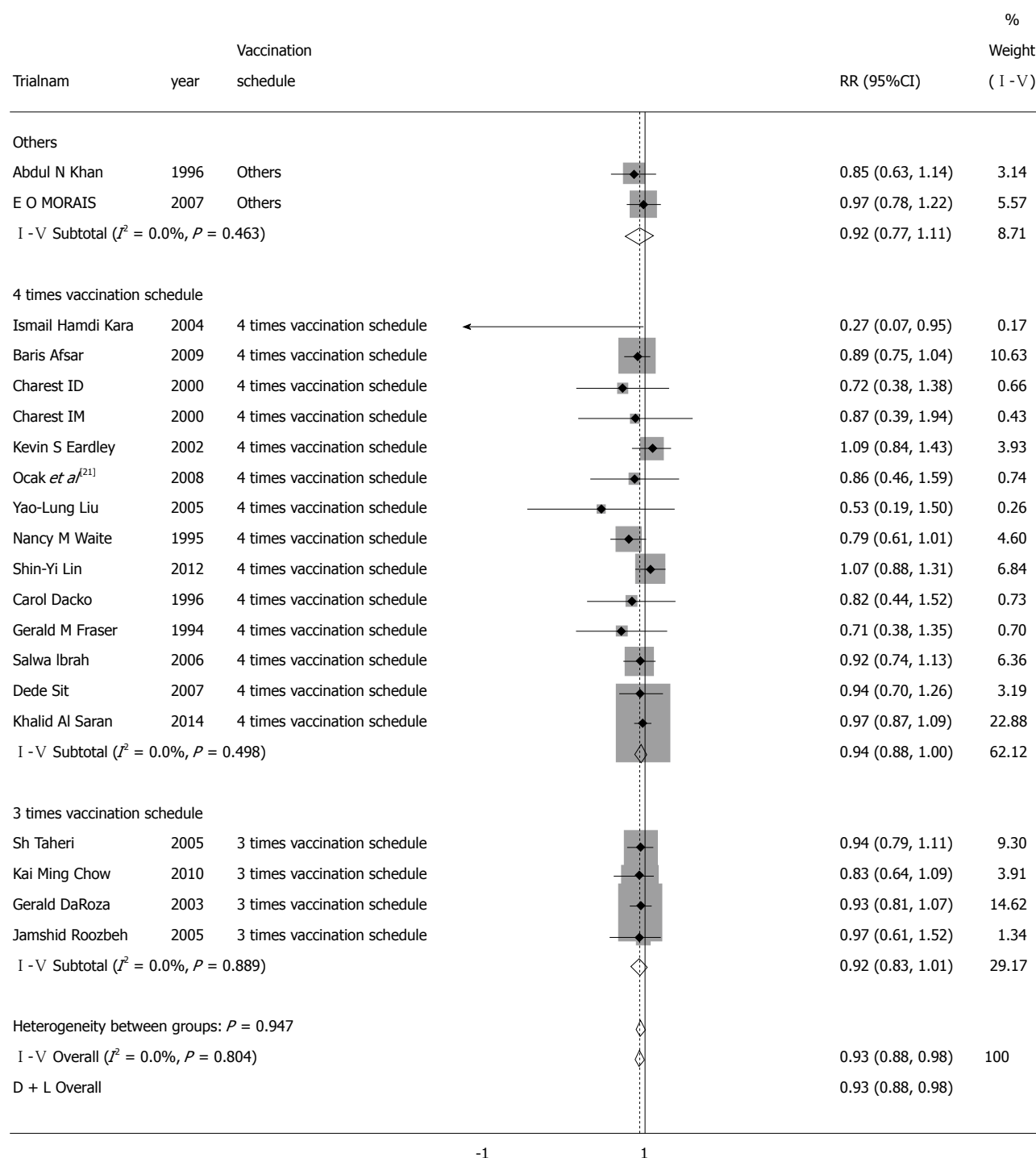


Figure 3 Forest plot: Meta-analysis of the association between gender of the end-stage renal disease patients and seroresponse to hepatitis B vaccination in patients with different vaccination schedules.

also significantly associated with poorer response to HB vaccination.

Very limited data coming from the previous clinical trials proposes that gender is a major interfering factor in the context of HB vaccine immunogenicity^[9]. On the other hand, most of the existing clinical trials represent no significant role for gender on response to HB vaccination, either in kidney disease patients^[7,10] or other end-stage organ disease patients^[39]. However, the patient population in each of the clinical trials was

limited, and in case there is a delicate difference in seroresponse to HB vaccine between the two genders, it can be easily lost. In fact, looking to most of the included clinical trials, males had relatively but not statistically significantly less percentages of response rate to HB vaccination^[10,13]. This urged us to conduct this meta-analysis to pool the existing data to represent a universal outlook to the issue.

This meta-analysis showed that in the kidney disease setting, males significantly represent lower

seroconversion due to HB vaccination than females. This finding is of clinical relevance. In a previous study, it had been proposed that immunization against HB in dialysis patients should be individualized based on factors that significantly affect seroresponse in these patients^[6]. So, according to the data derived from the current meta-analysis, male patients should be more rigorously surveyed after HB vaccination in dialysis setting. Moreover, future studies are recommended to find more potent immunization programs especially in this vulnerable population.

For having a more precise view on the subject, the data has been reanalyzed after stratifying the included trials based on their patients' dialysis mode, and found that the observed sex bias in the seroconversion due to HB vaccine was only significant in hemodialysis patients, and no significant difference has been observed for patients on peritoneal dialysis or CKD patients not on dialysis. Although on one hand this finding may urge us to pay more attention in men under maintenance hemodialysis therapy, we should have in mind that lack of detecting any sex discrimination in other study groups may be simply due to the comparatively limited sample size in the latter groups.

Once again, the data has been stratified based on their vaccination schedule, mainly in patients receiving 3 or 4 doses of vaccination. Although in none of the two schedules any significant difference in the seroresponse to HB vaccination has been detected regarding patients' sex, those on 4 times vaccination schedule represented a *P* value of 0.055 for sex; which might be of some value for some investigators.

Although this study is of some limitations, we believe that the findings of this study add significantly to the literature, and helps specialists to monitor their kidney disease patients more effectively and protect them against HBV infection attainment. This systematic review represents the strongest evidence on the significance of sex on the seroresponse to HB vaccination in kidney disease patients with males having more impaired immune response to the vaccination. Moreover, this sex bias was significantly more prominent among hemodialysis (vs other therapeutic procedures) patients, and in those on 4 times vaccination schedule (vs 3 times), although the latter failed to reach the significance level. It should also be mentioned that the age range of the included patients in the current meta-analysis (44-61 years) is much younger than the general age of the dialysis population, which might put some limitations in the globalization of our study results. In conclusion, this Meta analysis showed significant effect for the sex of CKD and dialysis patients on the immunogenicity of HB vaccine, with a better response for females. This sex discrimination was most prominent among hemodialysis patients. This finding suggests us to specify a sex-dependent vaccine dosage administration for patients with kidney disease. Future studies directing to find strategies with more efficacy, as well as surveys directing to find other interfering factors in this regard

are recommended.

COMMENTS

Background

Dialysis patients are substantially at higher risk of developing hepatitis B virus (HBV) infection, so preventive measures are of extreme importance in this population. Anti-HBV vaccination has been the most popular preventive strategy in this population for a long time; nonetheless, its feasibility in this population has been under serious doubt. Several factors have been documented as players of significant roles in the seroresponse to HBV vaccination.

Research frontiers

During the past decades, several surveys have been performed to unveil the potential associations between dialysis patients demographic data and their seroresponse to HBV vaccination. Moreover, several systematic reviews as well as meta analyses were published to investigate these associations using pooled data of the randomized trials. To the authors' knowledge, this is the first meta-analysis that have ever investigated an citation between dialysis patents gender and their seroconversion rate after HBV vaccination.

Innovations and breakthroughs

Based on the current meta-analysis, gender is a significant factor determining response to HBV vaccination in kidney disease patients, with females significantly better responding to the vaccination. This may led future scientists to develop some individualized vaccination protocols that improve the response rate of the males to the vaccination.

Applications

Sex is a significant factor predicting seroresponse to HBV vaccination. Cumulation of data of different factors playing roles in this context can help authors to develop specific vaccination protocols for specific groups that maximizes immunization rate in this population.

Terminology

Hemodialysis is a type of renal replacement therapy which purifies the blood from unwanted materials in a way similar to kidney function. Peritoneal dialysis is a type of renal replacement therapy that uses peritoneal space for purification of the blood contents using dialysates getting injected into it. Chronic kidney disease patients are those who have significant renal function disturbance without a need to renal replacement therapy.

Peer-review

The paper is well-written and the results have potential clinical applications.

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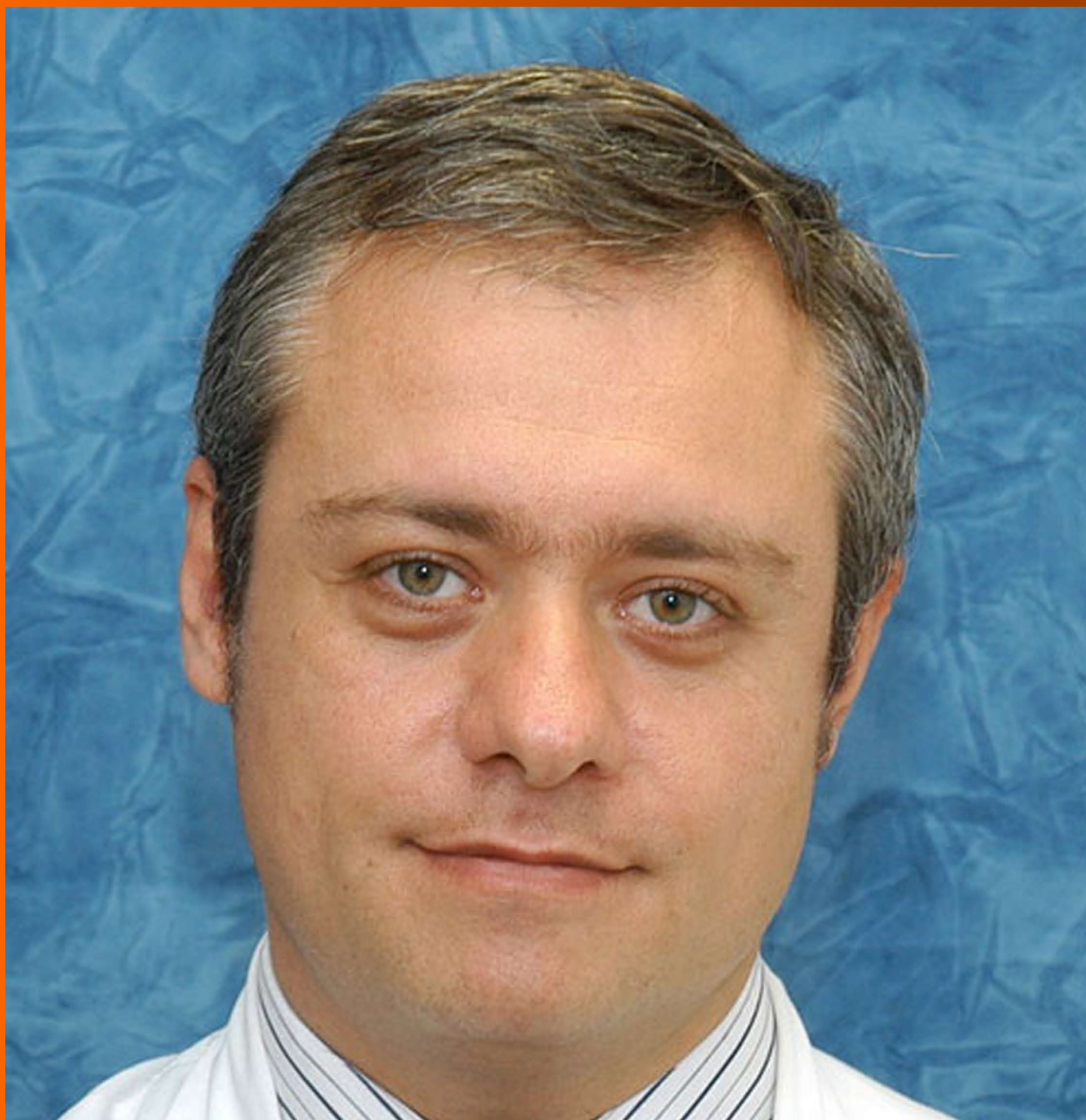
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Extracellular vesicles as mediators of vascular inflammation in kidney disease

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Abstract

Vascular inflammation is a common cause of renal impairment and a major cause of morbidity and mortality of patients with kidney disease. Current studies consistently show an increase of extracellular vesicles (EVs) in acute vasculitis and in patients with atherosclerosis. Recent

research has elucidated mechanisms that mediate vascular wall leukocyte accumulation and differentiation. This review addresses the role of EVs in this process. Part one of this review addresses functional roles of EVs in renal vasculitis. Most published data address anti-neutrophil cytoplasmic antibody (ANCA) associated vasculitis and indicate that the number of EVs, mostly of platelet origin, is increased in active disease. EVs generated from neutrophils by activation by ANCA can contribute to vessel damage. While EVs are also elevated in other types of autoimmune vasculitis with renal involvement such as systemic lupus erythematosus, functional consequences beyond intravascular thrombosis remain to be established. In typical hemolytic uremic syndrome secondary to infection with shiga toxin producing *Escherichia coli*, EV numbers are elevated and contribute to toxin distribution into the vascular wall. Part two addresses mechanisms how EVs modulate vascular inflammation in atherosclerosis, a process that is aggravated in uremia. Elevated numbers of circulating endothelial EVs were associated with atherosclerotic complications in a number of studies in patients with and without kidney disease. Uremic endothelial EVs are defective in induction of vascular relaxation. Neutrophil adhesion and transmigration and intravascular thrombus formation are critically modulated by EVs, a process that is amenable to therapeutic interventions. EVs can enhance monocyte adhesion to the endothelium and modulate macrophage differentiation and cytokine production with major influence on the local inflammatory milieu in the plaque. They significantly influence lipid phagocytosis and antigen presentation by mononuclear phagocytes. Finally, platelet, erythrocyte and monocyte EVs cooperate in shaping adaptive T cell immunity. Future research is needed to define changes in uremic EVs and their differential effects on inflammatory leukocytes in the vessel wall.

Key words: Extracellular vesicle; Atherosclerosis; Kidney disease; Glomerulonephritis; Macrophage

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Core tip: This review addresses the role of extracellular vesicles (EVs) in vascular inflammation that can cause renal damage and is also shaped by uremic mediators. Vasculitides are common causes of renal damage. Functionally, neutrophil EVs induced by anti-neutrophil cytoplasmic antibody contribute to endothelial damage. EVs are main distributors of shiga toxin in the circulation and into tissues in typical hemolytic uremic syndrome. In atherosclerosis in patients with and without kidney disease, endothelial EVs are elevated. Uremic EVs are deficient in mediating vascular relaxation. EVs modulate mononuclear phagocyte differentiation, cytokine production, lipid phagocytosis and antigen presentation, atherosclerotic inflammatory processes significantly altered in uremia.

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INTRODUCTION

Subcellular membrane vesicles collectively termed extracellular vesicles (EVs) are a third pathway of intercellular communication between direct cell-to-cell contact and secretion of soluble signaling molecules^[1]. EVs can be secreted by virtually all cell types and contain a variety of components^[2,3]. They are already present under physiologic conditions in a variety of bodily fluids^[4]. EVs critically modulate local and systemic inflammatory and immune processes^[4-7]. How EVs affect leukocytes and their function in the arterial wall in patients with kidney disease will be discussed for both acute vasculitis and chronic vascular inflammation in atherosclerosis.

LEUKOCYTES IN THE VASCULAR WALL

Leukocytes are an integral part of the healthy vessel^[8,9] and differentially increase in vascular inflammation^[10]. The arterial wall is invaded by blood leukocytes in inflammation both directly across the main vascular endothelium and through vasa vasorum of larger vessels. This process is a tightly regulated cascade of leukocyte activation, rolling, adhesion and transmigration, to date studied mostly in neutrophilic granulocytes^[11-13]. Vascular inflammation is mostly found in the arterial tree and microvessels including glomerular capillaries. Inflammation of the much thinner venous wall is rarely a clinical problem beyond reaction to intravascular thrombosis^[10]. This is remarkable as most endothelial leukocyte adhesion and transendothelial migration is observed in venules^[14]. Vascular inflammation is central in allo-immune processes such as transplant rejection. These have recently been reviewed (among others^[15,16]). This review focuses on native arteries and

glomerular capillaries.

Impaired renal function during both acute kidney injury and chronic kidney disease significantly influences the structure of the arterial wall, affecting arterial endothelial cells and smooth muscle cells^[17,18]. Structural changes are most obvious in enhanced atherosclerosis development^[19-21]. A prominent feature in humans and mouse models with end stage kidney disease is extraosseous calcification of the arterial media^[19,22]. Chronic inflammation in atherosclerosis occurs in normal and reduced kidney function, however, both innate and adaptive leukocytes are specifically altered by renal impairment^[23-25].

CHARACTERIZATION OF EVS

Since the first description of "platelet-dust" in 1967^[26], EVs were found in diverse biological fluids^[27]. Important factors of EV characterization are size and surface markers indicating their cellular origin^[5,28-30]. EVs are a very heterogeneous population as both characteristics additionally vary with mode of EV generation^[31]. In addition, most of the currently used flow cytometry instruments are not optimal for detection of particles of submicrometer size^[32,33]. Organizations such as the Society for Extracellular Vesicles, formed in 2011, and databases such as EVpedia (<http://evpedia.org>) are instrumental in establishing reliable standards, including specification of preanalytical procedures and basic clinical information^[4,27,34].

Currently, two main groups of EVs are distinguished by both size and mode of generation: Exosomes and microparticles^[1,3,28,29] (Figure 1). Exosomes are small EVs, ranging from 30-100 nm. They originate from endosome-derived multivesicular bodies and are released to the extracellular space when the multivesicular bodies fuse with the plasma membrane^[35,36]. Microparticles (also referred to as ectosomes, membrane vesicles, nanovesicles and shedding vesicles) measure 100-1000 nm^[3,30,35,37]. They directly bud off from the plasma membrane^[35,36]. Both types of vesicles are enclosed by a lipid bilayer, but due to the fact that microparticles directly bud from the plasma membrane, they have a more similar membrane composition to their parent cell than exosomes^[28,35]. For example, leukocyte surface proteins such as CD14, CD36 and CD11c are found on leukocyte microparticles^[38]. Phosphatidylserine was initially thought to be enriched on microparticles only, but was later also found on exosomes^[3]. Exosomes display endosome-associated proteins like annexins, flotillins or CD63 on their surface^[28]. However, the expression of these proteins on microparticles cannot be completely excluded^[3]. In addition to a possible biological overlap, this also reflects the technical challenge of multicolor fluorescence analysis of small particles^[32,33]. Principal intravesicular contents such as cytoplasmic proteins, metabolites, RNAs, microRNAs and lipids can be found in both, exosomes and microparticles, however, in different abundance^[2,3,35]. In addition to exosomes and microparticles, apoptotic bodies have been described as a separate entity by some authors^[35,36,39]. These have been defined as large (1-5 µm) vesicles

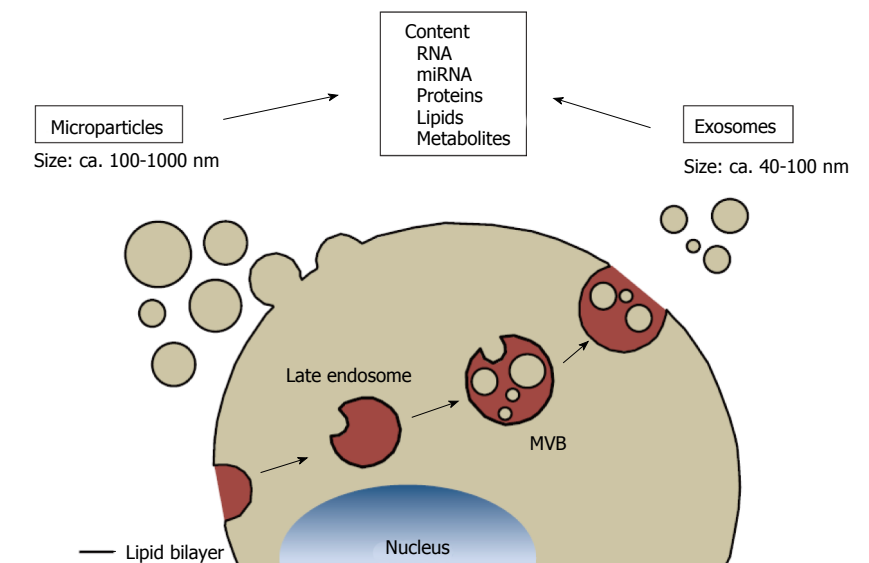


Figure 1 Classification of extracellular vesicles.

Types of extracellular vesicles are distinguished by their mode of generation. Microparticles directly bud off the plasma membrane and measure approximately 100-1000 nm. Exosomes are released by fusion of multivesicular bodies with the plasma membrane and their sizes range between 40-100 nm. Both types of extracellular vesicles can contain RNA, microRNA, proteins, lipids and metabolites. MVBs: Multivesicular bodies.

generated during apoptosis. However, other EVs also express the inner membrane marker Annexin V on their surface.

Following current recommendations^[29], the overarching term EV will be used for all secreted vesicles in this review and further characterization will be provided by naming specific surface markers.

EVS IN CHRONIC KIDNEY DISEASE

In end stage renal disease, both the uremic milieu and hemodynamic changes during the dialysis procedure can contribute to EV generation^[40]. Uremic toxins such as p-cresol and enoxylsulfate induced EV shedding from HUVECs^[41]. Hemoconcentration by dialysis increased blood viscosity, thereby decreasing shear stress and EV generation^[42]. In addition, morphologically similar EVs may serve different functions is generated in an uremic milieu - for example, EVs from healthy controls, but not patients with end stage renal disease conferred endothelium mediated arterial relaxation *in vitro*^[43].

Counts and provenience of circulating EVs have been characterized in patients with chronic renal impairment with and without renal replacement therapy. Some studies found elevated serum concentrations of total and CD42⁺ platelet EV^[44-46], total, endothelial (CD31⁺, CD114⁺)^[43,46], platelet (CD41⁺), and erythrocyte (CD235⁺) EVs^[43] in patients with end stage renal disease, while in others, total plasma EV concentrations were unaltered^[47,48] or only endothelial EVs were increased^[41]. Also, the effect of the hemodialysis procedure is controversial with an increase in some^[47] but not other studies^[44,45]. The currently available studies included relatively small patient numbers and discrepancies that are at least partly explained by pre-analytical variables such as different modes of blood draw, storage and anticoagulation, flow cytometry equipment and surface markers used. However, addressing a possible pathophysiologic cause, a recent study further stratified patients with moderate kidney disease (mean GFR 39 mL/

min) according to the presence of cardiovascular disease defined by significant stenosis on coronary angiography^[49]. EVs of both platelet (CD42⁺) and endothelial (CD31⁺) origin were significantly higher in patients with coronary artery disease, irrespective of renal impairment. Indeed, a large number of observational studies report increased concentrations of circulating EVs in atherosclerosis^[50-53]. Especially endothelial EV concentrations appear to be predictive for cardiovascular prognosis^[54]. This was confirmed in a recent observation in a large group of 844 individuals from the Framingham offspring cohort. Endothelial EV counts (CD31⁺ or CD114⁺) correlated with hypertension, elevated triglycerides the metabolic syndrome and an overall higher Framingham in patients inversely correlated with brachial artery flow-induced dilatation and positively correlated with indices of arterial stiffening^[43]. Endothelial (CD31⁺) EV concentration was associated with severe hypertension in a number of cohorts^[55,56]. Concentrations significantly correlated with renal damage manifesting as micro- or macro-albuminuria in this condition^[57].

The currently available data is also limited by a mostly cross-sectional study design that precludes detection of temporal changes in single patients^[52]. Measurement of EV concentration is evaluated as a predictive factor in a number of ongoing prospective trials^[58]. However, there are some longitudinal data for patients with end stage kidney disease. A follow up study of 81 hemodialysis patients for a mean of 50 mo revealed that endothelial (CD31⁺) EV concentration in serum obtained after the long interval was a significant predictor of all cause and cardiovascular mortality, an association that was not observed for CD41⁺ platelet, CD11b⁺ leukocyte or CD235⁺ erythrocyte EVs^[59]. Another prospective study investigated endothelial EV counts (CD31⁺) in a cohort of 227 patients with end stage renal disease who were scheduled for kidney transplantation^[48]. Endothelial EVs significantly decreased during 60 d of longitudinal follow up after kidney transplantation. However, they did not differ from

healthy controls at start of the trial^[48] which may reflect that these patients represent a subgroup with relatively few co-morbidities.

In summary, chronic elevation of endothelial EVs currently appears to be significantly associated with vascular dysfunction and atherosclerosis in renal disease.

THE ROLE OF EV IN RENAL VASCULITIS

Systemic inflammation is frequently associated with elevated EV concentrations. Pathophysiologically, monocytic and endothelial EVs can directly induce MCP1, interleukin (IL)-6 and VEGF production in human podocytes^[60] thus enhancing glomerular injury. Investigations of EVs in systemic lupus erythematoses (SLE), ANCA vasculitis and typical hemolytic uremic syndrome (HUS) will be reviewed. It is also of note that our literature review revealed no information on EVs in either the pathogenesis or regarding the circulating EV counts in other common forms of renal vasculitis, including postinfectious glomerulonephritis, a historically common cause of renal vascular inflammation, and IgA nephropathy as the currently most common entity in the Western world.

RHEUMATIC DISEASE WITH RENAL INVOLVEMENT

EVs function has been studied in systemic rheumatic disease^[61,62]. In SLE, a common rheumatic cause of glomerulonephritis, elevated levels of EVs, particularly of platelet origin, have consistently been detected in patients with active antiphospholipid syndrome^[63-66], and also in Sjögrens syndrome^[64] and closely been associated to intravascular thrombosis. Mechanisms of modification of inflammation of the vascular wall by EVs in SLE have not been reported to date. However, EVs in SLE display increased amounts of immunoglobulin and complement^[67] and it is conceivable that they may contribute to deposition of these in the renal glomerulum. Furthermore, the proteome of these EVs in SLE appears to differ from healthy controls^[68] and EVs constituents in SLE such as Galectin 3 binding protein have also been detected in glomerular deposits in individual patients with lupus-associated glomerulonephritis^[69].

ANCA ASSOCIATED VASCULITIS

In anti-neutrophil-cytoplasmic antibody (ANCA) associated vasculitis, a number of studies have shown elevated serum EV concentrations during active disease^[47,70-72]. Counts reverted normal during remission. In addition, counts were significantly higher than in patients with other glomerulonephritides such as IgA nephropathy, minimal change disease, diabetic nephropathy but also lupus nephropathy^[47,71]. Most EVs in ANCA disease were of platelet origin, but leukocyte and endothelial derived EVs were also found^[47,70-73]. Histologically, ANCA vasculitis presents as acute necrotizing vasculitis not only of the

glomeruli, but arteries of all sizes with predilection of small vessels^[74]. The most prominent infiltrating cell types are neutrophilic granulocytes and even more abundantly, monocytes^[75]. However, most research on leukocyte function within the vascular wall has concentrated on neutrophils. ANCA can induce generation of EVs from pre-activated, *e.g.*, tumor necrosis factor (TNF) α primed neutrophils^[72,76,77]. These particles increased CD54 surface expression and IL-6 and IL-8 production from human vein endothelial cells (HUVECs) *in vitro*, suggesting that they can promote inflammation of the vessel wall^[72]. ANCA induced EVs also contained tissue factor and may thus promote hypercoagulability and the increased rates of thrombosis observed in patients with ANCA disease^[76,77].

TYPICAL HUS

Typical HUS is a complication of enteral infection with shiga toxin producing strains of *Escherichia coli* (STEC). EVs are highly elevated in patients with active systemic disease and platelet EV attach to leukocytes, most abundantly monocytes in peripheral blood^[78-80]. Recent research shows that EVs are also generated from erythrocytes in this condition^[81], a type of EV that can activate monocytes to produce pro-inflammatory cytokines^[82]. Platelet monocyte complexes and EV generation from both can be induced by shiga toxin. These EVs contain tissue factor and can thereby contribute to the microthromboses characteristic of the disease^[80]. They also bore activated complement constituents, namely C3 and C9^[78]. Neutrophils phagocytosed them, a process that may further contribute to their activation, adhesion and vascular inflammation^[78]. Both leukocyte and platelet EVs contain shiga toxin and significantly contribute to its spreading into tissues including podocytes and tubular epithelium in the kidney^[83] thus contributing to toxicity. Whether or not shiga toxin increases or diminishes leukocyte lifespan appears to depend on experimental conditions *in vitro*^[84]. *In vivo*, increased rates of both monocyte and neutrophil cell death were observed during STEC-HUS^[79]. It is conceivable that shiga toxin transferred into the vascular wall by EVs will also influence vascular resident leukocytes^[83].

THE ROLE OF EVS IN VASCULAR INFLAMMATION IN ATHEROSCLEROSIS

EVs are abundant within the atherosclerotic wall which may enhance their biologic functions^[6]. EVs from human endarterectomy specimens have been isolated by serial centrifugation and analyzed by flow cytometry in comparison to material from macroscopically unaffected arteries^[85,86]. A detailed analysis determined that most plaque EVs are of leukocyte origin, including 29% macrophage (CD14⁺), 15% lymphocyte (CD4⁺), 8% granulocyte (CD66b⁺) provenience^[86]. No platelet, but erythrocyte and smooth muscle cell markers were detected in EVs from the plaque lysate, recent *in vitro* data providing first evidence of EV generation from smooth muscle cells in

contact with pro-atherogenic lipids^[87]. The analysis of plaque EV provenience was confirmed by subsequent studies including proteome analysis^[38,88].

Mechanistic roles of EV action in atherosclerotic inflammation have mostly been ascribed to their protein content^[50,51] including large cytoplasmic protein structures such as proteasomes and inflammasomes^[89,90]. In addition, other constituents such as nucleic acids, notably microRNA^[91,92], glycosylation pattern^[93] and lipids^[94] critically contribute to EV function in atherosclerosis^[6,90,91]. Elevated systemic lipid levels and local deposition in the plaque makes EV lipids likely candidates for modulation of plaque development^[95]. High levels of free cholesterol induce generation of phosphatidylserine and tissue factor rich EVs from human monocyte-derived macrophages, partly induced by caspase-3 mediated apoptosis. Systemically, circulating EV concentrations, mostly of platelet origin (CD41⁺) were significantly decreased after lipid apheresis in humans^[96]. In renal impairment, lipoprotein function is markedly changed and protective functions are lost^[97,98] making it a possible mediator of the observed functional shift in uremic EVs.

Patients with chronic kidney disease from any cause are at a markedly elevated risk of cardiovascular morbidity and mortality^[97,99-101]. Medial calcification is characteristic of end-stage kidney disease^[99,100]. Atherosclerotic plaques in moderate renal impairment are mostly found in the arterial intima and are histologically similar to lesions in normal renal function^[102], a phenotype that has been replicated in animal models of atherosclerosis^[103,104]. Given the high prevalence of cardiovascular disease already in the general population, the role of inflammatory leukocytes in atherosclerotic plaque development has been explored in human samples and atherosclerotic animal models with a variety of methods including histology, flow cytometry and live cell imaging^[105-107]. Numbers of both adaptive and innate leukocytes in the vessel wall markedly increase during atherogenesis. With specific regards to renal impairment, current data on EV effects on innate and adaptive leukocyte populations prominent in atherosclerotic lesion formation will be reviewed.

THE ROLE OF EVS IN LEUKOCYTE INTERACTION WITH THE ENDOTHELIUM

When entering the vascular wall and again with growing intimal plaques, leukocytes come into close contact with endothelial cells. As a possible mechanism of proatherogenic EV effects on endothelial cells, CD40 ligand on human carotid plaque EVs is required for endothelial cell activation and neoangiogenesis by promotion of endothelial cell proliferation^[88]. EVs isolated from human atherosclerotic plaques can transfer ICAM-1 to endothelial cells, thus facilitating leukocyte, mainly monocyte adhesion and transmigration^[108]. They also expressed TNF α converting enzyme and plaque EVs that increase shedding of both TNF α and activated protein C from activated HUVECs^[109]. The fact that monocyte and T cell EVs

induced matrix metalloproteinase in synovial fibrocytes in rheumatoid arthritis suggests that this is a general EV property^[110]. Neutrophil EVs increased endothelial cell IL-6 release *in vitro*^[111]. T cell EVs generated both in *in vitro* and *in vivo* and EVs from patients with myocardial infarction decreased flow induced endothelial relaxation and downregulate eNOS expression^[112,113]. As a potential positive feedback loop, NOS inhibition induces L-selectin and PSGL-1 expressing EVs from neutrophilic granulocytes seeded to HUVECs *in vitro*, that in turn increasing neutrophil transmigration^[114]. Given NO inhibition by a range of uremic toxins^[115], it is conceivable that these processes cooperate in renal impairment to impair vascular function.

Circulating EV counts are highly elevated during acute arterial thrombosis in a large number of studies. These have recently been reviewed and will therefore only been referred to in relation to vascular leukocytes in this manuscript^[116-119]. However, it is of note that EV phosphatidylserine surface expression as a pro-thrombotic mediator was significantly increased in patients with the nephrotic syndrome of different etiologies^[120] and the *in vitro* pro-coagulant effect of EVs from both hemodialysis and peritoneal dialysis patients was enhanced^[46].

GRANULOCYTES

Neutrophilic granulocyte concentrations in peripheral blood and even more so, the neutrophil/lymphocyte ratio, are well-documented predictors of cardiovascular mortality^[121,122]. This relationship is also highly significant in patients with end stage renal disease^[123]. Recent animal data suggest that neutrophils mechanistically promote hypertension associated vascular damage and endothelial dysfunction^[124]. Neutrophils are essential in early atherosclerotic plaque development, probably by NET formation^[125]. They also generate a variety of EVs with pro- and anti-inflammatory functions^[111,126-128]. Acting directly on the parental cell type, Annexin A1 present in neutrophil EVs inhibits neutrophil rolling, adhesion and migration in mice^[126]. Neutrophil extravasation is promoted by close neutrophil contact with platelets and platelet EVs^[12,13] (Figure 2). Both platelets and neutrophils generate long tethers during adhesion, some of which remain as free vesicles in the environment^[129,130]. The essential role of platelet particles for directed neutrophil migration through the vessel wall is under active *in vivo* investigation by advancing imaging techniques^[11-13,131,132]. Thrombus formation after plaque rupture directly activates neutrophils^[133], a process that continues to be mechanistically explored in experimental arterial lesions^[134]. Antagonizing either glycoprotein I b or II b III A on platelet EV inhibited neutrophil activation^[135,136]. This may be relevant beyond acute thrombosis, as enhanced platelet activation by junctional adhesion molecule A deficiency^[137] increased while deletion of glycoprotein Ib decreased myeloid cell activation and atherosclerotic lesion size^[138]. These data suggest that platelet and platelet EV interactions with granulocytes promote also chronic

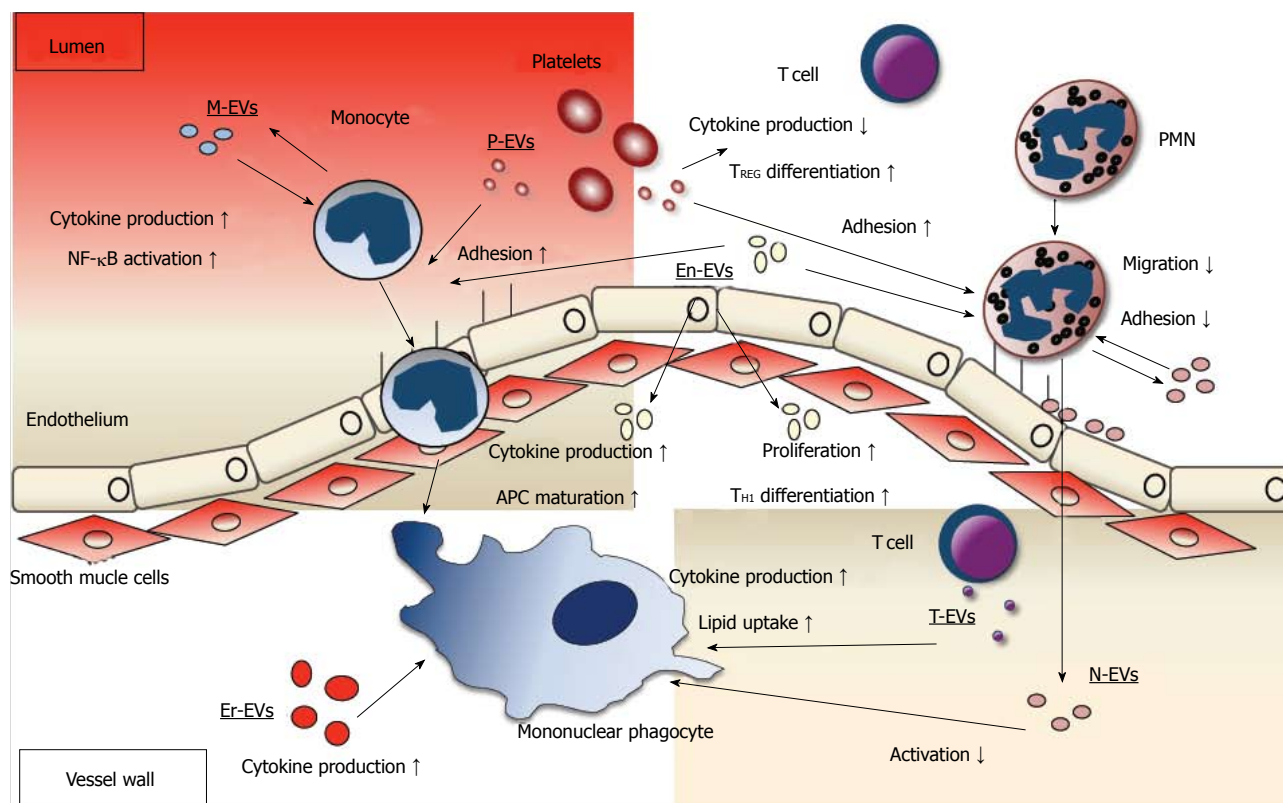


Figure 2 Roles of extracellular vesicles in leukocyte function in the atherosclerotic plaque. Data on interaction of EVs with neutrophilic granulocytes, monocytes and mononuclear phagocytes and T lymphocytes is summarized. Regarding neutrophilic granulocytes (PMN), platelet EVs (P-EV) promote neutrophil (PMN) adhesion to the endothelium, neutrophil EVs (N-EV) mostly decrease adhesion and migration through the endothelium. Regarding monocytic cells, endothelial (En-EV) and P-EVs promote adhesion to the endothelium. Inside the plaque, En-EVs promote antigen presenting cell (APC) maturation and cytokine production and erythrocyte EVs (Er-EVs), monocyte EVs (M-EVs) and T cell EVs (T-EVs) increase cytokine production. T-EVs also increase lipid uptake. N-EVs suppress activation. Regarding T cells, P-EVs decrease cytokine production, En-EVs promote T cell proliferation and TH1 differentiation. EVs: Extracellular vesicles.

atherosclerosis, in the absence of plaque rupture or thrombosis.

MONOCYTES AND MONONUCLEAR PHAGOCYTES

Myeloid phagocytes are central in atherosclerotic plaque development. They have a dual role with lipid uptake on the one hand, resulting in foam cell formation that can lead to cell death and thereby necrotic plaque cores and antigen presentation to cells of the adaptive system on the other hand^[139-143]. In atherosclerosis enhanced by renal impairment, lesional macrophage content increased^[104,144]. Angiotensin receptor I on myeloid cells^[144-146] and IL-17^[104] are instrumental in mediating this phenotype. Myeloid derived phagocytes in the atherosclerotic plaques differentiate from immigrating monocytes, but also proliferate locally, especially in mature plaques in which they are subject to the local milieu^[147]. Both processes are influenced by EVs (Figure 2).

Monocyte adhesion to the endothelium *in vitro* was enhanced platelet EVs, induced by storage, thrombin or shear stress^[148-150]. Platelet EVs also increased monocyte surface expression of adhesion molecules such as CD11a, CD11b integrins, platelet adhesion molecule 1 (CD31),

CD33 lectin, and receptors such as CD14 and CD32 Fc receptor^[148-150]. Endothelial EVs elicited by oxidized LDL or homocysteine from rat arterial endothelial cells contained high levels of heat shock protein 70 (HSP70) that increased monocyte adhesion *in vitro*^[151]. *In vivo* in murine atherosclerosis, RANTES from platelet EVs coated the endothelium resulting in enhance monocyte adhesion^[152].

Macrophage phenotype has a decisive role in plaque growth and stability of the lesion. In renal impairment, histologic analysis of the plaque showed that markers of M1 macrophage polarization were up-regulated with corresponding down-regulation of M2 markers^[153]. Erythrocyte EVs that are found atherosclerotic plaques^[86] induced TNF α production in monocytes in a CD40 ligand dependent fashion^[82]. Platelet EVs induced secretion of cytokines that promote atherosclerotic plaque formation such as TNF α , IL-1 β and IL-8 in a monocytic cell line *in vitro*^[149]. IL-1 β that is central atherogenesis^[154] is itself contained in EVs released from platelets^[155,156] and myeloid phagocytes^[157-159]. However, cytokine induction by platelet EVs is not universal as small platelet EVs inhibited human monocyte-derived phagocyte TNF α and IL-10 secretion while TGF β production was enhanced^[160]. Human granulocyte EVs increased macrophage TGF β 1, but not IL-6 or IL-8 expression and blocked pro-inflammatory responses induced by zymosan or LPS. The authors

also noted large donor variations in response to EVs suggesting that genetic factors may have a significant influence^[127]. Annexin 1 is a potential mediator of the anti-inflammatory effects of granulocyte EVs^[126]. Autocrine effects of monocytic EVs on monocyte differentiation and cytokine production varied with cell culture conditions. phorbol-12-myristate 13-acetate (PMA) elicited EVs from THP1 cells induced cell cycle arrest and macrophage differentiation TGF β 1 dependently^[161] while human monocyte EVs increased TNF α and IL-6, release reactive oxygen species production and induced nuclear factor (NF)- κ b activation^[162]. Interestingly, NO, a pathway that is significantly inhibited in uremia, markedly enhanced EV release from RAW264 macrophages *in vitro*^[163]. T lymphocyte EVs induced in both peripheral blood T lymphocytes and a human T cell line by phytohemagglutinin (PHA) and PMA increased TNF α , IL-1 β and soluble IL-1 receptor a production in monocytes in a dose-dependent manner. This was not observed for EVs from unstimulated T cells^[164,165]. Both TNF α and IL-1 β generation were inhibited by HDL, connecting these studies directly to regulation of inflammation in the atherosclerotic plaque.

Regarding lipid phagocytosis, lipid and cholesterol content in peritoneal macrophages from atherosclerotic mice with renal impairment was significantly higher than in control animals^[166] and the ability to take up labeled exogenous oxidized LDL particles significantly impaired in aortic macrophages^[104]. This was attributed to decreased cholesterol efflux, mediated by decreased expression of the transporter ABCA1^[166]. Platelet EVs increased uptake of oxidized LDL if present during macrophage differentiation *in vitro*. This protocol also increased CD14, CD36 and CD68 surface receptor expression^[150]. In contrast, small platelet EVs with less than 50 nm diameter decreased lipid uptake *via* reduction of CD36 surface expression by enhanced ubiquitination^[167] T lymphocyte EVs from PHA-activated human T lymphocytes increased cholesterol uptake in THP-1 cell and human monocyte derived macrophages^[168].

Regarding antigen presentation, expression of the antigen presenting cell marker CD11c significantly increased in atherosclerotic aortas of mice with renal impairment^[104]. T cell proliferation was significantly higher in their then aortas of atherosclerotic control mice. In addition, life cell imaging demonstrated that aortic T cell interactions with CD11c⁺ cells were significantly more frequent and longer in vessels from mice with renal impairment^[104]. There is a large body of evidence for a role of EVs in antigen presenting cell function^[6]. While many studies focused on tumor antigens, some may be directly relevant to atherosclerosis. Endothelial EVs from a human microvascular cell line induced by TNF α enhanced antigen presenting cell maturation, indicated by morphologic maturation, up-regulation of HLA-DR, CD83 and CCR7 and IL-6 secretion in a cell line and human plasmacytoid dendritic cell, but not in myeloid cells. While the stimulated cells were capable of inducing mixed lymphocyte reaction, interferon γ (IFN γ) was not induced by the co-incubation. Platelet and T cell EVs were used as controls and did not

elicit this response^[169]. Erythrocyte EVs enhanced T cell proliferation by modulation of monocyte maturation and induction of TNF α ^[82]. In a somewhat different setting, platelet EV recovered from thrombin-activated platelet supernatants induced HLA-DR expression in immature DCs during differentiation from human PBMC. This was mediated by CD40L^[170], a protein that has been detected on human carotid plaque EVs^[88]. Small EVs from resting platelets exerted a contrary effect and decreased HLA-DP, DQ, DR and CD80 expression during human PBMC differentiation^[160]. While CD14 expression decreased similar to control cells, platelet EV also decreased endocytic capacity. Neutrophil EVs decreased immature dendritic cell phagocytic capacity and increased TGF β release. Furthermore, LPS mediated maturation was severely impaired including surface marker expression, cytokine production and induction of T cell proliferation^[171] extending the protective neutrophil effect from endothelium to monocyte derived phagocytes.

In summary, EVs of different cellular origins modulate mononuclear phagocyte functions that promote atherosclerosis in renal impairment.

LYMPHOCYTES

T cells are major modifiers of plaque formation among adaptive immune cells while the role for B cells is controversial^[105-107]. B cell interaction with EVs can enhance or diminish B cell function^[172,173], however, a link to atherosclerosis remains to be defined.

Among T helper cells, IFN γ -producing T_{H1} cells strongly promote atherosclerotic lesion formation. In the current experimental models, there appears to be no major role for T_{H2} cells in atherogenesis, while regulatory T cells and their marker cytokines such as IL-10 can attenuate lesion formation^[105-107]. The impact of T_{H17} cells and their marker cytokine IL-17, which has a significant role in attraction of innate leukocytes such as neutrophilic granulocytes and monocytes^[174], appears to be highly context-dependent^[10,175]. Recent data show that proatherogenic lipoproteins can enhance T_{H17} polarization^[176]. IL-17 production in T cells is markedly enhanced by environmental chemicals *via* the aryl hydrocarbon receptor^[177-180]. Its ligands are well known uremic toxins^[181,182]. Indeed, the IL-17 production was significantly increased in a cohort of patients with end stage renal disease^[183]. Mechanistically, IL-17 was instrumental in increased myeloid cell accumulation and lesion burden in moderate renal impairment^[104].

The effect of EVs on T cell function *in vitro* significantly varies depending on the cell of origin (Figure 2). Endothelial EVs enhanced CD4⁺ T cell proliferation in mixed lymphocyte reaction *via* modulation of dendritic cell maturation, resulting in enhanced TNF α and IFN γ secretion^[69]. Similarly, EVs from TNF α -stimulated HUVECs induced T_{H1} differentiation in human PBMCs^[184]. Erythrocyte EVs induced T cell proliferation indirectly *via* monocyte derived antigen presenting cell polarization. This stimulated the production of the pro-atherogenic cytokines IL-1 β , IL-2,

IL-7, IL-17 and IFN γ during co-culture of human PBMCs^[82]. In contrast, small platelet EVs directly interacted with CD4⁺ T cells. They decreased IFN γ , TNF α and IL-6 production during polarization^[185]. This was at least in part due to an increase in regulatory T cells induced by EV TGF β . EVs from antigen presenting cells promote T cell priming^[186,187]. In atherosclerosis, plasma and plaque EVs contain MHC I, MHCII and CD40L as EV surface antigens and it is therefore conceivable that these processes are also active during atherosclerosis *in vivo*^[38,88].

CONCLUSION

Data on mechanisms how EVs modulate leukocyte adhesion, differentiation and vascular function in inflammation have greatly enhanced our understanding of these pathophysiological processes. Experimental results suggest a number of mechanisms that enhance EV generation and modulate their function in renal patients. While analytic tools continue to be optimized and therapeutic options are limited to inhibition of platelet EVs at this point, EV counts start to serve as activity and prognostic markers in different conditions.

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Major comorbid disease processes associated with increased incidence of acute kidney injury

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Abstract

Acute kidney injury (AKI) is commonly seen amongst critically ill and hospitalized patients. Individuals with certain co-morbid diseases have an increased risk of

developing AKI. Thus, recognizing the co-morbidities that predispose patients to AKI is important in AKI prevention and treatment. Some of the most common co-morbid disease processes that increase the risk of AKI are diabetes, cancer, cardiac surgery and human immunodeficiency virus (HIV) acquired immune deficiency syndrome (AIDS). This review article identifies the increased risk of acquiring AKI with given co-morbid diseases. Furthermore, the pathophysiological mechanisms underlying AKI in relation to co-morbid diseases are discussed to understand how the risk of acquiring AKI is increased. This paper reviews the effects of various co-morbid diseases including: Diabetes, cancer, cardiovascular disease and HIV AIDS, which all exhibit a significant increased risk of developing AKI. Amongst these co-morbid diseases, inflammation, the use of nephrotoxic agents, and hypoperfusion to the kidneys have been shown to be major pathological processes that predisposes individuals to AKI. The pathogenesis of kidney injury is complex, however, effective treatment of the co-morbid disease processes may reduce its risk. Therefore, improved management of co-morbid diseases may prevent some of the underlying pathology that contributes to the increased risk of developing AKI.

Key words: Acute kidney injury; Kidney disease; Human immunodeficiency virus; Co-morbidities; Diabetes; Cancer; Cardiac surgery; Acquired immune deficiency syndrome; Risk factors; Immune response; Cardiovascular disease

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Core tip: In order to prevent, diagnose, and prophylactically treat patients, healthcare providers must identify co-morbidities that significantly increase the likelihood of acute kidney injury (AKI). Any treatments that compromise cardiac output, renal perfusion pressure, and glomerular hemodynamics risk ischemic injury to the kidney. The innate and adaptive immune responses, which are activated by renal epithelial cell necrosis contribute to the

progression of AKI. These factors have been shown to be enhanced in diabetes, cancer, cardiac surgery and human immunodeficiency virus acquired immune deficiency syndrome patients.

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INTRODUCTION

According to the Acute Kidney Injury Network (AKIN), AKI is an abrupt loss in kidney function within 48 h, as defined by an increase in serum creatinine of 26.4 $\mu\text{mol/L}$ (0.3 mg/dL) or more; a percentage increase in serum creatinine of more than 50% from baseline; or a reduction in urine output, oliguria ($< 0.5 \text{ mL/kg}$ hourly for $> 6 \text{ h}$)^[1,2]. AKI can be characterized by severe changes in kidney function. The severity of these changes are time sensitive, thus, early treatment may minimize the complications associated with AKI^[3]. AKI is most often secondary to extrarenal events in critically ill patients, specifically those that are hospitalized and are suffering from progressive degenerative diseases^[4]. AKI has been shown to occur in 1% of patients admitted to the hospital and it has been shown that up to 7% of patients develop AKI during hospital stays^[1,5,6]. The incidence of AKI in intensive care units (ICU) has been shown to range from 20% to 50%^[7]. On average 5% of patients in the ICU with severe AKI require renal replacement therapy (RRT)^[8].

Patients are at an increased risk of death from postoperative AKI. According to Hobson *et al*^[9] the risk-adjusted 90-d postoperative mortality was 6.5% for patients with AKI (ranging from mild to severe) in comparison to 4.4% in patients without AKI. Some of these surgical procedures include thoracoabdominal aortic surgery^[10], bone marrow transplantation^[11] and cardiac surgery^[12]. AKI, as a result of ischemia, is also a frequent clinical event. In the hospital setting, ischemic-AKI occurs in 50% of patients with AKI^[13]. Ischemic-AKI occurs for a variety of reasons such as the use of vasoconstrictive drugs or radiocontrast agents and/or hypotension associated with sepsis or blood loss after surgery or trauma^[2]. Individuals who survive AKI have an increased risk of short and long-term complications. Some of these complications include a 10-fold greater risk of chronic kidney disease, a 3-fold greater risk of end stage renal disease and double the risk of death^[14,15].

Biomarkers have become a novel concept for the early diagnosis of AKI. A combination of two urinary cell-cycle arrest biomarkers, insulin-like growth factor-binding protein 7 (IGFBP-7) and tissue inhibitor of metalloproteinases-2 (TIMP-2) have been used to predict the risk of moderate

and severe AKI (defined by stages 2 and 3 respectively according to the KDIGO classification of AKI)^[16]. These biomarkers have been said to perform better than existing markers such as NGAL, KIM-1, interleukin (IL)-18, L-FABP and Cystatin C^[17,18]. In AKI, these biomarkers localize in the site of injury where they are involved in the process of the G1 cell-cycle arrest, which acts to prevent cells from continuous division when DNA is damaged^[19]. Two independent multicenter cohort studies conducted by Kashani *et al*^[17] and Bihorac *et al*^[18] allowed for the development of the FDA approved NEPHROCHECK® Test system. The test system is comprised of assays for TIMP-2 and IGFBP-7, which is to be used in conjunction with clinical evaluations. This system is used as a clinical aid in the risk assessment for moderate to severe AKI within 12 h of patient assessment^[17,18]. As such, these new advancements allow for the early detection of AKI.

Several epidemiological studies have proposed a wide array of risk factors for AKI. These include acute clinical conditions, diagnostic, or therapeutic procedures, and chronic disease states. However, they do not highlight the relationship of co-morbid diseases with the pathophysiology of AKI in a systematic manner. As such, this paper seeks to identify important co-morbidities and illustrate mechanisms by which these co-morbidities increase the incidence of AKI. Identifying co-morbidities that significantly increase the likelihood of AKI will allow healthcare providers to prevent, diagnose, and prophylactically treat patients, thereby reducing the long-term complications associated with AKI.

Pathogenesis and co-morbid disease processes in AKI

Renal blood flow is highly regulated to ensure oxygen delivery for normal renal function^[20]. Cardiac output, renal perfusion pressure, and glomerular hemodynamic factors are major determinants of renal blood flow autoregulation. If these factors are compromised, ischemic and toxic injury to the kidney can occur^[20,21]. The afferent arteriole plays an important role in autoregulation to maintain glomerular filtration rate (GFR). There are two mechanisms by which the afferent arteriole regulates GFR: (1) the myogenic reflex occurs when renal perfusion pressure rises causing the smooth muscle of the afferent arteriole to constrict; and (2) tubuloglomerular feedback (TGF) is sensitive to sodium delivery to the macula densa causing vasoconstriction of the afferent arteriole^[22]. Further, cyclooxygenase inhibitors such as aspirin and other nonsteroidal anti-inflammatory drugs (NSAIDs) that may be taken by patients with co-morbid diseases can cause severe intrarenal vasoconstriction, serious decline in GFR, and worsen AKI^[23-25].

The core pathology of AKI can be broken down into degenerative processes that target the tubular epithelium, vasculature and activate the immune response leading to a decline in kidney function. AKI associated with ischemia reperfusion injury, sepsis or toxins causes a rapid loss of proximal tubular cell cytoskeletal integrity and cell polarity^[26]. As a result, there is a shedding of the proximal tubule brush border and loss of polarity with the

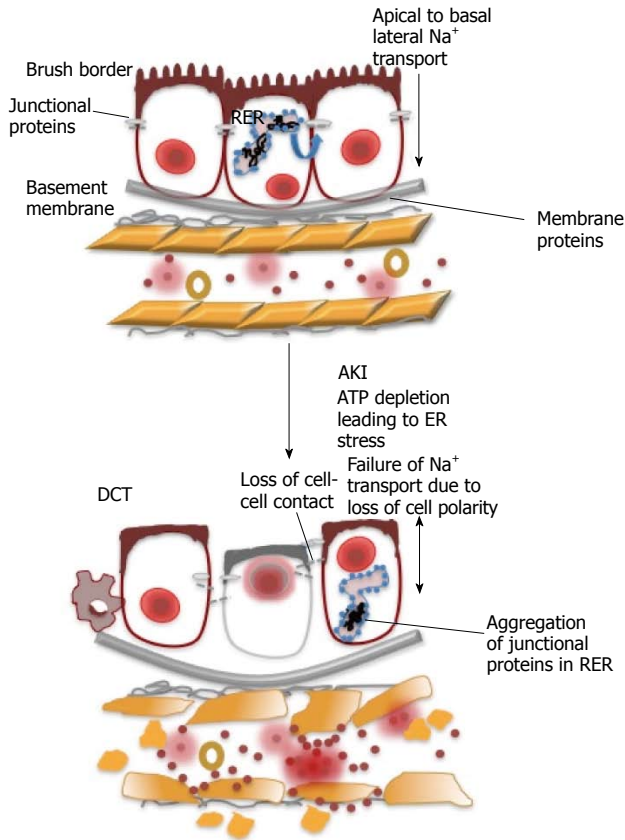


Figure 1 Epithelial cell damage. Ischemia reperfusion injury, sepsis or nephrotoxins are some the main causes of damage to epithelial cells resulting in AKI. The damage induces changes to the cytoskeleton, adhesion molecules and membrane proteins. ATP depletion results in the disruption of tight junctions causing back-leak of the filtrate as the actin cytoskeleton structure is altered. Endoplasmic reticulum stress caused by ATP depletion causes the aggregation of junctional proteins inducing an increase in the permeability of the endothelium. The loss of cell polarity due to AKI results in the failure of Na^+ reabsorption allowing high concentrations of Na^+ to reach the distal tubule stimulating an aberrant TGF response. AKI: Acute kidney injury; TGF: Tubuloglomerular feedback; ATP: Adenosine triphosphate; ER: Endoplasmic reticulum.

mislocalization of adhesion molecules and other membrane proteins such as Na^+/K^+ -ATPase and β -integrins^[26]. ATP depletion can cause ER stress, which causes protein misfolding, including epithelial junction proteins, leading to loss of cell polarity and failure of sodium reabsorption. This results in an aberrant TGF response (Figure 1)^[2,26] caused by loss of the ability of the proximal tubular cells to reabsorb filtered sodium, thus, increasing sodium delivery to the distal nephron. With an increase in the delivery of sodium to the macula densa, the TGF mechanism of autoregulation senses hyperfiltration, causing afferent arteriole constriction^[20]. However, the perfusion of the kidney may already be compromised by prerenal causes leading to an exaggerated TGF response, resulting in a sudden and substantial drop in GFR^[20].

AKI and inflammation

Both the innate and adaptive immune responses, activated by tubular epithelial cell necrosis, are key contributors to the progression of AKI^[1,21]. Activation of the inflammatory process triggers the expression of cytokines and

chemokines like tumor necrosis factor (TNF) and IL-6 through toll-like receptors that detect materials released in response to injury and interact with their ligand receptors to activate a proinflammatory response to the site of injury. Upregulation of chemokines and adhesion molecules in the endothelium results in the infiltration of inflammatory cells such as neutrophils, lymphocytes, and macrophages from blood vessels to the interstitium of the kidney^[1,21,27].

AKI and endoplasmic reticulum stress

AKI caused by ischemia, nephrotoxic drugs, or contrast agents has been associated with endoplasmic reticulum (ER) stress^[27]. The ER has a pivotal role in the maintenance of protein homeostasis where it controls the concentration, conformation, folding and transport of synthesized proteins^[27]. Disruptions such as hypoxia, glucose depletion, and oxidative stress can prevent the correct functioning of the ER where an accumulation of misfolded proteins in the ER lumen initiates the unfolded protein response (UPR)^[27,28]. The UPR serves as an adaptive response attempting to re-establish normal ER functioning through the activation of calcium-dependent molecular chaperones such as glucose-regulated protein-78^[1,27]. The UPR pathway can also induce the transcription of pro-apoptotic genes that cause cell death. Oxidative stress and inflammation are compounded by ER stress *via* the UPR, which contribute to glomerular and tubular damage in patients with AKI^[27].

AKI and endothelium and vasculature damage

When the endothelium is damaged, the arteriole responds to a local high concentration of vasoconstrictive agents with a greater magnitude, as the injured endothelial cells produce a decreased amount of vasodilatory substance. There is an increase in the permeability of the endothelium post-injury, consequently resulting in a loss of fluid into the interstitium, thereby compromising blood flow^[27]. Chronic hypoxia alongside the downregulation of angiogenic factors can cause a decline in the number of blood vessels and consequently lead to increased fibrosis that works in a positive feedback mechanism to reinforce its progression and ultimately cause epithelial cell injury and apoptosis^[27,28]. Smaller constrictive vessels respond more intensely to vasoconstrictive agents (e.g., angiotensin II, thromboxane A_2 , prostaglandins etc.), but have a decreased response to vasodilators (acetylcholine, bradykinin, NO)^[27]. These effects can be a consequence of alterations in the endothelium due to injury or enhanced leukocyte-endothelial adhesion. The latter effects can cause the obstruction of the small vessels and activate the inflammatory response, which becomes a vicious cycle of coagulation that prevents the delivery of vital nutrients and oxygen to the epithelial cells^[27].

Co-morbidities and AKI

Damage to the kidneys, as a result of AKI, may be enhanced with the presence of co-morbidities and thereby complicate the treatment procedure. One study defined

the incidence, risk factors and outcomes of AKI in a patient population from the Scottish Hip Fracture Audit database^[29]. These patients who sought treatment for femur fracture and developed AKI showed an increase in inpatient morbidity, mortality (within 30 and 120 d) and length of hospital stay with multiple co-morbidities^[29]. This study highlights the co-morbidities associated with the development of AKI including, diabetes mellitus, vascular disease, hypertension and pre-morbid chronic renal disease. The data presented in this study suggests that most cases of AKI occur post-surgery and the causes of AKI are multi-factorial comprising of pre, intra- and post-operative factors^[29].

Diabetes-associated AKI

Globally, in 2014, it is estimated by the World Health Organization (WHO) that 387 million people suffer from Diabetes mellitus (DM), where 90% of the cases are of Type II diabetes^[30]. The risk of AKI has been shown to be increased in patients with DM, with an adjusted odds ratio of 1.99, compared to non-DM controls with the same GFR^[31]. It was determined that individuals who require dialysis, which is indicative of the severity of AKI, were an older patient group with DM and included individuals who had other complications such as hypertension and proteinuria^[31]. A reason proposed for the higher risk of AKI in patients with DM is the frequent occurrence of complications associated with DM. Some of these complications include, cardiovascular disease; heart failure; exposure to medications such as diuretics and others that serve as nephrotoxic agents^[32].

A greater susceptibility to ischemic insults of the diabetic kidney has been shown in experimental rodent models and in diabetic patients^[33]. One study examined the influence of 30-min renal ischemia in rats with streptozotocin-induced DM. This study showed a complete recovery of the renal function in non-DM rats while DM animals showed a permanent loss of renal function^[34]. DM rats, 8 wk after ischemia was induced, became completely anuric with tubular atrophy, and had extensive inflammation and tubulointerstitial fibrosis, which became evident within 4-wk post-surgery^[34]. Another study led by the same investigators showed treatment of these rats with insulin prior to the ischemic event reduced ischemic injury^[35].

The mechanism by which diabetes increases the severity of AKI has not yet been well established, but a great deal of research supports the connection between obesity, inflammation, and insulin resistance^[36]. Inflammatory cytokines such as TNF- α and IL-6 are produced by adipocytes and have been shown to cause insulin resistance^[36,37]. In rodent models of diabetes and diabetic humans, the increased upregulation of inflammatory cytokines in the kidney and urine have been shown^[38]. These changes have been shown to result in long-term renal complications such as proteinuria and renal hypertrophy^[38]. To experimentally determine the mechanistic role of TNF- α in facilitating the heightened risk of ischemic injury in Type II diabetic mice, one study used

a neutralizing TNF- α antibody or nonimmune globulin control^[39]. The mice were pre-treated with TNF- α antibody or nonimmune globulin injections 20 min before bilateral renal ischemia^[39]. This study showed that the treatment with the TNF- α antibody was renal-protective against ischemic injury. Thus, the study concluded that diabetes increases the susceptibility to ischemic AKI due to an elevated TNF- α -mediated inflammatory response^[39].

Although a majority of the scientific community agrees that diabetes increases the severity of AKI, some controversy surrounding DM and susceptibility to AKI exists. A study conducted by Venot *et al*^[40] has shown no role of DM in increasing the risk of AKI or RRT. Instead, DM has been shown to only worsen the renal prognosis at discharge, determined by patients need for RRT, levels of serum creatinine and the recovery of renal function^[40]. Additionally, the data from another study has shown that the history of DM is based on unclear self-reports of patients or records, and thereby does not reflect the current glucose control. Thus, using diabetes as a marker for a heightened risk of AKI at baseline clinical assessment in patients undergoing cardiac surgery may not be a useful tool in predicting renal injury outcomes^[41]. Moreover, patients without a formal diagnosis of DM can suffer from chronic hyperglycaemia (CHG) due to pathological glycemic control or early stages of DM^[41,42]. This study highlights that hyperglycaemia is also associated with cardiac dysfunction, susceptibility to infections and endothelial dysfunction, which pose as risk factors of perioperative morbidity and mortality after coronary artery bypass grafting (CABG) surgery^[41]. The results of this study suggest that the measurement of Hemoglobin A1c (HbA1c) of $\geq 6.0\%$, which is an established tool used in the evaluation of diabetic control and CHG in patients with DM, is associated with a higher incidence of AKI after CABG^[41]. Thus, a patient's blood glucose levels should be evaluated for CHG, independent of DM, as it could be a strong determinant of AKI.

Cancer-associated AKI

AKI is an important complication of cancer and cancer-therapy where cancer patients are susceptible to a number of kidney lesions that can cause complications in the efficacy of treatment^[43]. Factors such as the type and severity of malignancy (a solid tumour or hematologic process), associated complications such as co-morbidities and illnesses, and types of cancer management and therapy cause variability in when AKI is acquired^[43]. One study conducted on Danish cancer patients reported the highest rates of AKI were in patients with kidney cancer at 44%, myeloma at 33% and liver cancer at 31.8%^[44]. The rate of AKI in critically ill cancer patients was shown to be between 12% and 49%, with 9% to 32% of these patients requiring RRT^[5,45,46], which is higher when compared to patient populations of an illness of similar severity^[45,47,48]. Thus, AKI management in cancer patients is essential for patient survival and recovery.

AKI in cancer patients can be divided into prerenal, intrarenal or postrenal causes. Prerenal AKI is most

commonly seen in cancer patients due to hypotension as a result of intravascular volume depletion caused by sepsis, vomiting, or diarrhea^[43]. Hypercalcemia due to parathyroid hormone release, which increases bone resorption and renal tubular resorption of calcium, is seen in 10% to 30% of malignancies^[49,50]. This can lead to a prerenal state of AKI due to vasoconstriction as well as volume depletion from natriuresis and diuresis^[49,50]. Additionally, prerenal causes can result from the use of medications such as diuretics, angiotensin-converting enzyme inhibitors, angiotensin receptor blockers or nonsteroidal anti-inflammatory agents for tumours, and/or other medical conditions such as hypertension or congestive heart failure^[43]. Intrarenal causes of AKI in cancer patients consist of primary glomerular disease, acute tubular necrosis attributable to toxins or ischemia, infiltrative processes due to immune system activation, and microangiopathic processes^[43]. Postrenal AKI is a result of kidney obstruction that is common in malignancies in the bladder, prostate, uterus, and cervix^[43].

Nephrotoxicity by means of cancer therapy is one of the leading causes of AKI in cancer patients^[51]. In a multivariate model, the OR for developing AKI from chemotherapy was 1.61, 4.55 for intravenous contrast and 1.52 for antibiotics^[51]. Renal injury can be induced in a variety of ways by nephrotoxic drugs. In general, intrarenal vasoconstriction, direct tubular toxicity and intratubular obstruction are damaging results of nephrotoxic agents^[51]. High levels of toxins are delivered and reabsorbed by the kidneys, which lead to increased intracellular concentrations of nephrotoxins in the tubular cell and medullary interstitium^[51]. Further, the kidney is a site for drug metabolism and clearance^[52]. Thus, the kidney can breakdown compounds that may be relatively harmless into toxic metabolites, or impairment of renal function can cause chemotherapeutic agents to concentrate in the kidneys without being cleared^[52,53]. Delayed drug metabolism and excretion, due to increased concentrations of nephrotoxins, can result in increased systemic toxicity requiring an adjustment of treatment dosage^[53]. As such, the nephrotoxic potential of anti-cancer agents can be significantly increased if there is pre-existing kidney damage and or a presence of concomitant co-morbidities such as heart failure and sepsis^[53].

Cardiac surgery-associated AKI

Cardiac surgery-associated (CSA) - AKI is an important clinical problem that stems from a complex multifactorial pathogenic process. The incidence of CSA-AKI is 25%^[54]. Mortality associated with the development of AKI can be as high as 60%, with an average of 15%-30% depending on the measurement and defining criteria of AKI^[55]. Factors that increase the risk of CSA-AKI can be divided into preoperative and intraoperative (associated with and followed by postoperative CSA-AKI) categories.

The preoperative period is a critical point wherein renal

injury can occur due to fluctuations in hemodynamics, exposure to nephrotoxic agents and the activation of the inflammatory response^[55]. Injury, as a result of the aforementioned, can be substantiated when a patient undergoes surgery that decreases renal perfusion and reduces renal functional reserve. Patients undergoing conventional coronary bypass (CCB) often present with renal injuries that can range from minor to severe^[55]. The pre-existing renal injury condition can be further amplified with the use of drugs such as diuretics, NSAIDs or angiotensin receptor blockers that can impair the autoregulation of renal blood flow^[56]. Additionally, incidents of preoperative hypotension may lead to endothelial injury that can impair the production of vasodilatory substances such as NO causing vasoconstriction as a result of catecholamines and angiotensin II to further exacerbate injury^[57,58].

The intraoperative period is when patients are exposed to anaesthesia and undergo CCB, these significantly impair hemodynamics and activate the innate and adaptive immune response^[55]. Hemodynamic changes can be controlled and regulated given that a patient's medical history is thoroughly assessed and the kidney is perfused accordingly during surgery. However, if not controlled, hemodynamic changes can lead to regional renal ischemia and can induce or extend renal injury^[55]. Additionally, the activation of inflammatory mediators can initiate in the preoperative period and extend into the intraoperative period. An elevation of TNF- α levels have been observed in patients with pre-existing congestive heart failure, which further amplifies the inflammatory response during CCB in intraoperative period^[59,60]. Neutrophils and the vascular endothelium are activated, inducing the upregulation of adhesion molecules such as platelets^[60]. These events activate the upregulation of cytotoxic free-radicals^[61], proteases^[62], cytokines^[63] and chemokines (IL-6, IL-8 and TNF- α)^[63,64].

Postoperative events that impair renal function are similar to causative factors of AKI that are frequently found in intensive care setting such as the use of vaso-active agents, hemodynamic instability, exposure to nephrotoxic medications, volume depletion, and sepsis. Postoperative cardiac performance may be compromised with ventricular dysfunction causing reduced blood flow to the kidney and subsequently resulting in AKI^[55].

Human immunodeficiency virus-associated AKI

Human immunodeficiency virus (HIV) infection that may progress to acquired immune deficiency syndrome (AIDS) creates an immunosuppressed state allowing for life-threatening opportunistic infections and cancers to thrive^[65]. In contrast to AKI as a result of pre-renal and post-renal causes, HIV-associated AKI is most often due to HIV-mediated viral or immunological disease and or nephrotoxicity from treatments^[66]. Risk factors for AKI in HIV infection include low CD4⁺ levels, AIDS, hepatitis C and liver disease^[67]. Additionally, medications used to treat HIV such as anti-retroviral therapy (ART) or highly active

antiretroviral therapy (HAART) may also increase the risk of developing AKI due to their nephrotoxic properties^[66]. The OR of HIV patients acquiring AKI in pre-HAART has shown to be 2.9 and substantially increased to 6.0 in post-HAART^[66]. ART causes severe immunosuppression where the CD4⁺ count becomes dangerously low at < 200 cells/mm³; normal values ranging from 500 cells/mm³ to 1200 cells/mm³^[68]. The decreased CD4⁺ count is an independent predictor of experiencing AKI and is a vital predictor of HIV related morbidity and mortality^[69]. Furthermore, co-viral-infections have been shown to increase the incidence of AKI. Hepatitis C virus co-infection occurs in 15%-30% of HIV-infected patients in the United States, where 30% of AKI events are a result of underlying liver damage^[69].

Although no reliable data exists on the incidence and causes of AKI especially amongst HIV⁺ patients, South Africa, where 5.6 million of the 34 million people infected with HIV reside^[67], faces problems of herbal intoxication, sepsis due to opportunistic infections, or severe gastroenteritis with dehydration^[68,70]. AKI has been shown to be a critical cause of mortality particularly amongst indigenous black communities where herbal remedies are prescribed by traditional healers as curative measures for problems such as AIDS-related abdominal pain, diarrhea or to eliminate HIV from the system^[70-72]. One of the most common nephrotoxic plants is the Impila (*Callilepis laureola*), found in regions of South Africa, Democratic Republic of Congo, Zimbabwe, and Zambia^[73,74]. Nephrotoxicity from herbal remedies can arise from direct causes such as renal injury due to acute tubular necrosis and acute interstitial nephritis or indirectly as a result of intravascular hemolysis and dehydration due to diarrhea^[68]. Therefore, HIV plays a major role in AKI from direct infection processes and treatment regimens.

CONCLUSION

AKI is an important clinical event that manifests in critically ill patients. AKI is associated with a multitude of risk factors that disrupt the homeostatic processes of the kidneys. Its complexity stems from pre-existing co-morbidities of patients that vary in severity, thereby making an overarching systematic treatment and management protocol difficult to deliver to patients suffering from AKI. A great deal of light has been shed upon the mechanistic basis by which AKI develops and progresses with the assessment of risk factors, however research efforts and emphasis should be placed on developing treatment interventions that can reverse or attenuate renal injury. To do this, therapeutic strategies need to be devised on a case-by-case basis where the identification of important co-morbid diseases such as DM, cancer, cardiac surgery and HIV takes place.

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Health literacy in kidney disease: Review of the literature and implications for clinical practice

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Abstract

Health literacy is the capacity of an individual to understand information related to a disease in order to make an informed decision. In patients with kidney diseases, studies have reported increasing impact of limited health literacy on health outcomes. Our paper discusses current literature

on health literacy in kidney diseases.

Key words: Health literacy; Kidney diseases; Rapid estimate of adult literacy in medicine; Hemodialysis; Peritoneal dialysis; Chronic kidney disease

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Core tip: Health literacy is an increasingly recognized cause of suboptimal care and management of chronic diseases in patients. Our paper reviews the current literature on its prevalence and impact in the population with kidney diseases. More studies are needed in patients with kidney diseases to better understand the effect of limited health literacy.

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BACKGROUND

According to the Institute of Medicine, health literacy is defined as "the degree to which individuals have the capacity to obtain, process, and understand basic health information and services needed to make informed health decisions"^[1]. It is a complex phenomenon including diverse communication skills of individuals beyond simply being able to read. It also involves oral understanding (speaking and listening skills), numeracy, and cultural and conceptual knowledge. The prevalence of limited health literacy is higher amongst the elderly, minorities, and those with lower socioeconomic status including income and education^[2].

Health literacy is particularly important in the large

and growing number of patients with chronic kidney disease (CKD) due to the complexity of the disease, which requires a high level of patient involvement and self-management skills. Patients with kidney disease must follow appropriate dietary restrictions, adhere to complex medication regimens, make decisions about dialysis, and keep up with multiple appointments in the health care system. Despite having data on methods to delay disease progression, kidney outcomes are suboptimal in part related to patient factors. Of these, there is increasing evidence that health literacy plays an important role in the care of patients with kidney disease^[3,4]. We present currently available health literacy screening tools, studies of health literacy in patients with kidney disease, and strategies to address health literacy in clinical practice.

HEALTH LITERACY MEASURES

There are a variety of health literacy screening tools available, many of which have been studied in patients with kidney disease. One of the most commonly used tools is the Rapid Estimate of Adult Literacy in Medicine (REALM), which is a 66-item word recognition test. Scores are based on the total number of words a patient can correctly identify and pronounce, categorized by grade-equivalent reading level. Scores range from 0 to 66 with lower scores representing more limited health literacy (0 to 18 = less than 4th grade reading level; 19 to 44 = 4th to 6th-grade reading level; 45 to 60 = 7th to 8th-grade reading level; > 60 = 9th-grade reading level or above). Limited health literacy is defined as a REALM score < 60. The REALM takes about three minutes to administer^[5]. It has been studied in patients with chronic kidney disease and kidney failure, including transplant, hemodialysis, and peritoneal dialysis patients^[6,7]. A kidney transplant specific version is also available, the REALM-T^[8].

Another commonly used health literacy screening tool is the Test of Functional Health Literacy in Adults (TOFHLA). This test uses the modified cloze procedure, where every fifth to seventh word is omitted from reading passages and subjects select the correct word from among a set of four options. The full version involves 50 reading comprehension items in 3 passages and 17 numeracy items, and takes about 22 min to complete^[9]. The shortened version (S-TOFHLA) includes 36 reading comprehension items and takes about 7 min to complete^[10]. Scores are categorized into inadequate, marginal, and adequate health literacy. Both versions are available in Spanish.

Routine use of the REALM and TOFHLA has been largely limited by administration time; therefore, shorter literacy screening instruments are increasingly being used. A 3-item brief health literacy screen (BHLS) is available^[11], which uses the following self-report questions: (1) How confident are you filling out medical forms by yourself? (2) How often do you have someone help you read hospital materials? and (3) How often do

you have problems learning about your medical condition because of difficult understanding written information? Answers are scored on a 5-point Likert scale. The BHLS takes approximately 1 min to complete and has been validated against the REALM and the S-TOFHLA in the hemodialysis population^[12]. The areas under the receiver operator curves comparing BHLS were reported as 0.71 (95%CI: 0.61-0.80) for the REALM, and 0.73 (95%CI: 0.59-0.88) for the S-TOFHLA. The use of a single item version using just one of these questions (confidence with forms) has been validated against the REALM in patients on peritoneal dialysis^[13].

The newest vital sign (NVS) is another instrument that can be used to assess health literacy. This test uses a nutrition label from a pint of ice cream and requires patients to answer six questions related to the information on the label^[14]. A score of 4 or more indicates adequate literacy, a score of 2-3 indicates possible limited literacy and a score of 0-1 indicates high likelihood of limited literacy^[15]. Devraj *et al.*^[16] have used this tool in the CKD population to examine the association between health literacy and kidney function. More recently, this tool has been used to assess literacy outcomes in the kidney transplant population^[17,18].

HEALTH LITERACY IN CHRONIC KIDNEY DISEASE

Despite the increased awareness of the importance of health literacy in kidney care^[19], there have been few studies examining health literacy in patients with chronic kidney disease not on dialysis. Wright *et al.*^[20] reported an 18% prevalence of limited health literacy in a single cohort of 401 patients with CKD stage 1-5 in an outpatient study. In this study, limited health literacy based on the REALM was associated with poorer CKD knowledge. Another study of 2340 patients with mild-moderate CKD reported a prevalence of limited health literacy of 28% in non-Hispanic Blacks and 5% in non-Hispanic Whites. This study used the S-TOFHLA as a tool to measure health literacy^[21]. The investigators reported that participants with limited health literacy had lower estimated glomerular filtration rate (eGFR) (34 mL/min vs 42 mL/min per 1.73 m²); higher urine protein/24 h (0.31 g vs 0.15 g); a higher self-reported cardiovascular disease (61% vs 37%); and were less likely to have blood pressure < 130/80 mmHg (51% vs 58%). Finally, Devraj *et al.*^[16] also found an association of limited health literacy with kidney function. In a small study of 150 patients with CKD stages 1-4, every unit increase in the Newest Vital Sign score was associated with a 1.9% increase in eGFR. Further studies are needed of health literacy in patients with earlier stages of kidney disease, since their care and needs are different than those with more advanced disease.

HEALTH LITERACY IN DIALYSIS

There has been more research on the impact of health

literacy in the dialysis population compared to those with earlier stages of kidney disease. In peritoneal dialysis, three studies have reported the prevalence of limited health literacy ranging from 6% to 50%^[6,7,13,22]. In previous studies by us, the prevalence of limited health literacy was similar in peritoneal dialysis^[13] and hemodialysis^[23] study populations. In addition, limited health literacy was not associated with an increased risk of infectious complications or hospitalizations. This suggests that the presence of limited health literacy should not preclude consideration of peritoneal dialysis for renal replacement therapy, provided patients receive appropriate hands-on training that is tailored to their individual literacy needs^[7].

In the hemodialysis population, the largest study by Cavanaugh *et al.*^[24] examined health literacy in 480 incident hemodialysis patients using the REALM. They reported a prevalence of 32% of limited health literacy in their cohort, which is similar to what was reported by Grubbs *et al.* in their study of 62 hemodialysis patients using the S-TOFHLA^[25]. On the other hand, Green *et al.* reported a prevalence of limited health literacy of 16% in their analysis of 260 patients on maintenance hemodialysis using the REALM^[23]. Variations in prevalence of limited health literacy may be due to differences in patient populations or the use of alternative health literacy assessment tools. In all these studies, limited health literacy was seen more often in non-white people and those with lower educational status and lower income levels^[23,24,26].

In terms of outcomes, Green *et al.*^[26] reported that limited health literacy was independently associated with an increased incidence of missed dialysis treatments, emergency department visits, and dialysis related hospitalizations. Another study of 72 patients on hemodialysis reported that people with limited health literacy had worse blood pressure control than those with adequate health literacy^[27]. Moreover, limited health literacy has been associated with an increased risk of death in hemodialysis patients^[24].

HEALTH LITERACY IN KIDNEY TRANSPLANT

Limited health literacy may be a barrier to kidney transplantation. Grubbs *et al.* reported that the access to kidney transplantation is reduced in patients with inadequate health literacy. They reported, in a cohort of 62 dialysis patients, that participants with inadequate health literacy had 78% lower hazard of referral for transplant work up than those with adequate health literacy (AR = 0.22; 95%CI: 0.08-0.60; $P = 0.003$)^[25].

In another study by Dageforde *et al.*^[28], living kidney donors and recipients were compared with the deceased donor recipients. They reported that the deceased donor kidney transplant recipients were more likely to have moderate or low health literacy than living donor kidney transplant recipients (OR = 1.911; $P = 0.022$).

Table 1 Clinical "red flags" for limited health literacy

Patient registration forms that are incomplete or inaccurately completed
Non-adherence with medications or treatments
Frequently missed appointments
Lack of follow-through with labs, imaging tests, or referrals
Unable to name medications, explain what medications are for, or explain timing of medication administration
May offer excuses to deflect reading tasks
"I forgot my glasses"
"Let me bring this home so I can discuss it with my children"
Seldom have questions
Seek help only when illness is advanced
Have difficulty explaining medical concerns

They also found that the living donors had a higher level of health literacy.

More recently, Kazley *et al.*^[17] examined health literacy and its impact on kidney transplant outcomes. They used the REALM-T (REALM modified for the transplant population), NVS and Decision Making Capacity Assessment Tool (DMCAT). They reported that each of these tools significantly predicted whether or not a patient was listed for transplant. However, the NVS and DMCAT tool significantly predicted whether a patient actually received a transplant.

IMPLICATIONS FOR CLINICAL PRACTICE

There is now convincing evidence that limited health literacy is common in patients with kidney disease and associated with a variety of adverse outcomes. In clinical practice, providers can consider routinely screening for health literacy in order to identify at-risk patients who may need more tailored care. In fact most studies till date have looked at the impact in chronic kidney disease and dialysis population. The data in population with acute kidney injury is sparse and it will be interesting to see future studies looking at prevalence and influence of health literacy in this subset of patients with renal failure. However, consideration must be given to time constraints and the potential to induce shame^[29]. Clinical "red flags" can also be used to predict which patients may have limited health literacy (Table 1)^[30], but it is important to note that many patients who struggle with understanding may not exhibit any of these signs. A better approach recommended by most experts is to implement the use of health literacy "universal precautions", which encourages the systematic use of clear health communication principles to promote better understanding for all patients^[31,32]. Key clear health communication principles are shown in Table 2. Communications skills training have been shown to be effective at increasing the use of a variety of these skills^[33]. A health literacy universal precautions toolkit is available online at <http://nchealthliteracy.org/toolkit>. Additional resources include an online plain language medical dictionary (www.lib.umich.edu/plain-language).

Table 2 Clear health communication techniques

Explain things clearly in plain language
Avoid medical jargon (for example, state "long-term" rather than "chronic")
Avoid vague terms such as "negative" test result
Slow down
Focus on 1-3 key points or messages - and repeat
Confirm understanding using teach-back
"I want to be sure that I explained your medication correctly. Can you tell me how you are going to take this medication?"
Effectively encourage patients to ask questions
"What questions do you have?" rather than "Do you have any questions?"
Use analogies and pictures
Use patient friendly educational materials
4 th -6 th grade reading level
Picture-based
Write down important instructions

language-dictionary) and readability formulas (www.readabilityformulas.com) to target written materials at the appropriate reading level (4th-6th grade). Several recently published reviews of the readability of patient education materials in chronic kidney disease are also available^[34,35].

CONCLUSION

In summary, health literacy is an important consideration in kidney disease care. Tools are available to help providers address health literacy in clinical practice. Collective efforts are critically needed to reduce the impact of limited health literacy and improve the quality of care and outcomes of this high-risk population^[36].

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Proton-pump inhibitor-induced hypomagnesemia: Current research and proposed mechanisms

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Abstract

Since the early reports nearly a decade ago, proton-pump inhibitor-induced hypomagnesemia (PPIH) has become a well-recognized phenomenon. While many observational studies in the inpatient and outpatient populations have confirmed the association of PPI exposure and serum magnesium concentrations, there are no prospective,

controlled studies to support causation. Molecular mechanisms of magnesium transporters, including the pH-dependent regulation of transient receptor potential melastatin-6 transporters in the colonic enterocyte, have been proposed to explain the effect of PPIs on magnesium reabsorption, but may be a small part of a more complicated interplay of molecular biology, pharmacology, and genetic predisposition. This review explores the current state of research in the field of PPIH and the proposed mechanisms of this effect.

Key words: Hypomagnesemia; Proton-pump inhibitor; Magnesium; Nephrology; Renal

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Core tip: Proton-pump inhibitor (PPI)-induced hypomagnesemia has become a well-recognized phenomenon over the past decade, through the publications of case reports and larger observational studies in the inpatient and outpatient populations. However, there are no prospective, controlled studies to support causation. Molecular mechanisms of magnesium transporters, including the pH-dependent regulation of transient receptor potential melastatin-6 transporters in the colonic enterocyte, have been proposed to explain the effect of PPIs on magnesium reabsorption, but may only comprise a small part of a more complicated interplay of molecular biology, pharmacology, and genetic predisposition.

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MAGNESIUM HOMEOSTASIS

Magnesium is the fourth most abundant intracellular

ion, and despite its relatively low extracellular concentration, it has numerous essential functions in intracellular metabolism and ion transport. The majority of total body magnesium is housed within bone cells, while the remaining 1% circulates in the blood. As with most electrolytes, the balance of intake, absorption, and excretion in the gastrointestinal and renal systems, as well as the constant flux between the circulating and storage compartments within the serum and bone, respectively, are the determinants of magnesium homeostasis.

The small and large bowels are responsible for magnesium absorption *via* passive and active transport. Paracellular movement between intestinal epithelial cells, or enterocytes, occurs across a concentration gradient. Though the magnesium permeability of the small intestine is poorly understood, its relatively ion-permeable nature is presumed to be due to the relatively low expression of tight junction proteins^[1]. Active transport of magnesium occurs more distally in the small intestine (*i.e.*, cecum) and large bowel. The enterocyte apical cell membrane proteins transport magnesium from the intestinal lumen into the circulating blood *via* dedicated ion channels. Located on the luminal surface, these proteins have been identified as transient receptor potential melastatin (TRPM) 6 and 7. Their high affinity for magnesium actively helps the body maintain adequate magnesium levels through upregulation of magnesium absorption, especially in times of decreased magnesium intake^[2-5]. The cydin M4 exchanger (CNNM4 Na⁺-Mg⁺) is located on the basolateral surface and may be responsible for ultimate magnesium reabsorption into the serum after luminal absorption into the cytosol of the enterocyte^[6]. During periods of decreased dietary magnesium, the active transport pathways can increase magnesium absorption significantly^[7].

After absorption through the gastrointestinal tract, about 20% of magnesium in the blood is protein-bound (mostly to albumin), while 15% is complexed to anions. The remaining majority (65%) of extracellular magnesium is unbound in its ionized form. As opposed to other cations, like calcium, acid-base disturbances have little effect on this distribution^[8]. Intracellularly, predominantly as the complexed, non-ionized form, magnesium has essential roles in the mechanisms behind the maintenance of DNA stability and repair as well as the regulation of the enzymatic activity of hundreds of enzymes^[9]. Over the past two decades, numerous magnesium transporters have been identified, facilitating magnesium flux across cell membranes into the intracellular environment to accomplish these roles. The rate of transport varies among tissue types and higher concentrations of magnesium measured within rapidly growing cells suggests that the rate of intracellular magnesium transport is likely associated with the relative metabolic activity of the cell and its capacity to proliferate through its activating effect on DNA, RNA, and protein synthesis^[10,11].

Approximately 80% of circulating magnesium is

filtered into the urinary space. While most other ions are predominantly absorbed *via* the proximal tubule, the majority of filtered magnesium is reclaimed in the thick ascending Loop of Henle (TAL). In the TAL, the passive reabsorptive pathway is dependent on "claudins", or tight junction proteins. Claudins 16 and 19, which are not expressed within the tight junctions of small intestine enterocytes, have been implicated as modulators of magnesium balance, and can lead to renal magnesium wasting when absent^[12]. Additional studies show that claudins demonstrate "interdependence", as they require each other for appropriate placement into the tight junctions within the distal convoluted tubule (DCT)^[13]. Active, high affinity transcellular magnesium reclamation occurs through these TRPM6 transporters. As such, the DCT determines the ultimate magnesium concentration in the urine, and along with appropriate gastrointestinal absorption, ensures magnesium homeostasis.

In contrast to other predominantly intracellular cations such as potassium and calcium, and despite its crucial roles in cell proliferation and intracellular enzymatic activity, there is no hormonal axis solely dedicated to magnesium homeostasis. In addition, alterations in circulating serum magnesium concentrations are offset by a much larger intracellular magnesium depot, so that a negative daily magnesium balance may not manifest with lower magnesium concentrations. Intracellular magnesium depletion and normal serum magnesium concentrations may coexist, and total magnesium deficiency might not manifest until cellular stores are exhausted^[9]. Therefore, using serum magnesium levels to diagnose magnesium deficiency is inherently challenging. The "intravenous magnesium loading test" is a method used to better approximate a magnesium deficit^[14]. The concentrations of intravenously infused magnesium and the magnesium excreted in the urine are carefully measured to estimate total body magnesium balance. In individuals with sufficient total body magnesium, only 10% of the intravenously infused magnesium should be retained, while the remaining 90% is excreted through the urine. Individuals with intracellular magnesium depletion are expected to increase the absorption rate to > 50%-60%^[15]. This test allows for a more accurate representation of the magnesium absorption required to achieve homeostasis. However, it is not performed in the context of patient care as a laboratory standard for accurate result interpretation does not yet exist. In the current state of the evaluation of magnesium disorders, despite the concerns noted above, clinicians and clinical researchers readily use serum magnesium concentrations to estimate total body magnesium.

EARLY REPORTS OF PROTON PUMP INHIBITOR INDUCED HYPOMAGNESEMIA

The link between proton pump inhibitor (PPI) use to hypomagnesemia was first recognized by the scientific community through a published case report in 2006^[16].

Since then, numerous case reports have demonstrated this relationship, independent of other electrolyte abnormalities. These reports typically describe patients with chronic PPI exposure, presenting with symptoms characteristic of hypomagnesemia, including arrhythmias and symptoms of neuroexcitability such as seizures and tetany^[17]. Numerous different formulations of PPIs have been implicated, indicating that the association is likely a drug class effect. Hypomagnesemia is typically improved after the PPI is discontinued, and PPI re-challenge results in hypomagnesemia recurrence^[18]. Conversely, in patients prescribed histamine-2 receptor antagonists, an older class of medications for gastric acid suppression, hypomagnesemia does not recur. Notably, a majority of these case reports could not account for additional etiologies of magnesium deficiency, including malabsorptive conditions, poor dietary intake (*i.e.*, malnutrition of alcohol use), or diuretic-related renal magnesium excretion, prompting the more recently published observational studies.

Estimating the exact usage of PPIs is difficult given its availability both over-the-counter and with a doctor's prescription. It has been suggested that prescriptions for PPIs are in excess of 100 million per year. Increased reporting of the proton-pump inhibitor-induced hypomagnesemia (PPIH) phenomenon to the United States Food and Drug Administration's Adverse Events Reporting System in combination with the early published case reports, resulted in the release of a "drug safety communication" in 2013^[19]. The announcement alerted health care professionals to the risk of hypomagnesemia among chronic PPI users, particularly among those on a diuretic or other medications known to affect magnesium levels, with the consideration of obtaining baseline and regular follow-up serum magnesium concentrations over time. While large studies have confirmed this increased risk with concomitant diuretic use^[20], others have encountered the PPIH phenomenon among both "casual" (intermittent) PPI users, and chronic users alike^[21].

LIMITATIONS OF PUBLISHED OBSERVATIONAL STUDIES

Subsequent larger observational studies further support this association in both inpatient and outpatient populations^[22-24], but all have significant limitations, and to date, no well-designed study to accurately describe the potential hypomagnesemic effect of PPIs has been done. While the FDA communication states that these effects occur in longer-term PPI use, duration of exposure to PPI therapy has been difficult to quantify in retrospective studies. PPIs are widely available without a prescription and this may lead to under-reporting to medical providers. Additionally, it is likely that some patients may be taking them on an "as-needed" basis rather than daily or twice a day, making any subsequent measurements of hypomagnesemia uninterpretable. Among the limitations of the observational data, residual confounding due to

indication is perhaps the most challenging. Since PPIs are primarily used to treat disorders of the gastro-intestinal tract, and likely are also associated with alterations in dietary behavior, the PPI-associated hypomagnesemia could simply reflect less dietary intake. Furthermore, despite the widespread use of PPIs, the overall reported incidence of PPIH remains low. Although studies that examine the effect of PPI on either ionized magnesium concentrations, intracellular magnesium stores, or magnesium balance, have yet to be performed, a large observational study suggests the PPI exposure is not associated with an increased risk of arrhythmias, as one might expect in the setting of intracellular magnesium deficiency^[25]. Uncertainty about the causal relationship of PPI use and magnesium will remain without carefully designed, prospective studies that clearly address PPI therapy duration and magnesium balance through the measurement of magnesium intake and magnesium excretion before and after PPI exposure.

THE PPIH PHENOMENON: POTENTIAL MECHANISMS

Early reports suggested that PPIH was not due to renal magnesium wasting, but rather decreased gastrointestinal absorption^[26], and additional studies further support renal magnesium conservation in the setting of PPIH^[27]. A recent study examining 24-h urine magnesium excretion in PPI-exposed patients showed that PPI users had lower urinary magnesium. The statistical models controlled for other measures of dietary intake, implicating decreased intestinal magnesium uptake^[28]. These clinical observations highlighting decreased intestinal magnesium uptake are supported by more recent mechanistic studies, which have focused on the potential effect of PPI use on the TRMP6 transporter, the major pathway of intestinal magnesium absorption.

Intracellular magnesium regulates TRPM6 activity along with pH^[29,30] whereby a more acidic milieu increases TRPM6 activity. Since PPI therapy decreases gastric hydrogen proton secretion, thereby increasing lumen pH, PPI use could potentially decrease TRPM6 activity, resulting in decreased magnesium absorption^[31-33]. Figure 1 demonstrates this hypothesis. Adding complexity to this hypothesis, longer term PPI use actually increases the amount of intestinal protons in the distal small bowel^[34] and significantly decreases basic pancreatic secretions^[35]. However, since the majority of active magnesium reabsorption occurs in the cecum and colon, this effect may dissipate before reaching these locations further along the gastrointestinal tract.

PPI exposure may also lead to upregulation of the distal colon's H⁺-K⁺ ATPase (CHK- α), a homolog of the gastric H⁺-K⁺ ATPase targeted by PPIs, increasing its activity by 30%^[36]. However, when studied, increased CHK- α expression did not lead to any changes in serum magnesium levels, urinary magnesium excretion, or fecal magnesium excretion. Given this finding, it is plausible

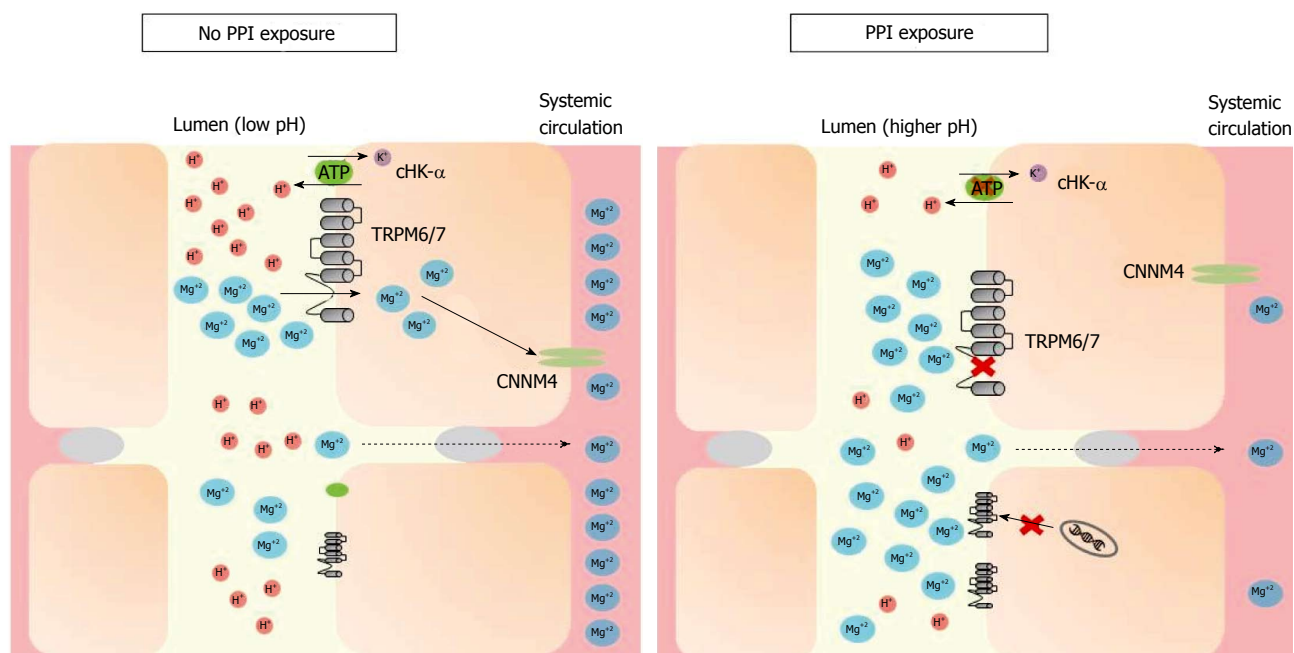


Figure 1 Under conditions of magnesium deficiency, proton-pump inhibitors may inhibit magnesium absorption by increasing the pH of the intestinal lumen, through both gastric and non-gastric antagonism of the H^+-K^+ ATPase pump (“proton pump”). TRPM6/7 affinity for magnesium decreases in a higher pH environment. While this may trigger mRNA transcription of TRPM6 channels in most individuals, hypomagnesemia may develop when this compensation is incomplete or the individual has additional risk factors. TRPM: Transient receptor potential melastatin; cHK- α : Colonic hydrogen-potassium ATPase; CNNM4: Cyclin M4; PPI: Proton-pump inhibitor.

that this increased mRNA expression level of colonic TRPM6 may compensate for the reduced TRPM6 currents caused by the pH-dependent downregulation described above^[31]. While this appropriate overexpression of TRPM6 in times of magnesium deficiency may help ameliorate intestinal magnesium malabsorption and maintain total body magnesium balance, individual epigenetic variations in this response could explain why PPI-induced hypomagnesemia is not uniformly seen among PPI users^[37,38].

While never evaluated in the setting of PPI use, early studies have employed a radiolabeled magnesium challenge and suggest that the magnesium secretion from the intestine is a small component of magnesium homeostasis^[39]. It is currently unclear whether the lack of a “sensing mechanism” within enterocytes that could prompt upregulation of magnesium absorption in times of dietary magnesium restriction could contribute to the clinically significant hypomagnesemia seen in some with PPIH.

Although most have focused on a potential effect of PPI therapy on the TRPM6, and potentially, the Cyclin M4 (CNNM4), transporters in the intestine, there are a number of other ubiquitously expressed transporters previously identified as contributory to magnesium homeostasis including TRPM7^[4], magnesium transporter 1 (MagT1)^[40], Cyclin M3 (CNNM3)^[41], solute carrier family 41 member 1 (SLC41A1)^[42], nonimprinted in Prader-Willi/Angelman Syndrome family (NIPA)^[43], and membrane magnesium transporters 1 and 2 (MMgT1/2)^[44]. While these transporters may play important roles in overall magnesium homeostasis, their role in magnesium

transport and specific locations within the colon is unclear. Therefore, they remain potential targets for investigation of how PPIs may interact with them and ultimately lead to hypomagnesemia.

Genome-wide association studies (GWAS) have also identified potential loci that may influence serum magnesium levels^[45]. Follow-up studies in individuals of African-American ancestry specifically focused on two loci, *MUC1* and the aforementioned *TRPM6*, and analyzed gene-environment interactions, finding significant effect modification of insulin levels with *TRPM6* and progesterone levels with *MUC1*^[46]. While the mechanism of TRPM6-associated hypomagnesemia is better characterized, the influence of transcriptional variations of *MUC1*, which normally encodes a transmembrane mucin forming part of the mucosal barrier of the intestine^[47], remains unexplored.

FUTURE RESEARCH DIRECTIONS

Nearly a decade after the initial reporting of PPIH, there is increasing awareness of the phenomenon. Case reports and observational studies have contributed to our understanding of the prevalence of PPIH among a variety of patient populations with unique risk factors for its development. In conjunction with the clinical findings of preserved renal magnesium reabsorption in periods of magnesium deficiency, molecular physiology studies have proposed a viable pH-dependent mechanism for the role of TRPM6 within colonic enterocytes. However, much uncertainty regarding the relatively rare occurrence of PPIH remains, with individual genetic variation at specific

loci and under-characterized magnesium transporters among the highest-yield unexplored research directions. Additionally, prospective studies that carefully control for nutritional intake among PPI users and accurately measure total body magnesium are needed to help determine causality of the association of PPI use and hypomagnesemia.

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Upper tract urothelial carcinoma: Paradigm shift towards nephron sparing management

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worse prognosis, with a 5 year overall mortality of 23%. To date, the gold standard management of UTUC has been radical nephroureterectomy (RNU), with nephron sparing techniques reserved for solitary kidneys or cases where the patient could not tolerate radical surgery. Limited data from these series, as well as select series where nephron-sparing endoscopic management has been offered to a broader patient base, suggest that minimally invasive, nephron sparing techniques can offer comparable oncologic and survival outcomes to RNU in appropriately selected patients. We review the current literature on the topic and discuss long term outcomes and sequelae of the gold standard treatment, RNU. We also discuss the oncologic outcomes of minimally invasive, endoscopic management of UTUC. Our goal is to provide the reader a comprehensive overview of the current state of the field in order to inform and guide their treatment decisions.

Key words: Urothelial carcinoma; Ureteroscopy; Upper tract; Endoscopy; Minimally invasive

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Core tip: In the appropriate patient population, minimally invasive endoscopic treatment of upper tract urothelial carcinoma provides comparable oncologic and survival outcomes to the gold standard radical nephroureterectomy.

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Abstract

Upper tract urothelial carcinoma (UTUC) is relatively rare compared to urothelial carcinoma of the lower tract, comprising only 5%-10% of all urothelial cancers. Although both entities share histologic properties, UTUC tends to be more invasive at diagnosis and portend a

INTRODUCTION

Urothelial cell carcinoma (UCC), a common malignancy encountered by urologists, is the 4th most common

overall neoplasm and the 8th most common cause of cancer death in men. Upper tract urothelial carcinoma (UTUC), however, is a relatively rare neoplasm, comprising only 5%-10% of all UCCs and 5%-7% of all renal neoplasms^[1-3]. Despite their histologic similarities, UTUC and lower tract UCC may represent two distinct oncologic entities. The natural history of both disease states differs, in that 60% of UTUCs are invasive at diagnosis compared to 15%-25% of lower tract UCCs. UTUC portends a worse prognosis, with an overall 28% 5-year extra vesicle recurrence rate and a 23% 5-year mortality^[4]. The prognosis for muscle invasive UTUC is particularly grim, with a 5 year cancer specific survival (CSS) less than 50% for pT2 /T3 lesions and less than 10% for pT4 lesions^[5,6].

Given the wide body of lower tract UCC literature and the well documented bladder tumor recurrence rate following UTUC, management and surveillance of lower tract disease is standardized and well adhered to. In contrast, most of the recommendations for management of UTUC and subsequent surveillance have been extrapolated from current guidelines for lower tract UCC. Only one specific guideline from the European Association of Urology (EUA) currently exists for the surgical management of UTUC, as well as no randomized controlled trials (RCT) compared to 238 RCTs for bladder cancer^[7,8]. UTUC is, at best, included as a subset in guidelines for bladder cancer amongst other professional societies such as the American Urological Association (AUA) and International Consultation on Urological Diseases (IDUC)^[9-11].

The gold standard treatment for UTUC is radical nephroureterectomy (RNU) with ipsilateral bladder cuff excision^[4]. As our instrumentation technology improves, endoscopic management of UTUC has become feasible. Early experience with endoscopic management of UTUC has been limited to patients with solitary kidneys, bilateral disease, or those who are not surgical candidates to undergo RNU. Data from these cases, though limited to retrospective, unmatched comparative studies, demonstrates no short and mid-term difference in overall survival and CSS between endoscopic management and RNU^[12].

The lack of concrete management guidelines for UTUC, as well as the feasibility of nephron sparing treatment techniques, raises questions of the appropriateness of our current management strategies. In this article we review existing treatment options for UTUC, their effectiveness from an oncologic standpoint, as well as the morbidity incurred long term due to impaired renal function. Though we encourage the reader to come to their own conclusion, we propose that in appropriately selected patients, endoscopic treatment of UTUC is as effective as RNU with lower long term renal complications.

We performed a review of the literature from January 1980 to January 2015, including all English language articles using the search terms "endoscopic management", "ureteroscopic management", "percutaneous management"

and "UTUC". A total of 236 articles were reviewed, yielding 66 articles pertinent to the topic. Outcome measures of upper tract recurrence, overall survival, and CSS were extracted from retrospective and prospective studies.

EPIDEMIOLOGY

As previously discussed, UTUC represents a relatively rare subset of urothelial carcinoma. Bladder tumors represent 90%-95% of all UCs, while UTUCs account for only 5%-10% of all UCs, with an annual incidence in western countries of 2 new cases per 100000 people^[3]. Among UTUCs, pyelocaliceal tumors are twice as common as ureteral tumors. Concurrent bladder tumors are diagnosed with UTUC in 17% of UCC patients. Bladder recurrence after UTUC is common, occurring in 22%-47% of patients, while contralateral upper tract recurrence occurs in only 2%-6%^[13]. Upper tract recurrence after a primary bladder tumor is reported as rare, with an incidence of 1.7%-3.1%^[14,15]. UTUCs have a peak incidence in the elderly population, between age 70 and 80, and are three times more prevalent in men than in women^[16]. Hereditary UTUC exists as a component of hereditary nonpolyposis colorectal carcinoma (HNPCC) or Lynch syndrome^[17].

DIAGNOSIS AND STAGING

The most common presenting symptom of UTUC, occurring in 70%-80% of cases, is either gross or microscopic hematuria^[18]. Flank pain is less common, occurring in 20%-40% of cases, while presentation with a lumbar mass is even more rare, occurring 10%-20% of the time. Both of these entities likely represent advanced disease with worsened prognosis^[19,20].

CT imaging with and without IV contrast has replaced IV excretory urography and ultrasound as the gold standard imaging modality with the highest accuracy for diagnosing UTUC. Its sensitivity ranges from 67%-100% and specificity from 93%-99%, depending on the technique used^[18]. CT imaging cannot accurately stage UTUC, as staging relies on depth of invasion, which is difficult to determine on imaging alone. However, the presence of hydronephrosis in conjunction with known or suspected UTUC portends a worse prognosis, as it is associated with advanced stage disease^[21,22]. Other imaging modalities, such as contrast enhanced MRI, are still in their infancy for diagnosis of UTUC, with a limited sensitivity of 75% for tumors < 2 cm^[23].

Cytology alone is of limited utility as it is less sensitive for UTUC than for bladder tumors. If utilized, it should be performed in situ, with samples being taken directly from the collecting system or ureter *via* the ureteroscope^[24]. Flexible ureteroscopy is a highly effective means of diagnosis, either through direct visualization of tumor in the ureter, renal pelvis and collecting system, or *via* ureteroscopic biopsies, which approach 90% accuracy regardless of the total volume of tissue sample

obtained^[25]. As with CT imaging, accurate staging is difficult with ureteroscopy and biopsies, as the nature of the biopsy forceps makes obtaining muscle in the specimen difficult. Tumor grade is often used as a proxy for stage given that most high grade tumors are also high stage^[5]. Though there are some who advocate for use of imaging findings alone for diagnosis of UTUC, this makes determining the prognosis difficult, as one is not able to determine tumor grade (and thus, by proxy, estimate stage) without tissue specimens. Our recommendation is thus to perform ureteroscopic biopsies on all patients with suspected UTUC.

TREATMENT OPTIONS

RNU

The gold standard treatment for UTUC is RNU with concomitant management of the ipsilateral intramural ureter^[4]. Traditionally this was performed as an open procedure, adherent to standard oncologic principles, namely avoiding entry into the urinary tract to prevent gross spillage of tumor. With the evolution of laparoscopic and robotic surgery, minimally invasive variants of RNU have been developed. Thus far, short to mid term oncologic outcomes seem to be equivalent between laparoscopic and open techniques; however, we currently lack the follow up to prove long term oncologic equivalence between these modalities^[26]. Management of the ipsilateral intramural ureter is critical for adequate recurrence free survival (RFS), as this is the area of highest recurrence. Various methods exist for excising the intramural ureter - extravesical, transvesical, and endoscopic (the "pluck" technique). All three have shown no difference in CSS and OS; endoscopic management techniques have, however, shown higher local bladder recurrence rates^[27]. It is not currently standard practice to perform a retroperitoneal lymph node dissection (LND) along with RNU; a growing body of data suggest it increases median time until recurrence and improves CSS^[28].

LONG TERM IMPACT OF RNU

Aside from the immediate perioperative complications of RNU, which do not differ greatly from any large oncologic resection, patients undergoing this procedure must contend with the long term impact of losing an entire renal unit. Initial studies on creatinine clearance and GFR, performed on the donor nephrectomy population, did not show a long term decrease in renal function^[29-31]. However, one could argue that these donor nephrectomy patients represent a carefully selected cohort of patients that lack the risk factors for renal deterioration after major surgery. A study of patients undergoing nephrectomy for renal cell carcinoma, arguably a patient cohort more closely matched to that of the UTUC population, showed that 10% of patients had significant deterioration of their creatinine post nephrectomy^[32]. A study of 131 patients undergoing nephroureterectomy showed an 18% decrease in GFR at a median of 5 year

follow up^[33]. Another retrospective study of 374 patients undergoing RNU showed an even higher decrease in GFR, at 32%, with no significant trend towards GFR recovery over time^[34]. It would seem apparent from the data that nephroureterectomy does indeed lead to significant impairment of renal function.

Renal impairment, end stage renal disease (ESRD) in particular, accounts for a large percentage of health care spending in the elderly^[35]. Cost analysis data from UTUC patients undergoing either RNU or renal sparing treatment for UTUC demonstrates a 3-fold to 10-fold cost savings of nephron sparing treatment over RNU over a 10 year period with similar oncologic outcomes^[36]. Perhaps more importantly, overall survival and quality of life of patients whose renal insufficiency necessitates dialysis has been proven to be greatly diminished compared to the non dialysis dependent population^[37]. Urologists have globally accepted the aforementioned arguments as strong reasons for renal preservation in the management of small renal masses - could these principles be selectively applied to UTUC?

NEPHRON SPARING TREATMENTS FOR UTUC

The rationale for conservative surgery for UTUC stems from the fact that most UTUC is superficial and low grade^[38]. Thus, coupled with the aforementioned drawbacks of renal loss and decreased GFR, as well as improvements in endoscopic technology, allow for pursuit of renal sparing techniques. Currently available nephron-sparing treatments for UTUC include ureteroscopic retrograde tumor ablation, percutaneous antegrade tumor ablation, or segmental ureterectomy. As the focus of this review is endoscopic management of UTUC, segmental will not be discussed further here.

Patient selection is critical, as currently endoscopic management techniques are only advisable for low grade, small volume tumors or for patients who would otherwise not be fit to undergo RNU^[7] (Figure 1). The decision between retrograde ureteroscopic tumor management and antegrade percutaneous ablation depends primarily on tumor size and location. Large tumors in the renal pelvis are best approached in a percutaneous fashion, while ureteral tumors lend themselves to a ureteroscopic approach. Small tumors in the collecting system may be approached by either fashion^[12,38].

Currently no randomized controlled trials exist comparing endoscopic management techniques to the gold standard radical nephroureterectomy. Most of the published data come from small, retrospective and unmatched comparative studies. A 2014 meta-analysis of eight retrospective series, totaling 1002 patients, demonstrated no statistically significant difference in overall survival and CSS between the two modalities. The authors hesitated to conclude oncologic equivalence given the low level of the evidence^[12]. Additionally, patients tended to be selected for favorable tumor characteristics, such as low grade features and small tumor size. Analysis

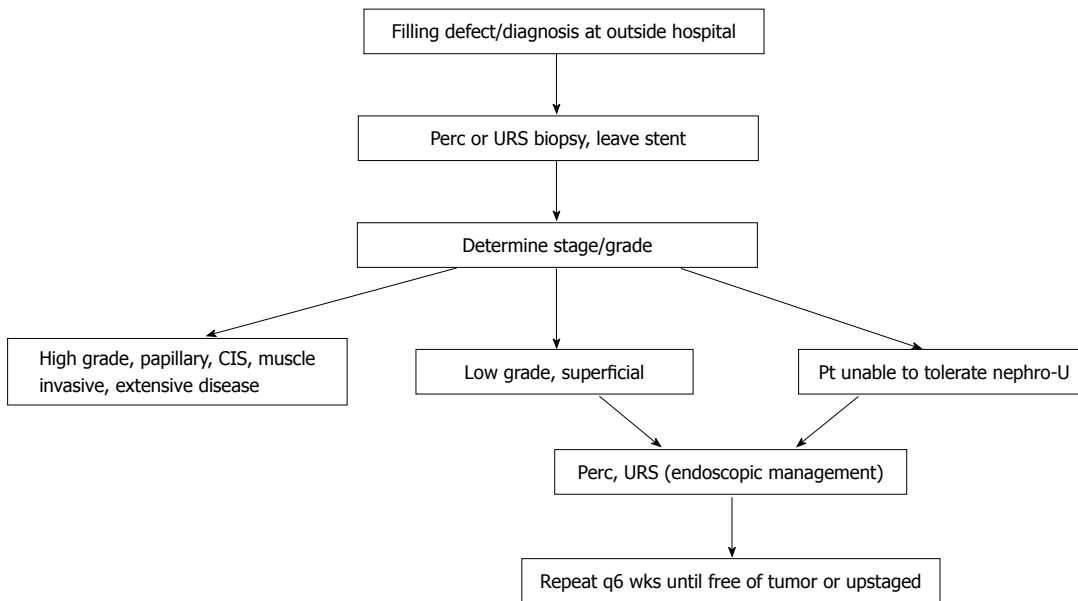


Figure 1 Sample treatment algorithm for patients with upper tract urothelial carcinoma.

of all the existing literature reveals that ureteroscopic ablation of the tumor is associated with high rates of upper urinary tract recurrence (15%-90%) and intravesical recurrence (12%-70%). Tumor grade, size and multifocality predict upper tract recurrence while previous history of bladder cancer predicts intravesical recurrence (Table 1)^[39-57]. The large variations in population size, initial tumor characteristics and length of follow up likely explain the broad range of observed outcomes.

Similarly, the only data on outcomes of percutaneous management of UTUC come from retrospective series (Table 2)^[58-66]. Overall, patients managed percutaneously had similar clinical features to those managed ureterscopically - namely low grade, small focal tumors. Those undergoing percutaneous ablation had lower rates of upper tract recurrence (10%-65%) and intravesical recurrence (10%-42%) than those treated with the ureterscopic approach. Given the high rate of comorbidities, much like in the ureterscopic population, the overall survival was poor (68%-96%) while the CSS was high (75%-100%).

DISCUSSION - BROADENED INDICATIONS FOR NEPHRON SPARING TREATMENTS?

Currently, the only "imperative" indications for systematically offering nephron sparing treatment of UTUC include anatomically or functionally solitary kidneys, substantial renal insufficiency with the impending threat of hemodialysis or bilateral UTUC^[7]. We believe that, though limited in its retrospective nature, the existing data indicate that the patient population to whom nephron sparing treatment is routinely offered as a first line option should be expanded.

UTUC continues to challenge urologists as a potentially

devastating disease that tends to affect older, sicker patients. As this review of the literature demonstrates, patients treated with ureterscopic or percutaneous means have a much higher CSS than OS, meaning that they eventually succumb to their comorbidities, and not their cancer. Thus, we believe that patients with significant comorbidities make excellent candidates for first line nephron sparing options. Ureteroscopic and percutaneous approaches offer similar CSS, at least according to medium term data, while avoiding the morbidity and potential of a RNU for an already unhealthy patient population.

Amongst otherwise healthy UTUC patients, we believe nephron sparing treatments should still be offered to those patients with low grade, low stage disease. Although UTUC is more often invasive at diagnosis, truly low grade and low stage disease seems to follow a similarly indolent course, with frequent recurrence but rare progression, as low grade bladder cancer^[2,3,7]. Thus, as endoscopic technology and techniques improve, allowing for better ureterscopic evaluation and biopsy, we should be better able to separate low grade from high grade disease. Patients with low grade disease have shown excellent CSS in the existing endoscopic management literature; using these treatments would allow us to spare them the morbidity of losing a renal unit.

Post-treatment surveillance is critical for achieving excellent CSS outcomes. Thus, patients considered for endoscopic management of their UTUC must be compliant. At our institution we repeat ureterscopic or percutaneous surveillance ever 3-6 mo for 2 years and then annually; similar variations on this surveillance protocol exist throughout the literature. Additionally, CT imaging allows for detection of progression to metastatic disease and should be performed at regular time intervals.

Table 1 Outcomes of ureteroscopic management of upper tract urothelial carcinoma

Ref.	n	Grade on biopsy (G1/G2/G3 vs LG/HG)	F/U (mo)	Outcomes (%)
Schmeller <i>et al</i> ^[39] (1989)	16	6/10/0	14	19 UTR, 100 OS, 100 CSS
Andersen <i>et al</i> ^[40] (1989)	10	NA	35	NA
Gaboardi <i>et al</i> ^[41] (1994)	18	12/6/0	15	50 UTR, 100 OS, 100 CSS
Engelmeyer <i>et al</i> ^[42] (1996)	10	9/1/0	10	70 UTR, 90 OS, 100 CSS
Chen <i>et al</i> ^[57] (2000)	23	22LG/21HG	23	64 UTR, 12 IVR
Daneshmand <i>et al</i> ^[43] (2003)	30	7/6/14	31	90 UTR, 23 IVR, 77 OS, 97 CSS
Matsuoka <i>et al</i> ^[44] (2003)	27	10/2/0	33	26 UTR, 15 IVR
Iborra <i>et al</i> ^[45] (2003)	23	NA	NA	35 UTR, 96 CSS
Johnson <i>et al</i> ^[46] (2005)	35	35/0/0	32	68 UTR, 100 CSS
Rouprêt <i>et al</i> ^[47] (2006)	27	19LG/8HG	52	15 UTR, 22 IVR, 77 OS, 81 CSS
Reisinger <i>et al</i> ^[48] (2007)	10	10/0/0	73	50 UTR, 70 IVR, 100 OS, 100 CSS
Krambeck <i>et al</i> ^[49] (2007)	37	2/13/7	32	62 UTR, 37 IVR, 35 OS, 70 CSS
Painter <i>et al</i> ^[50] (2008)	45	NA	NA	89 CSS
Lucas <i>et al</i> ^[51] (2008)	39	27LG/12HG	33	46 UTR, 62 OS, 82 CSS
Pak <i>et al</i> ^[36] (2009)	57	NA	53	90 UTR, 93 OS, 95 CSS
Cornu <i>et al</i> ^[52] (2010)	35	16LG/6HG	24	60 UTR, 40 IVR, 100 OS, 100 CSS
Gadzinski <i>et al</i> ^[53] (2010)	34	NA	58	84 UTR, 75 OS, 100 CSS
Cutress <i>et al</i> ^[54] (2012)	73	34/19/6	54	69 UTR, 43 IVR, 60 OS, 90 CSS
Grasso <i>et al</i> ^[55] (2012)	80	66LG/14HG	38	81 UTR, 59 IVR, 74 OS, 87 CSS
Fajkovic <i>et al</i> ^[56] (2013)	20	14LG/3HG	20	25 UTR, 15 IVR, 45 OS, 95 CSS

G1: Grade 1; G2: Grade 2; G3: Grade 3; LG: Low grade; HG: High grade; F/U: Follow up; UTR: Upper tract recurrence; IVR: Intra-vesical recurrence; OS: Overall survival; CSS: Cancer specific survival; NA: Not available.

Table 2 Outcomes of percutaneous management of upper tract urothelial carcinoma

Ref.	n	Grade on biopsy (G1/G2/G3 vs LG/HG)	F/U (mo)	Outcomes (%)
Tasca <i>et al</i> ^[58] (1992)	10	1/5/0	19	50 UTR, 90 OS, 100 CSS
Fuglsig <i>et al</i> ^[59] (1995)	26	NA	21	31 UTR, 96 OS, 100 CSS
Plancke <i>et al</i> ^[60] (1995)	10	6/3/11	28	10 UTR, 10 IVR, 90 OS, 100 CSS
Patel <i>et al</i> ^[61] (1996)	26	11/11/1	45	35 UTR, 42 IVR, 75 OS, 91 CSS
Clark <i>et al</i> ^[62] (1999)	17	6/7/4	24	33 UTR, 75 OS, 82 CSS
Goel <i>et al</i> ^[63] (2003)	20	15LG/5HG	64	65 UTR, 15 IVR, 75 CSS
Palou <i>et al</i> ^[66] (2004)	34	7/21/5	51	44 UTR, 74 OS, 94 CSS
Rouprêt <i>et al</i> ^[64] (2007)	24	17LG/7HG	62	13 UTR, 17 IVR, 79 OS, 83 CSS
Rastinehead <i>et al</i> ^[65] (2009)	89	50LG/39HG	61	33 UTR, 68 OS
Fiuk (current study)	65	34LG/33HG	28	55 OS, 87 CSS

G1: Grade 1; G2: Grade 2; G3: Grade 3; LG: Low grade; HG: High grade; F/U: Follow up; UTR: Upper tract recurrence; IVR: Intra-vesical recurrence; OS: Overall survival; CSS: Cancer specific survival; NA: Not available.

Urinary cytology is not as useful in UTUC and thus is left to the surgeon's discretion.

We thus propose that nephron sparing treatment of UTUC, either ureteroscopic or percutaneous, be offered as a first line therapy to the following patient populations: (1) any patient with an anatomically or functionally solitary kidney; (2) any patient with renal insufficiency great enough to impose the threat of hemodialysis with any further renal insult; (3) any patient with multiple bilateral UTUC tumors; (4) any patient with comorbidities great enough to be life limiting or to incur additional risk with nephroureterectomy; and (5) any patient with low grade, low stage disease who can be trusted to commit to 3-6 mo surveillance.

By using a risk-adapted strategy for expanding current indications for first line endoscopic treatment of UTUC, we hope to minimize renal unit loss without compromising oncologic safety. Development of improved

biopsy techniques, urothelial cancer biomarkers, and improved prediction nomograms may help further delineate these indications in the future.

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Cooling dialysate during in-center hemodialysis: Beneficial and deleterious effects

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Abstract

The use of cooled dialysate temperatures first came about in the early 1980s as a way to curb the incidence of intradialytic hypotension (IDH). IDH was then, and it remains today, the most common complication affecting chronic hemodialysis patients. It decreases quality of life on dialysis and is an independent risk factor for mortality. Cooling dialysate was first employed as a technique to incite peripheral vasoconstriction on dialysis and in turn reduce the incidence of intradialytic hypotension. Although it has become a common practice amongst in-center hemodialysis units, cooled dialysate results in up to 70% of patients feeling cold while on dialysis and some even experience shivering. Over the years, various studies have been performed to evaluate the safety and efficacy of cooled dialysate in comparison to a standard, more thermoneutral dialysate temperature of 37 °C. Although these studies are limited by small sample size, they are promising in many aspects. They demonstrated that cooled dialysis is safe and equally efficacious as thermoneutral dialysis. Although patients report feeling cold on dialysis, they also report increased energy and an improvement in their overall health following cooled dialysis. They established that cooling dialysate temperatures improves hemodynamic tolerability during and after hemodialysis, even in patients prone to IDH, and does so without adversely affecting dialysis adequacy. Cooled dialysis also reduces the incidence of IDH and has a protective effect over major organs including the heart and brain. Finally, it is an inexpensive measure that decreases economic burden by reducing necessary nursing intervention for issues that arise on hemodialysis such as IDH. Before cooled dialysate becomes standard of care for patients on chronic hemodialysis, larger studies with longer follow-up periods will need to take place to confirm the encouraging outcomes mentioned here.

Key words: Hemodialysis; Dialysate temperature; Cool dialysate; Intradialytic hypotension; Hypotensive episodes;

Hemodynamic stability; Cool temperature dialysis

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Core tip: Cooled dialysate is commonly employed to reduce the incidence of intradialytic hypotension (IDH) in patients on chronic dialysis. The studies to date that have evaluated cooled dialysate are limited by small sample size and it has not become the standard of care for managing IDH. However, the small studies that exist are promising and suggest that cooling dialysate improves hemodynamic tolerability of dialysis, minimizes IDH, and exerts a protective effect over major organs including the heart and brain. More studies are needed to assess the long-term effects of cooling dialysate in this population.

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INTRODUCTION

According to the KDOQI Clinical Practice Guidelines, intradialytic hypotension (IDH) is defined as a symptomatic decrease in systolic blood pressure of ≥ 20 mmHg or a decrease in mean arterial pressure (MAP) by 10 mmHg with symptoms being characterized by abdominal discomfort, nausea, vomiting, muscle cramps, dizziness, restlessness, and anxiety, amongst others^[1]. It occurs in approximately 20%-30% of dialysis sessions^[2] and is the most frequent complication of renal replacement therapy^[3]. IDH is multifactorial in etiology but is largely attributed to the rapid reduction of blood volume that occurs with ultrafiltration and the inadequate cardiovascular response to the reduction in blood volume^[2,4]. Populations predisposed to IDH include the elderly and those with diabetes and cardiovascular diseases^[3].

IDH is an independent risk factor for mortality in patients on hemodialysis^[5,6] and may negatively affect their quality of life^[7]. It is also associated with increased morbidity including frontal lobe atrophy^[8], myocardial injury^[9], and mesenteric ischemia^[10]. Nephrologists employ various methods on dialysis to minimize IDH including sodium modeling, ultrafiltration profiling, addition of calcium or bicarbonate to the dialysate bath, and dialysate cooling. While each method has its positive and negative attributes, the focus of this review will be on dialysate cooling.

Extracorporeal cooling of blood on dialysis first came about over three decades ago when it was noted to curb the incidence of IDH. Since then, it has been employed as a measure to mitigate IDH although it has not become standard of practice in chronic hemodialysis

units.

DISCUSSION

Core body temperature and the effect of cooled dialysate

Humans keep their core body temperature (CBT) within a narrow range. When CBT rises, the body increases peripheral blood flow or initiates sweating in an attempt to remove heat from the body by convection or radiation, respectively. Shivering is usually an involuntarily thermoregulatory mechanism employed by the body to generate heat when CBT falls. While on dialysis, the dialysate temperature is set to an arbitrary standard of 37 °C (98.6 °F) in an effort to achieve a "normal" CBT and maintain isothermia. However, a significant amount of variability exists amongst individuals when it comes to CBT. First, CBT follows a circadian pattern that peaks between 4 and 9 pm and nadirs between 2 and 8 am^[11,12]. CBT tends to be lower in elderly individuals^[12], higher in women than in men, and is highest in black women^[13]. In hemodialysis-dependent individuals, CBT is usually lower than in the non-dialysis population^[11,14], with nearly 40% having a CBT less than 36.5 °C^[15] compared to the mean CBT of 37 °C (range, 36.2 °C to 37.5 °C) in non-dialysis dependent individuals^[12]. The importance of this becomes evident when one considers that even a slight change in CBT on dialysis initiates thermoregulatory mechanisms which may be detrimental on dialysis. For example, a supraphysiologic dialysate temperature, such as 37 °C, could raise the CBT in any given individual resulting in vasodilation and consequent cardiovascular instability. The vasodilation may be in direct competition with the expected vasoconstriction which occurs in the setting of ultrafiltration and could further lead to hemodynamic compromise.

Since supraphysiologic dialysate temperature was viewed as suboptimal and potentially detrimental, the idea that subphysiologic dialysate temperature might be beneficial arose, specifically in those individuals that suffered from IDH. Cooled dialysate temperature was postulated to be beneficial for the following reasons: First, it avoided heat accumulation and hence counterproductive thermoregulatory vasodilation; second, it likely led to a catecholamine surge which induced both peripheral vasoconstriction and cardiac inotropy^[16]. However, at the time various potential consequences surrounding cooled dialysate remained unclear. Would dialysis adequacy be inferior? Would it cause prolonged vasoconstriction potentially placing vulnerable vascular beds at risk for ischemia? Would patients be tolerant of the cooled CBT on dialysis? Finally, would it be effective at minimizing IDH?

Various studies have since been performed to address the above issues. Kaufman *et al.*^[17] aimed to evaluate the efficacy of cooled dialysate during short-time, high Kt/V dialysis treatments. He postulated that cooled dialysate might increase urea compartmentalization during dialysis

treatment leading to increased urea rebound post-dialysis and hence decrease dialysis efficacy. The study was performed in 15 patients who underwent a total of 56 dialysis sessions. Each participant served as their own control. Dialysate temperatures were adjusted to either lower CBT (cooled dialysis) or keep CBT at a thermoneutral temperature. Dialysate cooling resulted in -266 ± 15 kJ heat-energy exchange per treatment whereas thermoneutral dialysis averaged 5 ± 31 kJ per treatment; dialysate temperature averaged $35.7^\circ\text{C} \pm 0.02^\circ\text{C}$ and $37.1^\circ\text{C} \pm 0.02^\circ\text{C}$, respectively. Cooled dialysis resulted in statistically greater increases in the peripheral vascular resistance index and MAP. It also reduced the maximum intradialytic fall in MAP and necessary interventions by staff to address hypotensive symptoms. There were no statistical changes in blood volume, cardiac index, urea rebound, or effective Kt/V. The authors concluded that cooling dialysate stabilized hemodynamics during dialysis, reduced the number of staff interventions required to address IDH symptoms, and did so without affecting the efficacy of high-efficiency dialysis. A systematic review that evaluated 22 studies comprising of 408 patients has since concluded that using cooling dialysate temperature does not reduce dialysis adequacy^[18].

Ayoub *et al.*^[19] aimed to gauge patient perception of cooled dialysate. Five patients known to have IDH were dialyzed for three sessions using cooled dialysate (35°C) followed by another three sessions with dialysate temperature set at 36.5°C . The same was done in a second group of five patients known to have stable blood pressures during and after their dialysis sessions. Their results demonstrated that cooling dialysate resulted in a statistically significant increase in ultrafiltration in the group known to have IDH. This group also experienced significantly higher intra- and post-dialysis MAPs with cooled dialysate. While the IDH-prone group had no episodes of hypotension with cooled dialysate, they had a total of seven episodes of hypotension with neutral temperature dialysate, all requiring nursing intervention ($P < 0.001$). There was no statistical difference in intradialytic pulse rates between the two groups nor did cooling dialysis have an effect on urea removal between the two groups. Patients' perception about cooled dialysis was assessed by a questionnaire designed specifically for this study. It comprised of the following questions: "How did you feel while being dialysed on cool temperature? Compared with normal temperature dialysis of 36.5°C , did you feel any differences while being dialysed on cool temperature? If yes, what were the differences? Would you like to continue cool temperature dialysis?" The results of the questions were as follows: 80% of patients felt more energetic after being dialyzed with cooled dialysate; 80% felt a dramatic improvement in their general health with cooled dialysate; 80% requested to always be dialyzed with cooled dialysate; 20% reported feeling cold during dialysis. The authors concluded that for patients prone to IDH, cooled dialysate improved hemodynamic stability during and after dialysis,

improved tolerance of dialysis, reduced the number of nursing interventions required to address IDH, and had an overall positive impact on patients' energy and activities of daily living. This is the only study to date that has specifically assessed patient perception of cooled dialysate temperature. However, a systematic review by Selby *et al.*^[18] pooled the results of five studies in which symptoms were reported during cooled dialysis. Their analysis demonstrated that patients undergoing cooled dialysis were 1.98 (95%CI: 0.38-3.57) times more likely to become symptomatic than patients dialyzed with standard dialysate temperatures. When the analysis omitted the study by Ayoub and Finalyson^[19] due to milder symptoms being reported compared to the other four studies, the results were non-significant with symptoms occurring 1.5 (95%CI: -0.2-3.2) times more often with cooled dialysis than during standard dialysis.

A similar study by Jost *et al.*^[20] compared cooled dialysate to thermoneutral dialysate to specifically evaluate its efficacy on "problem" patients. The design used a double-blinded, cross-over protocol to evaluate 12 patients, six of whom were prone to IDH and six known to have large interdialytic weight gains defined as consistently gaining > 4 kg in the interdialytic period. Each patient served as their own control and was randomly assigned to one session of dialysis at 35°C and one at 37°C . Results demonstrated significantly lower blood pressures at 1, 2, and 3 h of dialysis at a thermoneutral dialysate temperature when compared to the cooled dialysate temperature. A total of 18 episodes of symptomatic hypotension occurred during the study period, 16 of which occurred in the IDH-prone group. Furthermore, no episodes of symptomatic hypotension occurred during cooled dialysis ($P < 0.01$). The authors concluded that cooling dialysate significantly improved hemodynamic tolerance during dialysis and also significantly reduced the incidence of IDH during dialysis in patients prone to IDH. These studies added to the literature supporting cooled dialysate as an effective way of reducing IDH.

Cooled dialysate compared to other modalities used to minimize IDH

Dheenan and Henrich^[21] were the first to compare cooled dialysate to other methods that are commonly employed to mitigate IDH. They used a single-blinded, cross-over protocol to evaluate 10 patients on chronic hemodialysis with a history of IDH. Patients were randomized to one week periods (three dialysis sessions) of five varying dialysis protocols performed in a random and blinded fashion. Each patient underwent four protocols commonly employed to minimize IDH in addition to a standard dialysis protocol which served as a control. The protocols were as follows: A standard dialysis group with dialysate sodium of 138 mEq/L (served as the control group), high sodium dialysate (patient dialyzed using a steady dialysate sodium of 144 mEq/L), sodium modeling using a step function design (dialysate sodium declined from 152 to 140 mEq/L in the last 30 min of

dialysis), ultrafiltration (one hour of isolated ultrafiltration in which 50% of the target weight loss was removed followed by three hours of isovolemic dialysis), and cool temperature dialysis in which dialysate was cooled to 35 °C (sodium concentration was 140 mEq/L in this group). The results revealed indistinguishable weight losses with each protocol suggesting that the volume of ultrafiltration was consistent across each protocol. However, the results demonstrated superiority of sodium modeling and cooled dialysate groups over the other groups, and multiple similarities between these two methods. Both had significantly fewer hypotensive signs and symptoms per treatment and fewer hypotensive episodes per treatment when compared with standard treatment. Both also had significantly fewer nursing interventions for IDH per treatment when compared to the ultrafiltration and control group. The nadir MAP was significantly lower in the control and ultrafiltration groups whereas the upright post-dialysis blood pressure was best preserved in the sodium modeling and cooled dialysate groups. Sodium modeling was tolerated by all but one patient who developed hypertension, headache, and nausea; 6 out of the 10 reported increased thirst sensation however this did not translate into increased interdialytic weight gain during the one week follow-up period. Cooled dialysate, however, was not well tolerated. Seven of 10 patients reported a “cold” sensation and two patients were noted to be shivering on dialysis.

A similar study by Rezki *et al.*^[22] evaluated 16 patients in a two-phase protocol. The first phase consisted of three standard HD sessions with a sodium concentration of 140 mEq/L with dialysate temperature at 37 °C and served as the control for each patient. During the second phase, patients were dialyzed successively under the following conditions: Fixed sodium dialysate concentration at 144 mEq/L, sodium modeling from 152 to 138 mEq/L, one hour of ultrafiltration alone followed by three hours of standard dialysis, dialysis with cooled dialysate ($T < 37^{\circ}\text{C}$), and a combination of sodium modeling with cooled dialysate. When compared to the control protocol, there was a statistically significant decrease in the signs and symptoms of hypotension and in the incidence of IDH when patients were dialyzed with sodium modeling, cooled dialysate, or the combination protocol. When compared to the control protocol, fewer medical staff interventions were required when patients were dialyzed with the combination protocol or cooled dialysate. There was no increase in subjective thirst or in interdialytic weight gain when a protocol employing sodium modeling was performed. In this study, four of the 16 patients noted shivering when dialyzed with cooled dialysate.

Both of these studies suggest that cooling dialysate temperature is as effective a method as sodium modeling when it comes to mitigating IDH. They also suggest that cooling dialysate may be poorly tolerated and associated with patient discomfort on HD. However, sodium modeling has been associated with a number of side effects including worse hypertension and increased

interdialytic weight gain due to increased thirst^[23]. Whether one method is superior at reducing IDH or is better tolerated than the other remains to be seen in a larger trial with longer follow-up periods.

Effect of cooled dialysate on vulnerable vascular beds

One of the questions that arose when cooled dialysate was first introduced was whether vasoconstriction would also occur at an arteriolar level and potentially place vulnerable vascular beds at risk for end-organ injury. Since that time, it has become apparent that dialysis itself is a hemodynamic stressor^[24] which triggers circulatory stress and consequently damages vasculature in the heart, mesentery, and brain^[25-27] amongst other organs. Two recent trials demonstrated that cooled dialysate imparts a protective effect in these organs.

Eldehni *et al.*^[25] hypothesized that ultrastructural injury to the white matter in the brain might be mitigated by cooling dialysate hence reducing dialysis-induced circulatory stress. This was evaluated by randomizing 38 incident dialysis patients to dialyze for 12 mo at either 37 °C or 0.5 °C below their core body temperature; the latter was determined by averaging each patient's temperature by tympanic thermometer during six sessions prior to commencing the trial. An individualized temperature was chosen as it is thought to be better tolerated than an arbitrary temperature of 35 °C^[28]. A form of magnetic resonance imaging (MRI) called diffusion tensor imaging (DTI) was used to evaluate the structural integrity of the brain white matter at baseline and after 12 mo of thrice-weekly dialysis. DTI was chosen as an imaging modality as it has previously been used to detect clinically significant changes in cerebral small vessel disease^[29]. Additionally, MAP extrema points were measured over the course of 12 mo. MAP extrema points measure the frequency and amplitude required to maintain optimal organ perfusion; higher extrema points correlate with high variation in organ perfusion and translates to detrimental perfusion of vulnerable vascular beds^[25,30]. After 12 mo, patients dialyzed at 37 °C exhibited patterns of ischemic brain injury on MRI that were not noted in the cooled dialysate group. Additionally, patients dialyzed at 37 °C had a notable worsening of their MAP extrema points that was not seen in the cooled dialysate group. Both of these results were statistically significant. The authors concluded that cooling dialysate minimized injurious perfusion of cerebral vascular beds and consequently decreased the degree of brain injury noted on DTI. An advantage to this study is the long-term follow-up over the course of one year. However, despite having a larger sample size than in earlier studies evaluating the effects of cooled dialysate, it was still limited by a small sample size. Additionally, the study suffered from a high dropout rate of 47.9%, although this was primarily due to difficulty in recruiting patients on incident HD; there were no dropouts reported as a result of the intervention.

Odudu *et al.*^[24] used the same patient population and study design as Eldehni *et al.*^[25] to evaluate whether cooled dialysate would have cardioprotective effects

over the course of a 12 mo follow-up. Fifty-four incident dialysis patients were randomized to a dialysate temperature of either 37 °C or 0.5 °C below their core body temperature and followed for 12 mo. Tagged cardiac magnetic resonance imaging was performed at baseline and at 12 mo; the imaging modality was chosen for its high reproducibility and use as a reference standard technique to evaluate regional left ventricular (LV) strain. While there was no statistically significant change in the study's primary outcome, change in resting ejection fraction, there were multiple significant secondary outcomes of note. The cooled dialysate group experienced a significant reduction in both LV mass as well as LV end-diastolic volumes. The control group had a significant reduction in peak systolic strain, diastolic function, and segmental LV strain whereas these functions were preserved in the cooled dialysate group. As markers of subclinical cardiomyopathy, these findings suggest that cooled dialysate had a protective cardiac effect over the one year study period. Lastly, aortic distensibility, an independent marker for future cardiovascular events, was also preserved in the cooled dialysate group and significantly decreased in the control group. Whether these findings suggest that cooled dialysate may one day be linked to a decreased risk of cardiovascular events in the dialysis population remains to be seen.

CONCLUSION

Cooling dialysate first came into practice three decades ago after it was noted to curb the symptoms of patients suffering from IHD on dialysis. Since then, many benefits of cooled dialysate temperatures have come to light. Multiple studies have demonstrated improved hemodynamic tolerance on dialysis specifically in patients prone to IDH without any adverse effect on dialysis adequacy. It is an inexpensive intervention that also reduces the frequency of nursing involvement to address IDH in patients on chronic hemodialysis. More recently, studies suggest that one year of cooling dialysate temperature in incident dialysis patients mitigates features of subclinical cardiomyopathy and ischemic brain injury when compared to patients dialyzed at the standard 37 °C.

While the cooler temperatures may cause discomfort in some patients, recent studies suggest that a temperature of 0.5 °C below an individual's CBT is better tolerated than an arbitrary temperature of 35 °C. Additionally, Ayoub and Finlayson^[19] demonstrated that cooled dialysate may actually improve a patient's energy following dialysis, and in their cohort of patients, individuals requested to be dialyzed with cooled dialysate temperatures following the study. Finding a "sweet spot" for cooled dialysate may allow for increased patient satisfaction and in turn, improve patient compliance with dialysis. The advantages and disadvantages of cooling dialysate are summarized in Table 1.

Unfortunately, all of the studies performed to date on

Table 1 Advantages and disadvantages of cooling dialysate temperatures

Advantages	Disadvantages
Improved hemodynamic tolerance during and after dialysis	Patient discomfort
Improved patient energy	Shivering
Preservation of vulnerable vascular beds (subclinical cardiomyopathy and ischemic brain injury)	
Fewer nursing interventions needed	

cooled dialysate have been limited by small sample sizes. Larger studies are needed in order to be generalizable to a greater portion of the chronic hemodialysis population. From a patient perspective, it will be important to study perception and comfort with dialysis at cooler temperatures since there appears to be a trend toward a higher incidence of symptoms with cooled dialysis when compared to standard dialysis. Studies with longer follow-up times would be useful to evaluate the effects of sodium modeling and cooled dialysate on incidence of IDH as well as its effects on interdialytic weight gain and hypertension. Longer follow-up would also allow for assessment of the effects of cooled dialysate on vulnerable vascular beds and their clinical correlates, for example cardiovascular events, dementia, memory, executive function, *etc.* Encouraging results in such studies would have the potential to change the standard of care in patients on chronic hemodialysis.

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Use of percutaneous nephrostomy and ureteral stenting in management of ureteral obstruction

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Abstract

The management options for ureteral obstruction are diverse, including retrograde ureteral stent insertion or antegrade nephrostomy placement, with or without eventual antegrade stent insertion. There is currently no consensus on the ideal treatment or treatment pathway for ureteral obstruction owing, in part, to the varied etiologies of obstruction and diversity of institutional practices. Additionally, different clinicians such as internists, urologists, oncologists and radiologists are often involved in the care of patients with ureteral obstruction and may have differing opinions concerning the best management strategy. The purpose of this manuscript was to review available literature that compares percutaneous nephrostomy placement *vs* ureteral stenting in the management of ureteral obstruction from both benign and malignant etiologies.

Key words: Percutaneous nephrostomy; Urinary diversion; Ureteral obstruction; Quality of life; Ureteral stents; Pelvic malignancy; Urinary drainage

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Core tip: Ureteral obstruction as a consequence of malignant or benign etiologies is a common urologic entity that is often challenging for clinicians to determine the optimal method of urinary decompression. There is no consensus on the use of stents *vs* percutaneous nephrostomy in the management of ureteral obstruction as well as a lack of clear superiority of stenting over percutaneous approach in terms of complications and quality of life considerations. Therefore, treatment decisions must be individualized using a multidisciplinary approach involving the patients, their family and members of the treatment team.

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INTRODUCTION

Ureteral obstruction is a heterogeneous clinical entity, and it is often challenging for the clinician to determine the optimal method of decompression. Malignant ureteral obstruction can arise from intrinsic urologic malignancy such as prostate or bladder cancer, or extrinsic involvement from another primary malignancy, most commonly of gynecologic or colorectal origin^[1-3]. The therapeutic goal of urinary drainage in malignant disease is to adequately drain the upper urinary tracts for symptomatic relief with maintenance of renal function, allowing the initiation of systemic therapy while minimizing further urologic intervention, hospitalization and negative impact on the quality of life^[2-4]. On the other hand, the etiology of benign ureteral obstruction is generally a consequence of intraluminal pathology, such as ureteropelvic junction obstruction, ureteral stones or ureteral stenosis. Extraluminal benign obstruction can arise from localized mass effect of benign tumors such as uterine leiomyomas or retroperitoneal fibrosis^[5-7]. Benign ureteral obstruction caused by ureteropelvic junction obstruction is primarily managed with definitive treatment of the underlying condition^[8].

There are no clear guidelines regarding optimal methods of urinary decompression in the management of ureteral obstruction. The purpose of this article is to review recent literature assessing outcomes of retrograde ureteral stenting and percutaneous nephrostomy (PCN) insertion in the treatment of ureteral obstruction resulting from malignant and benign etiologies to elucidate the associated morbidity, effects on quality of life and variability in technical success.

DATA ACQUISITION

PubMed was used to search for articles addressing the management of malignant and benign ureteral obstruction using key phrases "ureteral stent" and "nephrostomy". This yielded 850 articles that were screened by title and abstract. Screened articles were then independently evaluated by two authors (HL and LH) for inclusion in the review. Manuscripts were included if they reported original research comparing PCN and ureteral stenting. Exclusion criteria included a study focus on pediatric populations, no differentiation between antegrade vs retrograde stenting, or study population totaling < 10. A total of 16 articles were included in the final review.

PCN VS URETERAL STENTING IN THE SETTING OF MALIGNANCY

Malignant ureteral obstruction may occur secondary to contiguous tumor invasion, extrinsic ureteral compression by pelvic malignancies, or by pelvic metastases of tumors that originate from outside the pelvis such as breast, gastric or pancreatic cancers. Obstruction can also occur in the setting of retroperitoneal or pelvic lymphadenopathy due to metastatic disease, or as a consequence of treatment resulting in retroperitoneal fibrosis or ureteral stricture^[9,10].

Obstruction may be evident during staging of the disease or workup for impaired renal function as evidenced by hydronephrosis with renal cortical atrophy on abdominal imaging. Additionally, patients may experience acute flank pain, renal failure, uremia or sepsis secondary to urinary tract infections. The rationale for decompression aims to offer relief of the above symptoms, to alleviate complications from renal insufficiency and to facilitate systemic therapy.

Determining the etiology of obstruction may be helpful in planning treatment approaches as tumors involving the bladder, uterine cervix and prostate cancer are known to have lower retrograde stenting success rates^[11]. The etiology of obstruction is also important for estimating patient prognosis. Non-urologic malignancies such as gastric and pancreatic cancers have a worse prognosis with shorter overall survival than urologic malignancies^[12,13].

Prognostication and quality of life: To decompress or not to decompress?

Although the intention of diversion is to prolong patient survival, this goal is often not achieved with diversion. Malignant ureteral obstruction can be a sign of advanced disease^[14,15] and patients with ureteral obstruction secondary to advanced malignancies traditionally have poor life expectancy measured in months even if relief of ureteral obstruction is achieved. In a prospective study of 205 patients with obstructive uropathy secondary to advanced cervical cancer, urinary diversion with PCN drainage or ureteral stenting was found to be associated with modest survival advantage in the months immediately after diversion^[16]. However, there was no significant difference in quality of life when compared to patients who elected not to undergo diversion^[16]. In contemporary studies, the median survival of patients with ureteral obstruction secondary to pelvic malignancies after urinary diversion ranges from 96 to 144 d^[17,18], with 88% mortality within one year of decompression^[18].

Objective criteria have been studied to prognosticate survival after urinary diversion in patients with ureteral obstruction secondary to advanced malignancy. Ishioka *et al*^[18] studied survival in 140 patients with urinary obstruction secondary to advanced incurable malignancies

and identified predictors of poor prognosis associated with shorter survival time after palliative urinary diversion by PCN: Serum albumin before diversion (≤ 3 g/dL), degree of hydronephrosis (grade 1 or 2) and three or more events related to disseminated malignancy^[18]. Patients with 2 or 3 of these predictors had a 2% survival rate at 6 mo while patients with none of these characteristics had a 69% survival rate^[18]. Cordeiro *et al*^[17], in a prospective study of 208 patients who underwent ureteral stenting or PCN for malignant ureteral obstruction, identified the number of events related to malignancy ≥ 4 and Eastern Cooperative Oncology Group (ECOG) index ≥ 2 to be significantly associated with poor prognosis after urinary diversion with a median survival rate of 7.1% at 12 mo in the unfavorable risk group^[17]. On the basis of these findings, ureteral stenting and nephrostomy tubes may not be indicated in poor risk patients.

Following ureteral stenting or PCN placement, quality of life may be impaired secondary to irritative urinary symptoms, pain, need for tube changes on a regular basis and often worse performance status^[19,20]. In a prospective direct comparison of the quality of life after nephrostomy or stent placement in 46 patients with malignant urinary tract obstruction, Monsky *et al*^[19] found no significant difference in the quality of life between treatment groups based on standardized validated surveys. In this study, patients managed with stenting reported more irritative voiding symptoms and pain while patients undergoing nephrostomy placement required more frequent tube changes secondary to complications.

In summary, there is no clear evidence that urinary diversion in the setting of malignant urinary obstruction improves the quality of life. Additionally, no significant difference has been reported between the two diverting modalities. Urinary decompression may be justified if improvement in renal function will facilitate systemic therapy and alleviate symptoms of ureteral obstruction. However, with an understanding that this specific condition entails poor prognosis, all treatment decisions must be decided on an individual basis with a multidisciplinary approach involving the patients, their family, and members of the treatment teams.

Complications of PCN and retrograde ureteral stenting

The complications profile differs for ureteral stents and nephrostomy tubes and warrants consideration when managing malignant ureteral obstruction. Patients with ureteral stents commonly experience irritative lower urinary tract symptoms and somatic pain, requiring some form of analgesia in up to 70% of patients within seven days of the procedure^[21]. Other complications such as stent failure from encrustation and obstruction, ureteral perforation, stent migration, stent fracture and the forgotten stent have been well documented^[22-24]. Mild hematuria is common after ureteral stenting as a result of urothelial irritation. Significant hematuria after ureteral stenting can be caused by arterio-ureteral fistula between

the ureter and the common or internal iliac arteries. This rare phenomenon has been reported in the setting of pelvic malignancies treated with surgery and radiation^[22]. On the other hand, external tubes and drainage bags as a part of daily PCN care have associated complications involving tube blockage, leakage and dislodgement requiring additional tube changes in up to 83% of patients compared to 16% with ureteral stents^[19]. Inadvertent bowel transgression is a rare complication of PCN when the colon lies in a retrorenal position. Pleural complications including pneumothorax, hemothorax, empyema, and hydrothorax may occur in less than 0.1%-0.2% of patients^[25]. Bleeding and gross hematuria may occur from puncture of intercostal vascular structures or parenchymal vessels, which are usually self-limited, requiring transfusion in 2%-4% of standard nephrostomy insertions^[22]. Late arterial bleeding occurs from pseudoaneurysms, arterio-venous and arterial calyceal fistulas secondary to injury of renal arterial branches^[22]. In a study by Song *et al*^[26] of 70 patients managed with PCN vs ureteral stenting for gynecologic malignancies, 14% of patients who underwent stenting were noted to have gross hematuria one week after insertion and 8% of patients had severe hematuria after PCN insertion secondary to cancer-related poor coagulation states. These complications were managed conservatively without need for acute intervention. In addition, there was no statistically significant difference in the overall complication rates between the two groups of patients^[26].

Inflammatory systemic complications such as sepsis, febrile urinary tract infections and pyelonephritis may develop as a consequence of drainage and manipulation of potentially infected, obstructed urinary systems, which are further compounded by the immunosuppressive state of advanced malignancy and subsequent systemic treatments. In studies of ureteral obstruction in advanced malignancies, Cordeiro *et al*^[17] reported a higher proportion of pyelonephritis in patients treated with PCN vs ureteral stenting ($P = 0.002$). Conversely, Ku *et al*^[27] reported acute pyelonephritis affecting 5.9% of patients managed with ureteral stents and 3.8% of patients with percutaneous nephrostomies, and febrile episodes in 10% and 15%, respectively. There was no statistically significant difference in the overall stent-related or nephrostomy-related complications as well as the accumulated incidence of inflammatory systemic complications between the two groups^[27]. Similarly, no significant difference was observed in the incidence of urinary tract infections between the two treatment modalities^[19].

While complications of PCN and ureteral stenting are well documented in the literature with variable incidences, data from comparative analyses of the two modalities are limited (Table 1), PCN and ureteral stent placement have comparable overall complication rates based on available evidence. Neither cystoscopic stent placement nor PCN insertion is exempt from major complications such as bleeding and sepsis or minor complications associated

Table 1 Percutaneous nephrostomy *vs* retrograde stent in malignant ureteral obstruction

Ref.	Study design	Cohort	Diagnosis	Stent	Complications	Nephrostomy	Complications	Mortality	Conclusions
Feng <i>et al</i> ^[1] , 1999	Retrospective, 1984-1996	<i>n</i> = 37 (20 female) patients with ureteral obstruction due to pelvic malignancy	Diuretic renogram or abdominal CT scan	22/31 underwent successful stent placement, 13/31 (42%) remained successfully diverted with stents	Migration (1), encrustation requiring cystolitholapaxy (1), intractable pain requiring repositioning (2)	6 had primary PCN placement, 9/31 had PCN placed due to unsuccessful placement of stent, 6/22 had PCN placed due to failed internal stent, 3 failed stents but did not have PCN placement	Dislodgement requiring reinsertion (3)	NR	33% of patients with disease confined to primary organ or locally advanced disease were managed successfully by stents <i>vs</i> 36% of patients with distant metastases, 92% of cervical cancer patients required PCN (89% failed initial internal stents), 50% of prostate cancer patients required PCN but 100% of patients who initially had successful stent placement did not require PCN at average follow-up of 15 mo, 100% colon cancer patients required PCN due to failure of internal stents
Hyppolite <i>et al</i> ^[2] , 1995	Retrospective, 1989-1994	<i>n</i> = 34 females with gynecologic malignancy	US and serum cr > 1.5 mg/dL	8 (3 had PCN as well)	6/7 (86%) developed urosepsis	17 (unilateral/bilateral)	7/17 (41%) (1 urosepsis, 3 bleeding, 3 urine leak)	2/34 died within 2 wk and declined intervention, 3/7 who underwent stent placement died from urosepsis from procedure	Stenting predisposes to urosepsis and should be avoided. Bilateral nephrostomy allows significant improvement of renal function
Kanou <i>et al</i> ^[48] , 2007	Retrospective, 1990-2003	<i>n</i> = 75 (45 female) patients with pelvic malignancy, patients with normal excretion from 1 kidney excluded	NR, need for primary PCN reported to be based on CT, MRI, or cystoscopic evaluation	37/51 underwent successful stent placement, 29/37 (78%) remained successful	Earlier replacement (5), discomfort requiring no intervention (2)	24 had primary PCN placement, 14/51 had PCN due to inability to place stent, 8/37 had PCN placed after failed stent	Dislodgement (9), obstruction requiring exchange (4), difficulty in exchange (2), pain/dermatitis (3), minor hemorrhage (2)	66/75 with mean survival of 5.9 (PCN) and 5.6 mo (stent)	Higher percentage (78%) of success may be related to utilizing stents without shaft side holes
Ku <i>et al</i> ^[27] , 2004	Retrospective, 2000-2002	<i>n</i> = 148 (80 female) patients with advanced malignancy causing ureteral obstruction	US, CT, or MRI with high grade obstruction, impaired renal function, clinical symptoms, or febrile UTI	68 (5 had antegrade stent placement), 60/68 (89%) remained successful	8 (11.8%)	80 (5 secondary PCN after failed stent placement), 1/80 failed PCN	7 (8.8%)	NR	Stenting and PCN placement have similar outcomes in terms of decreases in serum creatinine, complications, and incidence of pyelonephritis but significant differences in failure (11% stent <i>vs</i> 1.3% PCN) suggesting that patients with retrograde stenting may have ongoing obstruction requiring eventual PCN placement
Monsky <i>et al</i> ^[19] , 2013	Prospective survey	<i>n</i> = 30 (16 female) patients with malignancy-related ureteral obstruction	Initially evaluated by symptoms of urinary obstruction such as pain, deterioration of renal function, or infection and confirmed by CT	15 patients (22 stents)	Dislodgement (1), Pain (1), Infection (1), Fistula (1)	15 patients (24 PCN)	Dislodgement (7), Pain (4), Infection (3) Obstruction (4), Leak (1)	2/30	Patients with PCN or stents have similar QOL. Patient with stents have more irritative symptoms while PCN may experience more minor complications requiring more frequent changes
Song <i>et al</i> ^[26] , 2012	Retrospective, 2006-2010	<i>n</i> = 75 females with gynecologic malignancy	US, CT, or MRI with hydronephrosis, elevated cystatin(c), or clinical symptoms	61/75 underwent stent placement, 50/61 (82%) were managed with stents successfully	16/25	14/75 underwent PCN after unsuccessful stenting, 11/61 required PCN after failure with stent management	24/50	61/75 with mean survival of 9 mo for stent and PCN cohort	Progression to PCN was noted in patients with bladder invasion and severe hydronephrosis. Multivariate analysis revealed that obstruction > 3 cm and elevated cystatin(sic) > 2.5 mg/L predicted stent failure. Stenting was less expensive and required less procedural time as compared to PCN

CT: Computed tomography; PCN: Percutaneous nephrostomy; MRI: Magnetic resonance imaging; NR: Not reported/studied; US: Ultrasonography.

with impaired quality of life.

Efficacy of PCN and ureteral stenting in malignant ureteral obstruction

In the setting of malignant obstruction, ureteral stent placement has well described technical limitations. Cystoscopic placement of ureteral stents may be technically difficult in the setting of advanced malignancy and is associated with high failure rate when extrinsic obstruction is secondary to pelvic or retroperitoneal tumors^[28,29]. However, PCN requires an external collection device that often results in quality of life impairment for which some patients may initially refuse the procedure. As such, the most efficacious management of malignant ureteral obstruction has not been well established and remains controversial. In many instances, the type of urinary diversion may depend on clinician bias and expertise, procedure availability, and urgency of the diversion^[30,31].

It is well reported that a percutaneous approach to malignant urinary decompression has high technical success rates^[1,27,32]. When urgent relief of ureteral obstruction is the only factor in determining the modality of drainage, PCN appears to be the more reliable approach in the setting of advanced malignancy. Ku *et al*^[27] reported a greater chance of progressive loss of patency after ureteral stenting compared to PCN in which the incidence of failed diversion secondary to obstruction was 11% and 1.3%, respectively. Feng *et al*^[1] demonstrated initial success of stent placement in 71% of patients with pelvic malignancies with late stent failure in 41%, necessitating PCN placement with 100% success rate. In this study, 89% of cervical cancer patients failed initial stent placement and 92% ultimately required percutaneous drainage^[1]. In a similar setting, Ganatra *et al*^[11] reported late stent failure in more than one third of patients within 6 mo of initial stent placement. Gross tumor invasion evident at cystoscopy was a significant risk factor for stent failure with progression to PCN^[11]. Song *et al*^[26] reported successful management of ureteral obstruction secondary to gynecological malignancies by ureteral stenting in 67% of patients with greater trend toward PCN progression noted in patients with tumor invasion of the bladder. Other studies by Docimo *et al*^[29], Cheung *et al*^[33] and Yossepowitch *et al*^[34] demonstrated post-procedural stent failure rates in extrinsic malignant ureteral obstruction ranging from 42%–45%. Despite the high rate of ureteral stent failure, a difference in median survival between the two treatment modalities has not been demonstrated^[26].

In general, variables such as the type and level of obstruction, renal insufficiency, degree of hydronephrosis, systemic treatment post-stenting, cystoscopic evidence of bladder invasion and length of obstruction greater than 3 cm have been found to be predictors of stent failure in the setting of malignant ureteral obstruction^[11,26,33,34]. Furthermore, prostate, cervical and bladder cancers causing ureteral obstruction due to tumor invasion of the trigone have a higher primary stent failure rate compared to non-pelvic malignancies^[1,20]. Therefore, primary PCN placement should be advocated in these patients. Patients

with prostate cancer who underwent successful internal stent placement, however, were found to have long duration of stent function and low late failure rate^[20].

In summary, PCN is an effective method of diversion in patients with ureteral obstruction secondary to advanced malignancies. This should be the primary method of decompression in patients whose tumors are visualized to involve the urinary bladder. When adequate urinary decompression has been achieved, conversion of a PCN to an antegrade stent is possible, thus eliminating the need for nephrostomy collection devices to minimize complications and improve patient independence. For patients with other pelvic or non-pelvic malignancies, retrograde ureteral stenting may be attempted. If successful, long-term drainage may be expected, however close monitoring is required for late stent failures. If stent placement is unsuccessful, percutaneous drainage remains an option and is nearly always technically successful.

PCN VS STENTING IN THE SETTING OF NON-MALIGNANT URETERAL OBSTRUCTION

Non-malignant causes of ureteral obstruction can be intrinsic such as stone disease, ureteral stricture or congenital ureteropelvic junction obstruction, or extrinsic such as idiopathic retroperitoneal fibrosis.

Nearly all clinicians agree that obstructing stones, with a concern for sepsis require immediate decompression of the urinary system^[31]. Though large epidemiologic studies of the management of obstructed infected nephrolithiasis demonstrate higher rates of sepsis and mortality associated with PCN placement relative to ureteral stenting, the observational nature of the analysis highlights the need for prospective analyses of PCN vs stenting for obstructive nephrolithiasis^[35]. Despite this obvious need, there are few studies comparing the efficacy of ureteral stenting vs PCN in the setting of obstructive urolithiasis (Table 2). The choice between PCN and stenting is often made by the urologist at initial presentation and can be influenced by factors including disease severity, stone size, location of stone, eventual modality of definitive stone management, or even availability of in-house interventional radiology services^[31]. Retrospective studies reveal that both procedures have high success rates^[23,36]. In the setting of unsuccessful stenting, PCN is often successful, but the contrary is not always true. Furthermore, patients are often selected for PCN over ureteral stenting in the setting of larger stones and if they are more severely ill^[36,37]. Goldsmith *et al*^[36] studied 130 patients who underwent decompression for obstructing ureteral stone with PCN or stent placement. Although patients who underwent PCN placement had longer hospital stay, other outcomes such as time to definitive stone management, rates of spontaneous stone passage, and initiation of stone metabolic workup were not statistically different. The authors noted that the

Table 2 Percutaneous nephrostomy vs retrograde stent utilization in ureteral stone disease obstruction

Ref.	Study design	Cohort	Diagnosis	Stent	Complication	Nephrostomy	Complications	Conclusions
Ahmad <i>et al</i> ^[23] , 2013	Retrospective, 2010-2011	<i>n</i> = 300 (20/100 (stent) and 36/200 (PCN) had malignant obstruction)	NR	97/100 had successful placement	37/97 (38%) complication rate 3 (7 fever/sepsis, 10 bleeding/hematuria, 1 ureteral perforation, 2 stent migration, 5 stone encrustation) NR	195/200 had successful PCN placement	25/195 (12.8%) complication rate (7 fever/sepsis, 9 bleeding/hematuria, 9 dislodgement) NR	PCN had lower incidence of complications as compared to stenting
Goldsmith <i>et al</i> ^[36] , 2013	Retrospective, 1995-2011	<i>n</i> = 130 patients with infected urolithiasis who underwent procedural decompression	CT and 2/4 SIRS criteria	69/71 successful stent placement 2 proceeded to PCN	NR	58/59 successful PCN placement, 1 proceeded to retrograde stent	NR	Patients selected for PCN had larger stones and were more severely ill. Patients who underwent PCN had longer hospital stay on multivariable analysis. Time from septic event to definitive treatment, rates of spontaneous stone passage, and initiation of metabolic stone workup were the same between the two groups
Joshi <i>et al</i> ^[41] , 2001	Prospective, non-randomized	<i>n</i> = 34 patients (22 male) with obstructing ureteral stones	X-ray, US, IV urography	21	NR	13	NR	Stent patients were more likely to report hematuria, dysuria, urgency as compared to PCN patients. Stent patients required analgesics more frequently than the PCN group. Patients in the PCN required more daily care as compared to stent patients. EuroQOL questionnaire revealed differences in mobility, self care, and problems with usual activity and pain between the two cohorts but no significant differences in overall QOL
Mokhmali <i>et al</i> ^[38] , 2001	Prospective, randomized, 1996-1998	<i>n</i> = 40 patients with ureteral stone and evidence of infection	Imaging modality NR and 1 major (renal colic, fever, stone > 15 mm, sepsis and elevated Cr > 1.7 mg/dL) or 2 minor criteria (lower UTI, wbc change, diminished patient compliance)	16/20 successfully underwent stent placement	Fluoroscopy exposure > 2 min (40%), IV analgesics (35%)	20/20 underwent initial PCN, 4/20 underwent subsequent PCN due to failed attempted stent	Fluoroscopy > 2 min (10%), IV analgesics (10%)	Time to definitive therapy was longer in stent group as compared to PCN group due to persistent signs of urinary tract infection. Unsuccessful stent placement occurred in older patients and with stones located in proximal ureter. No statistical differences in QOL but a trend to lower QOL was seen in stent patients who were male or < 40 yr
Pearle <i>et al</i> ^[40] , 1998	Prospective, randomized, 1995-1997	<i>n</i> = 42 patients with ureteral stone and evidence of infection	IV pyelography, US, X-ray, CT, or retrograde pyelography with WBC > 17000 mm or temperature > 38 °C	21 underwent successful stent placement		20/21 underwent successful PCN, 1 proceeded to undergo retrograde stent placement		Fluoroscopy and procedural times shorter in stent vs PCN cohort. Higher number of positive urine cultures post-PCN was noted as compared to post-stent placement. Length of stay, blood culture positivity, and time to WBC and temperature normalization were not statistically different. Costs associated with stent placement more than twice of that of PCN. Increased back pain noted in PCN group
Yoshimura <i>et al</i> ^[37] , 2004	Retrospective, 1994-2003	<i>n</i> = 53 (59 events) patients underwent emergency drainage with ureteral stones and SIRS criteria	NR	35 stent events	NR	24 PCN events	NR	Patients who underwent stent had smaller stones but similar rates of ICU management as compared to PCN

SIRS: Systemic inflammatory response syndrome; QOL: Quality of life; US: Ultrasound; IV: Intravenous; WBC: White blood cell count; NR: Not reported/studied; ESWL: Extracorporeal shock-wave lithotripsy.

method of initial decompression correlated with eventual approach selected for definitive stone management. Patients treated with PCN were more likely to undergo percutaneous definitive management, while patients managed with ureteral stenting were more likely to be treated with a ureteroscopic approach^[36].

Two prospective studies comparing PCN vs stent management of obstructing ureteral stones have conflicting outcomes. Mokhmalji *et al*^[38] in 2001 prospectively randomized 40 patients to receive either PCN or stent. Sixteen out of twenty stents were successfully placed while all twenty PCNs were successfully placed initially. All unsuccessful stents were successfully managed by PCN. Their results demonstrated that stent utilization was less successful as compared to PCN and there was a trend for longer antibiotic therapy due to persistent signs of urinary tract infection in patients who underwent stent placement. Consistent with Mokhmalji *et al*^[38], a large epidemiologic survey reveals that stent failure as evidenced by the need for nephrostomy placement has been noted to be related to male gender, renal stone location, and acute kidney injury^[39]. In contrast, Pearle *et al*^[40] randomized 42 patients to receive PCN vs stents. This study failed to demonstrate one procedure to be more successful than the other^[40]. All 21 stents and 20 out of 21 PCNs were successfully placed. One failed PCN successfully underwent stent placement. Their results demonstrated an increased incidence of bacterial urinary colonization post-procedure in the PCN group as compared to the stent group, but overall no differences in time to clinical improvement or length of stay were noted.

In stone disease, the decision for PCN vs stent appears to be dictated by stone size and clinical presentation. The prospective studies looking at both procedures revealed no definitive best practice and nearly all of the studies reported on different outcomes making direct comparison impractical.

Quality of life with short-term PCN vs stent

Unlike malignant ureteral obstruction, decompression with PCN or stenting in stone disease is often short-term with eventual removal. In light of this, quality of life considerations for these patients are not necessarily the same as for those requiring long-term decompression, and should be studied in this population as well. Joshi *et al*^[41] prospectively surveyed 21 stent and 13 PCN patients using the EuroQol, a validated general quality of life questionnaire, as well as procedure specific questions focusing on symptoms in three categories - dysuria, pain, and daily care. Patients were surveyed by a single interviewer on the day of definitive therapy. Patients who had stents were more likely to require analgesic medications and reported urinary symptoms such as dysuria, hematuria, and urgency. PCN patients required more daily care of nephrostomy, but overall there were no statistically significant differences in utility scores calculated from the five EuroQol domains encompassing mobility, self-care, usual activity, pain/discomfort, and

anxiety/depression. Mokhmalji *et al*^[38] confirmed that there were no statistically significant differences between the two procedures in terms of general well-being and state of mind when assessing patients who underwent stent vs nephrostomy immediately post-operatively and 2-4 wk subsequently^[38]. These studies suggest that both stents and PCN decrease the quality of life and although patients did not prefer one procedure over the other, they should be made aware of potential discomforts associated with each procedure given the options.

PCN vs ureteral stent for idiopathic retroperitoneal fibrosis

Idiopathic retroperitoneal fibrosis is a rare disease of unknown etiology and is characterized by chronic inflammation within the retroperitoneum resulting in ureteral obstruction in up to 50% of cases^[42-45]. Mertens *et al*^[46] conducted a retrospective study of 30 patients with idiopathic retroperitoneal fibrosis involving 44 renal units from January 2002 to April 2010 with a median nephrostomy or stent dwelling time of 9.3 mo. PCN was placed as the first intervention in 27% of the entire cohort (12/44), and ultimately the majority of these patients (9/12) received subsequent ureteral stent placement^[46]. In contrast, the majority of renal units (32/44) initially underwent attempted stent placement, which was successfully initiated in 79% (25/32) and successfully maintained in 80% (20/25). The authors found that the overall rate of complication (obstruction, dislodgment, bleeding requiring transfusion, acute pyelonephritis, and urosepsis) was similar for both cohorts (PCN 21% vs stent 17.9%; $P = 0.79$). Ultimately, the investigators concluded that both stents and PCN were safe methods of urinary tract drainage with similar complication profiles. Complementary advantages were noted and the authors concluded that both methods of drainage may be utilized given the relapsing/remitting course of disease^[46].

CLINICIAN PREFERENCES FOR PCN VS URETERAL STENTING

Patient choice is heavily influenced by physician recommendations^[47], and as there are no clinical guidelines and little published evidence directing the use of PCN vs ureteral stenting, physicians often rely on their personal experience and preference in advising their patients. Further, patients may receive conflicting advice from various providers, as the clinician advising intervention is often not the clinician who also performs the intervention (medical oncologist vs urologist vs interventional radiologist).

In 2006, Lynch *et al*^[31] conducted a postal survey amongst 153 radiologists and 132 urologists residing in the United Kingdom to determine current opinion regarding utilization of PCN vs ureteral stent for acute renal obstruction. Despite a meager response rate of 19.3% (18.3% of radiologists and 19.3% of urologists), the authors demonstrated 90%-100% consensus for urinary

tract decompression for the clinical scenarios of “clinical sepsis” and “elevated creatinine and potassium”, while only 50% of clinicians felt unobstruction was indicated in the scenario of “ureteral obstruction with hydronephrosis with advanced malignancy for palliation”^[31]. Additionally, clinicians disagreed on the method of decompression with urologists favoring PCN over ureteral stent placement more often than radiologists for all clinical scenarios (74% vs 49%; median preference rate urologist vs radiologist) other than patients with “uncomplicated benign disease” and in those patients with “coagulopathy”. The authors speculated that the results were driven by logistical (availability of operating rooms and anesthesia) and patient factors (evidence of pelvic malignancy, radiotherapy, chronic upper tract stricture) rather than financial motives, given the absence of monetary incentives to providing care in the United Kingdom health system^[31].

Similarly, Hyams *et al.*^[30] sought to compare intervention preferences for malignant external ureteral obstruction utilizing a web-based survey sent to 3000 American clinicians (1500 urologists and 1500 medical oncologists). While only 15% of urologists and 12.4% of medical oncologists responded, there was significant disagreement between urologists and medical oncologists in regards to management of hypothetical clinical scenarios. For example, oncologists were more likely to recommend PCN as the next option after stent failure in unilateral obstruction (79% vs 62%, $P < 0.0001$), where as urologists were more likely to suggest stent manipulation including upsizing, stent exchange, internalizing, *etc.*, (37% vs 17%). Further, perception of complication varied between both groups. Urologists reported the greatest risk of dislodged PCN (48% vs 18%, $P < 0.0001$), while medical oncologists primarily feared infection (40% vs 8%). In regards to indwelling ureteral stents, urologists were most concerned about the negative impact on quality of life (65% vs 13%, $P < 0.0001$) while oncologists were again primarily concerned with risk of infection (43% vs 3%). Of note, both urologists and oncologists agreed that indwelling ureteral stents afford greater comfort (87% vs 93%, $P = 0.07$) and quality of life (95% vs 93%, $P = 0.46$)^[30]. Taken together, both studies indicate consensus amongst clinicians for urinary tract unobstruction in certain clinical scenarios (sepsis and AKI), yet significant divergence of opinion in other scenarios (malignant external ureteral obstruction). Additionally, the preference of PCN vs ureteral stenting varies both by clinician specialty and nationality^[30,31]. Both groups of investigators advocate for additional prospective studies, clinical guidelines, and ultimately increased communication between specialists^[30,31].

CONCLUSION

This review sought to find consensus on the use of PCN vs stents in the treatment of ureteral obstruction. There were no prospective studies that compared PCN vs stent utilization in the management of malignant ureteral

obstruction. Of the retrospective studies reviewed, the majority could not find a difference in complication rates or quality of life between the two procedures. Due to the retrospective nature of the studies, success rates could not be effectively compared between the two methods as patient selection for either procedure was based often on clinician and/or patient preference. In summary, most authors recommended stent utilization as a first step if possible and nephrostomy drainage if there is concern for difficulty in retrograde access of the ureters, or in the setting requiring immediate relief of kidney dysfunction. This area would certainly benefit from additional prospective studies as often the reasoning behind initial ureteral stent placement is driven by clinician preference arising from the belief that ureteral stents provide a decreased risk of infection and increased quality of life despite studies citing no statistical differences in these areas^[19,27].

Both retrospective and prospective studies were reviewed for management of obstruction due to stone disease. The retrospective studies were of heterogeneous quality demonstrating significant differences in stone size between patients who underwent PCN and stent placement. The prospective studies revealed that overall quality of life was similar although with different bothersome aspects in each of the two groups. Overall, stent utilization tended to require more analgesia as compared to PCN. The available studies revealed conflicting results on rates of infection between the two procedures, as well as time to definitive therapy, and length of hospital stay.

Although PCN and stent utilization appear to be mostly clinician-driven, certain patterns of practice are notable. Most clinicians prefer stent utilization due to presumed benefits associated with decreased rates of infection and improved patient comfort, while PCN utilization is noted for more definitive efficacy of urinary drainage. This review has revealed multiple studies showing either no difference or conflicting evidence regarding infection rates and we urge clinicians to be aware of this lack of clear superiority of ureteral stenting over PCN. Finally, given that quality of life studies have not demonstrated a clear superiority of ureteral stenting over PCN, when long term PCN and stent management is being considered, the patient should be centrally involved in the discussion, and the decision for either procedure will need to be agreed upon mutually.

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Sentinel lymph node biopsy in renal malignancy: The past, present and future

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Abstract

Sentinel lymph node biopsy (SLNB) is now an established technique in penile and pelvic cancers, resulting in a lower mortality and morbidity when compared with the traditional lymph node dissection. In renal cancer

however, despite some early successes for the SLNB technique, paucity of data remains a problem, thus lymph node dissection and extended lymph node dissection remain the management of choice in clinically node positive patients, with surveillance of lymph nodes in those who are clinically node negative. SLNB is a rapidly evolving technique and the introduction of new techniques such as near infra-red fluorescence optical imaging agents and positron emission tomography/computed tomography scans, may improve sensitivity. Evidence in support of this has already been recorded in bladder and prostate cancer. Although the lack of large multi-centre studies and issues around false negativity currently prevent its widespread use, with evolving techniques improving accuracy and the support of large-scale studies, SLNB does have the potential to become an integral part of staging in renal malignancy.

Key words: Sentinel lymph node biopsy; Dynamic sentinel node; Renal malignancy; Lymphoscintigraphy; Near infra-red fluorescence; Penile cancer; Lymphatic drainage

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Core tip: A number of studies have examined the use of sentinel lymph node biopsy in urogenital malignancies. In penile and prostate cancer it has been found to be a valuable tool to aid staging and accurately predict prognosis. Its use in renal cancer is poorly explored and would benefit from a better understanding of the lymphatic drainage of the kidney. It is also proposed that modifications of the technique such as use of positron emission tomography/computed tomography scanning and near infra-red fluorescence optical imaging agents may further improve the technique making it a feasible option for use in renal malignancy.

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INTRODUCTION

Renal cancer is now the 8th most common cancer in the United Kingdom and its incidence is rising^[1]. Advancements in imaging modalities and easy access to ultrasounds mean that tumours are often detected earlier and consequently with a smaller size than previously. Whilst size of tumour and haematogenous spread are acknowledged to be proportionately linked, small tumours do have the potential for early lymphatic spread and distant metastases^[2]. Unlike other urogenital malignancies such as penile cancer, lymphatic spread in renal cancer is often unpredictable making it unsuitable for en-block lymph node dissection^[3-5].

Sentinel lymph node biopsy (SLNB) offers a well-recognised alternative to lymph node dissection and is already widely used in melanoma and breast cancer^[6,7]. It is also already an accepted part of management in certain urogenital malignancies such as penile and pelvic malignancy^[8-12]. Associated with a lower mortality and morbidity cost than the traditional alternative, it still offers clinicians the opportunity to stage disease and equally importantly, to identify patients in whom tumour resection alone may not be curative^[10,13,14].

In renal cancer however, lymph node dissection and extended lymph node dissection still remain the management of choice in clinically node positive patients with renal malignancy, with surveillance of lymph nodes in those who are clinically node negative^[15]. Here, we examine the potential of SLNB in renal malignancy and some of the techniques that may be implemented in the future.

LYMPH DRAINAGE AND THE KIDNEY

The use and success of SLNB is reliant on the ability to reliably predict the lymphatic drainage of the organ and the dissemination of disease in a stepwise fashion. Of all urogenital malignancies, penile cancer exhibits the most reliable lymphatic drainage, allowing us to predict with some accuracy areas where the sentinel nodes will reside^[16]. Conversely, renal cancer, with the potential for both haematogenous and lymphatogenous spread is the least reliable, and it is only by using a mixture of cadaveric and sentinel lymph node mapping that basic patterns have been observed^[17]. Lymph node involvement in the absence of other metastases is common in pelvic and penile cancers, but uncommon in renal cancer.

Lymphatic drainage of the kidney can be grouped into three categories relative to their position to the renal vein: Anterior, posterior and intravascular. From the right kidney, the anterior bundles drain to the paracaval, precaval, retrocaval and interaortacaval nodes. Importantly the retrocaval nodes provide a route

of entry to the thoracic duct, facilitating more distant lymphatic spread. Posterior bundles drain to paracaval, retrocaval and interaortacaval nodes. Drainage from the intravascular bundles remains poorly understood^[18-20].

Different from the right kidney, anterior bundles in the left kidney drain to the para-aortic and pre-aortic nodes, while posterior bundles drain to the para-aortic and retro-aortic nodes. In the case of the left kidney, it is direct from the posterior bundle, rather than *via* nodes from the anterior bundle that connection to the thoracic duct is made^[17,21,22]. Lymphatic drainage from both kidneys may also run to the retroperitoneal lymph nodes, and from these spread to the thoracic duct. Overall, lymph node involvement is reported at rates of 4%-5% and considered to be a poor prognostic indicator^[23,24].

Despite not offering therapeutic benefit in renal malignancy, SLNB does offer the opportunity to histologically confirm the presence of positive nodes without full lymphadenectomy. In the absence of such clarity, the current European recommendation is to wait for nodes to become clinically palpable before excision, this can have significant implications on mortality. In penile cancer, since the introduction of SLNB and immediate lymphadenectomy for node positive patient, 3 year cancer survival increased to 84%, compared to just 35% for those who had lymph nodes excised only after they became clinically palpable^[25]. A further study reported a 5 year cancer survival of 91% for patients with penile squamous cell carcinoma after introduction of SLNB, compared to 82% before its introduction^[13].

THE SENTINEL NODE CONCEPT IN UROGENITAL CANCER: HOW DID WE GET HERE?

The concept of a sentinel node was introduced by Halstead who proposed that tumour cells spread from the primary lesion sequentially along the lymph chain, only spreading beyond the first node once it has been overwhelmed by tumour^[26]. It was Gould however, in a paper on parotid malignancy, who initially described these first nodes as sentinel nodes^[27].

When SLNB was first introduced, sentinel nodes were identified solely using either intraoperative or preoperative lymphangiograms. This was first trialled for urogenital malignancy by Cabanas^[28] in 1977. In a study of 100 patients he successfully proved the existence of a sentinel node in disseminated penile malignancy. In 46 of those patients he was able to perform lymphangiogram guided SLNB and from this concluded that a positive SLNB was a good indicator for further surgical intervention in the form of a full regional lymph node dissection^[28]. However, this technique was associated with a high failure rate and poor reducibility as nodes were often difficult to identify and locate and the technique did not allow for anatomical variation between patients^[29].

This concern was addressed with the introduction of blue dye allowing for cutaneous lymph node mapping. Once injected at the primary tumour site, the blue dye travels along the lymphatic chain to the sentinel node, making it easier for the surgeon to identify. Introduced in 1989 for melanoma, cutaneous lymph node mapping now has since been explored for use in breast, penile and cervical cancer^[30-34].

The concept of cutaneous mapping was rapidly followed by the introduction of radiolabelled tracer using a gamma probe. Proposed by the team at Vermont medical centre, their study, on 16 feline models, found that the use of radiolabelled tracer detected with a gamma probe was comparable to blue dye tracer but additionally allowed the surgeon to confirm excision of the correct node and determine possible presence of residual lymph nodes^[34].

In 2000, Horenblas *et al.*^[32] examined the feasibility of dynamic SLNB (DSNB) in penile cancer. Using a combination of lymphoscintigraphy, patent blue dye and a gamma probe they concluded that DSNB held potential as a promising staging technique^[35]. Their conclusion, supported by Tanis *et al.*^[8] who cited an 80% sensitivity for this procedure, cemented the role of DSNB in penile cancer. It was in this form that the Augsberg group introduced DSNB to prostate cancer^[36]. They successfully demonstrated the validity of DSNB for use in prostate cancer and in a further study of 117 patients, the same group demonstrated a sensitivity of 96% for the procedure, a validation replicated in bladder cancer in Sherif *et al.*^[37]'s study of 13 patients^[38]. They concluded that not only can DSNB be used to identify sentinel nodes in patients with known bladder cancer but that it has the additional advantage over traditional lymphadenectomy of identifying nodes outside the standard lymphadenectomy areas.

TAILORING THE SENTINEL NODE CONCEPT FOR RENAL CANCER

It was Bernie *et al.*^[39] in 2003 who introduced DSNB to renal cancer. Combining the use of blue dye and intra-operative gamma probes they successfully demonstrated that in 40 porcine models, excised sentinel nodes exhibited an increased radioactive count when compared to controls^[39].

In 2010 Bex *et al.*^[40] continued the work of Bernie, confirming the use of sentinel node mapping in renal malignancy in human models. They successfully demonstrated that the use of pre-operative lymphoscintigraphy combined with the injection of technetium 99m under either ultrasonography (US) or computed tomography (CT) guidance can be used to identify sentinel nodes in renal malignancy.

Single-photon emission computed tomography (SPECT) CT combines single photon emission computed tomography with CT in order to provide more precise information about the presence and location of sentinel

nodes. The concept of such anatomical fusion imaging, as an alternative to planar lymphoscintigraphy was first introduced for use in prostate cancer in 2005. That study successfully demonstrated that images from CT scan and SPECT scanning could be superimposed in all 12 of the patients studied and successfully identified 87% of lymph nodes^[41]. A Swedish study in 2006, expanded this work to bladder cancer when they successfully demonstrated that SPECT CT scanning detected 21 sentinel nodes in five patients, compared to just two with planar lymphoscintigraphy^[42].

In 2011, Sherif *et al.*^[37] trialled SPECT CT for use in lymph node mapping for renal cancer. Their study of 13 patients introduced pre-operative SPECT scanning to lymph node mapping in renal malignancy. They combined lymphoscintigraphy and SPECT CT imaging, with both radiolabelled tracer and patent blue dye in order to identify sentinel nodes. This study successfully detected 32 sentinel nodes in 10 of 11 patients, 28 of which were detected by the use of radiolabelled tracer. The patent blue dye was used in 8 patients but only identified sentinel nodes in one patient^[43].

SLNB IN RENAL CANCER: WHERE NEXT?

SLNB in renal cancer, still lags well behind its penile and pelvic counterpart and has some way to go before a widespread implementation can be considered. In addition to concerns about small studies, concerns about sensitivity-in particular false negatives, and patient selection remain.

Renal cancer is not alone in these concerns, with many papers initially raising similar concerns around false negative rates in penile and pelvic cancers. A study of 2020 patients undergoing SLNB for prostate cancer cited a false a negative rate of 6.2%, whilst a study in 2011 of SLNB in penile malignancy cited an even higher rate of 15%^[44,45]. In both cases, figures are controversial and highly variable, and measures such as pre SLNB CT to exclude macrometastases, a potential cause of false negatives, have been implemented^[8,44,46,47]. More importantly, SLNB has overcome these problems to become part of the accepted management for both penile and pelvic cancers.

Below, we discuss alternative or additional techniques that are currently being explored in other urogenital malignancies. These may hold the solution for the redemption of SLNB for use in renal malignancy.

IMPROVING SENSITIVITY

Near infra-red fluorescence optical imaging agents (NIRF) is a non-radioactive, more penetrative alternative to radiolabelled tracers and patent blue, which may provide the solution to concerns around sensitivity. First introduced in 2003 in mice models, it was initially studied in breast cancer, with Melancon *et al.*^[47] successfully demonstrating that NIRF provided a superior alternative

to T1 weighted MR, identifying all six cervical nodes, compared to just four^[48]. The first use of NIRF in urogenital malignancy was in 2011, when lymphatic pathways in prostate cancer were mapped with indocyanine^[49]. NIRF has since been used bladder cancer and in robot assisted SLNB in both bladder and prostate cancer^[50,51].

The introduction and acknowledgement of NIRF as a tracer, has led to the potential for a hybrid tracer, combining the fluorescence of NIRF with the well-established pharmacokinetics and bio-distribution of radiocolloids such as technetium 99m. The use of a multimodal tracer was first studied in mice in 2011^[52]. Since then its use has been studied in prostate and melanoma with the finding that it is equally effective tracer with faster distribution than blue dye^[53,54]. Similarly in penile cancer, a study of 65 patients, cited an increased sensitivity (96.8%) compared to patent blue dye alone (55.7%)^[55].

The use of positron emission tomography/CT (PET/CT) as part of the SLNB procedure has also been explored as a means of improving false negative rates. Here fluorodeoxyglucose PET/CT scan was performed routinely preoperatively in patients undergoing SLNB for penile squamous cell carcinoma. In a study of 129 patients, involving 254 basins, use of both techniques, reduced false negative rates to 5.6%, proving that it may have potential to improve the SLNB technique^[56]. PET/CT has been more vigorously explored in breast cancer, where a study of 191 patients concluded that it had the highest specificity of Ultrasound and MRI, but that it required all 3 in combination to reach the highest sensitivity^[57]. There is no current available work on its role in SLNB for renal cancer and its impact here remains to be seen.

IMPROVING PATIENT SELECTION

Patient selection remains one of the challenges of lymph node disease. At present all patients who are clinically node positive in all urological malignancy undergo full regional lymphadenectomy. Historically, those with node negative disease in penile and bladder cancer were undergoing SLNB despite concerns that a high false negative rate means that disease may go unidentified. To address this, colleagues in the Netherlands introduced an ultrasound scan for patients with clinically node negative disease. Any suspicious nodes visualised underwent fine needle aspiration and cytology. Those with a negative FNAC or absence of suspicious nodes proceeded to SLNB procedure, consisting of lymphoscintigraphy and injection of patient blue, whilst those with a positive FNAC proceeded straight to inguinal lymph node dissection. The introduction of the pre-operative ultrasound, combined with a decision to explore all groins after lymphoscintigraphy, rather than those with suspicious nodes, reduced their false negative rate from 19.2% to 4.8%^[58]. Similarly, a study of 500 inguinal basins, cited a 91% sensitivity rate with blue dye and radiolabelled tracer, which rose to a 94% with the introduction of the

pre-operative ultrasound^[59].

An alternative solution would be the introduction of mathematical algorithms such as the Partin table and Briganti nomograms used in prostate malignancy. These algorithms calculate the likelihood of lymph node involvement, and only those with a high calculated risk proceed to lymphadenectomy^[60,61]. The concept of identifying risk factors for positive lymph nodes in renal malignancy was first introduced in 2004 but it was Hutterer who created the first nomogram in 2007^[62,63]. In 2015 local symptoms, clinical node stage and lactate dehydrogenase were identified as independent predictors of lymph node disease, using all of these as determining factors in their nomogram which they cited as having a concordance index of 0.89^[64]. Further work and external validation has yet to be published and there is no current evidence to suggest that it could be extrapolated for an incorporation into use for SLNB.

IMPROVING MORBIDITY

One of the acknowledged benefits of SLNB when compared to the traditional alternative of lymphadenectomy is a reduced morbidity^[65]. This could be reduced further with the introduction of laparoscopic sentinel nodes, a theory explored by Kamprath *et al*^[66] in 2000, when they proved that laparoscopic sentinel nodes in cervical cancer would result in lower morbidity and also reduce post-operative pain, with shorter duration of stay when compared to an open procedure. Such a procedure has already been trialled in prostate cancer with good effect^[67].

Similarly, the SLNB has the potential to be performed robotically. This concept was explored by Rossi *et al*^[68], who concluded that a robotic lymph mapping procedure for use in endometrial and cervical cancer was not only feasible, but an efficient and reliable technique. A further study successfully used NIRF to identify sentinel drainage in pelvic cancers in robot assisted procedures^[50,51]. Whilst no direct comparisons have been made between traditional SLNB techniques and the robotic technique, a study comparing robotic and open surgical staging for endometrial cancer, demonstrated a lower incidence of post op ileus, duration of stay, infection and cardiopulmonary complications in patients who underwent a robot procedure whilst still achieving similar lymph node yields^[69]. If such findings can be extrapolated to SLNB and in particular to renal cancer, this may have a positive impact on morbidity.

CONCLUSION

SLNB offers the potential for accurate staging in renal cancer, the accuracy of which may have huge implications for prognosis. In its current form however, SLNB lacks not only the support of large, multi-centre studies but, like its predecessors in penile and pelvic malignancy, continues to be plagued by concerns around high false negative rates. With the investigation

and implementation of enhanced techniques, and support from large cohort size studies, SLNB does have the potential to become an integral part of staging in renal malignancy.

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Experimental models of renal calcium stones in rodents

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Abstract

In human nephrolithiasis, most stones are containing calcium and are located within urinary cavities; they may contain monohydrate calcium oxalate, dihydrate calcium oxalate and/or calcium phosphates in various proportion. Nephrolithiasis may also be associated with nephrocalcinosis, *i.e.*, crystal depositions in tubular lumen and/or interstitium, an entity which suggests specific pathological processes. Several rodents models have been developed in order to study the pathophysiology of intrarenal crystal formation. We review here calcium rodent models classified upon the presence of nephrolithiasis and/or nephrocalcinosis. As rodents are not prone to nephrolithiasis, models require the induction of a long standing hypercalciuria or hyperoxaluria (thus explaining the very few studies reported), conversely to nephrocalcinosis which may occur within hours or days. Whereas a nephrotoxicity leading to tubular injury and regeneration appears as a critical event for crystal retention in nephrocalcinosis models, surprisingly very little is known about the physiopathology of crystal attachment to urothelium in nephrolithiasis. Creating new models of nephrolithiasis especially in different genetic mice strains appears an important challenge in order to unravel the early mechanisms of urinary stone formation in papilla and fornices.

Key words: Nephrolithiasis; Nephrocalcinosis; Oxalate; Crystal; Urothelium

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Core tip: We review here calcium rodent models classified upon the presence of nephrolithiasis or nephrocalcinosis which appear as two different entities. Nephrocalcinosis appears related to tubular cell injuries in the setting of urinary supersaturation whereas the pathophysiology of nephrolithiasis is mostly unraveled. Though few models are available, attachment of crystals in the fornix or in the

papilla appear as a striking feature. Creating mice models of nephrolithiasis are thus required to understand the interaction between crystals and urothelium.

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INTRODUCTION

Renal stone is a common disease, occurring in 8% of the population. This disease is multifactorial and mainly considered related to environmental factors, especially western diet^[1]. Calcium stones are encountered in 80% of cases and contain calcium oxalate (72%), phosphate oxalate (14.7%) and often a mixture of the two^[1,2]. Among calcium oxalate crystals, calcium oxalate monohydrate crystalline form is oxalate dependent, whereas calcium oxalate dihydrate crystalline form is calcium dependent. Calcium deposits can be located within urinary cavities, in papilla and also in medullar collecting ducts as described in Cacchi-Ricci disease^[3]. Thus, though urine supersaturation is a prerequisite and accounts for crystal composition, it does not explain the diversity of calcium stone localization^[4]. Several animal models have been developed to investigate the pathophysiology of calcium oxalate nephrolithiasis, in rodent but also in larger animals such as porcine model^[5]. However, a majority of studies were performed in rodents due to an ease of access though most models lead to nephrocalcinosis instead of nephrolithiasis. Surprisingly, very few studies were performed in mice despite the possibility to create transgenic animals in order to study the early mechanisms of urinary stone formation in papilla and fornices.

We review here rodent models of calcium renal stone according to the presence of nephrocalcinosis, nephrolithiasis or the presence of the two features.

MODELS OF NEPHROCALCINOSIS

Oxalate minipumps

Administration of potassium oxalate (1.5 mol/L) by subcutaneous osmotic minipumps induces nephrocalcinosis in male Harlan-Sprague Dawley rats^[6,7]. Hyperoxaluria is detected as soon as day 1 and intrarenal deposits of COM crystals (birefringent crystals) are present mostly in tubules by day 14 with no reported renal failure. Interestingly, renal morphology of kidneys is normal although localized regions of inflammation are present. In some focal sites, tubular debris with cytoplasm vacuolization and also some regenerating tubules are present. At the time of crystal retention osteopontin (OPN), tumor necrosis factor (TNF) and kidney injury molecules (KIM) synthesis are significantly increased as assessed by northern blot^[6]

further assessing tubular injuries. No crystal retention within urinary cavities is reported (Table 1).

Intraperitoneal administration of oxalate

A single injection of sodium oxalate solution (7 mg/100 g body weight) in male Sprague Dawley rats is responsible for hyperoxaluria and CaOx crystals within tubules^[8]. Some small crystal aggregates are indeed present by 1 h in the loop of Henle, by 3 h in collecting ducts and by 6 h on papillary tips. Interestingly, crystals are initially intraluminal and later are located in tubular cells and also in the interstitium. CaOx crystals aggregate into tubular lumen leading to obstruction and lumen dilatation, with tubular cells necrosis, luminal cellular debris and exposure of basal lamina^[8]. The authors suggest that calcium oxalate crystals would appear in the lumen within proximal tubular segment and would coat cellular debris and urinary macromolecules, thus enhancing self aggregation and finally leading to tubular obstruction. Simultaneously, at the papillary tip, urothelial cells are injured with crystal deposits forming lesions suggestive of Randall Plaques^[8].

Ethylene glycol administration

Ethylene glycol (EG) administration is a well-known model of nephrocalcinosis: EG metabolizes into glycolate, glyoxylate, and oxalate leading to COM crystals in both urine and kidneys^[9]. Rats receiving EG-supplemented drinking water (0.75% vol/vol) develop hyperoxaluria and hypercalciuria one day after initiation^[10]. Moreover, intra tubular crystal deposits are detected as soon as day 1 both in medulla and cortex altogether with tubular injury, dilatation, regeneration and interstitial inflammation. Several macromolecule such as OPN, bikunin or Tamm-Horsfall (TH) protein that could either inhibit or promote calcification are also induced^[11]. Of notice, glycolate and glyoxylate metabolites seem to modify normal tubular epithelium into a crystal-binding epithelium^[10]. This EG model is currently used to study crystal binding molecules, crystal clearance and the relevance of several macromolecular inhibitors such as OPN in crystal retention^[10,12,13].

Hydroxyproline administration

The amino acid hydroxyproline (HyP) is a precursor of oxalate. In physiological conditions, HyP is first metabolized in mitochondria into glyoxylate and further metabolized to glycine by alanine glyoxylate amino-transferase (AGT) or to glycolate by glycolate reductase. Finally, glycolate is oxidized to oxalate by lactate dehydrogenase (LDH)^[14,15].

Diet supplements: After 7 d of exposure, Sprague-Dawley rats supplemented with HyP 5% in diet, develop a hyperoxaluria and many urinary crystals (a mixture of COD, COM, struvite and CaP crystals)^[16]. Noteworthy, after 28 d of supplements, all rats develop CaOx deposits in both medulla and cortex tubules, with some plaques

Table 1 Rodent models of nephrocalcinosis

Ref.	Species	Crystal main compounds	Crystals location	Animal Model	Read out	Urinary phenotype	Renal injury
Tawashi <i>et al</i> ^[17] , 1980	Rats	CaOx	Tubules	HyP injection <i>i.p.</i> (10 mL/kg),	1 d	Hyperoxaluria	Kidney dilatation, oedema
Khan <i>et al</i> ^[8] , 1982	Rats	CaOx	Tubules, interstitium, papillary tips	Sodium oxalate Injection (7 mg/100 g body weight)	1 h	Hyperoxaluria	Tubular obstruction and dilatation
Marengo <i>et al</i> ^[6] , 2004	Rats	COM crystals	Tubules (cortex, medulla)	potassium oxalate SC (Minipump)	14 d	Hyperoxaluria	Tubular injury, dilatation, regeneration, interstitial inflammation
Vervaeke <i>et al</i> ^[10] , 2009	Rats	COM crystals	Tubules	Ethylene glycol (0.75%)	1-6 d	Hyperoxaluria	Tubular injury, dilatation and regeneration, interstitial inflammation
Khan <i>et al</i> ^[16] , 2006	Rats	CaOx	Tubules (cortex, medulla), Papillary tips (plaques & stones)	5% Hyp supplement	28 d	Hyperoxaluria	Inflammation, tubular injury
Mo <i>et al</i> ^[20] , 2004	Mice	CaOx	Collecting ducts (medulla, papilla)	Tamm-Horsfall KO mice	2-4 mo		ND
Chau <i>et al</i> ^[23] , 2003	Mice	-CaP	Tubules (cortex, medulla), Papilla	Npt2 KO mice + HyP supplement (4 wk)	From birth	Hypercalciuria, Hyperphosphaturia	ND
Knight <i>et al</i> ^[24] , 2012	Mice	CaOx (COM + COD)	Tubules (Cortex, medulla) interstitium	GRHPR KO mice	After 4 wk (HyP supplements)	Hyperoxaluria	ND

CaP: Calcium phosphate; CaOx: Calcium oxalate; COM: Calcium oxalate monohydrate; COD: Calcium oxalate dihydrate.

and stones in papillary tips^[16]. In this model, crystal deposits are also associated with inflammation and damaged tubules; OPN is also up-regulated in tubules surrounding crystals; and hypercalciuria does not seem mandatory for renal CaOx deposits.

Intraperitoneal administration: In Sprague-Dawley rats *i.p.* administration of high dose of HyP (2, 5 g/kg), is followed by a massive deposition of calcium oxalate in renal parenchyma within 24 h with presumably an acute renal failure: Increased kidney volume and weight kidney, inflammation and oedema^[17].

Genetically modified animals

TH KO mice: Tamm Horsfall protein is synthesized by renal tubules and abundant in mammalian urine^[18,19]. TH is considered as a critical nephrolithiasis inhibitor, acting against crystal growth and aggregation^[1]. Indeed, TH KO mice between 2 and 4 mo old have crystal deposits located in medullar collecting ducts^[20]. Additional treatment with vitamin D and EG (1% in drinking water) for one month is responsible for a significant increase of crystal deposits especially in ascending limb of Henle loop (outer medulla), where TH is normally expressed. This model demonstrates the physiological relevance of TH protein in nephrocalcinosis prevention. Some studies suggest that the presence of Ca²⁺-binding domains and negatively charged sialylated residues would explain TH crystal inhibiting property^[21].

Npt2 KO mice: Co-transporters sodium/inorganic phosphate (Na/Pi) located in proximal tubules mediate 60%-70% of filtered phosphate reabsorption^[22]. In this

nephron segment, Na/Pi Type II a (Npt2) represents 80% of Na/Pi cotransporter mRNA and plays a major role in the maintenance of Pi homeostasis^[23]. Npt2^{-/-} mice are hypophosphatemic due to a renal Pi leak which in turn increases calcitriol synthesis resulting in the occurrence of hypercalciuria. Renal sections of Npt2^{-/-} pups mice display crystal deposits within tubules both in cortex, medulla and papilla. Interestingly, renal calcifications are increased in newborns and weaning pups and decrease thereafter in adult mice suggesting that the magnitude of urinary calcium and phosphate would be critical factors in this model. Accordingly, crystal deposits are mainly composed of hydroxyapatite (a calcium phosphate species) which is consistent with both a renal phosphate wasting and hypercalciuria. In this model, OPN is also up-regulated and expressed in tubules in the vicinity of apatite crystals. The reversal of crystal deposits appears also here as a remarkable feature.

GRHPR KO mice: Primary hyperoxaluria is a monogenic disease resulting from a liver enzyme deficiency. In type 1 primary hyperoxaluria, alanine glyoxylate aminotransferase, which catalyze transamination of glyoxylate to glycine in physiological conditions is deficient. In type 2, two enzymes are dysfunctional (glyoxylate reductase and hydroxypyruvate reductase), which normally catalyse the reduction of glyoxylate to glycolate and hydroxypyruvate to D-glycerate^[14]. Hence, increased glyoxylate which is finally oxidized to oxalate^[24] is responsible for a massive hyperoxaluria leading to nephrocalcinosis in human. Mice deficient in glyoxylate reductase (GR)/hydroxypyruvate reductase (GRHPR) or in alanine glyoxylate aminotransferase (AGT KO) develop as expected an inadequate

Table 2 Rodent models of nephrolithiasis

Ref.	Species	Crystals main compound	Crystals location	Animal model	Read out	Urinary phenotype	Renal injury
Bushinsky <i>et al</i> ^[25] , 2006	Rats	CaP ² CaOx ¹	Renal cavity (fornices) Papilla	Hypercalciuric rats (Genetic selection) under supplements ³ calcium 1.2% ⁴ calcium 1.2% + HyP 5%	18 wk after birth	Hypercalciuria Hyperoxaluria	None
Unpublished personal data	Mice	CaOx	Renal cavity (fornices)	Water supplement: HyP 4% + vitamin D (1000 UI) + ammonium chloride (0.28 mol/L) + calcium (0.25%)	15 d	Hypercalciuria Hyperoxaluria Hypocitraturia	None

¹Indicate the CaP or CaOx nature of crystal obtained either with ³calcium 1.2% or ⁴calcium 1.2% + HyP 5% as indicated column 5 above; ²Have been erased. CaP: Calcium phosphate; CaOx: Calcium oxalate; HyP: Hydroxyproline.

removal of glyoxylate^[24], with nephrocalcinosis occurring only in 25% of untreated GRHPR mice (crystal deposits are mostly intraluminal and few are located in the interstitium). However, after Hyp administration (1% in the diet) for one month, all GRHPR KO mice develop severe nephrocalcinosis, but only 20% for AGT KO mice. These data further strengthen the remarkable resistance of mice to renal crystal retention.

MODELS OF NEPHROLITHIASIS

Genetic hypercalciuric stone forming rats

Hypercalciuria is present in many patients with kidney stones (40%) and is often considered idiopathic. Hypercalciuria leads to urine supersaturation and thus increases calcium renal stone risk factors^[1,25]. Genetic hypercalciuric stone forming rats (GHS) (selected for 70 generations) have urine calcium excretion 8 to 10 fold above normal values. The authors demonstrated that hypercalciuria stems from 3 mechanisms: (1) an increased calcium intestinal absorption; (2) an decreased (Calcium sensor dependent) calcium tubular reabsorption; and (3) an increased bone resorption^[25]. As a matter of fact, after 18 wk of age, all GHS rats develop kidney stones. Noteworthy, stone composition is mostly apatite (CaHPO₄) when animals are fed with a standard 1.2% calcium diet, probably explained by urine CaHPO₄ supersaturation which increases faster than CaOx supersaturation^[26]. Conversely, an additional diet supplement of HyP 5% induces CaOx stones formation^[27] with crystal deposits mainly in contact with urothelial cells lining the papilla and in the fornix areas. Interestingly, similarly to tubular cells surrounding crystals, some urothelial cells in contact with crystals are indeed proliferating and also expressing high levels of OPN^[28]. This model thus appears very close to human renal stone disease as no nephrocalcinosis is observed though very rare and scattered crystal deposits may be detected within renal parenchyma (Table 2).

Hydroxyproline supplement in drinking water

Whereas Hyp administration in the diet induces nephrocalcinosis, Khan *et al*^[16] reported crystal deposits in renal pelvis and fornices after rat exposure to Hyp in drinking water for 28 d. A very few crystals are also seen in the tubules and in the papillary base. This model, thus also

looks very similar to human nephrolithiasis disease since most crystals are located within urinary cavities. Conversely to rats, mice develop mainly nephrocalcinosis after Hyp administration in drinking water (see below).

MODELS OF NEPHROCALCINOSIS ASSOCIATED WITH NEPHROLITHIASIS

Glycolic acid administration

Administration in male Wistar-strain rats of a glycolic acid diet during 4 wk leads to hyperoxaluria and CaOx tubular crystals both within cortex and medulla but also in pelvic cavities^[29] (Table 3).

Vitamin B-6 deficient diet

The liver enzyme alanine/glyoxylate amino-transferase which plays a key role in the conversion of glyoxylate (oxalate precursor) to glycine is vitamin B6 dependent; thus, vitamin B6 deficiency causes its dysfunction^[30]. Indeed, vitamin B6 deficient diet induces hyperoxaluria and hypocitraturia. Urinary oxalate excretion increases within 2 h after an intravenous hydroxypyruvate load in vitamin B6 deficient rats. These rats develop both calcium oxalate deposits in tubules, plaques on papillary tips and stones in renal fornices, pelvis and bladder^[31]. Another study slightly differs and shows only nephrocalcinosis with CaOx and CaP crystals in the papillary and parenchyma, tubular atrophy, interstitial fibrosis and chronic inflammatory infiltration^[32].

Small bowel resection

Ileal resection (IR) or bypass in humans may lead to massive hyperoxaluria and nephrolithiasis due to increased intestinal oxalate absorption^[33,34]. Indeed, CaOx nephrolithiasis has been estimated to occur in 15%-30% of patients after intestinal bypass surgery^[35]. The surgery decreases bile and pancreatic actions which trigger a poor fat absorption resulting into decreased calcium oxalate complexes and increased free oxalate (and oxalate salts which are efficiently absorbed in the colon segment) in intestinal lumen. Thus, dietary oxalates absorption increases leading to increased oxalate urine excretion^[35]. Moreover, these patients share a tendency to chronic volume contraction due to loss of water and

Table 3 Rodent models of nephrocalcinosis/nephrolithiasis

Ref.	Species	Crystals main compound	Crystals location	Animal model	Read out	Urinary phenotype	Renal injury
O'Connor <i>et al</i> ^[35] , 2003	Rats	CaOx CaP	Collecting ducts in cortex, medulla and papillary tips Urinary pelvis	Intestinal resection (40-45 cm)	4 mo after surgery	Hyperoxaluria, Hypocitraturia	Interstitial inflammation
Ogawa <i>et al</i> ^[29] , 1990	Rats	CaOx	Cortex and medulla tubules	Glycolic acid supplements	4 wk	Hyperoxaluria	
Di Tommaso <i>et al</i> ^[32] , 2002	Rats	CaOx CaP	Tubules, plaques on papillary tips, renal fornices, pelvis and bladder	Vitamine B6 deficient diet	12 wk	Hyperoxaluria, hypocitraturia	Tubules, inflammation

CaP: Calcium phosphate; CaOx: Calcium oxalate.

salt in diarrheal stool, which leads to decreased urine volumes. They also have decreased absorption, and therefore diminished urinary excretion, of citrate and magnesium, which normally act as inhibitors of CaOx crystallization^[34].

Intestinal resection (distal ileum) performed on male Sprague-Dawley rats fed individually with a low calcium and high oxalate diet (0.02% calcium, 18% lipid, 1% sodium oxalate) reproduces hyperoxaluria, hypocitraturia and nephrocalcinosis (by 4 mo). Calcium deposits are located in the cortex, medulla and papillary tip and contain CaOx, apatite and calcium carbonate^[35]. Of notice, crystal deposits are present in several collecting ducts associated with interstitial inflammation; crystal aggregates are detected near the fornix and 87% of kidneys display some calculi within pelvic lumen, measuring 0.5-2 mm^[35]. This model thus appears very similar to human enteric hyperoxaluria with both nephrocalcinosis and nephrolithiasis.

CONCLUSION

Concerning renal calcium stones, the most striking difference between rodents and humans lies in a special resistance of rodents to crystal retention noteworthy in female mice^[36]. Among all models, a hydroxyproline enriched diet responsible for both nephrocalcinosis and nephrolithiasis appears close to conditions encountered in a clinical setting when patient intakes of proteins are high. Nephrocalcinosis appears in several models due to the severity of oxalate burden but focusing data also epithelial phenotypical changes following injuries and/or crystal exposure would be a requirement for the onset of crystal adhesion and intratubular nephrocalcinosis, a mechanism called "fixed particle theory". *In situ* macromolecular inhibitors would in supersaturating condition unexpectedly promote crystal aggregation. The models associating nephrolithiasis and mild nephrocalcinosis suggest that such processes may be, at various degrees, more frequent in humans than expected. To date, no reliable models for Randall plaques are available with only two nephrolithiasis models but all in rats. Despite the differences between humans and rodents mentioned above, creating a nephrolithiasis model in mice appears indeed an important challenge

in order to better understand the early of urinary stone formation in papilla and fornices, the weight of calcium intake or absorption, enhanced bone resorption, and relevant macromolecules at play. It should also allow to test whether the "fixed particle theory" also applies to urothelium. Studying a nephrolithiasis model in specific genetically modified mice could also provide a deep insight into the very efficient rodent crystal clearance processes with potential translational applications.

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How botulinum toxin in neurogenic detrusor overactivity can reduce upper urinary tract damage?

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Author contributions: Baron M did the literature search and drafted the manuscript; Grise P did the literature search and added critical revision and comments to the manuscript; Cornu JN did the literature search, finalized the manuscript, provided critical revision of intellectual content, and supervised the work.

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Abstract

Intradetrusor injections of botulinum toxin are the

cornerstone of medical treatment of neurogenic detrusor overactivity. The primary aim of this treatment is to ensure a low pressure regimen in the urinary bladder, but the mechanisms leading to long-term protection of the urinary tract remain poorly understood. In this paper, we highlight the potential benefits of intradetrusor injections of botulinum toxin regarding local effects on the bladder structures, urinary tract infections, stone disease, vesico ureteral reflux, hydronephrosis, renal function based on a comprehensive literature review.

Key words: Botulinum toxin; Urinary tract infection; Kidney function; Neurogenic detrusor overactivity; Hydronephrosis; Urolithiasis

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Core tip: Intradetrusor injection of botulinum toxin prevent damage of the upper urinary tract *via* several potential mechanisms including reduction of bladder pressure, urothelium and suburothelium modifications, sensory receptors expression, and hypoxia reduction. These data could explain the favourable effects of intradetrusor injection of botulinum toxin on urinary tract infections, stone disease, vesico ureteral reflux, hydronephrosis, renal function.

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INTRODUCTION

Neurogenic lower urinary tract dysfunction is a highly prevalent disease, impairing significantly patient's quality of life, and results in a huge medico-economic burden^[1]. In particular, neurogenic detrusor overactivity (NDO)

is a common feature in the context of neurological diseases, resulting most of the time in urgency, increased frequency and urge urinary incontinence (UUI)^[2]. NDO (which can be associated with sphincter dyssynergia) is also at high risk of upper urinary tract deterioration in the long term, because of high bladder pressure, low BC and low bladder capacity. A bladder pressure > 40 cmH₂O has indeed been stated as the major urodynamic warning for upper urinary tract deterioration^[3]. This increase in bladder pressure can be due to NDO but also to detrusor sphincter dyssynergie in itself, that may need a specific treatment. The current review will only deal with the effect of botulinum toxin injection in the detrusor to treat NDO.

The management of NDO has two aims. While patients often focus on symptom relief (especially UUI), another objective is the long term protection of the upper urinary tract, in order to preserve renal function. To achieve these goals, NDO management must restore a continent, low pressure reservoir without vesico ureteral reflux (VUR), along with an adequate capacity and a good compliance. The first line treatment of NDO is antimuscarinics but many patients do not respond to antimuscarinics therapy, and require further treatment^[4].

Intradetrusor injections of botulinum toxin are one of the options available in patients who do not respond to medical therapy. This approach has now been approved and is extensively used for NDO management^[5]. Two types of botulinum toxin are available on the market: Abobotulinum toxin-A (Dysport) and onabotulinum toxin-A (Botox). Whilst the clinical efficacy of the treatment has been assessed in well-designed prospective studies and meta-analyses, the long term effect on prevention of upper urinary tract disease such as urinary tract infections (UTIs), VUR, hydronephrosis, stones, and chronic kidney disease is not fully understood yet.

The aim of this review was to summarize available evidence about how botulinum toxin can prevent upper urinary tract disease in a context of NDO.

EVIDENCE SYNTHESIS

Bladder effect

Urodynamic data: In addition to relief of lower urinary tract symptoms, intradetrusor injections of botulinum toxin have been shown to substantially modify the results of urodynamic studies in patients with NDO. Schurch *et al*^[6] in 2000, for the first time, injected 300 UI of botulinum-A toxin (Botox) in the detrusor of 21 patients with spinal cord injury (SCI) who had UUI by DO refractory to antimuscarinics. At 6 wk, 17 out of 19 patients (89.4%) had complete continence. The overall mean reflex volume (RV) significantly increased from 215 ± 90.4 mL before the injections to 415.76 ± 211.1 (P = 0.016) after the injections. The mean maximum cystometric bladder capacity (MCC) significantly increased from 296 ± 145.2 to 480 ± 134.1 (P = 0.016), respectively. There was also a significant decrease after treatment in mean maximum detrusor voiding pressure

(MDP) from 65.6 ± 29.2 cm water to 35 ± 32.1 (P = 0.016). Mean post-void residual urine volume (PVR) catheterized at the end of the urodynamic examination increased significantly from a mean of 261.8 ± 241.3 mL to 490 ± 204.8 (P = 0.016).

Following this first proof of concept, a number of other trials have investigated the changes in UDS after Intradetrusor injections of botulinum toxin. Through another prospective randomized, placebo controlled, double-blind, multi-center in 2005, Schurch *et al*^[7] have found no significant difference between 200UI and 300UI of onabotulinum toxin A injections. However this comparative study was focused on incontinence episodes as a primary endpoint and do not allow conclusion about the comparison of urodynamic features. Reitz *et al*^[8] in a prospective, open labeled study, used 300 U of Botox in the detrusor of 231 neurologic patients [167 SCI, 11 MS, 22 myelomeningocele (MMC)]. The evaluation was made at 12 and 36 wk. At 12 wk, the mean MCC increased significantly from 272 to 420 mL (P < 0.0001) while the mean MDP decreased from 61 cmH₂O to 30 cmH₂O (P < 0.0001). The mean bladder compliance (BC) also increased significantly from 32 mL/cmH₂O to 72 mL/cmH₂O and the mean PVR increased from 236 to 387 mL (P < 0.0001). At 36 wk, the results were quite similar although the mean BC was not significantly different from baseline (32 mL/cmH₂O to 51 mL/cmH₂O). These urodynamic data were correlated with a rate of 132 (73.3%) patients fully continent at 12 wk.

In a long term follow-up of 17 SCI patients at 6 years after 300 UI botulinum-toxin A injections, Giannantoni *et al*^[9] in 2009 showed persistent urodynamics modifications. The uninhibited detrusor contraction (UDC) first volume increased significantly from 213 ± 40.8 at baseline to 344 ± 32.6 at 4 mo, 365.4 ± 49.7 at 1 year, 410.8 ± 60.2 at 3 year, 413.7 ± 58.9 at 6 years (P < 0.001 between baseline and 6 years follow-up). Correspondingly the UDC maximum pressure decreased from 97.6 ± 32.4 to 23.8 ± 10.8 at 6 years (P < 0.01) whereas maximum cystometric capacity increased from 243 ± 64.7 to 420.8 ± 55.7 (P < 0.001).

The results are quite similar with Abobotulinum toxin A (Dysport). Del Popolo *et al*^[10] in 2008 have retrospectively evaluated three Dysport doses (500 U, 750 U and 1000 U) in 199 patients with SCI and NDO refractory to antimuscarinics. The evaluation was made at 3, 6 and 12 mo. The mean MCC increased significantly from 226 to 407 mL after the first injection and was still at 380 after seven injections while the mean BC significantly increased from 27 mL/cmH₂O to 41 mL/cmH₂O after one and seven injections. There was no significant difference between all doses. In 2010, our team has reported the comparison of two Dysport doses (500 U and 750 U) in a prospective, double-blind, randomized, comparative trial^[11]. Seventy-seven patients were included, 49 had SCI, 18 had MS and 11 had other neurological causes. At four weeks, the mean MCC increased from 242 to 434 mL with Dysport 500 U and from 180 to 423 mL with Dysport

750 U. The BC also increased from 32 to 37 mL/cmH₂O and from 23 to 59 mL/cmH₂O for Dysport 500 U and 750 U respectively. There were no significant differences between two groups.

These studies highlight the important urodynamic modifications induced by intradetrusor botulinum toxin injections.

Pathophysiology: Botulinum toxin act at the neuromuscular junction level by temporarily blocking acetylcholine (ACh) presynaptic release from parasympathetic nerves. It induces a paralysis of the detrusor smooth muscle that induce urodynamic changes and symptoms relief.

Serotype type A cleaves the SNAP-25 protein complex which plays an important role in the fusion of neurotransmitter-filled transmitter vesicles with the plasma membrane and their release during exocytosis. This induces an highly specific blockage of acetylcholine release at the neuromuscular junction of somatic and autonomic presynaptic nerve terminals^[12].

Those fragments of SANP-25 protein complex are detectable in the bladder for longer periods that would be expected in striated muscle^[13]. However, this motor effect does not entirely explain all the bladder changes. In fact, at the bladder level, BoNT/A seems to have a role in modulating both efferent and afferent neurologic activity, *i.e.*, both motor and sensitive fibers^[14].

Apostolidis *et al*^[15] showed that BoNT/A injections for human DO decrease sensory receptors P2X3 and TRPV1 levels in suburothelial nerve fibers. Those sensory receptors are overexpressed in neurological bladder suburothelium and are believed to play a role in sensory signal transduction in normal animal bladder^[16]. At 4 and 16 wk after BoNT/A intradetrusor injections in 38 patients (22 with neurologic DO and 16 with idiopathic DO, there was a significant decrease in P2X3-immunoreactive and TRPV1-immunoreactive (-IR) ($P < 0.0004$ and $P < 0.0008$, respectively), when significant improvements were observed in clinical and urodynamic parameters. P2X3-IR and TRPV1-IR fibers decrease were significantly correlated with reduction of urgency episodes at 4 and 16 wk ($P < 0.0013$ at 4 wk and $P < 0.02$ at 16 wk), but not maximum cystometric capacity or detrusor pressures.

Conte *et al*^[17] also showed that, after BoNT/A injections for detrusor overactivity, patients with Parkinson disease or SCI, significantly reduced at MCC, the expected soleus Hoffman reflex (H reflex) inhibition, whereas in those with SCI, it turned the H reflex facilitation into a slight inhibition. This reflex (basically defined as a reflexory contraction of muscle after stimulation of the related sensory fibers) tests the afferent information from the bladder (C and A δ fibers) that modulates the spinal motoneuron excitability. Those results highlight the fact that BoNT/A might influences H reflex modulation at MCC by reducing bladder afferent signalling.

However, motor effect seems to play a major role in increasing MCC and BC and decreasing MDP significantly. It creates a low pressure bladder during

filling and storage phases. The ureteral outlet may be affected by a bladder pressure over 40 cmH₂O or by a BC under 10 mL/cmH₂O leading to upper urinary tract functional obstruction^[3]. Prolonged periods of elevated detrusor pressure during bladder filling or voiding have been found to put the upper urinary tract at risk^[18]. Primary aim of therapy in patients with such problems is conversion to a low pressure bladder during filling even if this leads to incomplete emptying and the need to supplement emptying with catheterization.

Effect on UTIs

Clinical results: The impact of intra-detrusor botulinum toxin injections on UTIs has been investigated in various clinical trials. Gamé *et al*^[19] in 2008 has evaluated the impact of BoNTA 300 U on symptomatic UTIs (sUTIs). sUTIs were defined by the association of bacteriological criteria and symptoms such as fever, intensification of spasticity, intensification of autonomic hyperreflexia, pain and worsening of the neurological status. Of the thirty patients, 15 had SCI, 14 had MS and 1 had myelitis. All had at least one episode of sUTIs during the 6 mo prior to the injection (mean number 1.79 ± 0.39 per patient). At 6 mo, the number of sUTIs decreased significantly (0.2 ± 0.41) ($P = 0.003$) with only three patients having sUTIs (one pyelonephritis, one prostatitis, one orchitis). Of those three patients, two had SCI and one suffered from MS and they were those in whom BoNTA injections had the least effect on urodynamic changes. The overall incidence of bacteriuria was 43%.

In 2009, Giannantoni *et al*^[9], at 6 years follow-up of 300 U botulinum toxinA injections, in 17 SCI patients, reported a decreased in UTIs episodes from 6.7 ± 2.1 per year at baseline to 1.6 ± 1.3 at 4 mo, 3.3 ± 2.1 at one year, 1.7 ± 2.0 at 3 years and 1.8 ± 0.5 at 6 years ($P = 0.001$ between baseline and 6 years). However the definition of symptomatic UTI used in the trial is not specified.

Cruz *et al*^[20], evaluated in 2011 the safety of onabotulinumtoxinA, in a randomized, double-blind, controlled study vs placebo. 275 patients (121 SCI, 154 MS) were randomized in three groups (92 to placebo, 92 to onabotulinumtoxinA 200 U, and 91 to onabotulinumtoxinA 300 U). The mean rate of UTI was similar between all treatment groups, including placebo, in the SCI population (50%, 52%, 56.4% for placebo, 200 U and 300 U groups) whereas in the MS population, it was higher in the onabotulinumtoxinA groups compared with placebo (32%, 58%, 70% for placebo, 200 U and 300 U groups). Twelve percent, 30%, and 42% of patients in the placebo, onabotulinumtoxinA 200-U, and 300-U groups respectively, initiated CIC after the first injection. However, this level 1 study presented a major pitfall, that is the absence of clear definition of UTIs. Indeed the authors confused symptomatic UTIs (with clinical signs, including fever, and a positive urine culture) and asymptomatic bacteriuria (colonization), that is obviously increased by the high rate of self catheterization. In another level 1

study, Ginsberg *et al.*^[21] evaluated the safety of BoNTA in a randomized, double blind, controlled placebo trial in 416 patients (227 MS, 189 SCI). Two doses of Botox were used (200 U and 300 U). At 12 wk evaluation, the most frequent adverse effects reported were UTI and urinary retention. In MS population, the rate of UTI was higher after BoNTA than placebo (51% and 50% in 300 U and 200 U groups vs 28% for placebo) while it was similar in all groups in patients with SCI (42%, 48%, 50% in placebo, 200 U, 300 U groups respectively). But again, the authors disclosed that there was no clear definition between symptomatic and asymptomatic UTIs, so these studies cannot result in valuable hypotheses.

In a more focused study, Kuo *et al.*^[22] reported in 2011, among 132 onabotulinumA 200 U injections in 33 SCI patients, nine episodes of febrile UTIs (6.8%) and 37 (28%) episodes of asymptomatic UTI. Herschorn *et al.*^[23] in 38 patients with SCI and 19 with MS found a similar rate of UTI between placebo and 300 UI of onabotulinum A: 55 and 57% respectively. However, he didn't separate MS and SCI patients. Jia *et al.*^[24] in 2013 found similar results in men with SCI receiving 300 U of botox. The mean number of sUTIs prior to surgery was 1.49 ± 1.43 per patient over 6 mo and decreased to 0.78 ± 0.96 ($P = 0.023$) at 6 mo post-operatively. However, the overall sUTIs frequency had the tendency to decrease in patients who developed two or more UTIs before injection and to increase in patients who presented one or zero UTI before injection. The sUTIs included two acute epididymitis episodes. The others were acute pyelonephritis.

Physiopathology: UTIs are a major cause of morbidity and one of the main reasons for hospitalization in neurologic patients^[25]. It must be distinguished from asymptomatic bacteriuria, which is not threatening for the patient. One important confounder in clinical studies about intradetrusor injections of botulinum toxin is that treated patients often practice self catheterization, that increases the risk of *asymptomatic bacteriuria*. But the overall rate of *symptomatic* UTIs is thought to be decreased.

In the neurogenic patient, there are some structural and physiological factors that can be related to an increased risk of UTIs including: Over-distention of the bladder, vesicoureteral reflux, high pressure voiding, large post-void residuals, presence of stones in the urinary tract, and outlet obstruction (detrusor-sphincter dyssynergia, urethral stricture, enlarged prostate)^[26]. The method of bladder drainage has also a strong influence on UTI. The use of clean intermittent catheterization (CIC) has permitted to significantly overall decrease the mean rate of UTI in patients with neurological disorders^[26], despite the fact that CIC are associated with asymptomatic bacteriuria^[27].

Botulinum toxin injections and CIC (when needed) result in both a low pressure bladder regimen and minimal post-void residual, that are two conditions lowering the risk of symptomatic UTIs. Indeed, the

major factor of UTI is DO (eventually combined with outflow obstruction) which induces maximum detrusor pressure^[28], resulting in reduced blood flow as shown by animal models^[29]. Focal bladder hypoxia is associated with further deterioration of the detrusor function and fibrosis^[30,31], and has been postulated to favor adherence of bacteria to the urothelium^[32].

Many other mechanisms have been proposed as key factors influencing occurrence of UTIs (Figure 1). Wöllner *et al.*^[33] have shown that BoNT/A had a no direct antibacterial effect. Thirunavukkarasu *et al.*^[34] demonstrated a high modulation of genes and pathways involved in neuroinflammation, focal adhesion, cell adhesion molecules and gap junctions genes in intestinal epithelial cell lines treated with botulinum toxin A. Although it has not been studied, there might be the same effects in the urothelium that could decrease bacterial adhesion.

Moreover, the symptoms of UTI presented by neurological patients may be induced by a local inflammation arising from the local release of inflammatory mediators such as substance P (SP), neurokinin A, glutamate and calcitonin gene-related peptide (CGRP) from afferent nerves. Bacteria could cause a direct stimulation of afferent A-delta and C-fibres with an increased release of those neurotransmitters inducing dysuria, urgency, frequency and general symptoms such as malaise, fever and increased spasticity. *In vitro*^[35] and *in vivo*^[36] analysis have shown an effect of botulinum toxin in reducing glutamate release and decreasing pain. This might alleviate bladder symptoms and the awareness of sUTI by the patients. Furthermore, CGRP is a potent vasodilator, and SP enhances vascular permeability. These substances are involved in the physiological control of blood flow. The potential effect of botulinum toxin on modulation of inflammation and sensory pathways and its potential influence on UTIs occurrence remains to be elucidated.

EFFECT ON UPPER URINARY TRACT

Effect on VUR and hydronephrosis

VUR causes UTI, hydronephrosis and alters the upper urinary tract by mechanically delivering infected urine to the renal pelvis. BoNTA injections have been postulated as having a positive influence on VUR through various ways.

Clinical data: Very few studies have evaluated the impact of BoNTA on VUR and renal pelvis dilatation. To our knowledge, no studies have ever reported on VUR nor renal pelvis dilation induced by botulinum toxin as a primary outcome. Classically, trigonal injections are avoided owing to the potential risk of precipitating VUR from inhibition of the active trigonal antireflux mechanism. Nevertheless, according to the literature review by Davis *et al.*^[37] in 2015, no study has shown new onset of RVU nor worsening of preexisting RVU, induced by trigonal injections.

In the opposite, RVU treated by BoNTA injections

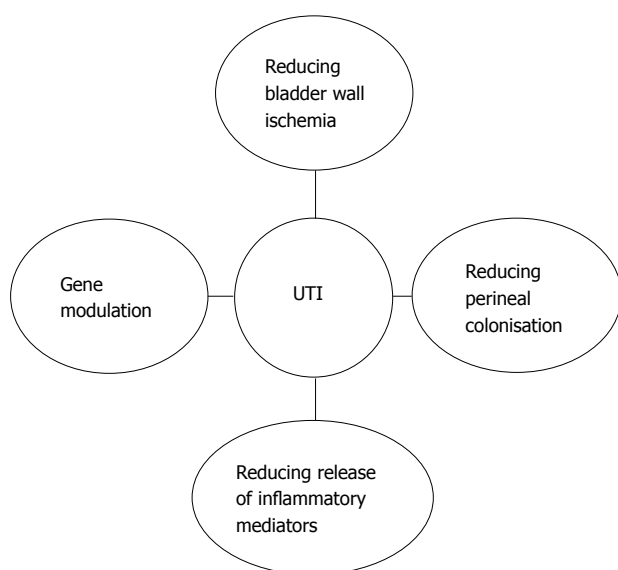


Figure 1 Pathophysiological explanation of how botulinum toxin A might decrease urinary tract infection occurrence. UTI: Urinary tract infection.

has been described. In a randomized, single-blinded study, Abedl-Meduig^[38] compared initial detrusor vs combined detrusor-trigone 300 IU BTX-A injections in 38 adults with SCI and refractory neurogenic urinary incontinence due to NDO. At baseline 2 patients in the detrusor arm had unilateral grade 2 and 3 VUR while 2 in the combined arm had unilateral grade 1 and 3 VUR, respectively. At week 8 no patient had new onset VUR or upgrading of preexisting VUR. Mascarenhas *et al*^[39] in 2008, performed trigonal injections in 21 neurological patients (12 SCI, 8 viral myelitis, 1 MS), 20 had no VUR previous to the injection and 1 had VUR grade II unilateral. At 8 wk evaluation, no cases of *de novo* VUR were detected and the patient with preinjection VUR had complete resolution of the reflux. For Gamé *et al*^[19] who used 300 U of BOTOX in 30 neurologic patients, 6 patients had VRR previously to the injections, and only 2 had one reflux remaining after the injections. But the difference was not significant. None of them had infections after treatment. Giannantoni *et al*^[9] in 2009 studied 17 SCI patients with DO. Three had VUR of grade III prior to treatment. At one year post injection, no one had persistent VUR.

Arrabal-Polo *et al*^[40] in 2012 have presented the case of a children non neurologic who presented a primary reflux and was successfully treated with botulinum toxin after failure of endoscopic treatment (Deflux and Macroplastique).

Giannantoni *et al*^[9], detected on kidney and bladder ultrasound, bilateral and monolateral renal pelvis dilatation in six and five patients, respectively, before the injection. At for weeks after the 300 U Botox injection, the dilatation disappear in all patients. Those results were maintained at 3 and 6 years follow up. In Mascarenhas study^[39], of 21 neurological patients, four (19.0%) had mild hydronephrosis and one (4.8%) had moderate hydronephrosis at baseline. Postoperative ultrasound after 8 wk of BoNTA injections,

showed no hydronephrosis in 20 (95.2%) patients and mild hydronephrosis in 1 (4.8%, $P = 0.125$).

Pathophysiology: The occurrence of VUR result from different mechanisms which defines whether reflux is considered as primary or as secondary. In general, VUR is considered primary if there is a deficiency of the uretero-vesical junction (UVJ). Secondary reflux is caused by overwhelming of the normal function of the UVJ. Bladder neurological dysfunction is often the root cause of secondary reflux^[41]. Chronic increases in intravesical pressure resulting from bladder outlet obstruction or detrusor overactivity can distort bladder architecture and UVJ. It can cause herniation of the bladder mucosa through the weakest point of the hiatus above the ureter and produce a "Hutch diverticulum" and secondary reflux^[42].

Uretero-hydronephrosis is also induced by high bladder pressure. Thus, reducing bladder pressure by botulinum toxin may improve VUR and hydronephrosis. Although low bladder pressure is achieved, a major deterioration of the UVJ might lead to persistent VUR. Indeed, increase wall tension in the ureter might lead to a significant decrease in smooth muscle perfusion and cause ischaemic lesion in the ureter^[43].

This emphasizes the importance to control the bladder pressure at the initial stage of an overactive neurogenic bladder in order to avoid secondary damages on bladder and upper urinary tract. These data also point out the potential interest, notably in children, of an urodynamic evaluation as primary VUR can be due to an anatomical defect but also a severe voiding dysfunction, especially if bilateral.

Effect on bladder and renal stone

Clinical data: No study has evaluated the relationship between botulinum toxin and renal stones. Only Wefer *et al*^[44] in 2010 reported less than 6 patients out of 214 (2.8%) presenting bladder stones. However he was not able to determine whether these disorders was BoNTA treatment related. Ginsberg *et al*^[21] reported in 2012, out of 416 patients, only one case of bladder stone formation after 300 U botox injection.

Pathogenesis: Renal and bladder calculi are an important source of morbidity for patients with neurogenic bladder. The incidence of renal stones in neurogenic patient is about 6.8%^[45] higher than in the common population. Old series have reported that most of the calculi were of struvite, induced by UTI^[46]. However, more recent trials have established that stones may also be of metabolic origin. For instance, Matlaga *et al*^[47] in 2005 has evaluated 32 renal calculi in a population of MMC and SCI, and found only 37.5% of struvite calculi and 62.5% of metabolic calculi. This modification of the origin of the stones might be due to a decrease in UTI in neurological population over the years, due to improvement of the urinary conditions in those patients.

By decreasing the mean rate of UTI and UUT

Table 1 Urinary tract infections after botulinum toxin injections in contemporary series

Ref.	Type of toxin	Patients	sUTI before injections	sUTI after injection	P	Bacteriuria % (n)	Symptomatic and asymptomatic UTI after injections	P
Gamé <i>et al</i> ^[19] , 2008	Botox 300 UI	30 15 MS 14 SCI 1 Myelitis	1.79/pp/6 mo	0.2/pp/6 mo	0.003	43		
Giannantoni <i>et al</i> ^[9] , 2009	Botox 300 UI	17 SCI	6.7/pp/yr	1.8/pp/yr	0.001			
Cruz <i>et al</i> ^[20] , 2011	Placebo Botox 200 UI 300 UI	154 MS					Placeb: 32% 200 UI: 58% 300 UI: 70%	P < 0.05 (vs placebo)
Cruz <i>et al</i> ^[20] , 2011	Placebo Botox 200 UI 300 UI	121 SCI					Placebo: 50% 200 UI: 52.6% 300 UI: 56.4%	
Kuo <i>et al</i> ^[22] , 2011	Botox 200 UI	33 SCI		6.80%		28 (37)		
Herschorn <i>et al</i> ^[23] , 2011	Placebo Botox 300 UI	57 38 SCI 19 MS					Placebo: 55% 300 UI: 57%	
Ginsberg <i>et al</i> ^[21] , 2012	Placebo Botox 200 UI 300 UI	227 MS					Placebo: 28% 200 UI: 51% 300 UI: 50%	
Ginsberg <i>et al</i> ^[21] , 2012	Placebo Botox 200 UI 300 UI	189 SCI					Placebo: 42% 200 UI: 48% 300 UI: 50%	
Jia <i>et al</i> ^[24] , 2013	Botox 300 UI	SCI 41	1.49/pp/6 mo	0.78/pp/6 mo				

sUTIs: Symptomatic urinary tract infections; SCI: Spinal cord injury.

dilatation, BoNTA injections may lead to decrease the incidence of struvite calculi but further studies are warranted.

Patients with neurogenic bladder are at increased risk of bladder stone formation. According to Chen *et al*^[45], within 10 years after SCI, 15% to 30% of patients will have formed at least one stone. The risk of forming a subsequent stone quadruples when a patient has already formed one stone^[48]. Furthermore, the manner in which the bladder is managed in SCI appears to have a significant impact on the risk of stone formation. One large study of over 450 patients noted that the use of CIC was associated with a significant reduction in the risk of bladder stone formation, with an annual risk of 0.2%, compared with 4% in those patients managed by a chronic indwelling catheter^[48].

CIC in patients treated by BoNTA might be beneficial for decreasing bladder stones formation. In the opposite, in patients who were not using CIC previously to the injections, there might be and increase risk of developing bladder calculi. However, this remains hypothetical and needs to be further established by dedicated, well performed clinical trials.

Effect on chronic kidney disease

Clinical data: In a long term follow-up of 17 patients during 6 years after 300 U of botulinum injections,

Giannantoni *et al*^[9] didn't show any impairment of renal function. Kuo *et al*^[21] evaluated the impact of botulinum toxin 200 U on renal function in 33 patients with supra sacral SCI. Videourodynamic and 99mTc-DTPA renal scanning for glomerular filtration rate (GFR) were performed at screening and every 3 mo during 24 mo of assessment. Onabotulinum toxin injections were repeated every 6 mo. GFR significantly decreased throughout the treatment course (96.27 ± 22.50 at baseline vs 83.51 ± 23.96 at 24 mo, $P = 0.028$). There was no significant change in mean serum Cr levels during the same period (0.623 ± 0.183 vs 0.675 ± 0.175 , $P = 0.802$).

In 2014, the same team^[49] evaluated the effect of 300 U vs 200 U of onabotulinum toxinA on renal function in 72 SCI patients. During the follow-up period, the changes in GFR from baseline to all time points did not differ significantly within each group or between the two groups. At baseline, the GFR was 94.2 ± 22.1 mL/min and 84.2 ± 19.6 mL/min in 200-U and 300-U groups, respectively. At the end-point, the GFR was 90.5 ± 24.2 mL/min and 88.0 ± 28.2 mL/min in the 200-U and 300-U groups, respectively.

There were no significant difference between 300 U group and 200 U group ($P = 0.197$) neither between group with compliance > 30 and group with low compliance (< 30).

Four patients had improved their renal function (2 in 200 U and 2 in 300 U group) at the end of the study. Inhibited detrusor contracture decreased significantly after the second detrusor injection of 300-U of onabotulinumtoxinA compared to that in the 200-U group.

Pathophysiology: The ultimate consequence of all upper urinary tract complications in neurological patients is the impairment of renal function. Although bladder management methods have evolved in recent decades, chronic renal insufficiency remains a significant cause of morbidity and it is one of the major concern to have in mind when treating those patients^[50].

In urodynamics studies, a bladder pressure > 40 cmH₂O mostly due to detrusor hyperreflexia and low BC are the major risk factors for renal damage in SCI patients^[3]. However, CIC, antimuscarinic therapy, and regular urodynamic monitoring have been reported to reduce the risk of renal failure^[51].

These studies show that renal function remains stable when patient have urodynamics modifications after botulinum toxin injections but without significant improvement (Table 1). However, the median term in follow-up of these series may be a limit for renal function study. The neurological disorder is also an important point to consider and SCI patients are more at risk of renal deterioration than multiple sclerosis patients. It highlights the fact that patients must be followed carefully on long term after botulinum injections.

Early and repeated detrusor onabotulinumtoxinA injections could therefore be beneficial to SCI patients before upper urinary tract deterioration.

An explanation why detrusor botulinum injection may not improve renal function is that anatomical renal damages may be irreversible, and also that renal deterioration may be caused by other factors. In particular, SCI patients are at higher risk to develop cardiovascular disease than others^[52]. Many other confounding factors in neurological patients can induce renal impairment such as, diabetes, obesity, lipid disorders, metabolic syndrome, and disturbances of the autonomous nervous system, which may result in blood pressure abnormalities, arrhythmias and cardiac disease^[51,53].

All these factors have to be taken into account when evaluating the long-term impact of on kidney function in the neurological patients. For the moment, this has not been correctly assessed and BoNTA are postulated as protective for the urinary tract in the long term, mainly through indirect benefits.

CONCLUSION

Botulinum toxin injections regulate urodynamic parameters in a context of neurogenic OAB. It furthermore may have a positive effect on UTIs, but this has to be put in perspective with the increased use of CIC. There is also an anticipated positive effect of BoNTA injections on hydronephrosis, VUR and stone disease, but with

a weaker level of evidence. Long term effects on renal function are also probably positive, but this parameter remains multifactorial.

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Observational Study

Matrix metalloproteinase-2 as a superior biomarker for peritoneal deterioration in peritoneal dialysis

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Author contributions: All authors contributed to the drafting and approval of the manuscript; Kusano E and Nagata D managed this multicenter clinical study; Morishita Y, Inoue M, Akimoto T, Saito O and Muto S collected the patients' clinical data and samples; Hirahara I analyzed the biomarker levels and data and wrote this paper.

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Abstract

AIM: To investigate the efficacy of effluent biomarkers for peritoneal deterioration with functional decline in peritoneal dialysis (PD).

METHODS: From January 2005 to March 2013, the subjects included 218 PD patients with end-stage renal disease at 18 centers. Matrix metalloproteinase-2 (MMP-2), interleukin-6 (IL-6), hyaluronan, and cancer antigen 125 (CA125) in peritoneal effluent were quantified with enzyme-linked immunosorbent assay. Peritoneal solute transport rate was assessed by peritoneal equilibration test (PET) to estimate peritoneal deterioration.

RESULTS: The ratio of the effluent level of creatinine (Cr) obtained 4 h after injection (D) to that of plasma was correlated with the effluent levels of MMP-2 ($\rho = 0.74$, $P < 0.001$), IL-6 ($\rho = 0.46$, $P < 0.001$), and hyaluronan ($\rho = 0.27$, $P < 0.001$), but not CA125 ($\rho = 0.13$, $P = 0.051$). The area under receiver operating characteristic curve for the effluent levels of MMP-2, IL-6, and hyaluronan against high PET category were 0.90, 0.78, 0.62, and 0.51, respectively. No patient developed new-onset encapsulating peritoneal sclerosis for at least 1.5 years after peritoneal effluent sampling.

CONCLUSION: The effluent MMP-2 level most closely reflected peritoneal solute transport rate. MMP-2 can be a reliable indicator of peritoneal deterioration with functional decline.

Key words: Encapsulating peritoneal sclerosis; Matrix

metalloproteinase-2; Peritoneal dialysis; Peritoneal solute transport; Peritoneal deterioration

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Core tip: Peritoneal effluent samples were obtained from 218 peritoneal dialysis (PD) patients with end-stage renal disease at 18 centers. The effluent levels of biomarkers matrix metalloproteinase-2 (MMP-2), interleukin-6, hyaluronan, and cancer antigen 125 were measured. Peritoneal solute transport rate was assessed by peritoneal equilibration test (PET) to estimate peritoneal deterioration. Among the biomarkers in the effluent, MMP-2 level correlated most significantly with peritoneal solute transport rate. The area under the receiver operating characteristic curve analysis for effluent MMP-2 level against high PET category was 0.90. MMP-2 may be a superior biomarker for peritoneal deterioration during PD.

Hirahara I, Kusano E, Morishita Y, Inoue M, Akimoto T, Saito O, Muto S, Nagata D. Matrix metalloproteinase-2 as a superior biomarker for peritoneal deterioration in peritoneal dialysis. *World J Nephrol* 2016; 5(2): 204-212. Available from: URL: <http://www.wjgnet.com/2220-6124/full/v5/i2/204.htm> DOI: <http://dx.doi.org/10.5527/wjn.v5.i2.204>

INTRODUCTION

Long-term peritoneal dialysis (PD) causes peritoneal deterioration with structural changes and functional decline such as ultrafiltration loss and increased solute transport rate, resulting in the cessation of PD treatment; thus, these are the major problems related to PD. At worst, peritoneal deterioration may even cause encapsulating peritoneal sclerosis (EPS), a serious but rare complication of PD with extremely high mortality rate^[1-4]. To undergo PD safely and adequately, preventing the progression of peritoneal deterioration is important.

The mechanism of peritoneal injury is not well-known, but it may develop through multiple factors such as infectious peritonitis and continuous exposure to non-physiological PD fluid with low pH, high osmolarity, and high glucose and glucose degradation product levels^[2-5].

Solute transport rate through the peritoneal membrane is often measured by peritoneal equilibration test (PET) to estimate PD efficiency and peritoneal deterioration^[3,4,6,7]. Transport rate increases with peritoneal deterioration and a state of higher transporter membrane is a factor contributing to the development of EPS in patients on PD. However, the D/P creatinine (Cr) value from a single test of the PET is not sufficiently predictive of EPS, and monitoring the time-course changes are necessary^[4]. In addition, PET is invasive because it requires blood sampling and patients need to spend half a day in hospital or clinic because the test takes a long time. Therefore, the PET is not performed routinely in many medical centers. It is thus necessary to

evaluate peritoneal deterioration using an easy and non-invasive method.

Some biomarkers such as matrix metalloproteinase-2 (MMP-2), interleukin-6 (IL-6), hyaluronan, and cancer antigen 125 (CA125) in the peritoneal effluent are often measured non-invasively to estimate peritoneal deterioration^[2,4,8-20].

MMP-2 degrades extracellular matrix components such as fibronectin and type IV collagen, which comprise the basement membrane. MMP-2 is produced by mesenchymal cells, macrophages, and endothelial cells in the peritoneum and plays important roles in angiogenesis, epithelial-to-mesenchymal transition (EMT) of mesothelial cells, inversion of transdifferentiated mesothelial cells, and migration of cells that promote inflammation or fibrosis^[2,5,8,10,21-23]. Effluent MMP-2 level correlates with peritoneal solute transport rate and has a possibility to predict EPS^[8-10]. On the other hand, Lopes Barreto *et al.*^[20] recently reported that the time course of MMP-2 appearance rates, studied by mean values from a model of repeated linear measures 4 years prior to EPS diagnosis, showed no difference between long-term controls and patients with EPS. IL-6 is a cytokine involved in acute phase inflammation and its effluent level is associated with high peritoneal solute transport rate^[2,4,13,14]. Goodlad *et al.*^[19] reported that effluent levels of IL-6, monocyte chemotactic protein-1, and CCL15 (eukotactin) did not improve prediction of future EPS compared with a model that used known clinical risk factors although these cytokines were found at higher levels in the effluent of patients who subsequently developed EPS. Hyaluronan, a large glycosaminoglycan that is constitutively synthesized by mesothelial cells, plays important roles in the maintenance of mesothelial cell morphology, re-mesothelialization, and wound repair^[15]. Intraperitoneal hyaluronan production increases with membrane permeability and length of time on PD^[16]. Effluent hyaluronan level may be a useful biomarker to assess functional and morphological changes of peritoneum^[4,16]. CA125 is a high molecular weight glycoprotein that is secreted from mesothelial cells; its concentration in effluent and appearance rate can indicate a change of the peritoneal mesothelial cell mass^[4,16-18]. According to the guidelines for peritoneal dialysis of Japanese Society for Dialysis Therapy^[4], it is very hard to evaluate peritoneal deterioration by a single biomarker, and, at present, no examination can be an absolutely reliable diagnostic method alone. For this reason, comprehensive judgment based on the results of multiple examinations is needed. Presently, the establishment of a simple and highly reproducible method with high sensitivity and specificity is extremely important.

The aim of this study was to compare many biomarkers and understand their individual properties and to confirm the efficacy of effluent biomarkers for peritoneal deterioration in peritoneal membranes with high solute transport rate.

MATERIALS AND METHODS

Design

The trial was conducted as a prospective, observational study at 18 centers in Japan. All patients were followed for at least 1.5 years after the measurement of effluent levels of the biomarkers.

Patients

PD patients with end-stage renal disease were analyzed based on peritoneal effluent biomarkers during the period of January 2005 through March 2013. Patients with bacterial peritonitis at the time of the analysis or in the preceding 4 wk were excluded from this analysis. After analysis of biomarkers, all patients were followed-up for more than 1.5 consecutive years to confirm for development of EPS.

Analysis of biomarker levels in the peritoneal effluents

The peritoneal solute transport rate was assessed by PET^[3,4,6]. Intraabdominal fluid was drained and 2 L of PD fluid containing 2.27%-2.5% glucose was injected intraperitoneally. The Cr level of peritoneal effluents obtained 4 h after the injection (D) was divided by that of plasma (P) to obtain the D/P Cr ratio. The glucose level of peritoneal effluents obtained 4 h after injection (D) was divided by that obtained immediately after injection (D0) to obtain the D/D0 glucose ratio. The effluent levels of MMP-2, IL-6, hyaluronan, and CA125 obtained at PET were measured by enzyme-linked immunosorbent assay (MMP-2: GE Healthcare, NJ, United States; IL-6: RD System, Inc., Minneapolis, MN, United States; hyaluronan: Seikagaku Biobusiness, Tokyo, Japan; CA125: Immuno-spec Co., CA, United States).

Statistical analysis

Statistical analyses were performed using R statistical software version 2.15.1 (R Foundation for Statistical Computing). Receiver operating characteristic (ROC) curve analyses were performed to evaluate the diagnostic accuracy of MMP-2, IL-6, hyaluronan, and CA125. Comparisons between two groups were performed by Wilcoxon's test. Relationships between clinical variables and effluent biomarker levels were analyzed by Spearman's correlation test. A *P* value of < 0.05 was considered to be significant.

Clinical trial registration

This study was registered as the MAJOR IN PD study (Multi-center Analysis in Japan, ORiginal INdicator of Peritoneal Deterioration) in the University Hospital Medical Information Network-Clinical Trials Registry (UMIN-CTR), which was approved by the International Committee of Medical Journal Editors (No. UMIN000010572).

RESULTS

A total of 218 PD patients, including one patient with EPS, were analyzed and their characteristics are sum-

Table 1 Characteristics of peritoneal dialysis patients

Characteristics of patients	
Sex (male/female)	122/96 (56% male)
Etiology (non-DM/DM)	179/36 (17% diabetes)
Age (yr)	56 (20, 46, 64, 84)
PD duration (mo)	40 (1, 15, 63, 206)
Peritonitis episode (times)	0 (0, 0, 1, 9)
D/P Cr	0.65 (0.38, 0.59, 0.71, 0.88)
D/D0 glucose	0.40 (0.21, 0.34, 0.44, 0.59)

Data except sex and etiology of renal failure are expressed as median with minimum value, interquartile range, and maximum value. PD: Peritoneal dialysis; Cr: Creatinine; DM: Diabetes mellitus; non-DM: Non-diabetes mellitus.

Table 2 Biomarker levels in the peritoneal effluent

Biomarker	Level in the peritoneal effluent
Matrix metalloproteinase-2 (ng/mL)	159 (14, 107, 220, 681)
Interleukin-6 (pg/mL)	14.6 (1.5, 7.6, 29.6, 253.5)
Hyaluronan (ng/mL)	109 (5.1, 53, 204, 889)
Cancer antigen 125 (U/mL)	6.7 (0.7, 4.0, 12.1, 91.8)

Data are expressed as median with minimum value, interquartile range, and maximum value.

Table 3 Correlation between patient characteristics and effluent biomarker levels

	Biomarker levels in the peritoneal effluents			
	MMP-2	IL-6	Hyaluronan	CA125
Sex (male/female)	<i>P</i> = 0.92	<i>P</i> = 0.54	<i>P</i> = 0.48	<i>P</i> < 0.05
Etiology (non-DM/DM)	<i>P</i> = 0.18	<i>P</i> = 1.00	<i>P</i> = 0.56	<i>P</i> < 0.05
Age (yr)	ρ = 0.076	ρ = 0.12	ρ = 0.046	ρ = -0.096
	<i>P</i> = 0.27	<i>P</i> = 0.092	<i>P</i> = 0.50	<i>P</i> = 0.16
PD duration (mo)	ρ = 0.050	ρ = 0.15	ρ = 0.25	ρ = -0.062
	<i>P</i> = 0.47	<i>P</i> < 0.05	<i>P</i> < 0.01	<i>P</i> = 0.93
Peritonitis episode (times)	ρ = 0.17	ρ = 0.25	ρ = 0.092	ρ = -0.012
	<i>P</i> < 0.05	<i>P</i> < 0.001	<i>P</i> = 0.20	<i>P</i> = 0.86

ρ values were derived from Spearman's correlation coefficient. PD: Peritoneal dialysis; MMP-2: Matrix metalloproteinase-2; IL-6: Interleukin-6; CA125: Cancer antigen 125; DM: Diabetes mellitus; non-DM: Non-diabetes mellitus.

marized in Table 1. Biomarker levels in the peritoneal effluent of all patients are shown in Table 2. The relationship between the effluent levels of the biomarkers and the characteristics of the patients is shown in Table 3. Effluent CA125 level was affected by sex and etiology of end-stage renal disease. Effluent IL-6 and hyaluronan levels correlated significantly with PD duration (*P* < 0.05, *P* < 0.01, respectively). Effluent MMP-2 and IL-6 levels significantly reflected the number of peritonitis episodes. The peritoneal solute transport rate determined by PET was correlated with MMP-2, IL-6, and hyaluronan levels in the effluent and the correlation coefficient between D/P Cr and MMP-2 level was significantly the highest (Figures 1 and 2, Table 4). The effluent level of each biomarker was correlated with that of the remaining biomarker (Table 4). In the patient with EPS, MMP-2, IL-6, hyaluronan,

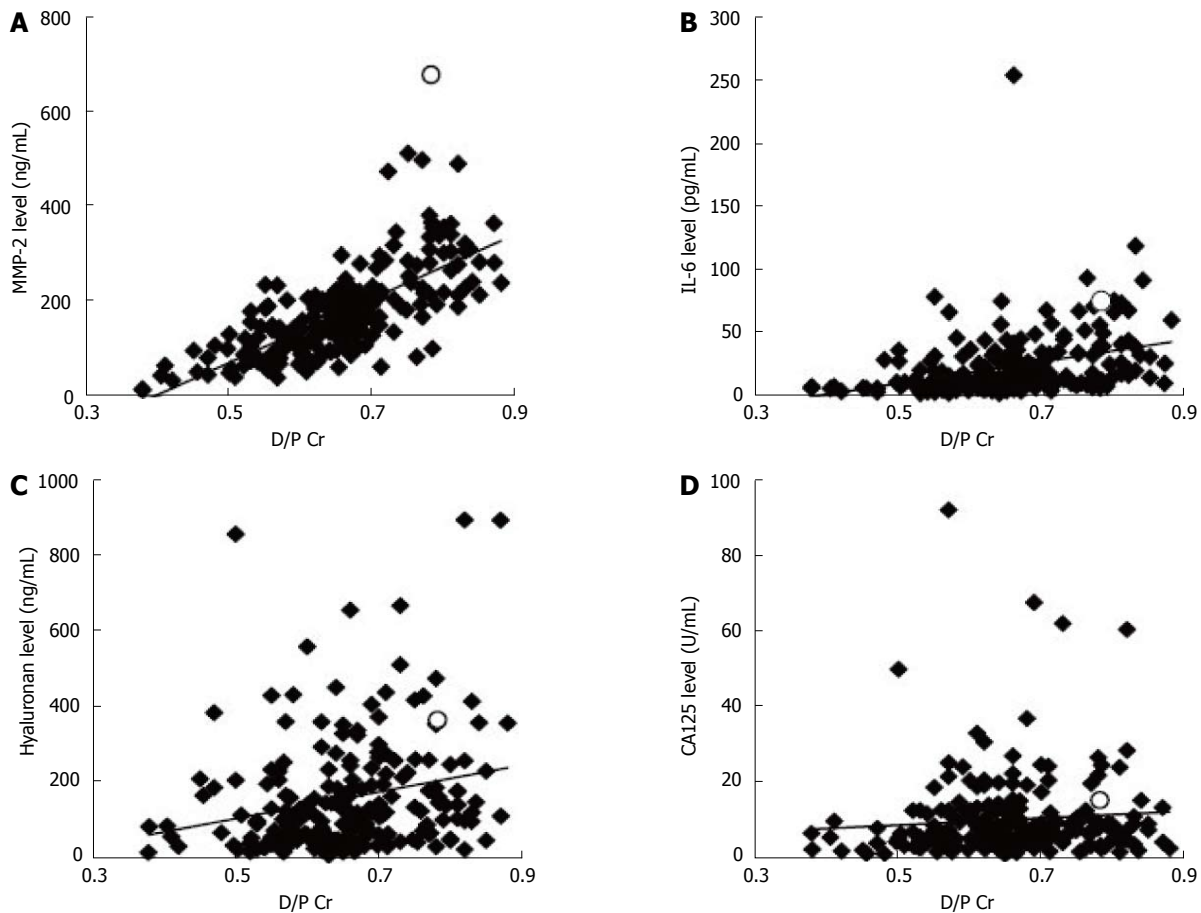


Figure 1 The relationships between peritoneal solute transport rate and effluent biomarker levels. Peritoneal transport rate was represented by D/P Cr ratio and was compared with effluent MMP-2 level (A), effluent IL-6 level (B), effluent hyaluronan level (C) and effluent CA125 level (D). The biomarker levels of the one patient with EPS are shown by open circles. MMP-2: Matrix metalloproteinase-2; IL-6: Interleukin-6; CA125: Cancer antigen 125; Cr: Creatinine; EPS: Encapsulating peritoneal sclerosis.

Table 4 Correlation between effluent biomarker levels and the results of peritoneal equilibration test

	D/P Cr	D/D0 glucose	MMP-2	IL-6	Hyaluronan	CA125
D/P Cr	$\rho = 1$	$\rho = -0.86$ $P < 0.001$	$\rho = 0.74$ $P < 0.001$	$\rho = 0.46$ $P < 0.001$	$\rho = 0.27$ $P < 0.001$	$\rho = 0.13$ $P = 0.051$
D/D0 Glucose		$\rho = 1$	$\rho = -0.65$ $P < 0.001$	$\rho = -0.28$ $P < 0.001$	$\rho = -0.22$ $P < 0.005$	$\rho = -0.094$ $P = 0.19$
MMP-2			$\rho = 1$	$\rho = 0.54$ $P < 0.001$	$\rho = 0.40$ $P < 0.001$	$\rho = 0.1$ $P < 0.05$
IL-6				$\rho = 1$	$\rho = 0.45$ $P < 0.001$	$\rho = 0.26$ $P < 0.001$
Hyaluronan					$\rho = 1$	$\rho = 0.18$ $P < 0.01$
CA125						$\rho = 1$

ρ values were calculated by Spearman's correlation coefficient. Cr: Creatinine; MMP-2: Matrix metalloproteinase-2; IL-6: Interleukin-6; CA125: Cancer antigen 125.

and CA125 levels were 681 ng/mL, 74.7 pg/mL, 362 ng/mL, and 15.1 U/mL, respectively; his effluent MMP-2 level was the highest among all PD patients. The highest MMP-2 level was 514 ng/mL among patients without EPS onset. In the patient with the highest IL-6 level (253.5 pg/mL), there was accumulation of ascitic fluid with C-reactive protein level of 1.3 mg/dL; his effluent MMP-2,

hyaluronan, and CA125 levels were 236 ng/mL, 651 ng/mL, and 4.0 U/mL, respectively.

In the present study, the proportion of PD patients with high PET category was 9.6%. ROC curves were constructed to assess the ability of the biomarkers in distinguishing high category of PET (Figure 3). MMP-2 and IL-6 levels in the peritoneal effluents from high PET

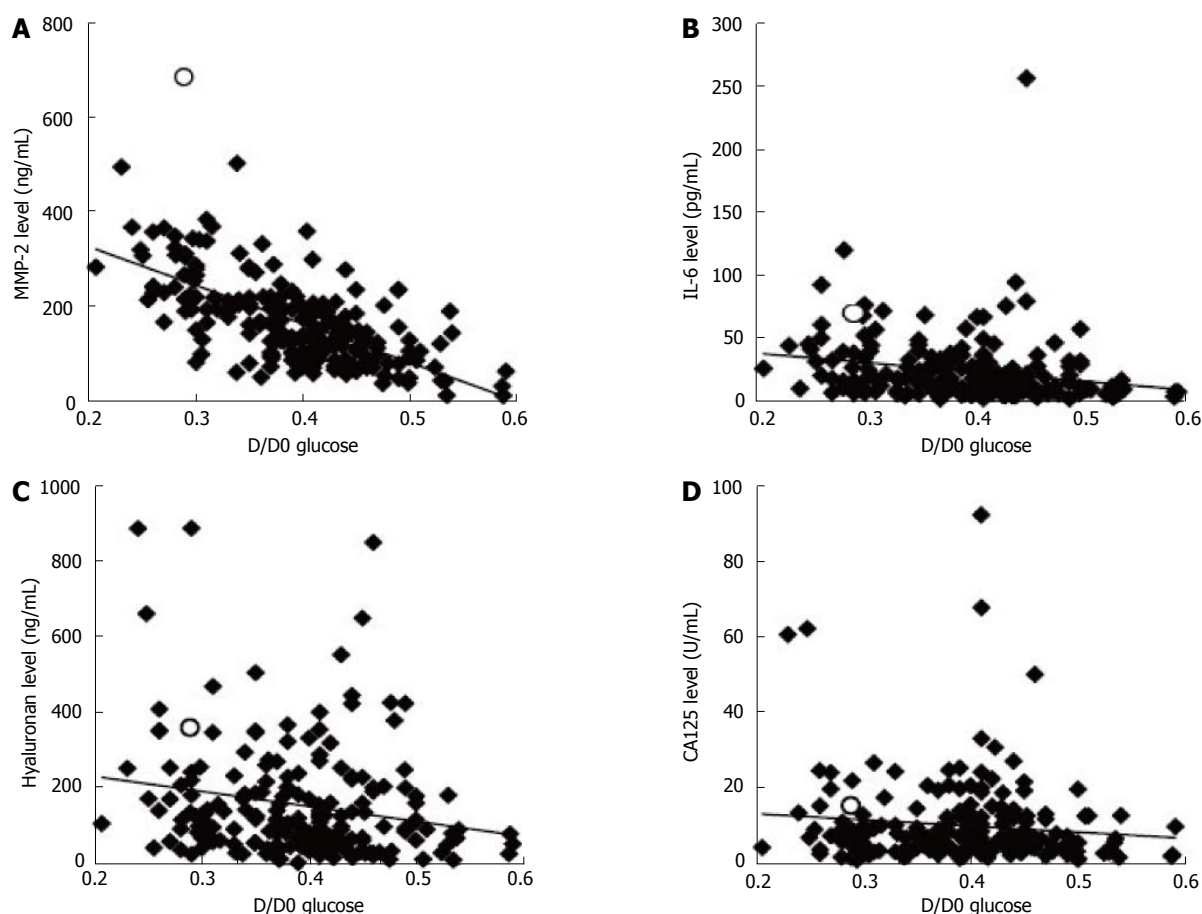


Figure 2 The relationships between D/D0 glucose and effluent biomarker levels. The glucose absorption rate was represented by D/D0 glucose and was compared with effluent MMP-2 level (A), effluent IL-6 level (B), effluent hyaluronan level (C) and effluent CA125 level (D). The biomarker levels of the one patient with EPS are shown by open circles. MMP-2: Matrix metalloproteinase-2; IL-6: Interleukin-6; CA125: Cancer antigen 125; EPS: Encapsulating peritoneal sclerosis.

Table 5 Test performance of effluent biomarkers cut-off levels to distinguish the high category of peritoneal equilibration test

Biomarkers	Cut-off level	Sensitivity (95%CI)	Specificity (95%CI)	AUC
MMP-2-	214 ng/mL	0.95 (0.86 to 1.00)	0.79 (0.74 to 0.85)	0.90
	219 ng/mL	0.86 (0.71 to 1.00)	0.81 (0.76 to 0.86)	
	228 ng/mL	0.81 (0.62 to 0.95)	0.84 (0.79 to 0.89)	
	238 ng/mL	0.76 (0.57 to 0.95)	0.87 (0.82 to 0.91)	
IL-6	16.8 pg/mL	0.81 (0.62 to 0.95)	0.60 (0.53 to 0.67)	0.78
	19.3 pg/mL	0.76 (0.57 to 0.90)	0.65 (0.58 to 0.73)	
	20.0 pg/mL	0.71 (0.52 to 0.90)	0.66 (0.60 to 0.73)	
	24.8 pg/mL	0.67 (0.48 to 0.86)	0.75 (0.66 to 0.79)	
Hyaluronan	94.5 ng/mL	0.81 (0.62 to 0.95)	0.47 (0.41 to 0.54)	0.62
	101.5 ng/mL	0.71 (0.52 to 0.90)	0.51 (0.44 to 0.58)	
	108.0 ng/mL	0.67 (0.48 to 0.86)	0.52 (0.45 to 0.58)	
	115.0 ng/mL	0.62 (0.43 to 0.81)	0.53 (0.46 to 0.53)	
CA125	3.85 U/mL	0.81 (0.62 to 0.95)	0.23 (0.17 to 0.29)	0.51
	4.45 U/mL	0.71 (0.52 to 0.90)	0.30 (0.23 to 0.37)	
	6.55 U/mL	0.62 (0.43 to 0.81)	0.50 (0.43 to 0.57)	
	7.05 U/mL	0.52 (0.33 to 0.71)	0.53 (0.46 to 0.59)	

AUC: Area under curve; MMP-2: Matrix metalloproteinase-2; IL-6: Interleukin-6; CA125: Cancer antigen 125.

category patients were significantly higher than these levels from non-high PET category patients (MMP-2: $P < 0.001$, IL-6: $P < 0.001$, hyaluronan: $P = 0.068$,

CA125: $P = 0.876$). The results from the ROC curve analysis and cut-off points for the high PET category are also shown in Table 5.

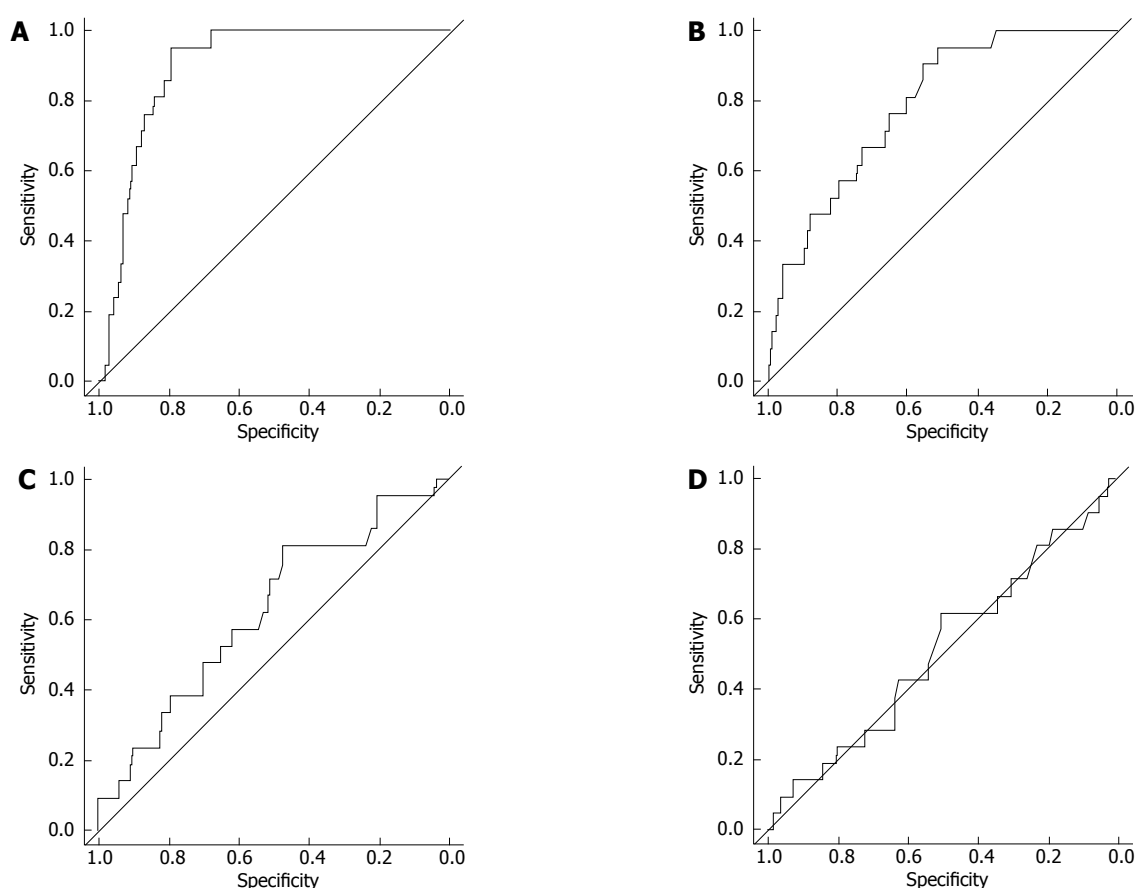


Figure 3 Receiver operating characteristic curve analysis of effluent biomarkers for the diagnosis of patients in the high category of D/P creatinine. A: Matrix metalloproteinase-2; B: Interleukin-6; C: Hyaluronan; D: Cancer antigen 125.

No patients developed new-onset EPS for at least 1.5 years after measurement of the effluent biomarkers.

DISCUSSION

For a safe and adequate PD, monitoring PD efficiency or peritoneal deterioration by increases in peritoneal solute transport rate is important. In addition to PET, some effluent biomarkers such as MMP-2, IL-6, hyaluronan, and CA125 are often measured to estimate peritoneal injury or progression to EPS during PD^[2,4,8-20]. But there are no absolutely reliable diagnostic method^[4]. Then, we analyzed the properties and efficacies of these biomarkers.

In the present multicenter clinical study, the results of PET most strongly correlated with effluent MMP-2 level among the biomarkers that we analyzed. High category of PET that is classified by high peritoneal solute transport rate was distinguished with high sensitivity and specificity by effluent MMP-2 level. In addition, effluent MMP-2 level of the patient with EPS was the highest among all PD patients that we analyzed. In our previous multicenter clinical studies, effluent MMP-2 level was high in patients with peritoneal injury^[8,9]. Cho *et al.*^[11] have reported that high effluent MMP-2 level is associated with peritoneal solute transport rate. Barreto *et al.*^[12] also observed significant correlations between

peritoneal transport parameters and effluent MMP-2 levels. Yamamoto *et al.*^[24] reported that high peritoneal membrane transport state may be a risk factor for EPS. Kawaguchi *et al.*^[3] summarized that an increase in D/P Cr ratio may constitute an independent and early marker of EPS. The Japanese Society for Dialysis Therapy Guidelines for Peritoneal Dialysis^[4] has recommended that PET should be routinely performed to evaluate peritoneal deterioration. In patients showing serial increase and persistently high D/P Cr for over 12 mo, progression of peritoneal deterioration could be suspected and discontinuation of PD should be considered. We previously reported that among 13 patients with MMP-2 level > 600 ng/mL except the patients, who had been diagnosed as EPS when MMP-2 levels were analyzed, 7 patients (54%) developed EPS and 3 patients (23%) died in a prospective study^[8]. In that study, only one patient with an MMP-2 level < 600 ng/mL developed EPS. In the present study, the effluent MMP-2 level of all patients without EPS onset was < 600 ng/mL and no patient developed EPS for at least 1.5 years after measurement of MMP-2 level. From these reports and results, MMP-2 may be expected to become a predictive marker for EPS. Recently, a retrospective clinical study by Lopes Barreto *et al.*^[20] was reported that the area under the ROC curve of MMP-2 appearance rate improved to 0.70 (95%CI: 0.51-0.899; $P = 0.06$) at one year prior

Table 6 Test performance of effluent matrix metalloproteinase-2 cut-off levels to predict the encapsulating peritoneal sclerosis in peritoneal dialysis patients

Cut-off level	Sensitivity (95%CI)	Specificity (95%CI)	AUC
458.5	1.00 (1.00 to 1.00)	0.96 (0.94 to 0.98)	0.99
609.5	0.88 (0.63 to 1.00)	0.99 (0.98 to 1.00)	
863.5	0.63 (0.25 to 0.88)	1.00 (0.99 to 1.00)	

AUC: Area under curve.

to the diagnosis of EPS. They compared 11 PD patients who developed EPS with 33 control PD patients using a 1:3 case control design. The absence of statistically significant findings may be explained by insufficient sample size. In this time, we additionally conducted ROC curve analysis to predict EPS by using eight PD patients with EPS vs 452 control PD patients who have been registered in our database^[8,9]. A summary of the results are shown in Table 6. In addition, as a result from ROC curve analysis using a 1:3 case control design as same as the analysis by Lopes Barreto *et al.*^[20], the area under the ROC curve for prediction of EPS in our study was 0.97 (95%CI: 0.92-1.00; $P < 0.01$) at 1 year prior to EPS diagnosis. The estimated sensitivity and specificity for prediction EPS development were 0.96 (95%CI: 0.88-1.00) and 1.00 (95%CI: 1.00-1.00), respectively, for a threshold effluent MMP-2 level of 414 ng/mL. Our data show higher sensitivity and specificity compared with the results by Lopes Barreto *et al.*^[20]; this discrepancy may be because of differences in the study designs (prospective and retrospective) or in methods of analysis. Our samples were prepared in the same manner at the 4-h PET and MMP-2 level was obtained as an absolute concentration. On the other hand, Lopes Barreto *et al.*^[20] analyzed effluent MMP-2 level as appearance rate. In the present study, effluent MMP-2 level significantly reflected the number of occurrence of peritonitis. Peritonitis, which induces peritoneal tissue injury, occurs 3.3 times more frequently in those who develop EPS than in those who do not^[3]. This suggests a close relationship of peritonitis with peritoneal injury and developing EPS. Thus, effluent MMP-2 may be a superior indicator of peritoneal injury or a predictive marker for EPS.

IL-6 that plays a critical role in inflammatory processes is secreted in large quantities by peritoneal mesothelial cells in response to inflammatory stimuli and is modulated by exposure to PD solutions^[13]. In the present study, effluent IL-6 level correlated with the results of PET and PD duration. On the other hand, although effluent IL-6 level in the EPS patient was high, the highest effluent IL-6 level was observed in another patient with accumulation of ascitic fluid and C-reacted protein-positive. This suggests that effluent IL-6 level reflected strong inflammation rather than EPS development. Cho *et al.*^[13] reported that effluent IL-6 level predicted increasing peritoneal solute transport rate and significantly increased with longer PD duration. Pecoits-Filho *et al.*^[14] also reported that effluent IL-6 level was

correlated with high peritoneal solute transport rate. PD duration, in addition to high peritoneal solute transport rate, is a risk factor for EPS^[25,26]. Abovementioned studies and our results suggest the efficacy of IL-6 as a biomarker of peritoneal deterioration. On the other hand, Goodlad *et al.*^[19] reported that although IL-6 was at higher levels in the effluent of patients who subsequently developed EPS, it did not improve prediction of future EPS compared with a model that used known clinical risk factors. Also in our study, a patient with the highest IL-6 level did not develop to EPS. As mentioned above, effluent IL-6 level may reflect deterioration of peritoneal membrane but it has to be kept in mind that effluent IL-6 level strongly reflects inflammation with or without EPS development.

Hyaluronan is constitutively synthesized by mesothelial cells and plays a crucial role in the maintenance of mesothelial cell morphology and re-mesothelialization. In particular, low molecular weight hyaluronan promotes angiogenesis, matrix protein synthesis, and transcription of MMPs^[15]. In the present study, effluent hyaluronan level also significantly reflected PET results and PD duration. Yamagata *et al.*^[16] reported that intraperitoneal hyaluronan production increased with both higher membrane permeability and longer time on PD. Monitoring of hyaluronan in the peritoneal effluent may be useful as a marker to assess functional and morphological changes in the peritoneum in long-term PD patients. Effluent hyaluronan level may reflect deterioration of peritoneal membrane.

The concentration or appearance rate of CA125 in PD effluent has been used as a biomarker for mesothelial cell mass in patients on PD. In the present study, although there was no association between effluent CA125 level and peritoneal solute transport, effluent CA125 level correlated weakly with all other biomarkers. Effluent CA125 level of male patients was significantly lower than that of female patients; in addition, the level of diabetic patients was significantly lower than that of non-diabetic patients. In several previous studies, the concentration and appearance rate of CA125 in peritoneal effluent had significant negative correlation with the duration of dialysis^[17]. In contrast, three cross-sectional studies found that duration of PD did not affect the CA125 level in the peritoneal effluent. Peritoneal transport parameters and a history of peritonitis in PD patients were not related to effluent CA125^[16,17]. A few previous studies did not observe a relationship between patient sex and effluent CA125^[17]. However, in a prospective study, effluent CA125 level was significantly lower in male patients than in female patients at 6 and 12 mo after PD initiation^[17]. Ditsawanon *et al.*^[17] reported that effluent CA125 level and appearance rate can also be used to follow-up individual patients who are not infected to evaluate peritoneal fibrosis, which is characterized by loss of mesothelial cells. Krediet^[18] described that effluent CA125 in stable PD patients without acute peritonitis is a marker of mesothelial cell mass, but has large inter-individual variability. Serial measurements over time can

be used for assessment of peritoneal mesothelial mass in individual patients, but effluent CA125 level may depend on individual characteristics of patients.

In conclusion, the peritoneal solute transport rate was most strongly correlated with the effluent level of MMP-2 among the biomarkers that were measured in the present study. MMP-2 may be useful as an indicator of peritoneal deterioration and can also potentially become a predictive marker with high sensitivity and specificity for EPS. Future studies should examine the serial changes of effluent MMP-2 level in relation to the progression of peritoneal injury. More patients should be tested to confirm the efficacy of MMP-2 as a biomarker and to eliminate selection bias of patients.

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COMMENTS

Background

Peritoneal dialysis (PD) is a blood purifying method for patients with end-stage renal disease. Long-term PD causes peritoneal deterioration with structural changes and functional decline, resulting in the cessation of PD treatment; these are the major problems associated with PD. At worst, peritoneal deterioration causes encapsulating peritoneal sclerosis (EPS), a serious complication of PD with extremely high mortality rate. To undergo PD safely and adequately, diagnosis of peritoneal deterioration is important.

Research frontiers

Solute transport rate through the peritoneal membrane is often measured by

peritoneal equilibration test (PET) to estimate PD efficiency and peritoneal deterioration. However, the results from PET are not sufficient predictors of EPS onset, and monitoring the time-course changes are necessary. In addition, PET is invasive and takes a long time. It is thus necessary to evaluate peritoneal deterioration using an easy and reliable non-invasive method.

Innovations and breakthroughs

In this study, the peritoneal solute transport rate most strongly correlated with the effluent level of matrix metalloproteinase-2 (MMP-2) among some biomarkers. MMP-2 could also predict EPS with high sensitivity and specificity at 1 year prior to EPS onset.

Applications

The data in this study suggested that effluent MMP-2 may be useful as a reliable indicator of peritoneal deterioration and a predictive biomarker with high sensitivity and specificity for EPS.

Terminology

MMP-2 degrades Type IV collagen and fibronectin, which are components of extracellular matrix, and plays a critical role in cell migration, angiogenesis, and epithelial to mesenchymal transition of mesothelial cells.

Peer-review

This is a good article.

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Observational Study

Chronic kidney disease in children and adolescents in Brunei Darussalam

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Informed consent statement: The authors confirmed that informed consent from patients is not needed for this study. The data were pooled and anonymized and would not reveal identities of any patients.

Conflict-of-interest statement: The authors confirmed that there is no conflict of interest.

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Abstract

AIM: To determine epidemiology of Bruneian paediatric chronic kidney disease (CKD) patients and factors that affect growth and progression of disease.

METHODS: A cross-sectional study conducted on all children below 18 years old who were diagnosed with CKD over a ten year period (2004 to 2013). The reference population was all children (< 18 years old) suffering from CKD and attending the tertiary paediatric nephrology clinic in Brunei Darussalam. Demographic (current age, age of diagnosis, gender, ethnicity), anthropometric (weight and height), diagnosis, laboratory data (serum creatinine and haemoglobin, urinalysis) and blood pressure were extracted from the patients' clinical case notes and recorded using a data collection form.

RESULTS: The study revealed a high national prevalence [736 per million child population (pmcp)] and incidence (91 pmcp) of CKD. If CKD was defined at Stage 1, 2, 3, 4 or 5, the associated prevalence figures were 736, 132, 83, 50 and 33 pmcp. Glomerulonephritis accounted for 69% of all prevalent cases, followed by congenital abnormalities of kidney and urinary tract (20%) and tubulointerstitial diseases (8%). Minimal change disease being the most common histological diagnosis. The median age of diagnosis was 4.5 years, with congenital disease patients experiencing an earlier onset of diagnosis. A large

proportion of patients were below the 5% percentile for height and weight. Non-glomerular diseases, adolescent and female patients were significantly associated with poor growth, but not glomerular filtration rate, age of diagnosis or steroid usage.

CONCLUSION: Brunei has a high prevalence of chronic kidney disease in the paediatric population with glomerulonephritis being the most common disease.

Key words: Brunei; Children; Adolescent; Chronic kidney disease; Epidemiology

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Core tip: This study provides demographic data for chronic kidney disease (CKD) in children and adolescents in Brunei Darussalam. Due to the small population, referral pattern and healthcare infrastructure of the country, the authors believe that the research has enabled a closer estimate of national prevalence and incidence of all stages of CKD than most countries. To our knowledge, this study is the first of its kind to report on epidemiology of CKD from the earliest stages.

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INTRODUCTION

Chronic kidney disease (CKD) affects almost 500 in 1 million people per year, among which 1%-2% are in the paediatric age range (0-17 years old)^[1]. Childhood and adolescent CKD, particularly in later stages, are associated with serious cardiovascular, neurologic, metabolic and other clinical complications. Further understanding in the epidemiology of CKD plays a fundamental role in identifying populations at risk as well to evaluate the interventions undertaken. Existing paediatric epidemiological data on incidence and prevalence are flawed by methodological differences between the various data sources in characterising age groups, degree of renal insufficiency and disease classifications^[2]. Most of the robust and available CKD data are extrapolated from national registries with adult ESRD cohorts in developed countries. To date, there are scarce epidemiological data on paediatric patients with earlier stages of CKD, especially in developing Asian countries where there are limited procedures and activities to collect and publish valid epidemiological data.

In Brunei Darussalam, there has been no published or collated information for this group of patients. Therefore, this study serves to determine the epidemiological

characteristics and clinical factors [gender, diagnosis, glomerular filtration rate (GFR), steroid usage] affecting growth and progression of kidney disease in the Bruneian paediatric CKD population. Additionally, comparisons were also made with data from pub-med listed literature on demographics and characteristics of international paediatric CKD patients.

MATERIALS AND METHODS

This was a cross-sectional study conducted on all children below 18 years old who were diagnosed with CKD over a ten year period (2004 to 2013). The reference population was all children (< 18 years old) suffering from CKD and attending the tertiary paediatric nephrology clinic in Brunei Darussalam. All available cases were included without sampling. Demographic (current age, age of diagnosis, gender, ethnicity), anthropometric (weight and height), diagnosis, laboratory data (serum creatinine and haemoglobin, urinalysis) and blood pressure were extracted from the patients' clinical case notes and recorded using a data collection form.

Weight and height retardation were defined as less than fifth percentile on the Disease Control and Prevention (CDC) growth charts^[3]. CKD was defined as GFR < 60 mL/min per 1.73 m² or the presence of kidney damage (structural or functional abnormalities other than decreased GFR) for more than three months^[4]. GFR was estimated by Schwartz's formula when height was recorded^[5]. The value of *k* varied with age and gender, being 0.33 in preterm infants (< 1 year old), 0.45 in full-term infants (< 1 year old), 0.55 in children (< 13 years old) and adolescent girls (\geq 13 years old), and 0.70 in adolescent boys (\geq 13 years old)^[5]. For children without height records (*n* = 3), eGFR-Pottel's formula was used^[6,7]. Anaemia was defined by the Clinical Practice Guidelines of the National Kidney Foundation's Kidney Disease Outcomes Quality Initiative (KDOQI guidelines) as haemoglobin level less than a specific threshold which varied with children's age and gender^[3]. Blood pressure readings were compared with blood pressure tables for children and adolescents, which were dependent on age and height. The diagnosis and classification of hypertension were accorded with National Heart, Lung, and Blood Institute (NHLBI)'s guidelines^[8].

RESULTS

The male to female gender ratio was 1.3:1. Socio-demographic characteristics and aetiological diseases of the study sample are shown in Table 1. The most common aetiology was glomerular diseases (*n* = 61) followed by congenital abnormalities of kidney and urinary tract (CAKUT) and tubulointerstitial diseases. Nine out of 61 cases were biopsied with minimal change disease (*n* = 4) being the most common histological diagnosis. The median age of diagnosis (data skewed to the right) was 4.5 (IQR = 6.0) years, with congenital disease patients

Table 1 Socio-demographic characteristics of study sample (89 patients)

Variable	<i>n</i> (%)	Mean (SD)
Current age (yr)		11.3 (4.12)
1-4	1 (1.1)	
5-9	30 (33.7)	
10-14	36 (40.4)	
15-18	22 (24.7)	
Gender		
Male	51 (57.3)	
Female	38 (42.7)	
Race		
Malay	76 (85.4)	
Chinese	9 (10.1)	
Others	4 (4.5)	
Aetiology		
Glomerular	61 (68.5)	
CAKUT	20 (22.5)	
Tubulointerstitial	8 (9.0)	

CAKUT: Congenital abnormalities of kidney and urinary tract.

Table 2 Anthropometric, clinical and laboratory characteristics of the study sample

Variable	<i>n</i>	<i>n</i> (%)	Mean (SD)	Median (IQR)
Weight in kilogram	87		-	29.90 (23.60) ¹
< 5 th percentile	22 (25.3)			
≥ 5 th percentile	65 (74.7)			
Height in cm	74		-	123.70 (27.60) ²
< 5 th percentile	23 (31.1)			
≥ 5 th percentile	51 (68.9)			
Systolic blood pressure (mmHg)	79		111.8 (13.86)	-
Diastolic blood pressure (mmHg)	79		70.9 (10.40)	-
Normal	40 (50.6)			
Pre-hypertensive	12 (15.2)			
Stage 1 hypertension	21 (26.6)			
Stage 2 hypertension	6 (7.6)			
Haemoglobin level (g/dL)	82		12.7 (1.65)	-
Normal	63 (76.8)			
Anaemia	19 (23.2)			
eGFR (mL/min per 1.73 m ²)	86		124.6 (52.99)	-
Stage 1 (> 90)	70 (81.4)			
Stage 2 (60-89)	6 (7.0)			
Stage 3 (30-59)	4 (4.7)			
Stage 4 (15-29)	2 (2.3)			
Stage 5 (< 15)	4 (4.7)			
Proteinuria	82		-	-
Yes	42 (51.2)			
No	40 (48.8)			
Haematuria	82		-	-
Yes	21 (25.6)			
No	61 (74.4)			

¹The distribution is skewed to the right; ²The distribution is skewed to the left. eGFR: Estimated glomerular filtration rate; IQR: Interquartile range.

experiencing an earlier onset of diagnosis.

Twenty-five point three percent and thirty-one point one percent of patients were under the 5% percentile

Table 3 Correlation between glomerular filtration rate, current age and age of diagnosis with weight and height

		Weight	Height
GFR	Correlation coefficient	0.168 ¹	0.176 ¹
	<i>P</i> value	0.125	0.140
	<i>n</i>	85	72
Current age	Correlation coefficient	0.609 ¹	0.541 ²
	<i>P</i> value	0.001	0.001
	<i>n</i>	87	74
Age at diagnosis	Correlation coefficient	0.450 ¹	0.368 ²
	<i>P</i> value	< 0.001	0.002
	<i>n</i>	84	71

¹Spearman's rank correlation; ²Pearson's correlation. GFR: Glomerular filtration rate.

for weight and height respectively. Patients with non-glomerular disease were found to be statistically most likely to have growth hindrance ($P = 0.001$ and $P = 0.003$ for weight and height respectively). A significant proportion of patients were hypertensive (34.2%) and anaemic (23.2%). Proteinuria and haematuria were present in 51.2% and 25.6% of patients respectively. The majority of patients were in Stage 1 CKD (81.4%) but 4 patients (4.7%) had end stage renal disease (on peritoneal dialysis). The anthropometric, clinical and laboratory characteristics of the study sample are shown in Table 2. The study found that there was no significant correlation between GFR and age of diagnosis with weight and height (Table 3). Steroid usage was not associated with growth attenuation ($P = 0.111$ and $P = 0.579$ for weight and height respectively) in patients with glomerular disease. However female, adolescent and non-glomerular patients were statistically more likely to experience growth attenuation. Details are shown in Tables 3 and 4.

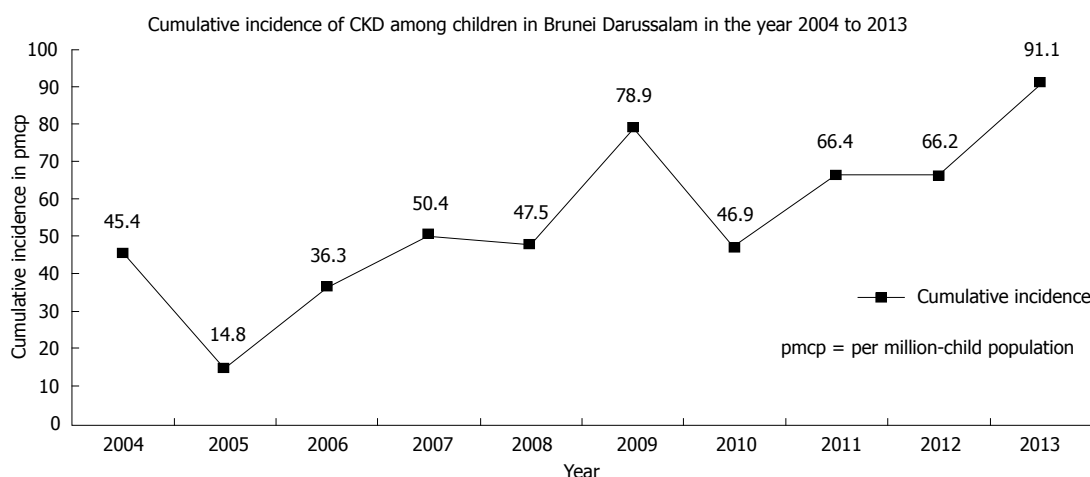
Based on population statistics for children of Brunei Darussalam, the age adjusted annual incidence of CKD over the 10 year range (2004-2013) is shown in Figure 1. The cumulative incidence and prevalence of all stages of CKD in 2013 were 91 and 736 per million child population (pmcp) respectively.

DISCUSSION

There is limited literature on the epidemiology of CKD in the paediatric population. Most paediatric patients with mild CKD are not represented in national registries, due to the asymptomatic nature of the condition. From our local experience, diseases with a more "dramatic" presentation like glomerulonephritis were more likely to be picked up and followed up by nephrologists. "Silent disease" may be missed especially in developing countries where screening, diagnosis and awareness of diseases may not be as extensive and comprehensive. The major difficulty in comparing studies is the inconsistent criteria used to define CKD. Our interpretation of the literature revealed that it is commonplace to disregard CKD stage

Table 4 Relationship between gender and aetiology with weight and height

	Weight		χ^2 statistic (df)	P value ¹	Height		χ^2 statistic (df)	P value ¹
	< 5 th percentile	≥ 5 th percentile			< 5 th percentile	≥ 5 th percentile		
	n (%)	n (%)			n (%)	n (%)		
Gender								
Male	9 (17.6)	42 (82.4)	3.81	0.051	10 (21.7)	36 (78.3)	4.95	0.026
Female	13 (36.1)	23 (63.9)	(1)		13 (46.4)	15 (53.6)	(1)	
Aetiology								
Glomerular	9 (15.0)	51 (85.0)	10.83	0.001	10 (20.0)	40 (80.0)	8.84	0.003
Non-glomerular	13 (48.1)	14 (51.9)	(1)		13 (54.2)	11 (45.8)	(1)	

¹ χ^2 test for independence.**Figure 1 Cumulative incidence of chronic kidney disease among children in Brunei Darussalam in the year.**

1 due to the lack of tangible physical and serological evidence of kidney impairment. However one can argue that such paediatric patients (e.g., nephrotic syndrome in remission) are debatably at higher risk of future kidney damage than their GFR-matched adult counterparts (with age related deterioration) due to the inherent reduced threshold for renal injuries and longer lifelong exposure to renal insults. For this research, we have made a conscious effort to include patients with all stages of CKD in an attempt to elucidate the true estimate for CKD prevalence and incidence in our population.

Our research revealed an estimated incidence and prevalence that is higher than most countries. However, if CKD definitions were set at Stage 1, 2, 3, 4 and 5, then the calculated prevalence would be 736, 132, 83, 50 and 33 pmcp respectively. The incidence of CKD in Brunei has shown progressive increments in the ten year period from 2004 to 2013, consistent with trends in the adult population^[9]. Local data from the Brunei Dialysis and Transplant Registry revealed a prevalence of 75 pmp in the young adult ESRD (CKD stage 5D) population (age 19-30), with a similar trend of increment over the last ten years^[10]. If only CKD stage III or lower patients are to be considered, our prevalence of 83 pmcp is on par with data from European studies with similar CKD profiles like Italy (74.7 pmcp)^[11], Belgium (56 pmcp)^[12] and Spain

(71.1 pmcp)^[13]. The prevalence of paediatric ESRD (33 pmcp) was also consistent with trends from developed Western and affluent Asian countries^[14]. Data from other developing Asian countries were available but direct comparisons on incidence and prevalence were difficult because of the difference in patients' population. Table 5 compares Bruneian data with PubMed listed literature (from the past 15 years) on prevalence, incidence and other demographic data from other countries.

There were some important differences between the aetiology of kidney diseases in our cohort with the local adult population and the international paediatric literature. The spectrum of disease differed markedly from our adult population, where diabetes mellitus and hypertension were the main aetiological diseases^[9]. There appeared to be a progression of importance for diabetes mellitus as an aetiological cause with increasing age. Glomerulonephritis (52%), diabetes mellitus (23%) and CAKUT (15%) were the three main causes of ESRD in our young adult population (age 19-30)^[10]. Globally, congenital causes (CAKUT) accounted for the most common aetiology among paediatric CKD^[2]; this was reported in developed countries including United States^[15], United Kingdom^[23] and Italy^[11]. However, only twenty patients (22.5%) were diagnosed with congenital anomaly in this study. On the contrary,

Table 5 Comparisons of paediatric epidemiological data from different countries from 1990-2015

	Period	No. of patients	Main aetiology	Male/female ratio	Mean age at diagnosis	GFR/CKD stage	Incidence	Prevalence
Brunei	2004-2013	89	GN (69%)	1.3	4.5	Mainly CKD 1 (81%)	91	736 (CKD1) 132 (CKD2 and above) 83 (CKD3 and above) 50 (CKD4 and above) 33 (CKD5)
Italy ^[10]	1990-2000	1197	CAKUT (58%)	2.0	6.9	GFR 42 (mean)	12.1	75
Belgium ^[11]	2001-2005	143	CAKUT (59%)	1.3	3.0	Mainly CKD 3 (67%)	11.9	56
Spain ^[12]	2007-2008	605		1.9	3.9	GFR 52 (mean)	8.7	71
United States ^[15]	1994-2007	7037	CAKUT (48%)					
Kuwait ^[16]	1996-2003	171	CAKUT 62%	2.7	33 mo	30% of patient reached ESRD within 18 mo of diagnosis	38-55	
Vietnam ^[17]	2001-2005	152		1.7	11.3	65% received RRT		5.1
Sudan ^[18]	2001-2006	205	GN 25%	1.7	9.8	63% of cohort reached ESRD during the follow up period		
Turkey ^[19]	2005	282	"Urological problem" 44.3%	1.3	8.0	CKD2-5	11.9	
Thailand ^[20]	1982-2005	101	GN 35%	1.6			Not rare	Double in last 6 yr of research
Jordan ^[21]	1988-2001	202	CAKUT 42%	1.3	7.5	59/202 patients require RRT	10.7	51
China ^[22]	1990-2002	1658	GN 52%	1.5	8.18	Mean serum creatinine 594.7 mmol/L		

CKD: Chronic kidney disease; GFR: Glomerular filtration rate; CAKUT: Congenital abnormalities of kidney and urinary tract; GN: Glomerulonephritis; ESRD: End stage renal disease.

there was a high prevalence of glomerular diseases (68.5%), comparable to published data from developing countries like Vietnam^[17], Sudan^[18], Thailand^[20] and Malaysia^[24]. It has been postulated that high proportions of glomerulonephritis may be related to high prevalence of bacterial, viral and parasitic infections that commonly affect the kidneys in developing countries^[14]. Furthermore, many paediatric CKD patients with CAKUT may have been referred directly to surgeons, with no subsequent follow up by nephrologists. As many of these patients do not have overt clinical symptoms, there may have been a delay in presentation of renal disease. This may have led to the patients being missed by this research leading to an underestimate of the prevalence of this disease.

Our study reports a male preponderance of 1.3. This is universally consistent with all the published studies in the literature which reported a range between 1.3 and 2.7. This gender disproportionality can be explained by the higher incidence of congenital disorders (obstructive uropathy, renal dysplasia and prune belly syndrome) in boys^[9]. Even after excluding these congenital defects, boys were still more likely to be affected by CKD^[11]. The median age of diagnosis (4.5 years) was similar to developed European countries (range of 3.3-6.9 years) but lower than developing Asian countries (range of 7.5 to 13 years). We suspect that this is related to healthcare infrastructure and health seeking behaviours of the population rather than the intrinsic characteristics of the disease in the population.

Poor growth in children with CKD is associated

with increased morbidity and mortality^[25]. A significant proportion of our patients were below the 5th percentile for weight (25.3%) and height (31.1%). This is not unusual for children with CKD due to congenital predisposition, electrolyte imbalances, malnutrition, bone disease and medications^[26]. Hamasaki *et al.*^[27] revealed that Asian CKD patients with congenital anomalies, lower GFR, being small for date and asphyxia at birth are more likely to have growth impairment. Our research showed that female and adolescent patients are more likely to experience growth attenuation. Adolescent patients usually experience growth spurts in their teenage years and it is not surprising to find that growth attenuation is maximal during this period. We observed other studies^[28,29] also derived similar results with females being more anthropometrically challenged than males, likely from a difference in age when they experience their growth spurts. Consistent with some literature reports^[30,31], this study did not find an association with steroid usage and growth attenuation in patients with glomerular disease. This suggests that GN diseases were predominantly steroid responsive and steroid regime was consistently kept to a minimum. This study also showed that there was no correlation between GFR and growth (weight and height), which corresponded with some reports from the literatures^[32,33]. We were not able to find an association between clinical and epidemiological factors linked with progression of renal disease.

We acknowledge that the sample size of this study is small and this may have affected the statistical evaluation of clinical and demographic factors, particularly in

association with growth. Since this is a retrospective study, there were some incomplete datasets from patients that were lost on follow up. We would also have like to scour paediatric, urological and general practitioner clinics for unreferred CKD patients but this would have gone beyond the realms of the ethical agreement set for the study.

This study is the first to describe the epidemiology of CKD among children and adolescents in Brunei Darussalam. The spectrum of disease is dissimilar to that in our adult population. We reported a higher incidence and prevalence than most countries because we were able to capture patients at earlier stages of their diseases. Our clinic is a one-stop referral centre for all cases of paediatric CKD in the country and we believe that it enables us to capture most of the symptomatic CKD patients in the country, regardless of stages of disease. We believe that this is unique as it allows us to predict the true scale of paediatric CKD and provide a closer estimate of national prevalence and incidence of CKD than most other countries. Furthermore, this study has heightened our awareness of growth attenuation and highlighted the need for early involvement of dieticians, nutritionists and social workers to improve the nutrition, education and social welfare of future paediatric CKD patients in our clinics.

COMMENTS

Background

Demographic data on paediatric patients with chronic kidney disease (CKD) are limited. This is especially true for patients with earlier stages of CKD and from developing countries. This study provided epidemiological characteristics of Bruneian patients from all five stages of CKD. In addition, analysis was done to elucidate factors that may have affected growth of patients and progression of renal disease. The study revealed a high national prevalence [736 per million child population (pcmp)] and incidence (91 pcmp) of CKD. If CKD was defined at Stage 1, 2, 3, 4 or 5, the associated prevalence figures were 736, 132, 83, 50 and 33 pcmp. Glomerulonephritis accounted for 69% of all prevalent cases. A large proportion of patients were below the 5% percentile for height and weight. Non-glomerular diseases, adolescent and female patients were significantly associated with poor growth, but not glomerular filtration rate, age of diagnosis or steroid usage.

Research frontiers

In comparison with other studies, the authors believe that we have a closer national estimate of CKD in the paediatric population due to our ability to capture CKD patients through our one-stop tertiary clinic and the relative small population in the country. Additionally, the authors identified that a significant proportion of the patients have attenuated growth, prompting us to advocate early interventions by nutritionists, dieticians and social workers to supplement treatment options by physicians to augment and intensify growth and physical development.

Innovations and breakthroughs

The authors believe that the authors are one of very few studies to predict the prevalence and incidence of CKD in the paediatric population, particularly for the earlier stages of diseases where patients may remain asymptomatic. More studies will be needed to evaluate this further.

Applications

Knowledge of national prevalence and incidence of CKD in the paediatric population can help service providers plan future population needs for renal

replacement therapy and can help public health promotion exercises to identify early disease and to delay progression to irreversible end stage renal disease.

Terminology

CKD: Chronic kidney disease; GFR: Glomerular filtration rate; KDOQI: National Kidney Foundation's Kidney Disease Outcomes Quality Initiative; CAKUT: Congenital abnormalities of kidney and urinary tract; GN: Glomerulonephritis; ESRD: End stage renal disease; Pmc: Per million child population.

Peer-review

A reasonable first analysis of congenital pediatric renal disease in a previously unstudied population with findings in the range of what might have been predicted.

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Comamonas testosteroni-associated peritonitis in a pediatric peritoneal dialysis patient

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Abstract

Comamonas testosteroni (*C. testosteroni*) has been rarely observed as an infectious agent in clinical practice. Few reports described its potential pathogenicity in bloodstream and abdominal infections. Here, we report our experience in the treatment of a *C. testosteroni*-associated peritonitis in a four-year-old girl receiving chronic peritoneal dialysis (PD). The organism was shown to be highly susceptible to appropriate antibiotic therapy. Infection responded promptly and the patient was managed conservatively without withdrawal from PD.

Key words: *Comamonas*; Peritonitis; Peritoneal dialysis; Comorbidity; Children

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Core tip: *Comamonas testosteroni* (*C. testosteroni*) has been largely overlooked as a potential pathogen in humans. This case reports not only the first description of a *C. testosteroni*-associated peritonitis in a pediatric patient, but also emphasizes the risk of uncommon causes of bacterial peritonitis especially in peritoneal dialysis children with severe comorbidities.

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INTRODUCTION

Comamonas testosteroni (*C. testosteroni*) is an aerobic Gram-negative organism with a widespread

environmental distribution. Infection by *C. testosteroni* is infrequent, however there are some reports describing its potential causative role in bacteremia, meningitis, urinary tract infections, endocarditis, cellulitis, and pneumonia^[1,2]. Isolation of *C. testosteroni* has also emerged in localized peritonitis as a complication of perforated appendicitis^[3,4].

Here, we present our experience in the treatment of a *C. testosteroni*-associated peritonitis in a four-year-old girl receiving chronic peritoneal dialysis (PD).

CASE REPORT

The girl was previously diagnosed with end-stage renal disease due to atypical haemolytic-uraemic syndrome, and she had been treated with automated PD for 10 mo. The girl was also affected by severe motor-cognitive impairment and idiopathic epilepsy.

She was admitted to our Department with a 24-h history of high-grade fever and complaining of abdominal pain. Physical examination revealed abdominal tenderness, along with cloudy peritoneal effluent. The patient's white blood cell count was normal ($6130/\text{mm}^3$), whereas the C-reactive protein was significantly increased (290 mg/L). The leukocyte in peritoneal effluent showed a count of $6600/\text{mm}^3$ (90% polymorphonucleated). One month before this event, the patient had experienced a *S. aureus* peritonitis, for which she had completed a 3-wk course of intraperitoneal therapy with glycopeptide.

After admission, empiric antibiotic therapy was started with both intravenous ceftazidime and teicoplanin. The fever subsided within 48 h and the leukocyte count in effluent resulted normal ($< 100/\text{mm}^3$) within 72 h from the start of antibiotic treatment. Signs and symptoms of peritonitis regressed within 48 h. On hospital day 3, cultures from peritoneal effluent resulted positive for *C. testosteroni*. Antibiotic treatment was then simplified, with single-agent intraperitoneal ciprofloxacin in order to complete a 3-wk course of therapy. The patient was discharged and a follow-up after 14 and 30 d showed persistent normalization of leukocyte in peritoneal effluent, negative control cultures, and regular PD course. To date, after 12 mo from the *C. testosteroni* peritonitis, the patient did not experience any other significant infectious episode. She is still on chronic PD and in the waiting list for renal transplantation.

DISCUSSION

Case reports provide important and detailed information for educational purposes related to clinical practice, which is often lost in larger studies^[5]. This concept is especially true if the disease described is rare. Clinical experience is the starting point of evidence-based medicine and the sharing of treatment outcomes represents a preliminary guideline for the future management of similar cases.

Peritonitis remains a frequent complication of PD in children and is the most common reason of technique failure. The microbiology is characterized by a predominance of Gram-positive organisms, with fungi responsible for

less than 5% of episodes^[6]. The vast majority of patients are treated successfully with antibiotics administered intraperitoneally and continue PD. The poorest outcomes are observed in patients with Gram-negative organisms or fungi peritonitis and in those with a relapsing infection. In this cases, early PD catheter removal with transient switch to haemodialysis is sometimes required^[7]. Despite the lower prevalence, fungal infections are associated with the highest mortality rate.

C. testosteroni is a gram-negative aerobic bacillus that is found in various environments, including soil, water, plants, and animals. In spite of its wide environmental distribution, there are few reports on its involvement in human infections^[1]. Most of the reported infections by this organism are community-acquired, however some authors suggested that it can also survive for a long time in the hospital setting. Indeed, it can colonize several devices, such as intravenous lines, respiratory equipment, and humidifiers^[8,9]. This seems mostly due to an extraordinary capability of this organism in both environmental adaptation and biofilm formation^[10]. Nevertheless, few molecular biological investigations were taken on the pathogenicity and virulence of *C. testosteroni*. Very recently, Liu *et al.*^[11] conducted a comprehensive genomic analysis among 10 *C. testosteroni* strains. They identified 24 types of virulence factors that were involved in several functions such as adherence, anti-phagocytosis, invasion, and secretion system. Moreover, the authors found that most of the virulence factors were owned by all of the strains and were highly conserved. These results supported the molecular biological basis of the potential pathogenicity of this bacterium.

Along with its own virulence factors, pathogenicity of *C. testosteroni* seems to be emphasized in patients with some degree of immunosuppression such as malignancy, prematurity, primary or secondary immunodeficiency induced by chronic liver disease and end-stage renal disease^[1,12]. Moreover, bacterial translocation from the gastrointestinal tract seems to play an important role in the pathogenesis of infections^[13].

Very recently, Altun *et al.*^[14] published the first continuous ambulatory PD patient treated for a *C. testosteroni*-associated peritonitis. The authors described a 29-year-old woman with end-stage renal failure secondary to hypertensive nephrosclerosis who had been treated with CAPD for 10 mo. In this case, along with the chronic dialysis status, the predisposing factor for peritonitis with this pathogen was probably a previous laparoscopic intervention because of incidental dislocation of an intrauterine device to the space between the peritoneum and the anterior abdominal wall. Signs and symptoms of peritonitis regressed rapidly during a 14-d period of oral ciprofloxacin.

C. testosteroni is usually sensitive to a broad range of antibiotics, including aminoglycosides, cephalosporins, fluoroquinolones, carbapenems, piperacillin-tazobactam, cephalosporins, and trimethoprim-sulfamethoxazole^[15]. According to the survey by Farshad *et al.*^[1], 32 out of 35 reported cases of human infection by this bacterium were

promptly responsive to antibiotic treatment. Outcome was fatal in three cases, including a 64-year-old woman on hemodialysis with a central venous catheter-related bacteremia^[12].

To our knowledge, the present case is the second report of a *C. testosteroni*-associated peritonitis in a PD patient, but is the first description in pediatric age. Information regarding immune function in children with chronic kidney disease or receiving dialysis are sparse. The incidence of infectious episodes in children on dialysis is higher than that found in adults; moreover, immaturity of the immune system may also contribute to its dysfunction especially in children with chronic diseases and several co-morbidities. In our patient, both dialysis status and severe motor-cognitive impairment may have increased the pathogenicity of *C. testosteroni*, similarly to previous adult case reports. Moreover, the previous and recent episode of *S. aureus* peritonitis might have represented a further predisposing factor to *C. testosteroni* infection. In fact, in PD patients, bacterial peritonitis induces a subsequent breakdown of intestinal barrier function and a transient impairment of host mucosal immune defense^[16]. This may have allowed further enteric low-virulence organisms to enter the peritoneal cavity by transmural migration and to cause peritonitis^[17].

C. testosteroni should be kept in mind as a rare cause of bacterial peritonitis in children receiving chronic PD. With the improvement in care of end-stage renal disease patients and given the potential for favorable outcomes, a higher number of children with severe co-morbidities is now started to PD. Clinical management of these children is demanding; in a report of the International Pediatric Peritoneal Dialysis Network, Neu *et al*^[18] showed that children on PD with comorbidity had a higher hospitalization rate than did patients without a comorbidity. Infections from both common and unusual pathogens were the most frequent reasons for hospitalization and mortality. Our experience confirms that *C. testosteroni* peritonitis responds promptly to adequate antibiotic therapy. A conservative management can be adopted without loss of the PD catheter and withdrawal from the PD.

COMMENTS

Case characteristics

A 4-year-old girl with severe motor-cognitive impairment, idiopathic epilepsy, and receiving chronic peritoneal dialysis (PD) was admitted with a 24-h history of high-grade fever and complaining of abdominal pain.

Clinical diagnosis

Physical examination revealed abdominal tenderness, along with cloudy peritoneal effluent.

Differential diagnosis

Bacterial (Gram-positive or Gram-negative) peritonitis, fungal peritonitis, culture negative peritonitis, encapsulating peritoneal sclerosis.

Laboratory diagnosis

WBC count was within normal limits, C-reactive protein was significantly increased (290 mg/L), and laboratory investigations in peritoneal effluent

showed a leukocyte count of 6600/mm³ (90% polymorphonucleated cells) with a positive culture for *Comamonas testosteroni* (*C. testosteroni*).

Treatment

Intraperitoneal ciprofloxacin in order to complete a 3-wk course of therapy.

Related reports

Infection by *C. testosteroni* is infrequent, however there are some reports describing its potential causative role in bacteremia, meningitis, urinary tract infections, endocarditis, cellulitis, and pneumonia. Isolation of *C. testosteroni* has also emerged in localized peritonitis as a complication of perforated appendicitis.

Term explanation

C. testosteroni-associated peritonitis represents a rare complication of chronic PD. The pathogenicity of this bacteria might be increased in immunodeficient patients, in children with severe chronic diseases, and affected by co-morbidities.

Experience and lessons

C. testosteroni should be kept in mind as a rare cause of bacterial peritonitis in children receiving chronic PD. *C. testosteroni* peritonitis responds promptly to adequate antibiotic therapy, therefore a conservative management can be adopted without loss of the PD catheter and withdrawal from the PD.

Peer-review

C. testosteroni-associated peritonitis in PD patients is a rare condition. This case-report can add some new information to clinical practitioners.

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Sleep disorders and chronic kidney disease

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Abstract

Sleep disorders have a profound and well-documented impact on overall health and quality of life in the

general population. In patients with chronic disease, sleep disorders are more prevalent, with an additional morbidity and mortality burden. The complex and dynamic relationship between sleep disorders and chronic kidney disease (CKD) remain relatively little investigated. This article presents an overview of sleep disorders in patients with CKD, with emphasis on relevant pathophysiologic underpinnings and clinical presentations. Evidence-based interventions will be discussed, in the context of individual sleep disorders, namely sleep apnea, insomnia, restless leg syndrome and excessive daytime sleepiness. Limitations of the current knowledge as well as future research directions will be highlighted, with a final discussion of different conceptual frameworks of the relationship between sleep disorders and CKD.

Key words: Chronic kidney disease; End-stage renal disease; Renal replacement therapy; Hemodialysis; Kidney transplantation; Sleep initiation and maintenance disorders; Disorders of excessive somnolence; Intrinsic sleep disorders; Parasomnias; Restless legs syndrome; Sleep apnea; Dyssomnias; Circadian rhythm disorders; Melatonin

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Core tip: Sleep disorders have a profound and well-documented impact on overall health and quality of life in the general population. In patients with chronic disease, sleep disorders are more prevalent, with an additional morbidity and mortality burden. The complex and dynamic relationship between sleep disorders and chronic kidney disease (CKD) remain relatively little investigated. This article presents an overview of sleep disorders in patients with CKD, with emphasis on relevant pathophysiologic underpinnings and clinical presentations.

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INTRODUCTION

Sleep disorders are prevalent in patients with chronic kidney disease (CKD) in particular those with end stage renal disease (ESRD)^[1]. It has been reported that 80% of ESRD patients receiving dialysis report sleep complaints, with daytime sleepiness to be the most common reported symptom^[2,3]. The reason for increased rates of sleep related issues and disorders in this population is likely multifactorial and will be discussed in this review. Sleep issues are not only related to decreased quality of life^[4,5], but are also associated with increased health related risks^[6], and mortality^[1,7] in CKD.

BIOLOGICAL EFFECTS OF CKD ON SLEEP

Although it is commonly accepted that patients with CKD experience poor sleep quality, not much is known about the physiological mechanisms underlying this phenomenon. According to Hildreth, patients with CKD often exhibit sympatho-vagal imbalance due to baroreceptor reflex function impairment in which there is hyperactivity of the sympathetic nervous system and decreased vagal tone^[8,9]. In healthy individuals, sleep is accompanied by a decrease in sympathetic activity and an increase in vagal tone that leads to a nocturnal dipping of blood pressure. However, patients who have sleep disorders resulting in hypoxemia and sleep fragmentation have been shown to have increased sympathetic nervous system stimulation and decreased parasympathetic activity, which results in a reduced fall in nocturnal blood pressure^[10].

Blood pressure regulation by the autonomic nervous system during sleep also affects the renin-angiotensin-aldosterone system. As blood pressure decreases during the normal sleeping period, there is a reflexive increase in plasma renin activity and aldosterone. As an individual goes through cycles of rapid eye movements (REM) and non-REM (NREM) sleep, there are oscillations of cardiac sympatho-vagal balance and plasma renin levels. Plasma renin activity and aldosterone peaks during NREM sleep, more specifically stages 3 and 4, and dips during REM sleep. This oscillatory nature of PRA is absent in patients who experience a night of sleep deprivation^[11]. However, decreased sleep duration is not the only factor affecting nocturnal PRA and aldosterone secretion. Sayk *et al*^[12] showed that decreased sleep quality induced by suppressing slow-wave sleep (stages 3 and 4) also reduced nocturnal blood pressure dipping, which would affect the RAA system as well. It is believed that the lack of nocturnal blood pressure dipping is an important risk factor for progression of

CKD^[13]. Reducing nighttime blood pressure by means of carefully timed antihypertensive therapy in the evenings may reduce the risk of progression of CKD to ESRD^[13].

CHRONOBIOLOGY OF MELATONIN IN CKD PATIENTS

Melatonin, a hormone secreted by the pineal gland, is responsible for the sleep - wake circadian rhythm. It is secreted in small amounts during the daytime but increases during the night, which correlates with the onset of nocturnal sleepiness. In a small cross sectional study comparing 30 ESRD patients undergoing hemodialysis (HD) and 20 healthy participants, nocturnal melatonin levels were significantly lower in patients with ESRD^[13]. About 22 of the 30 patients also lacked the circadian rhythm in melatonin secretion. HD did not correct or improve melatonin concentrations. In another study by Karasek *et al*^[14], melatonin concentrations released during the night did not improve with kidney transplantation, despite improvements in renal function. Sleep quality, as measured by actigraphy, did not significantly improve either.

CHANGES IN SLEEP ARCHITECTURE

Patients with ESRD typically exhibit poor sleep architecture as measured objectively on polysomnographic studies. In a comprehensive review, ESRD patients had short, fragmented sleep with total sleep times between 260-360 min^[15]. Sleep efficiencies ranged between 66%-85% and time spent awake ranged from 77-135 min. Sleep latencies were reported between 10-30 min and REM latencies between 92-64 min. There was a pattern of increased stage 1 and stage 2 sleep while slow wave sleep and REM sleep were decreased. Daytime sleepiness is a parameter not measured by polysomnographic studies but is still considered an important marker of inadequate sleep. Multiple sleep latency tests (MSLT) objectively measure daytime sleepiness by having the patient take five scheduled naps throughout the day separated by 2-h breaks. Time to onset of sleep, also known as sleep latency, of less than 5 min is considered to be pathological and may be exacerbated by various sleep disorders. A study conducted by Parker *et al*^[15] in 2003 also found that out of 46 ESRD patients, 46% had abnormal MSLTs. Another study conducted by Stepanski *et al*^[16] on peritoneal dialysis patients reported a MSLT of 6.6 ± 3.7 min.

SLEEP APNEA

Sleep apnea is a chronic sleep disorder which causes repeated cessation of breath while a person is sleeping. Characteristics of sleep apnea include loud snoring, breathlessness, waking up from sleep, and daytime sleepiness. Prevalence in the general population is

approximately 2%-4%^[17,18], compared to the prevalence in ESRD patients which is estimated between 50%-60%, through self-report questionnaires^[18-20], and about 70%-80% of ESRD patients when based on polysomnography^[17,19-21].

Sleep apnea is divided into three sub-types: Central sleep apnea (CSA), obstructive sleep apnea (OSA) or mixed^[22]. While OSA is the most common form of sleep apnea in the ESRD population^[18], CSA may be underreported in patients with ESRD, as it can only be diagnosed with polysomnography tests^[21]. OSA causes repeated episodes of apneas, arousals, and loud snoring. In contrast to CSA, OSA is commonly recognized by an individual's bed partner. The most conclusive method of diagnosing OSA remains overnight polysomnography studies^[21].

Sleep apnea in the ESRD can cause excessive sleepiness and cognitive impairment, diminishing daytime functioning. OSA is also commonly linked to depression, hypertension and increased cardiovascular morbidity and mortality^[17,21,22].

Pathology

The direct relationship between sleep apnea and ESRD is not clear. However, several studies have examined "rostral fluid shift" as a possible mechanism in the pathogenesis of OSA in CKD patients^[23,24]. Due to their reclined position overnight, excess fluid shifts from the legs towards the neck leading to upper airway restriction and collapse^[23].

Thus, when CKD patients accumulate excess fluid in the neck due to rostral shift, upper airway "collapsibility" increases leading to high rates of OSA occurrences^[23-25]. One study tested this theory by measuring the neck circumference (NC) and leg fluid volume (LFV) in ESRD patients with OSA^[23]. The change in LFV correlated with significant change in NC, supporting the notion that leg fluid is displaced into the neck overnight. Another study conducted by Elias *et al*^[24] confirmed the rostral fluid shift by measuring internal jugular vein volume (IJVVOL) and upper airway mucosal water content (UA-MWC). They found that greater IJVVOL and UA-MWC levels correlated to greater apnea-hypopnea index. Both studies concluded that fluid accumulation in the neck due to rostral shift predisposes ESRD patients to OSA.

Treatments

Similar to the general population, continuous positive airway pressure (CPAP) is the first line of treatment in CKD patients with OSA^[22,26]. Other treatment modalities in the general population include the use of dental appliances, oral surgery, and treating underlying medical conditions (*e.g.*, obesity or hypothyroidism). These modalities have not yet been extensively studied in the CKD population.

Research has shown that conversion from conventional HD to nocturnal HD (NHD) reduces the occurrence of apneas. One suggested mechanism is

that NHD aggressively removes more uremic toxins than conventional HD which may contribute to better sleep quality^[26]. Studies that examined ESRD patients before and after conversion to NHD, found that NHD was effective in lowering the heart rate and reducing the frequency of apneas and hypoxemias in all of the patients^[26,27].

INSOMNIA

Insomnia is the inability to fall asleep or stay asleep and is characterized by poor sleep quality and poor quality of life^[21]. It is a common sleep disorder in the general population and is significantly more common in ESRD patients on HD. The prevalence of insomnia in the general population ranges from 4% to 29%^[28]. Whereas in the ESRD population, approximately 50%-75% of ESRD patients experience symptoms of insomnia^[28-30].

Clinical significance

Poor quality of sleep and lack of sleep reduces overall quality of life and may lead to a host of other complications including impaired immune system and risk for cardiovascular disease^[21]. It is important to understand insomnia and its relationship associated with other complications in order to reduce mortality and improve quality of life and sleep in these patients.

Pathology

The causes of insomnia are both physiological and psychological and there are several factors that contribute to its onset. As compared to the general population, patients with insomnia have higher rates of anxiety, stress and relatively poor self-concepts^[31].

Insomnia is also commonly found in individuals with coexisting medical conditions. Other influences include low socioeconomic status, female gender, psychiatric conditions and conditions that cause chronic pain^[4,21]. In ESRD patients, the risk of insomnia is higher than the general population due to the physical stress of their condition. Chronic pain is a common problem in patients on dialysis and is a leading cause of insomnia in this population^[21]. Elder *et al*^[4] examined factors that affect sleep quality in a worldwide self-report study in 11351 patients on dialysis. Data showed that reports of poor sleep quality increased with reports of higher, more severe degrees of pain.

Sabbatini *et al*^[28] found dialysis shift time to be an important risk factor for the development of insomnia. Patients on dialysis during early morning shifts had higher rates of insomnia than patients on dialysis in the afternoon. Others have found that late night dialysis shifts play a role in insomnia as well^[21]. Additionally, the prevalence of insomnia is much higher in elderly patients with ESRD and patients who have been on dialysis for longer periods of time^[28].

Physiologically, individuals undergoing HD experience disturbances in the sleep-wake (circadian)

cycle^[32]. As discussed earlier, the process of dialysis influences secretion of melatonin, which is responsible for the regulation of the circadian cycle. In one study, 73% of patients on dialysis had no identifiable circadian rhythm at all^[14].

Additionally, high levels of parathyroid hormone (PTH) are linked to the prevalence of insomnia in patients with ESRD^[4,28]. PTH is associated with renal bone disease and bone pain. In a study of 654 patients, patients on dialysis had substantially higher levels of PTH than control patients.

In summary, research suggests that chronic pain, stress, older age, dialysis shift, melatonin, and high PTH all play a role in the development of insomnia in ESRD patients, although the mechanisms are not yet fully understood.

Treatments

There are pharmacological and nonpharmacological means of treating insomnia. Research suggests that it may be most beneficial to first treat the underlying conditions, such as pain or depression.

Sedative antidepressants and anxiolytics are effective in individuals who suffer from depression, worry and insomnia, however there is little research supporting their safety and efficacy in ESRD patients.

Melatonin is recommended for regulation and improvement of the sleep-wake cycle in patients with insomnia. The rather limited evidence base in ESRD patients supports this. In short-term studies on maintenance HD patients^[33,34], 3 mg of melatonin (administered at bedtime or 10 pm respectively) improved both subjective and objective sleep parameters, with no significant side effects reported. In one long-term study^[35], despite not sustaining its efficacy at one year, melatonin use continued to be a safe and well-tolerated option.

In a recent critical summary of the existing body of evidence, Yang *et al.*^[36] systematically reviewed the literature on such non-pharmacological interventions in dialysis-dependent patients, and identified 12 randomized controlled trials and one prospective cohort study. Four intervention modalities were studied; cognitive behavioral therapy (CBT), acupuncture, physical exercise and change of dialysis modality. None of the studies had a head to head design, and all RCTs were identified as having a high risk for bias, limiting their overall conclusion. They concluded that CBT for insomnia (CBTi) is helpful for patients on HD, and more studies are needed to further assess the potential of the other interventions.

From a cognitive behavioral perspective, acute insomnia is maintained through maladaptive coping strategies, resulting in a strong association between bed and arousal, not sleepiness. The core tenets of CBTi are stimulus control, sleep restriction, and sleep hygiene. CBTi usually starts with stimulus control, in which the association between bed and sleep is gradually re-

established, by following a set of behavioral instructions that may include keeping a fixed wake-time, using the bedroom/bed only for sleep or sex, sleeping only in the bedroom, and leaving the bed when not able to sleep. In sleep restriction, the total sleep time (the average time spent actually asleep) is estimated, and a fixed wake-time is established. The patient is then instructed to limit their time in bed to the estimated total sleep time, gradually "rolling back" their bedtime by 15 min increments. Sleep hygiene describes a broad set of "good sleep habits" that include exercising regularly (but generally not before bedtime), avoiding excessive liquids, caffeine, nicotine and alcohol in the evening, ensuring that the bedroom is comfortable and noise-free, as well as adjusting the timing of meals and snacks relative to bedtime.

Classically, CBTi is carried out in 6-8 wk sessions, starting with clinical evaluation and baseline assessment utilizing a sleep diary, followed by these three components, with gradual titration of sleep restriction. Chen demonstrated the effectiveness of CBTi in patients with ESRD^[36,37], but it is unclear from his publications how classic CBTi was adapted to the ESRD population.

Other studied techniques for insomnia include relaxation training, acupuncture and physical exercise. Relaxation training can be a helpful adjunctive therapy in treating chronic insomnia^[38,39]. It emphasizes progressive muscle relaxation and breathing exercises for the relief of chronic pain and insomnia. The Iranian group of Rambod *et al.*^[40] demonstrated that listening to an instructional relaxation audiotape for twenty minutes, twice a day, for 8 wk, after an initial training session, resulted in a statistically significant improvement of sleep quality in patients treated with HD, as measured by the Pittsburgh Sleep Quality Index.

In acupuncture, specific points along the pathways of energy are targeted, without using needles.

The evidence on the utility of acupuncture and acupoint massage in ESRD patients with chronic insomnia is largely derived from three RCTs conducted by a Taiwan-based group^[41-43], and two Iranian RCTs^[44,45]. The methodological concerns of these studies have been documented^[36,46], but acupuncture may be a safe alternative therapy for insomnia.

Physical exercise has beneficial effects on slowing the decline of renal function^[47-49]. In addition, aerobic or resistance exercise programs have been demonstrated to have moderately positive effects on sleep quality in the general population^[50]. The evidence base on such effects in the ESRD population is scant yet promising, and thus should be interpreted cautiously^[51-53]. Furthermore, little is described in the literature to guide the selection of ESRD patients for customized exercise interventions, or to ensure safety.

There is little that can be concluded about the treatment of insomnia in ESRD populations, however it is clear that more research is needed and that the

combination of pharmacological and nonpharmacological techniques are likely to work in tandem to provide the greatest relief to the patient.

RESTLESS LEG SYNDROME

Restless leg syndrome (RLS), also known as Willis-Ekbom syndrome, is a sensory-motor disorder manifested by unpleasant nocturnal sensations in the lower limbs that are relieved by movement. These sensations generally occur deep within the muscle of the leg, but patients occasionally report feeling them on the skin. Two-thirds of patients experience the sensation bilaterally; one-third of patients have unilateral symptoms. The most common site of symptoms is the upper calf, with 75% of patients reporting sensations there. About 80%-90% of RLS patients present with periodic limb movements of sleep (PLMS)^[54-57].

Epidemiology

In the general population, symptoms most frequently appear after the age of 45, with 38% of sufferers report onset of symptoms before age 20. RLS is twice as common in females than in males. Family history of RLS is common; 63% of patients report at least one first degree relative with RLS. No monogenic cause has yet been found, but studies show six different genes that may play a role^[58,59].

In HD patients, the prevalence of RLS is 20%-30%, compared to 3%-7% in the general population. In kidney transplant patients, the prevalence is close to 5%, approximately average for the general population^[2,3,57,60,61].

Clinical significance

RLS impacts sleep, which can lower sleep quality and efficiency as well as overall quality of life. Untreated RLS is highly associated with depression, both in the general population and in patients with CKD. In addition, RLS is associated with higher mortality in ESRD patients.

Pathology

Brain iron dysregulation plays a role in RLS^[54], possibly during transport across the blood brain barrier. Since iron is an essential cofactor in the production of dopamine, low iron levels could explain the changes in dopamine metabolism that occur in RLS. The syndrome is worsened by iron deficiency and symptoms are improved by iron supplementation. RLS sufferers show a drop in CSF ferritin levels throughout the night, while healthy controls do not. Circadian changes in brain iron status are what make this a circadian disease. Other possible factors associated with the condition are elevated serum calcium levels and PNS/CNS abnormalities^[61,62]. ESRD patients may be particularly susceptible to acquiring RLS because peripheral neuropathy complicates and overlaps the picture of RLS.

Assessment

Diagnosis of RLS is based on the 2012 revised International RLS Study Group criteria^[55]. These criteria include: Urge to move legs, usually because of an uncomfortable sensation; sensations are exacerbated when resting or lying down; urges and unpleasant sensations are at least partially relieved by motion, such as walking around; and symptoms cannot be accounted for by other medical issues or behavioral patterns. A levodopa test (50% improvement in symptoms after 25/100 mg of carbidopa/levodopa) can be used to help diagnose RLS, although not all patients respond to dopaminergic drugs. The PLMS Index - the number of leg movements per hour - can be recorded by polysomnography and is one measure used to assess severity of the syndrome.

Non-pharmacological treatments

Both aerobic exercise and resistance training have been shown to improve symptoms of RLS^[54,63]. Improvement of sleep hygiene is also thought to have some beneficial effect. There have been no controlled studies on the effects of alcohol, nicotine, and caffeine, but these substances are thought to aggravate the condition. Small studies have shown that pneumatic compression devices, acupuncture, and near-infrared light can be helpful to RLS sufferers^[54].

Pharmacological treatments

Dopamine agonists (DAs) are commonly considered to be the first pharmacological option, and they simultaneously address the symptoms of PLMS as well^[54]. Although DAs are an effective initial treatment, they are only shown to be effective in the long term in 25% of patients. In addition, long-term use brings about a worsening of symptoms, known as augmentation, in a large percentage of patients. About 6%-17% of RLS patients who take DAs develop impulse control disorders. Correcting iron deficiency has been shown to improve RLS in HD patients^[64]. Other pharmacological therapies include calcium channel alpha-2-delta ligands (gabapentin, and pregabalin), opioids, and iron therapy. Gabapentin, an alpha-2-delta ligand, is a good choice for patients with polyneuropathy in addition to RLS. In general, both gabapentin and pregabalin appear to be helpful in improving sleep quality in ESRD patients with painful peripheral neuropathy. However, dosages of both medications need to be renally-adjusted, and side-effect profile has not been adequately described in CKD studies^[65,66].

Augmentation

Augmentation refers to the severe exacerbation of RLS symptoms, sometimes up to 24 h a day, caused by the medication used to treat initial symptoms^[67-70]. It is thought to be the result of pharmacological treatment, not a natural progression of the disease. This is a common complication seen in patients treated with

dopaminergic drugs. Augmentation is characterized by gradually earlier onset of symptoms, greater severity of symptoms, increasingly shorter periods of rest between symptoms, expansion of symptoms to upper limbs, and shorter periods of effectiveness of medication^[54]. One study found prevalence of augmentation in patients treated with DAs to be as high as 76%^[54]. Because of this, DAs should be prescribed only when necessary and patients' symptoms should be monitored closely.

Large, methodologically sound studies are still needed to further assess the effectiveness of both pharmacological and non-pharmacological treatment options, as well as the impact of different renal replacement modalities.

EXCESSIVE DAYTIME SOMNOLENCE

Excessive daytime somnolence (EDS) is defined as the inability to stay awake or alert throughout the course of the day, resulting in sleepiness or inadvertent dozing during passive (reading, watching television) or active (driving, conversation) daily activity.

Compared to the 10%-12% prevalence in the general population^[71-73], EDS is significantly more common in CKD patients, especially those on HD^[3,15,74,75]. Parker *et al.*^[15] estimated that two-thirds of their HD subjects listed daytime sleepiness as a main complaint. Moreover, one-third had abnormal levels of objective sleepiness, and an additional 13% showed pathological levels of sleepiness on the MSLT and the Epworth Sleepiness Scale (ESS).

Pathogenesis

Multiple factors may contribute to daytime sleepiness^[15,76,77]. These factors include uremia, high prevalence of periodic limb movements and high prevalence of sleep apnea. Studies have shown all of these to be correlated with more severe daytime sleepiness. Other possible contributors include subclinical uremic encephalopathy, tyrosine deficiency (tyrosine being important for dopamine production), release of inflammatory cytokines during dialysis, high daytime melatonin levels, and change in body temperature rhythm. NHD may alleviate daytime sleepiness.

The approach to the assessment and treatment of EDS in CKD patients is generally the same as that to in the general population. An additional intervention is to switch to NHD^[76]. One study assessing patients after kidney transplant found that three months after surgery ESS scores had dropped significantly^[78].

CLINICAL RECOMMENDATIONS

While there is not sufficient data to make evidence-based recommendations, there is still a need for the practicing clinician to address sleep complaints in their patients with renal disease. Our recommendation is to first do an appropriate clinical assessment which may include a referral for a full evaluation including

somnography, if sleep apnea is suspected. Clearly, patients with sleep apnea should be aggressively followed and encouraged to use their CPAP machines, as recommended. Patients with conventional insomnia or sleepiness should have their sleep hygiene evaluated. Often the most basic components of sleep hygiene are being neglected and relatively minor changes in the patients' behavior can lead to substantial sleep change. If this approach is not successful the clinician may then consider referral for cognitive behavior therapy or the limited use of sleep agents. While sleep difficulty is very common in renal patients, the nephrologist should be encouraged to utilize the expertise of colleagues trained in sleep medicine and employ a team approach to care.

CONCLUSION

In patients with ESRD, the identification, diagnosis and treatment of sleep disorders is complicated by the overlapping presentation with CKD and other commonly comorbid conditions. One approach to conceptualizing this relationship is to consider sleep disorders as secondary or end product of multiple concurrent and interactive processes^[79]. Such processes include psychological disorders (depression, anxiety), lifestyle factors (coffee/nicotine use, sleep hygiene), treatment-related factors (timing of dialysis, daytime napping, production of cytokines, thermoregulatory changes, dialysis disequilibrium syndrome, disruptions in circadian rhythm, medication side effects) as well as intrinsic, ESRD-specific factors (anemia/OSA/RLS and other comorbidities, uremia, overall all health and quality of life, alterations in neurotransmitter production). This approach highlights the difficulty in separating sleep disturbances for either research or clinical purposes, and suggests that treatment of sleep disorders should be multi-layered and comprehensive.

An alternative simpler approach is to separate insomnia from concurrent medical/psychiatric comorbidities, and treat it as an independent co-occurring disorder^[80,81]. While this approach lacks the richness of a multifactorial conceptualization, it more readily allows for the targeted study and treatment of sleep dysfunction in ESRD populations.

Clearly, the high rates of sleep apnea, insomnia, and RLS in ESRD populations necessitate larger well-designed clinical trials. Future research should attempt to explain the complex interrelationships between sleep and kidney disease, test standard treatments in ESRD communities and develop novel treatments for sleep disorders that can take the complex psychosocial and physiological burden HD presents.

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Soy-based renoprotection

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Abstract

Chronic kidney disease (CKD) is a significant public

health problem as risk factors such as advanced age, obesity, hypertension and diabetes rise in the global population. Currently there are no effective pharmacologic treatments for this disease. The role of diet is important for slowing the progression of CKD and managing symptoms in later stages of renal insufficiency. While low protein diets are generally recommended, maintaining adequate levels of intake is critical for health. There is an increasing appreciation that the source of protein may also be important. Soybean protein has been the most extensively studied plant-based protein in subjects with kidney disease and has demonstrated renal protective properties in a number of clinical studies. Soy protein consumption has been shown to slow the decline in estimated glomerular filtration rate and significantly improve proteinuria in diabetic and non-diabetic patients with nephropathy. Soy's beneficial effects on renal function may also result from its impact on certain physiological risk factors for CKD such as dyslipidemia, hypertension and hyperglycemia. Soy intake is also associated with improvements in antioxidant status and systemic inflammation in early and late stage CKD patients. Studies conducted in animal models have helped to identify the underlying molecular mechanisms that may play a role in the positive effects of soy protein on renal parameters in polycystic kidney disease, metabolically-induced kidney dysfunction and age-associated progressive nephropathy. Despite the established relationship between soy and renoprotection, further studies are needed for a clear understanding of the role of the cellular and molecular target(s) of soy protein in maintaining renal function.

Key words: Chronic kidney disease; Diet; Proteinuria; Protein; Soy

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Core tip: This review summarizes the data, both animal and limited human studies, that support the hypothesis that a soy-enriched diet is protective against chronic kidney disease. While the clinical studies have small subject numbers, the data suggest that soy improves

renal function, or attenuates the progression of chronic renal dysfunction. The potential mechanisms of action, from both experimental and clinical studies, is also discussed, including positive effects on lipid and blood glucose profiles, improved vascular function and reduced inflammation. Consideration is also given to the potential active ingredients within soy, including both protein and isoflavones, that may mediate the renoprotective effect of the botanical.

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INTRODUCTION

Current prevalence of renal disease

Chronic kidney disease (CKD) is characterized by kidney damage and/or dysfunction that is present for more than 3 mo and that leads to complications in other organ systems^[1,2]. The leading causes of CKD are Type 2 diabetes and hypertension^[3-7] with less frequent causes being glomerulonephritis, nephrolithiasis, polycystic kidney disease (PKD), systemic lupus and hepatitis^[7,8]. Five stages of CKD severity are determined using measures of estimated glomerular filtration rate (eGFR), with measures of urinary albumin excretion rate (AER) conferring additional prognostic classification^[1,2]. These stages range from an asymptomatic condition (eGFR > 60 mL/min per 1.73 m² and urinary albumin < 30 mg/g) to end-stage renal disease (ESRD) (eGFR < 15 mL/min per 1.73 m² and urinary albumin > 300 mg/g) requiring kidney dialysis or transplantation. The prevalence of CKD varies between 2.5%-11.2% of adults in Europe, Asia, North America and Australia^[9]. In the United States the prevalence was reported to be 13.7% between 2007 and 2012^[10]. Hoerger *et al.*^[11] predicts the prevalence to increase to 14.4% in 2020 and 16.7% in 2030. Currently, incidence of CKD ranges from 200 cases per million to up to 400 cases per million in the United States and Taiwan^[12]. Studies show that CKD patients experience poorer quality of life and loss of function vs healthy individuals^[13-15].

Pathogenesis and etiology of renal disease

Hypertension is defined as systolic blood pressure greater than 140 mmHg and diastolic blood pressure higher than 90 mmHg^[16] and is the second leading cause of ESRD^[10]. Therapeutic goals for blood pressure are influenced by patient age as well as presence of comorbidities such as diabetes and CKD^[17]. Data collected from Chronic Renal Insufficiency Cohort study participants indicated that hypertension affects 67%-92% of CKD patients and its prevalence increases with deteriorating renal function^[18-20].

Besides CKD resulting from metabolic risk factors,

renal dysfunction can result from PKD. PKD is an inherited genetic disorder that is either autosomal dominant (ADPKD) or autosomal recessive (ARPKD) and is characterized by the development of fluid-filled cysts from the epithelial lining of the nephron which causes renal enlargement and obstructive tissue fibrosis that can lead to ESRD^[21]. As the fourth leading cause of kidney failure, PKD afflicts more than 500000 individuals in the United States^[22].

Mechanistically, CKD is marked by fibrosis, an accumulation of extracellular matrix (ECM)^[23]. Given the importance of renal fibrosis in the loss of renal function in CKD, much effort has focused on the mechanisms underlying fibrosis. Fibrosis results from abnormal accumulation of matrix, predominantly collagen, which is associated with loss of organ function as normal tissue is replaced by scar tissue^[24]. CKD is a prototypical example of progressive fibrosis leading to organ failure^[25,26].

The kidney may be uniquely sensitive to inflammation as it is a source for cytokine and chemokine synthesis within the tubular epithelium^[27], and due to the high blood flow, it is continually exposed to circulating pro-inflammatory mediators. There are significant data linking inflammation to the loss of renal function^[28]. A candidate signaling pathway that links inflammation and fibrosis in the kidney is nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB). This transcription factor has a long-recognized role as a pro-inflammatory mediator^[29]. A number of humoral stimuli induce NF-κB activity in the kidney, including tumor necrosis factor (TNF-α)^[30] and angiotensin II^[31] - both of which are associated with CKD^[32,33].

INTERVENTIONS IN RENAL DISEASE

Pharmacological interventions in renal disease

Primary interventions for treating and preventing the progression of CKD are focused on reducing the increased vascular disease risk in the early stages of this disease (Stages 1-3) and involve exercise, dietary changes, smoking cessation, glycemic and blood pressure control and lipid management^[7] which are also steps used in treating diabetes which itself is a risk factor for CKD^[5]. Dietary changes focus on managing salt/sodium, phosphorus, potassium and protein intakes^[2]. Glycemic control with various anti-diabetic drugs has been shown to reduce the risk and slow the progression of renal disease in both Type 1 and Type 2 diabetics^[5] with metformin being a first choice of therapy. Blood pressure reduction can dramatically reduce progression of renal disease^[20] and there is a good body of data showing that angiotensin-converting enzyme (ACE) inhibitors are effective in reducing proteinuria in non-diabetic^[34] and diabetic^[3] renal disease patients. Dual blockade of the renin-angiotensin-aldosterone-system is often used to achieve blood pressure targets in hypertensive subjects, however, combined use of ACE inhibitors and angiotensin-receptor-blockers are not

recommended as there is insufficient evidence of any further benefit to decrease CKD progression^[2], and several studies with combinations in subjects with diabetic kidney disease showed an increased risk of adverse events^[3,5].

Lipid management is of particular importance in CKD as many patients present with hyperlipidemia regardless of etiology^[8,35] and are at higher risk for cardiovascular events^[2,36-38]. Lipid lowering is particularly effective in the early stages of CKD where atherogenic risk factors have a much greater impact on disease progression^[7]. Moreover, it is now well recognized that dyslipidemia is an independent contributing factor in the progression of renal injury and dysfunction^[39-42]. There may be three pathophysiologic mechanisms of renal injury related to dyslipidemia^[41]. First, kidney mesangial cells exposed to oxidized lipoproteins (that are more prevalent in dyslipidemia) can stimulate the secretion of chemokines and adhesion molecules promoting the infiltration of macrophages which results in glomerulosclerosis and tubular fibrosis. This process has been suggested to be similar to the process of atherosclerosis^[42]. Second, uptake of oxidized lipoproteins by the infiltrating macrophages increases lipid deposition in the kidney and promotes the release of reactive oxygen species (ROS) and proinflammatory and proliferative cytokines [e.g., transforming growth factor- β (TGF- β) and platelet-derived growth factor-AB]. Lastly, macrophage secreted cytokines promote mesangial expansion through increased ECM protein synthesis^[41].

Dyslipidemia associated with CKD is most often characterized by elevated triglycerides, small dense low density lipoprotein (LDL) particles and dysfunctional high density lipoprotein (HDL)^[43-45]. Triglyceride-rich lipoproteins are elevated in CKD due to a variety of causes. Diabetic CKD patients tend to have more dysfunction of the vascular endothelium, leading to a relative deficiency of lipoprotein lipase (LPL)^[43]. Hepatic lipase is also lower in all CKD patients^[43] and may result from elevated parathyroid hormone in these patients, which increases hepatic calcium concentrations and deranges normal hepatocyte cell function^[37,46]. Finally, apolipoprotein (apo) CIII and angiopoietin-like 4 protein, both inhibitors of LPL function, are also higher in CKD patients^[43,47].

Various clinical practice guidelines for treating dyslipidemia in CKD have been published^[38] and most recommend the use of statins in early stages of the disease, as they are considered the safest lipid lowering agent in early stage kidney disease^[37,41]. The use of statins in early stage CKD has been shown to reduce the cardiovascular risk associated with the dyslipidemia, however the data do not support any benefit of statin in the long term reduction of renal disease progression^[37,48,49]. Statins can also, in certain cases, induce proteinuria^[49] so caution is recommended in the use of high dose or potent statin drugs. Clinical practice guidelines also do not recommend initiating statins in patients undergoing dialysis based on the results of

three pivotal trials (4D, AURORA and SHARP trials)^[50-52] which all failed to show efficacy of statins in reducing cardiovascular endpoints in dialysis patients as a whole. Nonetheless, statins have been shown to be helpful in reducing overall morbidity in renal transplant patients who are often on immunosuppressive medications which cause elevations in lipids^[37].

The lack of statin benefit to reduce overall cardiovascular mortality in dialysis patients has led some investigators to propose that other means of lipid lowering may be beneficial in this population^[53]. Since dialysis patients experience a high absorption of sterols, Silbernagel *et al.*^[53] suggest that other lipid lowering agents other than statins (e.g., bile acid binding compounds) should be considered in this population. To this end, it has been demonstrated that pharmacologic doses of omega-3 polyunsaturated fatty acids can be prescribed at all stages of CKD even in combination with a statin to successfully reduce hypertriglyceridemia^[36].

Nutritional interventions in renal disease

Nutritional interventions can be viewed as consisting of two phases in renal dysfunction - diet plans that address the underlying causes of kidney disease and slow the progression of kidney failure and those that support the patient and prevent complications arising from advanced kidney disease. The Academy of Nutrition and Dietetics, through their Nutrition Care Process advises using a nutrition diagnosis and potential disease etiology to develop an appropriate nutrition prescription for renal patients^[54]. Assessment tools that are validated for CKD include the Subjective Global Assessment (SGA) and Malnutrition Inflammation Score (MIS)^[54]. Nutritional interventions strive to address the comorbid conditions in CKD, such as hypertension, glucose and lipid homeostasis, inflammation and increased oxidative stress. Malnutrition in CKD (as assessed by the SGA or MIS) results in poor clinical outcomes and increased mortality rates in CKD patients^[54,55]. Protein energy wasting (PEW) is also common in CKD and can be distinguished from malnutrition (inadequate nutrient intake) since CKD-related factors may also contribute to PEW^[56]. Since low protein and low phosphorus diets have been shown to be effective in slowing the progression of kidney disease, careful consideration is required to design dietary interventions that maintain total energy intake while maintaining adequate but not excessive protein and phosphorus intake^[54].

The Dietary Approaches to Stop Hypertension diet is useful in reducing blood pressure^[57]; however, it may only be useful in very early stages of CKD since it recommends 4.5 g/d potassium, 1.7 g/d phosphorus and 1.4 g/kg per day protein which may be problematic in later stages of CKD^[58]. The International Study of Macronutrients and Blood Pressure study demonstrated that more vegetable protein, but not animal protein, was associated with lower blood pressure^[59] indicating that the source of protein may be important in renal diets. In the Nurses' Health Study, Knight *et al.*^[60] showed

that high non-dairy animal protein intake appeared to accelerate renal function decline in women with renal insufficiency, but not in women with normal kidney function. More attention has focused on vegetarian diets in the field of renal nutrition in general due to properties that ameliorate the factors contributing to renal dysfunction^[54,61-64]. Specifically, foods of plant origin tend to have higher energy/protein and energy/phosphorus ratios (*i.e.*, can satisfy energy requirements while maintaining a relatively low nitrogen and phosphorus intake), are low in saturated fats^[54,65], tend to improve glycemic control compared to omnivorous diets^[66] and may reduce oxidative stress and inflammation^[67]. A number of human studies have demonstrated renal benefits of dietary vegetable protein^[68-73].

Only a limited number of dietary intervention studies have been conducted in PKD patients. The Modification of Diet in Renal Disease Study showed no benefit over 18-45 mo follow-up (mean = 2.2 years) of protein restriction or improved blood pressure control on the rate of decline in GFR in ADPKD subjects with moderate renal insufficiency ($n = 141$; GFR 25 to 55 mL/min per 1.73 m²; mean GFR = 37.8 mL/min per 1.73 m²)^[74]. And, in some cases, deterioration of renal function actually seemed to be exacerbated by these interventions^[74]. A very low protein (0.28 g/kg per day)/low phosphorus (4-9 mg/kg per day) diet with keto acid-amino acid supplementation did however demonstrate limited efficacy ($P = 0.06$) in slowing disease progression in ADPKD patients with greater renal insufficiency ($n = 59$; GFR 13 to 24 mL/min per 1.73 m²; mean GFR = 17.4 mL/min per 1.73 m²). A key limitation of the study was that subjects may have already reached advanced stages of ADPKD such that maximal benefits of such interventions may not have been achievable^[74]. Retrospective analysis conducted by Choukroun *et al.*^[75] of data from a hemodialytic ADPKD population ($n = 109$) during follow-up care (average 6.7 ± 0.3 years) at Necker Hospital in Paris, France revealed a relationship between mean arterial pressure (MAP) and change in creatinine clearance ($r = 0.226$; $P = 0.01$) but observed no significant effect of protein intake ($r = 0.109$; $P = 0.33$). Despite the importance of nutrition in renal disease, there is a paucity of human clinical data in this area^[54]. Soybean protein has perhaps been the most extensively studied plant-based protein in subjects with kidney disease and has demonstrated renal protective properties in a number of clinical studies^[76,77]. Dietary intervention studies with soy protein have yet to be conducted in PKD patients.

SOY PROTEIN

Soy/soy protein consumption and renal health

The soybean consists of 36% (wt/wt dry matter) protein which can be extracted using differential processing techniques to yield a variety of soy protein ingredients such as soy flour, soy protein concentrate and soy

protein isolates that can be incorporated into a multitude of food forms^[78,79]. Soy protein is the only high quality plant-based protein that is widely available. Soy protein is a complete protein (provides adequate levels of all of the essential amino acids to support human nutritional needs) and is comparable in quality to milk, meat, and eggs as measured by the protein digestibility-corrected amino acid score, which is the globally recognized method for determining protein quality^[80]. Soy intake has been and continues to be greater in Eastern Asian rather than Western countries with tofu, natto and miso being the most common representatives/examples of traditional soy foods^[81]. Therefore, these populations have frequently been utilized to evaluate the health effects of soy consumption as part of the diet over the course of the entire lifespan.

Soybeans also contain mono/oligosaccharides (15% wt/wt dry matter), fiber (15% wt/wt dry matter), oil (18% wt/wt dry matter) and relatively small amounts of phytic acid, tocopherols, phytosterols, saponins and isoflavones^[78,79]. Soy isoflavones (genistein, daidzein and glycitein) are present in soybeans and in most soy protein products primarily as *b*-glycosides and their associated acetyl- and malonyl-ester forms and very little is present as non-conjugated isoflavones (aglycone form)^[82,83]. The aglycone form of isoflavones exhibit structural similarity to estrogen and these compounds are sometimes referred to as phytoestrogens, but have a limited ability to bind estrogen receptors, demonstrating a greater affinity for estrogen receptor β (ER- β) rather than estrogen receptor α (ER- α) and therefore may be better considered as selective estrogen receptor modulators^[84]. It should be pointed out that isoflavones are efficiently metabolized, once absorbed by the gut, to glucuronide and sulfate conjugates in humans and these conjugates account for 90% or more of circulating isoflavones in human plasma^[84,85]. Daidzein is converted to equol [(3S)-3-(4-hydroxyphenyl)-7-chromanol]] and/or O-demethylangolensin through the actions of certain commensal intestinal bacteria in some but not all human subjects^[63,81,86,87] and these metabolites are also present in plasma primarily as glucuronide conjugates^[88]. Conjugated isoflavones are generally very weak estrogen receptor ligands and may have as yet unidentified biologic activities *in vivo*^[89]. Finally, soy protein consumption leads to different target gene expression than is observed with estradiol treatment in animal models, confirming that the metabolic effects of soy protein cannot be equated with the hormonal effects of estrogen *per se*^[90].

Plasma and urinary concentrations of isoflavones increase somewhat proportionally with the consumption of soy foods in both animals and humans^[63]. Therefore, these parameters are frequently utilized as biomarkers of subject compliance in dietary intervention studies that include soy despite significant individual to individual variability which is also influenced by kidney function status, which will be discussed later.

SOY PROTEIN CONSUMPTION - HUMAN STUDIES

Soy protein consumption and renal health - human studies

A brief summary of human studies assessing the role of soy protein in renal function follows. Studies published in English were included if the main dietary intervention or subject of study was clearly identified as soy protein and was ingested as whole soy or as soy products.

Searches of the PubMed and SciFinder® database studies in English identified three single arm intervention studies (Table 1) and thirty-two placebo-controlled chronic interventions studies (Table 2) assessing the effects of soy protein on renal measures in subjects with varying degrees of renal dysfunction. Three studies studied the metabolism of soy isoflavones in subjects with ESRD or in renal transplant patients (Table 3). Two studies looked at the effects of soy protein consumption on renal calcium metabolism in healthy, normal subjects (Table 4). Eight studies assessed the renal response to single meal ingestions of soy protein in comparison to other proteins in healthy and Type 2 diabetic subjects with some renal dysfunction (Table 5). Citations in the Tables are listed in approximate order of severity of renal disease of study subjects.

Soy protein consumption and dyslipidemia

As previously mentioned, lipid management is particularly important in patients with renal disease as many patients present with hyperlipidemia regardless of the etiology of their kidney dysfunction. Soy protein lowers plasma cholesterol^[91] and, as of 2015, has an approved health claim based on this property in 13 countries^[92]. Three single arm dietary intervention studies (Table 1) demonstrated that consumption of 25 g or more of soy protein/day resulted in a significant lowering of total and LDL cholesterol (LDL-C) in renal transplant patients^[93,94] and in nephrotic patients with proteinuria^[95,96] when compared to their baseline diets. Soy protein consumption lowered plasma apoB concentrations in the nephrotic patients^[95,96]. Eight placebo-controlled chronic intervention studies in subjects with various degrees of renal dysfunction demonstrated that soy protein consumption resulted in significant lowering of plasma total cholesterol compared to the control diets^[97-104]. Five of these studies showed that soy protein diets resulted in significant lowering of LDL-C^[98,99,102-104], four demonstrated a reduction in plasma apoB^[100-103] and two studies reported a significant lowering of non-HDL-C^[100,101], with the latter being considered a more important prognostic biomarker for cardiovascular disease than LDL-C^[105]. Addition of 5 g/d fish oil to the soy diet in one study^[103] did not improve any of the lipid parameters, and, in fact, tended to raise LDL-C and apoB concentrations. Soy protein consumption tends to reduce plasma cholesterol more in renal

patients with elevated rather than normal cholesterol concentrations^[101] which is not unlike that observed in many other intervention trials with soy protein^[91]. Several studies summarized in Table 2 also noted that soy protein consumption tended to lower plasma triglycerides^[97-99,101] whereas other studies reported no significant change^[100,103,104]. Teixeira *et al.*^[106] observed a significant increase in HDL-C after soy consumption in Type 2 diabetic subjects with nephropathy, while Stephenson *et al.*^[104] reported no change in Type 1 diabetic subjects with early stage renal dysfunction. A decrease in apoA I and HDL-C was reported by Gentile *et al.*^[103] in non-diabetic nephrotic patients with renal dysfunction after soy protein consumption for 8 wk. While Tokede *et al.*^[91] concluded that soy protein consumption in mixed populations was associated with modest beneficial effects on HDL-C concentrations, the studies cited above on renal compromised subjects suggest that there may be differences in the ability of soy protein to modulate absolute concentrations of HDL-C depending on the nature of renal dysfunction. It should be also noted that absolute concentrations of HDL-C are probably less important than the ability of HDL-C particles to mediate cholesterol efflux from cholesterol-laden cells in the body^[107], so future studies on soy consumption should focus on this property of HDL particles rather than HDL-C concentrations alone.

Soy protein consumption and plasma glucose

Soy protein consumption was also associated with improvements in plasma glucose metabolism in several chronic intervention studies. Gentile *et al.*^[103] noted that fasting blood glucose concentrations were reduced significantly in non-diabetic subjects while on the soy diet alone compared to those values at baseline or on the soy protein plus fish oil diet. Azadbakht *et al.*^[98] also noted a significant decrease in fasting blood glucose concentrations in Type 2 diabetic subjects who had been on a soy diet for 4 years compared to the control (animal protein) diet. Teixeira *et al.*^[106] did not observe any improvements in blood glucose control following an 8 wk intervention with soy protein in Type 2 diabetic subjects in contrast to Stephenson *et al.*^[104] who noted that soy protein consumption in Type 1 diabetic subjects did not alter fasting glucose concentrations compared to the baseline diet but that the animal protein diet resulted in a significant elevation. Chen *et al.*^[101] noted that soy protein consumption decreased fasting insulin concentrations in hyperlipidemic subjects compared to milk protein consumption; in normolipidemic subjects soy protein consumption resulted in decreased plasma insulin concentrations compared to the baseline diet. The same authors also observed that serum insulin levels were significantly decreased by soy protein vs milk protein consumption in hypercholesterolemic subjects while plasma glucose levels were equivalent in both groups, suggesting an insulin sensitizing effect of soy protein consumption^[100].

Table 1 Single arm intervention studies of soy protein and kidney function

Ref.	Study design	Kidney function	Subjects/group	Amount of soy protein used	Control/comparator protein	Duration of intervention	Outcomes	Notes
Cupisti <i>et al</i> ^[93]	Single arm dietary intervention study	Renal transplant patients with moderate HC	13 subjects completed study (7M, 6F)	Goal was to replace 25 g/d animal protein with soy protein (dietary counseling only)	Animal protein (baseline)	5 wk on soy diet	Significant decrease in urinary creatinine after 5 wk on soy protein compared to baseline ($P < 0.05$) Soy protein resulted in significant decrease in TC ($P < 0.05$) and LDL-C ($P < 0.01$) after 5 wk compared to baseline; no change in HDL-C	
Cupisti <i>et al</i> ^[94]	Single arm dietary intervention	Renal transplant patients and age, sex-matched healthy controls (latter for vascular measure comparisons only)	20 per group (12M, 8F)	Goal was to replace 25 g/d animal protein with soy protein (dietary counseling only)	Animal protein (baseline and WO)	5 wk on soy diet followed by 5 wk WO	Renal transplant patients had significantly reduced FMD compared to age- and sex-matched control subject ($P < 0.001$) with no differences between groups in non-endothelium-mediated vasodilation Soy diet did not change total dietary protein intake, BW, renal function, urinary protein excretion, serum Ca or P Soy diet reduced TC and LDL-C and LOOH ($P < 0.01$) compared to baseline diet Soy diet resulted in improvement in FMD ($P = 0.003$) compared to baseline while reactive hyperemia and endothelium-independent vasodilation was unchanged; FMD returned to baseline after WO Increase in FMD correlated to increase in L-arg/ADMA ratio ($P < 0.05$) with soy diet	First study to show improvement in endothelial function in brachial arteries of renal transplant patients when animal protein substituted with soy protein
D'Amico <i>et al</i> ^[95,96]	Single arm dietary intervention	Nephrotic patients with proteinuria > 1.5 g/24 h over 25 mo and HL	20 subjects (13M, 7F)	0.7-0.8 g/kg per day mostly from soy protein in test diet; test diet also contained vegetable oils and no cholesterol	0.7-0.8 g/kg per day animal protein (baseline and WO)	8 wk baseline diet followed by 8 wk soy diet and then 8 wk WO	TC, LDL-C, HDL-C, apoAI and apoB decreased on soy diet compared to baseline diet ($P < 0.001$); no change in TG; lipids tended to revert to baseline during WO Urinary protein, urea, Na and P excretion were reduced significantly from baseline during the soy diet ($P < 0.001$) Soy diet results in significant decrease in CrCl with no change in serum creatinine; this persisted during WO BP did not change	Fibre, type of fat and no cholesterol were also other components of the soy protein arm that were different from the control diet; there was a modest but significant decrease in BW on the soy protein diet (no change in BMI)

ADMA: Asymmetric dimethyl arginine; BP: Blood pressure; BW: Body weight; BMI: Body mass index; CrCl: Creatinine clearance; FMD: Flow mediated dilation; HC: Hypercholesterolemia; HL: Hyperlipidemic; HDL-C: High density lipoprotein cholesterol; LDL-C: Low density lipoprotein cholesterol; LOOH: Lipid peroxides; WO: Washout; TC: Total cholesterol; TG: Triglycerides; M: Male; F: Female.

Soy protein consumption and vascular function

Hypertension contributes to deterioration of renal function as mentioned earlier, however, only a few soy protein intervention studies have evaluated soy protein's effects on vascular function or blood pressure in patients with renal dysfunction (Tables 1 and 2). Cupisti *et al*^[94] were the first to show that soy protein consumption

resulted in improvements in endothelial function in the brachial arteries of renal transplant patients compared to baseline diets. Increased flow mediated dilation after soy consumption correlated to increases in the plasma arginine/asymmetric dimethyl arginine (ADMA) ratios in the subjects^[94]. Arginine is the substrate for and ADMA is the endogenous inhibitor of endothelial nitric oxide

Table 2 Chronic controlled intervention studies of soy protein and kidney function

Ref.	Study design	Kidney function	Subjects/group	Amount of soy protein used	Control/comparator protein	Duration of intervention	Outcomes	Notes
Liu <i>et al</i> ^[128]	RC	Pre-hypertensive PM women	90 subjects/group (85, 87 and 81 completed study in the soy, daidzein and placebo groups, respectively)	40 g soy flour/d, 12.8 g soy protein/d	40 g lowfat milk powder (placebo) or 40 g lowfat milk powder with 63 mg/d daidzein	6 mo	No significant changes in most renal parameters were observed between groups Soy flour intake resulted in less decrease in eGFR _{Cockcroft} ($P = 0.044$) and % change in eGFR ($P = 0.031$) after 6 mo compared to the milk placebo group ($P = 0.044$) Effect of soy flour consumption to increase eGFR was greater in women with higher initial plasma cystatin C concentrations (Cys-C > 1.14 mg/L) ($P = 0.001$ for eGFR _{Cockcroft}) compared to milk placebo	All subjects were equal producers
Ahmed <i>et al</i> ^[131]	RC	Glomerulopathy with proteinuria (non-diabetic)	9 subjects/group, total 27 subjects (4M, 23F)	0.8 g/kg per day soy protein	0.8 g/kg per day an animal protein or 0.8 g/kg per day a soy protein + fiber	8 wk	No significant changes in anthropometric measures, serum lipids or proteinuria between diet groups	Significant decreases from baseline in overall energy and protein intake in all groups confounds end of study comparisons
Soroka <i>et al</i> ^[129]	RC	Non-diabetic, non-nephrotic CRF patients (urinary protein excretion < 3 g/d)	9 completed study (5M, 4F)	0.71 g/kg BW protein, mostly soy protein with egg (VPD)	0.85 g/kg per day APD (1:1, animal sources: grains)	6 mo	No difference in renal function between groups seen; both groups saw reduction in rate of GFR decline BUN, Urinary N excretion, PCR, 24 h urinary creatinine and phosphate were lower in VPD group	High dropout and small number of subjects Differences in total energy and protein intake in VPD and APD
D'Amico <i>et al</i> ^[102] , Gentile <i>et al</i> ^[103]	RC	Non-diabetic, nephrotic patients with proteinuria > 2.5 g/24 h for a mean of 24 mo and HC	20 subjects (9M, 11F)	Protein intake at end of study was calculated from urinary urea excretion to be 1.16 ± 0.04 g/kg per day (98% of this estimated to be soy protein)	Soy protein used in both experimental arms of study; baseline diet was comparator	8 wk for each arm (baseline diet, soy \pm 5 g/d fish oil in random order) followed by WO for 3 mo on baseline diet)	Soy diet significantly reduced TC, LDL-C, HDL-C, apoB ($P < 0.0001$) and apoA I ($P < 0.01$) compared to baseline; TGs were unaffected; lipids tended towards baseline values after WO Addition of 5 g/d fish oil to soy diet resulted in significant elevation of TC and apoB compared to soy diet alone ($P < 0.01$) Urinary protein, urea, P, Na and creatinine excretion was significantly decreased by both diet interventions ($P < 0.01$); measures tended towards baseline after WO Blood glucose was significantly reduced by both diet interventions ($P < 0.01$) however soy diet alone reduced blood glucose more than soy diet and fish oil ($P < 0.01$)	Both diet interventions resulted in modest decrease in BW and BMI (-4%) which was significantly different from baseline; both values tended towards baseline during WO
Anderson <i>et al</i> ^[97]	RC	T2D with proteinuria, obese, and HTN	8 M	1.0 g/kg per day protein, 50% soy protein in soy test diet	1.0 g/kg per day protein, 50% ground beef in animal test diet	8 wk, 4 wk WO	TC and TG decreased by soy diet ($P < 0.05$) vs animal protein diet SUN sig decreased by soy protein ($P < 0.05$) Change in GFR similar with both diets Urine protein excretion increased by soy vs animal protein diet ($P = 0.028$)	Low number of subjects

Azadbakht <i>et al</i> ^[98]	RP	T2D subjects with nephropathy, proteinuria, HTN	41 subjects: 18 M, 23 F	0.8 g/kg per day protein, 35% soy protein (textured soy protein), 35% animal protein, 30% vegetable protein	0.8 g/kg per day protein, 70% animal and 30% vegetable protein	4 yr	Decreased FPG in soy group ($P = 0.03$) Soy protein group decreased TC ($P < 0.01$), LDL-C ($P = 0.01$) and TG ($P = 0.01$) Serum CRP decreased ($P = 0.02$) on soy protein diet Soy protein diet reduced proteinuria ($P = 0.001$) and urinary creatinine ($P = 0.01$)	
Miraghajani <i>et al</i> ^[108,113]	RC	T2D subjects with nephropathy	25 subjects completed the study (10 M, 15 F)	2.5 g soy protein (240 mL soymilk/d)	3.3 g cow milk protein (240 mL milk/d)	4 wk interventions with 2 wk WO	Soy protein consumption resulted in a significant difference in % change of fibrin D-dimer concentrations compared to milk protein ($P = 0.04$); there were no differences in % changes in TNF α , IL-6, CRP, MDA or fibrinogen concentrations between groups Soy protein consumption resulted in significant decrease in systolic BP compared to cow milk protein (-4.50% vs +5.89%, $P = 0.02$)	Amount of soy protein used in diet intervention was low
Teixeira <i>et al</i> ^[106]	RC	T2D subjects with nephropathy	14 male subjects	0.5 g/kg per day soy protein (Approximately equal 50% of total daily intake)	0.5 g/kg per day casein	8 wk interventions with 4 wk WO	Urinary albumin-creatinine ratio was significantly reduced by ISP ($P < 0.0001$) and increased by casein ($P = 0.002$) Change in urinary albumin-creatinine ratio correlated inversely with plasma isoflavone levels ($P = 0.012$) CrCl did not change (GFR) with either diet HDL-C was increased after ISP ($P = 0.0041$) while it tended to be lower after casein ($P = 0.0847$) TC and LDL-C not changed by either diet Total and glycated hemoglobin did not change in either group No differences in BP between groups; however soy diet resulted in higher plasma arg/lys ratios ($P = 0.0097$) which persisted after fasting	
Stephens <i>et al</i> ^[104]	RC	T1D subjects with hyper-filtration GFR > 120 mL/min/1.73 m ²	12 subjects completed study (6 M, 6 F)	45-55 g/d soy protein to substitute for animal protein in control diet	45-55 g/d animal protein	8 wk interventions; no WO	GFR sig lower in soy group vs control group ($P = 0.02$) Excretion of urinary creatinine, urea and Na not diff between groups Microalbuminuria within normal ranges and unaffected by diet TC and LDL-C significantly reduced in soy group ($P < 0.02$, 0.05, respectively) whereas TG and HDL-C not diff between groups Serum glucose was not affected by soy protein diet but was significantly increased on the control diet ($P < 0.05$) compared to baseline Serum albumin did not change but total serum protein decreased in soy group ($P < 0.05$)	No washout between interventions

Chen <i>et al</i> ^[100]	RP	Nondiabetic hemodialysis patients	Soy group: 10 HL (7 F, 3 M) and 8 NL (6 F, 2 M) Control group: 9 HL (7 F, 2 M) and 10 NL (7 F, 3 M)	30 g/d soy protein	30 g/d milk protein	12 wk	No significant differences between groups in serum nutritional parameters or hemodialysis adequacy TC and TG decreased in HL subjects consuming soy <i>vs</i> milk protein over time ($P < 0.05$ at 12 wk) Non-HDL-C, apoB, TC/HDL-C ratio and insulin decreased in HL subjects consuming soy <i>vs</i> milk protein at 12 wk ($P < 0.05$) Non-significant differences between protein groups in NL subjects Soy protein resulted in significant decrease in fasting insulin in NL group at 12 wk compared to values at baseline ($P < 0.05$)	Test proteins consumed on top of usual hemodialysis diet
Chen <i>et al</i> ^[100]	RP	Non-diabetic hemodialysis patients with HC	Soy group: 13 (9 M, 4 F) Milk group: 13 (10 M, 3 F)	30 g/d soy protein	30 g/d milk protein	12 wk	No significant differences between groups in serum nutritional parameters or hemodialysis adequacy TC, non-HDL-C, apoB, TC/HDL-C and LDL-C/HDL-C ratios decreased in subjects consuming soy <i>vs</i> milk protein at 12 wk ($P < 0.05$) No differences in TG between soy and milk groups Soy protein resulted in significant decrease in fasting insulin at 12 wk compared to milk protein group ($P < 0.05$)	Not clear if some of the subject are the same as reported in Chen <i>et al</i> ^[100] as study protocols are the same
Imani <i>et al</i> ^[110]	RP	PD patients	18 subjects Soy group (9 M, 9 F), Control group (9 M, 9 F)	14 g soy protein at dinner each day	Meat instead of soy protein at dinner	8 wk	Soy protein diet resulted in significant 17% reduction in plasma coagulation factor IX activity compared to control group ($P < 0.05$) No significant changes in oxLDL, P, fibrinogen or activities of coagulation factors VII and X between groups	Study was not blinded Mean energy and protein intakes were less than recommended amounts (30 kcal/kg per day and 1.2 g/kg per day, respectively) which is common among PD patients
Fanti <i>et al</i> ^[112]	RP	ESRD patients on chronic HD with elevated CRP (> 10 mg/L)	Soy group = 15; control milk group = 10	25 g/d	25 g/d milk protein	8 wk	5 to 10-fold increase in mean serum IF concentration in soy group at end of study ($P < 0.001$) No significant change in CRP between groups, however, significant inverse correlation of CRP with IF concentration Significant positive correlation of serum IF concentration and serum albumin and IGF-1	Small number of subjects Test proteins provided as beverages, a cereal-type product and as snack bar

Siefker <i>et al</i> ^[109]	RP	HD patients	17 subjects	25 g soy protein (4 times per week)	Whey protein (exact amount not specified); provided 4 times per week	4 wk	No difference between groups on serum markers of renal function except creatinine; whey protein showed a significant decrease in creatinine from baseline ($P < 0.05$) whereas there was no change in the soy protein group from baseline oxLDL was significantly decreased after soy protein consumption ($P < 0.05$) compared to baseline; the % change in oxLDL compared to the whey group was significantly different ($P < 0.05$) No differences in plasma concentrations of 8-iso-PGF _{2α} , TNFα, or CRP between diet groups	Small number of subjects
			8 subjects on soy protein diet; 9 on whey protein					
Tomayko <i>et al</i> ^[114]	RP	MHD patients	Soy group = 12	27 g/d soy protein	27 g whey protein or noncaloric placebo powder (2 g Crystal Light)	6 mo	A significant time x treatment effect for IL-6 levels ($P = 0.036$) with both whey and soy protein groups decreasing compared to control group Soy diet resulted in a significant decrease in neutrophil-lymphocyte ratio (systemic inflammation marker) compared to control or whey diet ($P = 0.02$) Alkaline phosphatase, a marker of bone turnover, was increased in the control diet compared with both protein diet groups ($P = 0.04$) A significant time by treatment interaction was seen for gait speed when all 3 groups analyzed ($P = 0.048$); both soy and whey groups indicated improved gait speed while control diet had a decline Shuttle walk test time was significantly improved in the whey group ($P < 0.05$) and when protein groups were combined ($P < 0.05$) versus the control group; shuttle walk test time was increased in the soy group but was not significant compared to the control group (which had decreased test times)	First study to observe improvements in inflammation and physical function after intradialytic nutritional support in MHD patients with serum albumin ≥ 3.9 g/dL (<i>i.e.</i> , not malnourished)
			Whey group = 11					
			Placebo control = 15					

8-iso-PGF₂: 8-iso-prostaglandin F₂; APD: Animal protein diet; BP: Blood pressure; BW: Body weight; CrCl: Creatinine clearance; CRF: Chronic renal failure; CRP: C-reactive protein; eGFR: Estimated glomerular filtration rate; FPG: Fasting plasma glucose; F: Female; GFR: Glomerular filtration rate; GS: Glomerulosclerosis; HC: Hypercholesterolemia; HD: Hemodialysis; HL: Hyperlipidemic; HTN: Hypertension; HDL-C: High density lipoprotein cholesterol; IF: Isoflavones; IL-6: Interleukin-6; LDL-C: Low density lipoprotein cholesterol; M: Male; MDA: Malondialdehyde; MHD: Maintenance hemodialysis; Na: Sodium; NL: Normolipidemic; oxLDL: Oxidized LDL; P: Phosphorus; PD: Peritoneal dialysis; PM: Postmenopausal; RC: Randomized crossover trial; RP: Randomized parallel trial; SUN: Serum urea nitrogen; T1D: Type 1 diabetes; T2D: Type 2 diabetes; WO: Washout; TC: Total cholesterol; TG: Triglycerides; TNF: Tumor necrosis factor; VPD: Vegetable protein diet; ISP: Isolated soy protein; ESRD: End-stage renal disease.

synthase, so increases in this ratio would be expected to increase endothelial-dependent vasodilation. Interestingly, Teixeira *et al*^[106] also showed increases in plasma arginine/lysine ratios after soy vs casein diet, however, no differences in blood pressure between groups was noted. Compared to baseline diet, soy protein consumption by nephrotic patients resulted in significant decreases in blood pressure in a study by D'Amico *et al*^[95,96]. In a randomized crossover study by Miraghajani *et al*^[108], soy milk vs cow milk consumption

for four weeks also significantly reduced blood pressure in Type 2 diabetics ($n = 25$) with nephropathy. However, no significant differences in renal function as assessed by proteinuria, blood urea nitrogen (BUN), serum creatinine and eGFR, were observed^[108].

Soy protein consumption and markers of oxidation

Several chronic intervention studies showed that soy protein consumption was associated with decreases in measures of systemic oxidative processes (Tables

Table 3 Human studies with soy protein and renal isoflavone metabolism

Ref.	Study design	Kidney function	Subjects/group	Amount of soy protein used	Control/comparator protein	Duration of intervention	Outcomes	Notes
Fanti <i>et al</i> ^[115] , Franke <i>et al</i> ^[192]	3 separate protocols: Assessment of baseline serum concentrations of IFs Post-ingestion concentrations of IFs Effects of hemodialysis on IF concentrations	ESRD patients on HD and normal healthy subjects	23 HD subjects and 10 healthy subjects for baseline IF measures 7 HD patients and 8 healthy subjects for meal intervention study (8 h only); 2 healthy subjects and 3 HD subjects had multiple serum and urine timepts collected 5 HD patients for pre- and post-dialysis IF measures	20 g soy protein	Baseline diet is self-selected standard renal diet	Single meal interventions	55%-65% of HD patients had undetectable serum IFs and 35%-45% had concentrations > 200 nM on standard renal diet Serum concentrations of IFs greater post-soy protein ingestion compared to baseline for both groups ($P < 0.001$); concentrations in HD subjects after 8 h of soy protein consumption were greater than those in healthy subjects ($P < 0.05$) Half-lives of genistein and daidzein averaged 3.5 and 6 h in healthy subjects, respectively but were increased to an average of 47 and 58 h in HD patients HD did not effectively remove IFs from serum since (due to higher molecular weight of conjugates and large proportion of unconjugated IFs are bound to albumin)	First study to report blood levels of genistein and daidzein in ESRD patients Daidzein metabolites equol and O-DMA were not detected in sera of any of the subjects
Fanti <i>et al</i> ^[116]	Observational	Randomly selected HD patients residing in the United States, Japan or Thailand	Subjects from: United States = 20 Japan = 20 Thailand = 17	Habitual dietary intake of soy was assessed by questionnaires developed by their renal replacement therapy programme dieticians	Study aim was to compare habitual dietary intake of soy in 3 countries	N/A	Serum IF concentrations significantly higher in HD patients from Japan compared to United States or Thailand ($P < 0.0001$) Significant correlation between soya intake and genistein ($P < 0.0001$), daidzein ($P < 0.0001$), glycitein ($P < 0.001$) and O-DMA ($P < 0.01$) in subjects from all 3 countries ESRD HD patients displayed consistently higher concentrations of daidzein compared to genistein, while the reverse occurs in healthy subjects Concentrations of sulphated and unconjugated compounds in HD subjects (Japan only studied) are comparable to those detected in healthy subjects	
Locati <i>et al</i> ^[117]	Single arm intervention study	Renal transplant patients	16 subjects (11 M, 5 F)	25 g soy protein substituted for 25 g animal protein	25 g animal protein (as habitual diet)	5 wk	Serum IFs were measured and 5 different groups were identified on the basis of the IF profiles: (1) 4 subjects had no detectable IFs; (2) only genistein was quantifiable in 7 patients; (3) 3 patients had only detectable genistein and daidzein; (4) 2 subjects only had detectable genistein and equol; and (5) 1 subject had the highest observed genistein and daidzein with detectable dihydrogenistein and equol	Concentrations of serum IFs in the renal transplant patients were similar to those observed in healthy subjects

F: Female; HD: Hemodialysis; IF: Isoflavones; M: Male; O-DMA: O-desmethylangolensin; ESRD: End-stage renal disease; N/A: Not available.

1 and 2). Cupusti *et al*^[94] showed that soy protein consumption was associated with a reduction in plasma

lipid peroxides in renal transplant patients compared to baseline diet. Siefker *et al*^[109] observed that oxidized LDL

Table 4 Human studies with soy protein on renal calcium metabolism

Ref.	Study design	Kidney function	Subjects/group	Amount of soy protein used	Control/comparator protein	Duration of intervention	Outcomes	Notes
Breslau <i>et al</i> ^[119]	RC	Normal	15 subjects completed animal and ovo-vegetarian diet phases; 10 completed all 3 phases (including vegetarian)	Soy protein accounted for most of the 75 g protein/d in vegetarian phase; accounted for an unspecified but lower amount in ovo-vegetarian phase	Animal protein accounted for most of the 75 g per day in the animal protein phase; consisted of dairy, beef, chicken and fish	12 d No WO	Serum uric acid concentrations were significantly lower with the vegetarian and ovo-vegetarian diets compared to the animal protein diet ($P < 0.01$); urinary uric acid excretion was significantly lower in ovo-vegetarian diet vs animal diet only ($P < 0.02$) Urinary Ca and P were significantly lower in vegetarian diet compared to beef diet ($P < 0.02$); urinary oxalate was significantly higher in vegetarian vs beef diet ($P < 0.02$) Animal protein diet resulted in lower PTH level vs vegetarian diet ($P < 0.05$) Serum 1,25-(OH) ₂ D was higher in the vegetarian vs animal protein diet ($P < 0.01$)	Diets were constant for Ca, P, Na and total protein
Roughead <i>et al</i> ^[120]	RC	Normal PM women	13 female subjects	25 g soy protein substituted for 25 g meat protein	25 g meat protein in control diet	7 wk	Ca retention was not affected by substituting soy protein for meat protein Urinary pH was higher on the soy diet compared to the control diet ($P < 0.0001$); renal acid excretion was lower during soy diet ($P = 0.0001$) however urinary Ca excretion was similar between soy and meat diets Substitution of soy protein for meat protein did not affect bone metabolism as indicated by no differences between diets in a number of specific bone biomarkers No differences between soy and meat protein diets in plasma lipid or hemostatic measures	

Ca: Calcium; Na: Sodium; P: Phosphorus; PM: Postmenopausal; PTH: Parathyroid hormone; RC: Randomized crossover trial; WO: Washout.

concentrations were significantly reduced in hemodialysis patients after soy protein consumption (25 g/d for 4 day/wk for 4 wk) compared to a dairy protein control. Imani *et al*^[110] failed to see any difference in oxidized LDL concentrations in peritoneal dialysis patients after 8 wk on a soy vs meat protein intervention (14 g/d).

Soy protein consumption and inflammation

Inflammation is associated with increased morbidity and mortality in patients with advanced kidney disease^[111] and some intervention studies have indicated that soy protein may have anti-inflammatory properties. Azadbakht *et al*^[98] observed a significant decrease in serum C-reactive protein (CRP) after 4 years of a soy protein vs animal protein diet. Fanti *et al*^[112] did not see any significant decrease in CRP in ESRD patients after 8 wk on a soy vs milk protein supplement, however, this group did observe a significant inverse correlation between serum isoflavone and CRP concentrations. Serum isoflavone concentrations were also positively correlated with serum albumin and insulin-like growth factor concentrations which are markers of positive nutritional status. Miraghajani *et al*^[113], on the other hand, did not see any reductions in inflammatory markers CRP, TNF α or interleukin 6 (IL-6) in Type 2 diabetic subjects with nephropathy after 4 wk of a low

dose soy protein diet (< 5 g/d). Similarly, Siefker *et al*^[109], while observing a significant decrease in oxidized LDL concentrations after 4 wk of soy protein intake in hemodialysis patients, did not see decreases in CRP, TNF α or 8-iso-prostaglandin F $_{2\alpha}$. Miaghajani *et al*^[113] and Imani *et al*^[110] both reported effects of soy protein consumption on reducing markers of coagulation. Miraghajani *et al*^[113] observed reductions in D-dimer (fibrin degradation products that have been found to be correlated with renal dysfunction) after soy milk vs cow milk consumption in Type 2 diabetics with nephropathy, with no differences in fibrinogen levels between groups. Imani *et al*^[110] reported significant decreases in plasma coagulation factor IX activity in peritoneal dialysis patients after soy vs meat protein consumption, but no changes in fibrinogen or Factor VII or X activities. In a more recent study, Tomayko *et al*^[114] reported that 27 g/d soy vs whey protein for 6 mo resulted in a significant decrease in neutrophil-lymphocyte ratio, a marker of systemic inflammation, in adequately nourished maintenance hemodialysis patients.

Metabolism of soy isoflavones in subjects with renal dysfunction

It is probably of importance to researchers to note that the metabolism and excretion of isoflavones, derived

Table 5 Single meal intervention studies of soy protein and kidney function

Ref.	Study design	Kidney Function	Subjects/ group	Amount of soy protein used	Control/ comparator protein	Duration of intervention	Outcomes	Notes
Bilo <i>et al</i> ^[122]	Single meal intervention study (crossover)	Normal healthy subjects	6 normal subjects; 5 M, 1 F	Studies in normal subjects only: 80 g soy protein in single oral administration	Studies in normal subjects only: 80 g lactoprotein or beef protein or 36 g amino acids	Normal subjects: 8 individual renal function tests run on separate days	Soy protein ingestion induced significantly lower rises in GFR and ERPF compared to beef protein but not compared to lactoprotein or 36 g amino acid ingestion	Subjects with chronic renal insufficiency (PKD, NS, or MGP) were studied in a separate series of experiments in this publication, but were not used to evaluate soy protein
Buzio <i>et al</i> ^[127]	Single meal intervention study (crossover)	Normal healthy subjects	7 (gender not specified)	80 g (0.9-1.3 g/kg BW)	80 g red meat or 80 g dairy (cheese)	Single meal interventions conducted 1 wk apart	CrCl and urinary protein were not different between protein loads UAp was significantly lower after soy protein meal versus red meat or cheese meals ($P < 0.01$) (samples taken 4 h post-meal) Water excretion rate was higher after soy protein load versus meat ($P < 0.05$) or cheese ($P < 0.01$) Serum total protein was lower after soy protein load compared to meat ($P < 0.01$) or cheese ($P < 0.01$) loads	Publication describes 2 separate experiments; soy protein effects on renal function only assessed in second experimental protocol
Deibert <i>et al</i> ^[126]	Single meal intervention study (crossover)	Normal healthy and metabolic syndrome subjects; all with normal kidney function	10 subjects per group (All males)	1 st intervention: 1 g/kg/BW soy protein; Milk protein (83% soy protein); 2 nd intervention same protein source at 0.3 g/kg BW	N/A	Single meal intervention in normal healthy subjects; 2 meal interventions in subjects with metabolic syndrome (1 wk apart)	Patients with metabolic syndrome had significantly elevated baseline GFR and ERPF compared to healthy subjects ($P = 0.02$) After ingestion of 1 g/kg/BW protein, GFR and ERPF increased in both groups however the subjects with metabolic syndrome had significantly higher increases in GFR ($P < 0.002$) and ERPF ($P < 0.02$) compared to normal subjects; no significant effect of ingestion of 0.3 g/kg per BW protein on renal parameters in subjects with metabolic syndrome	0.3 g/kg/BW is amount of protein used in meal replacement therapy
Howe <i>et al</i> ^[118]	Single meal intervention study (Latin square crossover)	Healthy PM women	8 F subjects	45 g soy protein	0 g protein, 45 g beef or dairy protein (cottage cheese)	Single meal intervention; 6 meal interventions (1 wk apart)	Urinary Ca excretion was significantly greater after 45 g protein meal for all proteins compared to basal (0 g protein) meal ($P < 0.05$) % Ca resorbed by the kidney was significantly reduced after the dairy and soy protein meals ($P < 0.05$) Serum ionized Ca was unaffected, however, serum P was significantly lowered by all protein meals ($P < 0.05$) compared to 0 g protein meal Soy protein meal significantly reduced calcitonin versus baseline ($P < 0.05$) however, all protein means tended to lower calcitonin compared to baseline Dairy protein significantly increased PTH ($P < 0.05$) compared to baseline, however all protein meals tended to elevate PTH compared to baseline Serum insulin was significantly increased by all protein meals (over time) compared 0 g protein meal ($P < 0.05$)	

Kontessis <i>et al</i> ^[70]	Single meal intervention study (crossover)	Normal healthy subjects	7 M subjects	80 g soy protein	80 g lean beef	2 separate single meal interventions	GFR and ERPF increased significantly after acute beef protein load ($P < 0.005$ compared to baseline) but did not increase with soy protein load Renal vascular resistance fell significantly after beef load ($P < 0.05$) but was unchanged after soy protein load; plasma 6-keto-PGF1 α rose significantly after meat load ($P < 0.05$) but not after soy protein load Fractional albumin and IgG clearance rose after beef load ($P < 0.05$ and $P < 0.001$, respectively) but did not change significantly after soy protein load; plasma protein concentrations were not different between different protein loads; UAp was not different between groups Plasma glucagon increase was higher after meat load ($P < 0.05$) compared to soy protein load; no differences were seen between proteins on plasma insulin or growth hormone	Amount of soy protein in vegetable protein diet in the reported chronic study was not specified so is therefore not summarized
Nakamura <i>et al</i> ^[123]	Single meal intervention study (crossover)	Healthy and T2D subjects (T2D divided into 3 groups based on AER: Group A ≤ 20 $\mu\text{g}/\text{min}$ (Normal); B = 20-200 $\mu\text{g}/\text{min}$; C ≥ 200 $\mu\text{g}/\text{min}$)	11 healthy subjects (8M, 3F); 20 T2D patients (10 M, 10 F)	1g/kg soy protein (as bean curd)	1 g/kg tuna fish protein	Meals fed on separate days	In healthy subjects, eGFR increased ($P < 0.01$) after tuna meal but no significant difference after soybean curd meal In Grp A, eGFR increased with tuna meal ($P < 0.01$) but not after soybean curd In Group B there was no difference in GFR with either protein In group C, GFR sig decreased after tuna meal ($P < 0.05$) but not with soy protein No changes in AER with any protein in any group	
Nakamura <i>et al</i> ^[124]	Single meal intervention study (crossover)	Healthy and T2D subjects	10 healthy subjects and 6 T2D subjects	0.7 g/kg soy protein (as bean curd)	0.7 g/kg tuna fish protein or egg white protein or dairy protein (cheese)	Meals fed on separate days	eGFR was only significantly increased after ingestion of tuna fish protein ($P < 0.001$) and not after consumption of soy, egg white or dairy proteins	
Orita <i>et al</i> ^[125]	Single meal intervention study (crossover)	Healthy subjects	6 male subjects	86.9 g soy protein	86.9 g beef protein or fasting (0 g protein)	Meals fed 1 wk apart	Inulin clearance (GFR) was significantly increased over baseline at 2 h post beef or soy protein compared to fasting ($P < 0.005$ and $P < 0.05$, respectively) Creatinine clearance (GFR) was significantly increased by both beef and soy proteins at 2 and 3 h post-ingestion compared to fasting ($P < 0.01$) Plasma glucagon was significantly increased at 1 to 3 h post-ingestion by both beef and soy protein compared to fasting ($P < 0.01$)	First study to show an increase in GFR after a soy protein load in healthy subjects

AER: Albumin excretion rate; BW: Body weight; Ca: Calcium; CrCl: Creatinine clearance; ERPF: Effective renal plasma flow; eGFR: Estimated glomerular filtration rate; F: Female; GFR: Glomerular filtration rate; M: Male; MGP: Membranous glomerulopathy; NS: Nephrosclerosis; P: Phosphorus; PKD: Polycystic kidney disease; PM: Postmenopausal; PTH: Parathyroid hormone; T1D: Type 1 diabetes; T2D: Type 2 diabetes; UAp: Urinary urea appearance rate.

predominantly from soy in the diet, are mediated by the kidneys. Three studies have investigated the metabolism

of soy isoflavones in subjects with renal disease (Table 3). Fanti *et al.*^[115] noted that 55%-65% of hemodialysis patients in the United States had undetectable concentrations of serum isoflavones when they were on a standard renal diet. However, after a single soy meal ingestion, these levels were significantly increased in healthy subjects, but in ESRD patients on hemodialysis, serum isoflavone increases were on average increased three to four times higher than seen in the healthy subjects^[115]. Half-lives of genistein and daidzein averaged 3.5 and 6 h, respectively in healthy subjects but were increased to 47 and 58 h in hemodialysis patients. Fanti *et al.*^[116] also observed that hemodialysis patients in Asian countries have significantly higher serum isoflavone concentrations than that seen in the United States. Hemodialysis does not effectively remove glucuronide-conjugated isoflavones due to their relatively high molecular weight^[115]. Concentrations of sulfated and unconjugated isoflavones, however, tend to be similar between hemodialysis patients and healthy subjects^[116]. Fanti *et al.*^[116] also, not surprisingly, observed significant correlations between dietary intake of soy foods and overall serum concentrations of isoflavones. Ratios of daidzein and genistein in the serum also tend to be different between ESRD and healthy subject indicating the alterations in normal tubular excretion properties in the ESRD patients^[116]. It appears that renal patients who have undergone renal transplantation exhibit concentrations of serum isoflavones comparable to those observed in healthy subjects^[117].

Soy protein consumption and calcium metabolism

A study by Howe *et al.*^[118] cited in Table 4 evaluated the postprandial responses of calcium metabolism to single meal loads of varying protein sources in healthy postmenopausal women. High protein intake has been shown to increase urinary calcium excretion^[118]. Protein loads of 45 g of soy, beef or cottage cheese protein (but not 15 g) resulted in significant increases in urinary calcium excretion up to 3 h postprandially and the percent of calcium resorbed by the kidney was significantly reduced after the dairy and soy protein meals^[118]. Serum ionized (free) calcium was unaffected but serum phosphorus was significantly lowered by all protein meals compared to the non-protein meal^[118]. Soy protein significantly reduced calcitonin levels vs baseline and dairy protein significantly increased parathyroid hormone (PTH), however, all proteins tended to lower calcitonin and raise PTH^[118]. Thus, there may be subtle effects of protein source on calcium metabolism in the acute setting that involves renal metabolism. Table 4 summarizes two chronic intervention studies with soy protein that assessed its effect on calcium metabolism. Breslau *et al.*^[119] reported that serum uric acid concentrations were significantly lower after 12 d intervention on a largely soy-based vegetarian and ovo-vegetarian diet compared to the animal protein diet. The animal protein diet exhibited a significantly higher urinary uric acid excretion compared

to that observed with the ovo-vegetarian diet and is likely explained by the high purine content in animal foods^[119]. Excretion of calcium and phosphorus were significantly lower and urinary oxalate higher in the vegetarian diet compared to the beef diet^[119]. Breslau *et al.*^[119] reported lower PTH levels in the animal vs vegetarian diet group and serum 1,25-(OH)₂D was higher in the vegetarian compared to animal diet group^[119]. Roughead *et al.*^[120] did not observe any difference in urinary calcium excretion between soy and meat protein diets after 7 wk of intervention in healthy postmenopausal women, despite a significantly higher urinary pH in the soy group. Overall, no differences in measures of bone metabolism or body calcium retention were seen when women were ingesting the soy or meat protein diets^[120]. A recent study of maintenance hemodialysis patients (cited in Table 2) measured alkaline phosphatase (ALP), a measure of bone turnover and an independent predictor of mortality in these patients^[114]. The investigators noted that both soy and whey proteins gave rise to lower serum ALP compared to a non-protein placebo supplement^[114] which indicates that both proteins may be useful in improving health in these severely renal-compromised patients. This is further supported by the same authors' measures of physical function in these patients; both protein groups showed benefits in gait speed and shuttle walk test times on the protein vs placebo diets^[114]. In this study, subjects were not considered malnourished (mean serum albumin > 3.9 mg/dL) and yet, still sustained benefit from protein supplementation suggesting that there is the potential for enhanced effects in ESRD patients with nutritional deficiencies.

Soy protein consumption and renal function - acute studies

Table 5 summarizes studies conducted to evaluate the effects of single meal interventions with soy vs other proteins on renal function. It is well established that acute protein ingestion or infusion of amino acids results in a transient increase in GFR, or "hyperfiltration"^[121]. While this mechanism may be the normal response to protein ingestion, in patients with CKD, the hyperfiltration induced by a high protein diet is believed to contribute to the decline in renal function that deteriorates the undamaged nephron function^[121]. Acute ingestion of > 50 g soy protein has been shown to result in significantly lower increases in GFR compared to equal amounts of meat or fish protein, but not egg white, dairy protein or amino acid ingestion, in several studies^[70,122-124] while one study showed an increase in GFR after soy protein consumption that was equivalent to that induced by beef protein^[125]. Kontessis *et al.*^[70] determined that plasma glucagon was higher after a meat meal compared to a soy meal and plasma glucagon levels correlate with GFR^[121]. However, Orita *et al.*^[125] noted that both beef and soy elicited similar increases in plasma glucagon. Differences between these two studies may be in the way the proteins were

provided to the subjects since the protein loads were similar; the subjects in the Kontessis *et al.*^[70] study consumed soy as a powder dissolved in flavored water while the soy protein consumed by subjects in the Orita *et al.*^[125] study consumed the protein as a fried paste. The degree of renal function in subjects also affected the response to soy protein ingestion. Diebert *et al.*^[126] observed that subjects with metabolic syndrome had higher baseline GFR compared to healthy subjects and that there was a significantly greater increase in GFR in the subjects with metabolic syndrome after ingesting 1 g/kg body weight soy protein compared to healthy subjects. Notably, no differences from baseline GFR responses between subjects with metabolic syndrome and healthy subjects were seen when the protein load was reduced to 0.3 g/kg body weight which is the amount of protein commonly used in meal replacement products for weight management^[126]. Nakamura *et al.*^[123] also noted that when GFR data from subjects with Type 2 diabetes was divided by degree of urinary albumin excretion, that GFR was lower in the soy (bean curd) vs tuna protein meal only in the subjects with mild proteinuria and was not significantly different between proteins in the group with mid-range proteinuria. In subjects with significant proteinuria, the tuna protein meal caused a decrease in GFR but there was no significant change after intake of the soy protein^[123]. However, no differences in AER were observed between groups. The authors concluded that the amino acid compositions of the two proteins may be exerting differential effects on GFR based on elevated levels of circulating alanine, glycine and arginine observed after tuna fish meal consumption vs bean curd^[123].

In the acute protein loading study in normal subjects reported by Kontessis *et al.*^[70], fractional albumin and immunoglobulin G clearance (excretion divided by GFR) increased after a beef protein load but did not change after soy protein consumption. Urinary urea was increased similarly in both groups^[70]. In contrast, Buzio *et al.*^[127] reported significantly lower urinary urea appearance after soy compared to red meat or cheese consumption suggesting differences in soy protein digestion or absorption. Differences between these studies may again be related to the form of soy protein provided; in the study by Buzio *et al.*^[127] the soy protein was provided as soybeans and tofu and not as protein powder in the Kontessis *et al.*^[70] study.

Soy protein consumption and renal function - chronic studies

Soy protein consumption was shown to result in beneficial reductions in baseline GFR in chronic intervention studies in Type 1 diabetics with hyperfiltration compared to an animal protein diet^[104] and in pre-hypertensive postmenopausal women compared to a dairy protein supplement^[128] (Table 2). In the former study, the effect of 45-55 g/d soy protein to significantly reduce GFR in the subjects with demonstrated renal dysfunction compared to the same subjects on animal-based

diet could be seen in 8 wk of intervention^[104]. In the latter study, the postmenopausal women subjects had minimally compromised or normal renal function as assessed by GFR values, but after 6 mo on a soy protein diet (12.8 g/d) there was less of a decrease in eGFR than observed in subjects consuming the milk protein placebo^[128]. The effect of soy protein consumption to reduce eGFR was greater in women with higher initial cystatin C concentrations (indicating poorer renal function at study initiation)^[128]. No significant changes were observed in any other renal parameter in that study. Soroka *et al.*^[129] also evaluated GFR after a 6 mo intervention with a vegetable protein (mostly soy) or animal protein in non-diabetic, non-nephrotic chronic renal disease patients. No differences in GFR between groups were observed at the end of the study but both groups demonstrated reductions in the rate of decline of their renal disease^[129]. The high dropout rate and small number of subjects that completed the study as well as differences in the total energy and protein intakes between the diets makes it difficult to make any conclusions as to any benefits of soy protein on renal function. In another small study comparing 0.5 g/kg per day of soy or beef protein consumed for 8 wk by Type 2 diabetic subjects with proteinuria, Anderson *et al.*^[97] observed that both interventions resulted in similar decreases in GFR. Only the soy protein group demonstrated a significant decrease in serum urea nitrogen and an increase in urinary protein excretion^[97]. No other study has reported an increase in urinary protein excretion after a soy protein intervention. Other studies cited in Table 2 have demonstrated a reduction in proteinuria after soy protein interventions^[98,103,106,130]. Teixeira *et al.*^[106] also noted that changes in urinary albumin-creatinine ratios correlated inversely with plasma isoflavone concentrations. It is interesting to speculate that in subjects with more progressed renal dysfunction, elevated isoflavones resulting from inefficient renal clearance may help to protect the kidney from further damage.

Taken together, the data suggests that both short- (weeks) and long-term (years) consumption of soy-based diets is renoprotective in both healthy and renal compromised individuals. While this effect has not been seen in all studies^[97,108,131], the majority of studies show no negative consequences of soy protein consumption on renal function. A renoprotective effect of soy protein is supported by a recent meta-analysis of nine clinical trials comprised of 197 subjects, concluding that soy protein intake reduced serum creatinine in patients with pre-dialysis CKD^[77].

SOY PROTEIN CONSUMPTION - ANIMAL STUDIES

Soy protein consumption and renal function

The renoprotective effects of a soy-based diet were demonstrated 25 years ago using the male Fischer 344 rat

model of chronic, progressive nephropathy. In 1988, Kalu *et al.*^[132] demonstrated that life-long feeding of a soy-based diet (21 g soy/100 g diet) attenuated the late-life (21 mo and older) increase in serum creatinine^[132]. Interestingly, the soy-fed rats had similar renal function as the life-long caloric restriction - the gold standard for protection against age-related nephropathy. Iwasaki *et al.*^[133] used a similar protocol and demonstrated that median life span of the control rats was 730 d, compared to 844 in the soy-fed rats^[133]. In the control group, 41% of the rats that spontaneously died exhibited end-stage chronic nephropathy, which was reduced to 7% by the soy diet. A soy protein diet was shown to improve longevity in male Fischer 344 rats similar to life-long caloric restriction^[134]. This positive effect on survival was also seen in a study feeding male Wistar rats soy milk and normal rat chow beginning at 3 mo; the percentage of surviving animals at 18 mo increased from 55% to 87%^[135]. Importantly, the effect of a soy diet is not due simply to protein restriction as 40% diet restriction without restricting protein intake is highly effective at attenuating nephropathy^[136].

There is an extensive body of literature on the positive effect of soy on PKD in animal models. In Pcy mice, a model of PKD, a soy-based diet, both high and low protein diet (17.4 or 6 g/100 g diet, respectively, casein protein as control) for 13 wk reduced cyst size in low protein diets^[137]. The protective effect in this model was reproduced in another study^[138]. Han:SPRD-Cy rats, a PKD model, were fed 20% soy or casein diets *ad libitum* for 8 wk after weaning; the soy diet decreased serum creatinine and reduced cysts^[139]. In the same model, animals were fed 20% soy protein for 1 or 3 wk (casein protein as control) and at 3 wk, cyst area was reduced, and creatinine clearance improved^[140]. The positive effect of soy on creatinine clearance has also been observed in this model using heat-treated soy protein isolate^[141]. Interestingly, a soy diet 2 wk before mating - the diet is discontinued during pregnancy and lactation, afforded renoprotection in this model as assessed by proteinuria, but not serum creatinine or creatinine clearance^[142].

The renoprotective effects of soy protein have also been shown in metabolic models of renal dysfunction, including Zucker Diabetic Fatty (ZDF) and high-fructose fed rats^[143-145]. A 20% soy diet (casein control) for 160 d reduced proteinuria, as well as glomerulosclerosis and tubulointerstitial fibrosis, in obese Zucker rats^[146]. In female obese Zucker rats, a soy protein diet decreased proteinuria and glomerular damage, but did not affect creatinine clearance^[147]. In the male obese Zucker, a soy protein isolate diet (23.1 g/100 g diet) was begun 10 d after unilateral nephrectomy and renal damage was further induced by deoxycorticosterone acetate (DOCA). Proteinuria and urinary N-acetyl-beta-D-glucosaminidase (NAG) were reduced by soy at 1 and 2 wk after DOCA^[148].

The renoprotective effects of soy have also been observed in other models of renal dysfunction. A

soy protein diet (24.5%) reduced proteinuria in the male Imai rat model of spontaneous focal segmental glomerulosclerosis^[149]. However, when the control casein diet was supplemented with soy, protection was not seen. A soy diet also attenuated BUN and improved creatinine clearance in this model^[149]. However, soy has not been shown to be renoprotective in all studies. A 20% soy protein diet did not reduce cyst volume in Han:SPRD-Cy rats^[150]. In female PCK rats fed soy protein isolate 200g/kg diet (casein control) beginning at 5 wk and maintained on the diet for 12 wk, no positive effect on cyst size, inflammation, fibrosis, or BUN was seen^[151]. In a comparison of whey protein (13.8 g/100 g diet) and soy protein (13.1 g/100 g diet), Wistar rats were fed diets for 12 wk; no differences in renal morphology or function (albuminuria or BUN) were observed^[152]. In obese Zucker rats, a soy protein diet attenuated glomerular damage, but not proteinuria^[153]. Soy protein did not have a protective effect on GFR (assessed by creatinine clearance) in a canine model of nephropathy^[154]. The MRL/Mp-lpr/lpr mice are an autoimmune model of lupus; weanling female mice were fed soy (20% soy protein and 5% soybean oil) for 4-16 wk; in this model, soy increased proteinuria, reduced creatinine clearance, and increased serum creatinine^[155].

Mechanisms of action

Given the positive effects of soy in several animal models, and limited clinical studies, efforts have been made to identify the mechanism of the soy-based protection - both the specific soy constituent and the molecular/cellular pathways affected by soy. Beneficial effects have been seen in several of the animal models using a low-isoflavone diet^[156,157], suggesting that the isoflavones are not responsible for renoprotection. This is supported by the finding that genistein did not have a protective effect in the Pcy mouse model^[138] and that isoflavone-enriched soy protein diet did not enhance the effect of soy on decreasing cysts^[158]. However, genistein alone did reduce inflammation, oxidative stress and albuminuria and increased creatinine clearance in a high fructose model of renal dysfunction^[144,159]. In addition, in male obese ZDF × Spontaneously Hypertensive Hyperlipidemic rats, a high isoflavone, but not a low-isoflavone, diet attenuated BUN^[143]. In recent studies, β -conglycinin has been shown to have a positive effect on blood pressure, nephrin expression, proteinuria and lipid peroxidation in diabetic nephropathy in Spontaneously Hypertensive rats^[160].

Limited progress has been made in determining the cellular and molecular targets of soy in relation to renoprotection (Figure 1). Systemically, soy has been shown to have blood pressure lowering effects and positive effects on hyperlipidemia. Soy can reduce plasma^[161,162] and renal ACE activity^[163]. In 5/6 nephrectomized rats, Yang *et al.*^[164] showed that substitution of casein for 14 wk with either pepsin-hydrolyzed or intact soy protein in the diet ameliorated increases in systolic and diastolic

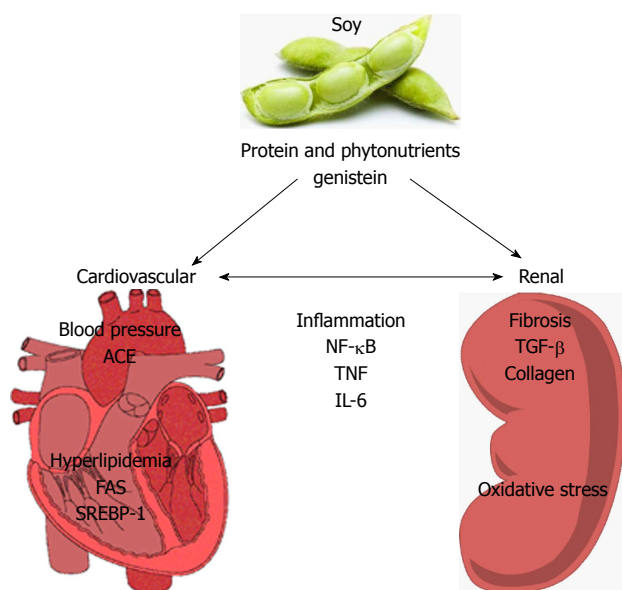


Figure 1 Potential mechanism(s) of soy-based renoprotection. Soy protein has been shown to have effects on the cardiovascular system, as well as direct renal effects, that improve renal function. The actual components of soy that mediate these effects are unclear; but some data suggest a contribution of soy isoflavones, including genistein. Lowering of blood pressure may occur *via* decreased angiotensin-converting enzyme levels. In addition, soy improves the lipid profile, putatively *via* a decrease in lipogenic pathways demonstrated in animal models (e.g., FAS and transcription factor SREBP-1). The anti-inflammatory effects of soy may occur systemically, or within the kidney; animal studies have shown decreased NF- κ B, TNF- α and IL-6 expression. Soy protein decreases renal fibrosis, possibly *via* a reduction in TGF- β signaling. There is also data suggesting that soy improves oxidant defense mechanisms in the kidney. Targets shown in *italics* have only been demonstrated in animal models. FAS: Fatty acid synthase; ACE: Angiotensin-converting enzyme; SREBP-1: Sterol regulatory element-binding protein 1; TGF- β : Transforming growth factor- β .

blood pressure coincident with significant decreases in plasma and renal ACE activities, kidney TNF α levels, proteinuria and plasma insulin concentrations^[164]. Yang *et al.*^[165] also evaluated the effects of 6 wk consumption of pepsin-hydrolyzed soy protein in a rat model of L-NAME (L-N^G-Nitro-L-arginine methyl ester)-induced hypertension, observing dose-dependent reductions in blood pressure, BUN, ACE activities and renal vascular damage^[165]. There is also data suggesting that soy may have beneficial effects on lipid profiles that correlate with renoprotection. In a puromycin-induced model of nephrotic syndrome, soy protein (20%) reduced both hypercholesterolemia, hypertriglyceridemia and proteinuria^[166]. In addition, the soy diet reduced sterol regulatory element-binding protein 1 and fatty acid synthase expression. In aging Wistar rats (18 mo), soy milk decreased total serum cholesterol, LDL-C and serum triglycerides and reduced renal lipid peroxidation^[135]. Soybean β -conglycinin has been shown to reduce cholesterol and improve renal function (albuminuria) in a streptozotocin-induced diabetes^[163].

Soy may also target the kidney itself to improve renal function, specifically *via* anti-inflammatory, antioxidant, or anti-fibrotic mechanisms. A reduction in renal NF- κ B has been observed in experimental studies^[145]. TNF- α

and IL-6 are decreased by soy^[145,164,165]. Chemokine (C-C Motif) receptor 2 (CCR2) is a chemokine receptor that is implicated in nephropathy^[167]; soy attenuates CCR2 expression^[168]. In a rat PKD model, a soy protein diet also decreases cyclooxygenase -1 and -2 activities^[169]. ROS are generated by normal cellular metabolic processes and include superoxide O $_2^{\cdot-}$, hydrogen peroxide (H $_2$ O $_2$) and the hydroxyl radical (OH $^{\cdot}$)^[170,171]. Reactive nitrogen species, such as peroxynitrite (ONOO $^-$), are formed through reaction of O $_2^{\cdot-}$ and NO $^{\cdot}$ ^[172]. As reviewed by Tucker *et al.*^[173], markers of oxidative stress have been shown to be significantly elevated in CKD patients and to be inversely correlated with eGFR^[173-176]. There is evidence that soy can reduce bromate- and iron-induced H $_2$ O $_2$ levels in the kidney, which corresponds with decreased lipid peroxidation^[177,178]. A soy diet (14.1% total energy) supplemented with genistein (40 mg/kg per day) has been shown to increase antioxidant capacity, including increased catalase, and decrease lipid peroxidation in a doxorubicin-induced renal dysfunction^[179]. Mechanistically, however, other studies have shown that soy did not affect superoxide dismutase, catalase, or glutathione-peroxidase activity, but did reduce nitrotyrosine levels^[180]. Thus, while soy may have antioxidant properties, the underlying mechanism has not been elucidated. There is data supporting the hypothesis that soy may inhibit the development of fibrosis, the common pathway in the development of CKD. Soy has been shown to reduce fibrosis, most notably in PKD^[181] and genistein reduces fibrosis in a high-fructose model^[145]. There is also evidence that soy decreases collagen expression^[145,182]. TGF- β is a potent pro-fibrotic mediator in the kidney^[183]; several studies have shown that soy attenuates renal TGF- β expression^[160,166,184]. In human HK-2 cells, parathyroid hormone-induced epithelial-to-mesenchymal transition α smooth muscle actin expression is attenuated by genistein (25-100 μ mol/L); in addition, there is reduced expression of pro-fibrotic connective tissue growth factor expression^[185]. Reviewed previously, Wnt/ β -catenin signaling is strongly implicated in renal fibrosis through its downstream induction of profibrotic gene expression^[186] as well as cyst formation in PKD^[187]. The effects of soy protein intake on Wnt signaling have only recently begun to be explored. Several studies conducted in rodent models of dyslipidemia have demonstrated effects of soy protein consumption on hepatic gene expression of Wnt pathway intermediates^[188-190]. Further investigation is needed to understand soy's impact on the Wnt/ β -catenin pathway in the kidney and how this may function in renoprotection.

CONCLUSION

Soy protein consumption has benefits in patients at risk for and who have demonstrated renal dysfunction and symptoms of early kidney disease. Soy protein can improve the dyslipidemia that contributes to and results

from renal disease. In addition, soy protein has been shown to reduce blood pressure and improve vascular health in subjects with renal disease and this may be related to its ability to reduce markers of oxidative stress and inflammation. Studies have shown that in the long term soy protein consumption can reduce deterioration of glomerular function and proteinuria, albeit in small-scale clinical studies. In acute studies, soy protein meals tend to increase GFR less than animal-derived protein (but not dairy) meals. The reasons for this remain to be elucidated and may be related to elevations of select amino acid profiles and/or micronutrients associated with soy vs animal derived proteins. Animal studies have begun to identify possible mechanisms of action of soy protein and its components in slowing the onset and progression of kidney dysfunction and more research, both human and animal, is needed to elucidate the mechanism of soy protein's renoprotective effects. Furthermore, studies, both preclinical and clinical, can further contribute to our knowledge of the role of dietary soy protein on renal health by more careful design and reporting of the interventions and outcomes as prescribed by Klein *et al.*^[191]. A large-scale clinical trial including detailed information on the soy source, analysis and reporting of analytical methodology used to determine bioactive constituents, both in the diet as well as in biological assessment following diets, and identifying dietary constituents that may interact with soy in the diet, is warranted based on the promising results summarized in this paper.

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Stop chronic kidney disease progression: Time is approaching

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Abstract

Progression of chronic kidney disease (CKD) is inevitable. However, the last decade has witnessed tremendous achievements in this field. Today we are optimistic; the dream of withholding this progression is about to be realistic. The recent discoveries in the field of CKD management involved most of the individual diseases leading the patients to end-stage renal disease. Most of these advances involved patients suffering diabetic kidney disease, chronic glomerulonephritis, polycystic kidney disease, renal amyloidosis and chronic tubulointerstitial disease. The chronic systemic inflammatory status and increased oxidative stress were also investigated. This inflammatory status influences the anti-senescence *Klotho* gene expression. The role of *Klotho* in CKD progression together with its therapeutic value are explored. The role of gut as a major source of inflammation, the pathogenesis of intestinal mucosal barrier damage, the role of intestinal alkaline phosphatase and the dietary and therapeutic implications add a novel therapeutic tool to delay CKD progression.

Key words: Chronic kidney disease; Progression; *Klotho*; Amyloidosis; Diabetic nephropathy; Micro RNA

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Core tip: The problem of chronic kidney disease (CKD) progression is a panic, affecting both patients and physicians. The fact that such patients will sooner or later need RRT terrifies them and makes these patients to survive a continuous mare. All the trials to stop this progression in the past only delayed this progression for some time. However, in the last 2 years many genuine experimental and clinical trials revived the hope to stop the progression almost completely in the vast variety of chronic renal diseases. In this review, we are highlighting most of these trials, stressing on the

different mechanisms that would stop CKD progression.

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INTRODUCTION

Chronic kidney disease (CKD) affects approximately one-seventh of adults above the age of 20 years^[1]. Progression of CKD is a major concern during managing patients in stages G1-4. The suppression of known "causes" of progression by targeting high blood pressure (BP) as well as the renin-angiotensin system (RAS) has achieved some success in REIN, RENAAL, IDNT, and other clinical trials^[2-4]. However, progression to end-stage renal disease (ESRD) is still inevitable. The recent discoveries of novel mechanisms underlying CKD progression opened the gate for more comprehensive understanding of the pathophysiology of CKD progression and the development of new therapeutic strategies. The role of chemokines in the recruitment of inflammatory cells into the kidney of a variety underlying diseases has opened the gate for new promising therapeutic modalities^[5,6]. The intensive studies done on *Klotho* and fibroblast growth factor 23 (FGF23) and their role in the control of renal phosphate handling^[7], and their unique anti-aging properties^[8,9] have disclosed appreciable data concerning their action on vascular calcification (V.C.)^[10], cardiac hypertrophy^[11], renal tubular epithelial- mesenchymal cell transformation^[12], and increased interstitial fibrosis^[13]. The last decade also witnessed the role of the gut in the pathogenesis of systemic inflammation in CKD patients^[14-16]. This chronic inflammatory status might add directly, through absorbed toxins or through its interaction with *Klotho* gene to the risk of V.C. and CKD progression^[17,18]. Therapeutic interventions manipulating such factors, besides the recent introduction of tolvaptan to treat autosomal dominant polycystic kidney disease (ADPKD)^[19], therapeutic IgG anti-SAP for the treatment of amyloidosis^[20,21], and anti-micro RNA for progressive interstitial fibrosis and/or glomerulosclerosis^[22] will expectedly improve the strategy combating CKD progression.

Epidemiology

CKD is inevitably progressive with the consistent decrease of glomerular filtration rate, leading finally to ESRD. In 2002, the United States National Kidney Foundation Kidney Disease Outcomes Quality Initiative clinical practice guidelines defined CKD as kidney damage or glomerular filtration rate lower than 60 mL/min per 1.73 m² or the presence of increased urinary

albumin excretion for 3 mo or longer, and proposed a classification scheme based on glomerular filtration rate^[23]. The important impact of albuminuria on CKD progression^[24] prompted the Kidney Disease: Improving Global Outcomes (KDIGO) Work Group on Evaluation and Management of CKD to include albuminuria in the revised 2012 classification^[25]. The estimated prevalence of CKD worldwide is 8%-16%^[26]. CKD is the 18th cause of death in 2010 (annual death rate 16.3 per 100000)^[27]. The 10 years all-cause mortality in diabetic nephropathy patient is around 5 times the rate in age and sex-matched nondiabetic personnel and triple the rate of diabetic patients without kidney disease^[28]. The risk of death increases as the GFR declines < 60 mL/min per 1.73 m² of body-surface area: The adjusted hazard ratio for death is 1.2 in CKD stage G3a, 1.8 in stage G3b, 3.2 in G4, and 5.9 in G5. The adjusted hazard ratio for cardiovascular events also increased inversely with the estimated GFR: 1.4, 2.0, 2.8, and 3.4 respectively. The adjusted risk of hospitalization with a reduced estimated GFR followed a similar pattern^[29]. These results indicate the serious impact of CKD progression on morbidity and mortality of CKD patients. It can also explain the marked discrepancy in the distribution of prevalence among different CKD stages^[30].

Proteinuria is an added risk for both CKD progression^[31] increased cardio-vascular and overall mortality^[32].

Pathogenesis

The mechanism of CKD progression among different CKD entities involves cytokine actions on renal hemodynamics, glomerular, and tubular functions. The characteristic pathologic feature of CKD is glomerular and interstitial infiltration by macrophages^[33]. Angiotensin II contributes to the hemodynamic and glomerular changes following the initial renal insult. This contribution results in progression of glomerular disease^[34]. Glomerular hypertension that follows renal insult results in increased angiotensin II activity. Angiotensin II activates transforming growth factor- β (TGF- β), macrophage chemoattractant protein (MCP-1), and vascular endothelial growth factor (VEGF) within the glomerulus^[35,36]. Accumulation of macrophages and lymphocytes; thus, ensues with further increase in production of IL-1, TNF- α , and MCP-1^[37,38]. Accumulating cytokines cause progressive glomerular damage by targeting podocytes. Although VEGF is a key player in the formation and maintenance of glomerular filtration barrier, elevated levels of VEGF are associated with glomerular hyperfiltration, hypertrophy, and proteinuria^[39]. Increased podocytes VEGF contributes to glomerular sclerosis in transgenic mice^[39]. Cytokines act also on mesangial cells inducing their proliferation or transforming them to fibroblast phenotype^[33]. The mesangial cell fibroblast phenotype secretes extracellular matrix components with consequent glomerular sclerosis^[33,40,41]. Endothelial cells generate endothelin,

TGF- β , and platelet-derived growth factor, in response to shear stress and glomerular hypertension. These cytokines and growth factors can also contribute to progressive glomerular sclerosis^[42,43]. Endothelial cells can also generate IL-1, TNF- α , and MCP-1 that ultimately result in attraction and proliferation of inflammatory cells^[44]. Intracellular adhesion molecule 1 (ICAM-1) secreted by endothelial cells facilitates neutrophil adhesion and enables macrophage infiltration^[35]. Although glomerular sclerosis is the key features of CKD progression; the tubulointerstitial damage correlates better with this progression than glomerular damage^[35]. Tubulointerstitial inflammation leads to tubulointerstitial damage. This inflammation starts as a consequence of glomerular hypertension and hypertrophy^[33]. Interstitial infiltration of inflammatory cells occurs in the early phases of renal diseases irrespective of the initial renal insult. These are primarily macrophages and T and B lymphocytes recruited to the interstitium by chemokines and adhesion molecules expressed by damaged tubular epithelium^[45]. Glomerular proteinuria is the postulated link between glomerular and renal tubular injury. Proteinuria may damage tubular lysosomes and increases MCP-1 release by proximal tubular epithelial cells^[46]. MCP-1 recruits and activates macrophages to release TGF- β . Tubulointerstitial fibrosis eventually starts and progresses^[47]. Fibroblasts maintain their activated phenotype even in the absence of the initial insult, *i.e.*, autonomous progression once the process starts^[48]. Tubular cells injured by lymphocytes and cytokines try to regenerate in a trial to replace damaged cells. This regeneration needs the transition of healthy epithelial cells into mesenchymal cells. This process is called epithelial-mesenchymal transition (EMT). Mesenchymal cells proliferate then transform back to epithelium if microenvironment becomes convenient (as occurs during recovery of acute tubular necrosis); otherwise, if inflammation is still there, mesenchymal cells transform into fibroblasts that continue the process of interstitial fibrosis^[49]. The anti-senescence protein, Klotho, favors epithelial regeneration and inhibits fibroblast phenotype transformation during EMT^[50]. Inflammation^[17,18,51,52], angiotensin II^[19,53,54], hyperphosphatemia and vitamin D deficiency^[55] suppress Klotho gene. Deficient Klotho activity enhances tubulointerstitial fibrosis^[56]. The attempt to repair damage begins with the recruitment of inflammatory cells but ends with an unchecked inflammatory response that activates matrix-producing cells leading to tubular cell apoptosis, irreversible scarring, loss of renal function, and ultimately ESRD^[57]. The extent of damage rather than the underlying disease determines the outcome^[58]. Progressive fibrosis is likely responsible for the disruption of glomerular and tubular architecture. Inhibition of the major mediators responsible for matrix accumulation might slow or arrest the progression of CKD. Support for this concept has been provided by the results of a number of studies in animal models of CKD, in which inhibiting factors that promote fibrosis, such as TGF- β , connective

tissue growth factor, and myofibroblast activation^[59-63] or enhancing factors that attenuate fibrosis, such as bone morphogenetic protein 7 and hepatocyte growth factor^[64,65] improved renal architecture and/or function. The present data indicate that TGF- β is the master regulator of the molecular events that result in renal fibrosis^[66]. So far, clinical trials using TGF- β antibodies did not achieve satisfactory results.

Standard of care management: Table 1

We do not have data to support the role of life style modification procedures (body weight control, exercise, and smoking quitting) on the course of CKD or cardiovascular impact in this population.

Protein restriction did not significantly affect CKD progression^[67]. Very low-protein diet does not delay CKD progression and may increase the risk of death^[68].

BP control significantly decreases the rate of decline in GFR in pre-dialysis CKD patients^[69]. RAS blockers should be used to control BP in CKD patients (diabetic and nondiabetic) with increased urine albumin excretion. RAS blockers have a significant impact on the rate of decline of GFR in CKD patients with proteinuria^[70-72]. They exert their action through many mechanisms including their hemodynamic effect on glomerular tuft pressure^[73,74], inhibition of cytokine overproduction^[75-79], increased serum and tissue angiotensin 1-7^[80-82] and stimulation of Klotho gene expression in CKD patients. The RAS-mediated renal damage might be through Klotho gene manipulation^[54]. This novel mechanism might clarify the vascular, cardiac and renal protective benefits of such agents^[53,56]. Manipulation of Klotho gene, adds a new exciting mechanism for the cardiovascular and renal protective actions of RAS blockers.

The addition of aldosterone antagonists whether non-selective (spironolactone) or selective (eplerenone or Finerenone) to anti-hypertension medications offered better BP and proteinuria control in mild to moderate CKD^[83-85].

Hyperkalemia is not infrequent with RAS blockers and/or aldosterone antagonists treatment in such patients. The use of bisacodyl laxative^[86], patiromer, the nonabsorbed potassium binder^[87] or sodium zirconium cyclosilicate^[88] can control hyperkalemia. These agents are not associated with the potentially serious adverse effects of potassium exchange resins^[89,90].

According to KDIGO guidelines, BP should be kept at 130/80 mmHg or lower^[91]. A much lower BP (less than 110/75 mmHg) is associated with slower rate of annual increase in kidney size and urine protein excretion rate in early cases of ADPKD as shown by a recent study, HALT-PKD^[92].

The strict control of blood sugar has a positive impact on survival of pre-dialysis diabetic CKD patients. Diabetic patients experienced the reversal of renal pathology after pancreas transplantation^[93]. Glycemic control might also delay CKD progression and postpones the need for dialysis^[94,95].

Statins reduce the risk of atherosclerotic cardiovas-

Table 1 Standard of care therapeutic management

Drug class	On-target parameter	Off-target parameters	Ref.
Antihypertensive			
RAS blockers	BP↓	UAE↓, GTP↓, K ⁺ ↑, AT1-7↑, cytokines↓, Klotho↑	[53,54,56,69-82]
Aldosterone antagonists	BP↓	UAE↓, K ⁺ ↑	[83-85]
K ⁺ binders			
Bisacodyl	K ⁺ ↓	Diarrhea	[86]
Patiromer	K ⁺ ↓		[87]
Na zirconium cyclosilicate	K ⁺ ↓		[88]
Blood sugar control	Blood sugar↓ HbA1c + 7	Progression↓ Postpones need of Dx	[93-95]
Hypocholesterolemic			
Statins	Cholesterol↓, LDL↓	Cardiovascular events↓	[96]
Hypouricemic agents			
Allpurinol	Uric acid↓	Renal events↓, CV events↓	[102,103]
Febuxostat	Uric acid↓	CKD progression↓	[104]
Sodium bicarbonate	HCO ₃ ⁻ ↑, PH ↑	Ptn catabolism↓, GFR decline↓	[105,106]
Phosphate binders			
Calcium based	P↓	PTH↓, Vasc calc.↑	[117-120]
Sevelamer	P↓	PTH↓, stop vasc calc, Mortality↓, Uric acid↓, Cholesterol↓, LDL↓, inflammation↓ Cardiovascular events↓	[121-131]
Lanthanum carbonate	P↓	PTH↓, stop vasc calc,	[123-139]
Iron compounds	P↓	Iron↑	[140,141]
Nicotinamide	P↓	TG↓, LDL↓, HDL↑	[142-144]

RAS: Renin angiotensin system; BP: Blood pressure; UAE: Urine albumin excretion; GTP: Glomerular tuft pressure; K: Potassium; AT1-7: Angiotensin 1-7; Dx: Dialysis; LDL: low density lipoprotein; CV: Cardiovascular; CKD: Chronic kidney disease; HCO₃⁻: Bicarbonate; Ptn: Protein; GFR: Glomerular filtration rate; P: Phosphorus; PTH: Parathormone; Vasc calc: Vascular calcification; TG: Triglycerides; HDL: High density lipoproteins.

cular disease in CKD patients; however, clinical trials have suggested a minimal effect of statins on CKD progression^[96].

The association between high serum uric acid (UA) and progression of CKD was suggested by many studies of stage G1 and G2^[97-99]. A more recent study denied this association in stages G3, 4 and 5^[100]. On the other hand, hyperuricemia was found as independent risk factor for CKD progression in children and adolescents^[101]. Treatment of CKD patients with estimated GFR of 40.6 ± 11.3 mL/min with allopurinol 100 mg/d was associated with significant decrease in renal events (need of dialysis, doubling of serum creatinine or > 50% reduction of GFR) and cardiovascular events in comparison to control CKD patients taking only their standard treatment (*P* < 0.004 and 0.02 respectively)^[102]. In addition, a recent meta-analysis showed a significant favorable effect of allopurinol on the rate of GFR decline^[103]. Another recent trial demonstrated the significant impact of febuxostat on CKD progression in stage G3 and G4 patients^[104].

Correction of chronic metabolic acidosis was originally recommended in CKD patient to inhibit excessive protein catabolism and calcium mobilization out of the bone. Sodium bicarbonate supplementation was found to slow the rate of progression of CKD to ESRD^[105]. In the more recent trial, a significant improvement in the rate of decline of GFR was encountered in stage G4 CKD patients treated with sodium bicarbonate to render serum bicarbonate level at 22 mmol/L or above^[106].

High serum phosphorus was suggested as a potential risk factor for a rapid decline in renal function

in CKD patients^[107]. The rate of progression of CKD (measured as 1/serum creatinine) was faster in hyperphosphatemic patients in stage G5 when compared to normophosphatemic patients in the same stage^[108]. In patients in stage G4 and G5, each 1 mg/dL higher serum phosphorus concentration, the mean decline in renal function increased with 0.154 mL/min per month^[109]. In addition, hyperphosphatemia is associated with increased mortality^[110]. Increased phosphate concentration leads to the formation of calcium-phosphate crystals, a process called "nucleation". If this process is left unchecked, calcium phosphate crystals undergo further aggregation to form monetite, brushite, octacalcium phosphate, amorphous calcium phosphate and finally hydroxyapatite. When exposed to such crystals, vascular endothelial cells increase production of reactive oxygen species and eventually undergo apoptosis^[111]. Endothelial cell death can expose underlying smooth muscle cells to the high ambient phosphate. Transformation of such cells to osteochondrocytes consequently develops^[112]. Fetuin-A is α-glycoprotein that binds calcium phosphate crystals, inhibiting the crystal growth and polymerization. Fetuin-A calcium phosphate complex is called calciprotein particles (CPP). In comparison to hydroxyapatite, CPP induce significantly less cytokine secretion when macrophages are exposed to equimolar concentrations of hydroxyapatite and CPP^[113]. In spite of the apparent protective effect of CPP, increased serum level of such particles reflects increased procalcific milieu^[114]. Higher CPP levels are thus associated with reduced renal function, higher scores of V.C., aortic stiffening and increased risk of death^[115].

When phosphate intake was restricted, the rate of decline in creatinine clearance was much less^[107]. Restriction of phosphate intake should start early in the course of CKD before the evident rise in serum phosphorus ensues. The restriction should initially be limited to food ingredients rich in inorganic phosphorus (like food preservatives and tasters). These food additives are found in sodas and processed foods^[116]. Bioavailability of organic phosphorus is higher in animal proteins compared to plant proteins. Phosphorus in the later is tightly bound to phytate, an indigestible ingredient found in plant foods. On the other hand, phosphate binders should only be used when serum phosphorus increases above normal limits. The very early use of the phosphate binders might be associated with progression of V.C. while lowering serum phosphorus and attenuating the progression of secondary hyperparathyroidism^[117]. Calcium-based phosphate binders are still very useful to control hyperphosphatemia, but can lead to hypercalcemia and/or positive calcium balance and cardiovascular calcification^[118]. The higher the dose ingested the greater the extent of V.C.^[119,120]. Thus, their use in cases suffering V.C., hypercalcemia, low level of parathormone and/or adynamic bone disease has to be restricted^[121]. When sevelamer was used in hyperphosphatemic stage 3-4 CKD patients, a significant impact on all-cause mortality and the need of dialysis was observed in comparison to calcium carbonate^[122]. Sevelamer is not just a calcium-free phosphate binder, but it has additional pleiotropic effects such as correcting certain abnormalities of lipid metabolism^[123], significant decrease in inflammatory parameters including interleukin (IL)-6, sCD14 and hs-CRP^[124,125], reduces serum UA concentration^[126], decrease serum FGF23^[127-129] and increases serum level of Klotho^[129]. The role of FGF23 and Klotho on the cardiovascular system and progression of CKD will be discussed later in this review. Compared to calcium-based phosphate binders, sevelamer improves endothelial function in CKD patients^[130]. Although sevelamer is more expensive compared to calcium-based phosphate binders^[131], the significant reduction in all-cause mortality and the significantly fewer hospitalizations in the sevelamer group can offset the higher acquisition cost for sevelamer^[132].

Lanthanum carbonate (LC) is another non-calcium based phosphate binder. LC had no impact on overall mortality in CKD patients^[133-135]. Contrary to sevelamer, LC does not have a consistent effect on FGF23. LC failed to cause reductions in FGF23 in patients with CKD stage G3-4^[136,137]. On the other hand, other studies showed that LC was effective in reducing FGF23 levels in CKD G3^[138] and CKD G4-5 patients^[139]. None of the trials on Lanthanum reported any effect on inflammation or inflammatory biomarkers. We are still waiting for such studies to assure non-inferiority of Lanthanum in this field.

Iron compounds represent the new class of phosphate binders. Ferric Citrate, Sucroferric oxyhydroxide,

and Fermagate (Iron-magnesium hydroxycarbonate) were tested in some clinical trials^[140]. Most of the clinical studies done so far were using ferric citrate, stressing on phosphate binding and ferrokinetics after short periods of trial. A single study looked for non-inferiority of Sucroferric oxyhydroxide (PA21) compared to sevelamer carbonate concerning phosphate binding^[141].

The value of nicotinamide in phosphate control (as well as its effects on lipid levels) was explored in some short-term trials on dialysis patients^[142-144]. However, such trials did not look for either pharmacokinetics or safety. None of these trials studied the impact on V.C., FGF23, Klotho or inflammatory mediators.

Novel therapeutic interventions: Table 2

Interstitial inflammatory cell infiltrates are a hallmark CKD of different etiology. Such infiltrates are the consequence of the interaction between chemokines locally produced when renal tissue is injured, and membrane receptors located on the cell membrane of leukocytes. Seven chemokine receptors are recognized, so far, on the surface of leukocytes^[145]. Such leukocytes potentially secrete pro-inflammatory, pro-apoptotic and pro-fibrotic cytokines that perpetuate renal tissue destruction and progression to CKD. A single chemokine receptor can respond and interact with different chemokine ligands. Therapeutic interventions targeting the receptors is thus much preferred to interrupt such renal leukocytes recruitment^[146]. The chemokine receptor CCR1 looks to play a pivotal role in leukocyte migration. This role extends to the interaction of other receptors with their chemokine ligands^[147]. While CCR1 is essential for leukocyte recruitment into the interstitium^[148], CCR2 and CCR5 do the job in case of glomerular infiltration^[149,150]. CCR1 antagonists proved to have a significant impact on leukocyte infiltration, interstitial fibrosis, tubular injury and kidney function tests in different rat models of renal injury (e.g., unilateral ureter ligation, lupus nephritis, Adriamycin-induced renal injury, and collagen 4A3 deficient mice; the synonym of human Alport's syndrome)^[146]. When the CCR1 antagonist, BL5923, was used in mice suffering diabetic nephropathy, the interstitial recruitment of *ex vivo* labeled macrophages was markedly decreased. This was associated with reduced numbers of proliferating tubular epithelial and interstitial cells, tubular atrophy, and interstitial fibrosis. Glomerular pathology and proteinuria were not affected by the CCR1 antagonist^[151].

A mirror-image (Spiegelmer) for MCP1 was *in vitro* built-up using non-natural nucleotides. This RNA oligonucleotide is called Emapticap Pegol. It binds and neutralizes MCP-1 (also called CCL2), a pro-inflammatory chemokine that plays an important role in diabetic kidney disease^[152]. A phase IIa study that looked for safety and efficacy of Emapticap Pegol in phase IV diabetic nephropathy showed statistically significant reduction in urinary albumin excretion after the use of Emapticap Pegol for 12 wk as 3 times/wk subcutaneous injections. The anti-proteinuric effect persisted for 12 wk

Table 2 Novel therapeutic interventions

Therapeutic modality	Mechanism of action	Primary end points	Ref.
Chemokine ligand and receptor antagonists			
CCR1 antagonists	Block CCR1 receptors on leucocyte surface	Leuc. Inf.↓, IF↓, TI↓, and improved KFTs	[146]
Emapticap pegol	Binds and neutralizes MCP-1	UAE↓, glycemic control in phase IV D.N.	[5,152,153]
CCX140	Block CCR2	UAE↓, glycemic control in phase IV D.N.	[6,154]
Pentoxifylline	Anti-inflammatory	UAE↓, eGFR loss↓	[156,157]
VDRA			
Paricalcitol	Improves G.M. sieving, antifibrotic	UAE↓, eGFR loss↓	[160-162]
IAP			
Mediterranean diet	Restores intestinal microbiota, IAP↑	eGFR loss↓	[184]
Bound phosphorus	IAP↑		[186]
Vitamin K	IAP↑		[188]
S.O.D. mimetic			
Tempol	Oxidative stress↓	UAE↓, GS↓, TID↓	[189]
SRA			
Sarpogrelate	Antiplatelet	UAE↓	[192]
V2RA			
Tolvaptan	V2 receptor blocker	No. of cysts↓, growth of cysts↓	[19]
IgG anti-SAP antibodies	Binds SAP within amyloid tissue	Clearance of tissue amyloid deposits	[20]
RG-012	Inhibitor of miR-21	GS↓, IF↓, TI↓, Infl.↓	[22]

Leuc. Inf.: Leucocyte infiltration; IF: Interstitial fibrosis; TI: Tubular injury; KFTs: Kidney function tests; UAE: Urine albumin excretion; D.N.: Diabetic nephropathy; eGFR: Estimated glomerular filtration rate; VDRA: Vitamin D receptor agonists; G.M.: Glomerular membrane; IAP: Intestinal alkaline phosphatase; S.O.D.: Superoxide dismutase; GS: Glomerulosclerosis; TID: Tubulointerstitial disease; SRA: Serotonin receptor antagonist; V2RA: Vasopressin receptor antagonist; SAP: Serum amyloid protein; miR: Micro RNA; infl.: Inflammation.

after discontinuation of treatment. It also succeeded to improve glycemic control^[5,153]. A novel CCR2 antagonist was tried in diabetic kidney disease patients having type 2 diabetes. This antagonist is called CCX140. The results of phase II showed that the use of CCX140 given orally in a dose 5 mg/d on top of the standard of care treatment was associated with an additional significant reduction of urine albumin excretion rate. This improvement started after 12 wk and continued for the whole period of the study (52 wk). These patients were already treated with RAS blockers. Significant improvement in the slope of decline of GFR over that achieved with the standard of care treatment was also observed beside the improved glycemic control^[6]. The results of phase 3, however, did not confirm the significant impact on GFR but did confirm the anti-proteinuric and the glycemic favorable outcomes reported in phase 2^[154]. CCX168 is another inhibitor that targets C5aR, the chemoattractant receptor that binds to the complement fragment C5a. Oral administration of CCX168 ameliorated anti-MPO-induced mesangiocapillary glomerulonephritis in mice^[155]. In addition, this inhibitor is in phase 2 trials in patients with aHUS, IgA nephropathy, and ANCA-associated vasculitis.

Pentoxifylline is a phosphodiesterase inhibitor with anti-inflammatory action. It is used as a treatment of peripheral vascular disease. The addition of low-dose pentoxifylline, 400 mg/d, to losartan plus enalapril resulted in a significant decrease of urine protein excretion rate from a baseline of 616 mg/d to 192 mg/d 6 mo later in type 2 diabetic patients^[156]. Another

clinical trial explored add-on pentoxifylline to maximized RAS blockade on renal disease progression in stage G3-4 CKD T2DM patients. Pentoxifylline dose in this trial is 1200 mg/d. After 24 mo of follow-up, treatment with pentoxifylline was associated with a slower rate of eGFR loss together with the significant reduction in urine protein excretion^[157].

An inverse relationship was observed between serum level of 25(OH) vitamin D and the rate of GFR decline in children suffering CKD. Serum levels higher than 50 nmol/L were associated with 75% renal survival at 5 years of observation in contrast to 50% in case of levels below 50 nmol/L ($P < 0.001$). Higher serum levels of 25(OH) vitamin D were associated with lower urine protein/creatinine ratio. Renal survival increased 8.2% for every 10 nmol/L increase in 25(OH) vitamin D ($P = 0.03$), independent of eGFR; proteinuria, and underlying renal diagnosis^[158]. It seems that activation of vitamin D receptors (VDR) on podocytes improves glomerular membrane sieving of proteins and has an anti-fibrotic effect^[159]. Paricalcitol in a dose of 2 µg/d showed a significant effect on urine albumin excretion in type 2 diabetic patients with overt nephropathy^[160]. PROCEED trial is another prospective controlled study of paricalcitol in type 2 diabetes patients in phase IV diabetic nephropathy on low or high salt intake and already treated with RAS blockers^[161]. This trial has already completed and results are expected within few weeks.

Paricalcitol treatment of uremic mice restores deficient Klotho synthesis in CKD renal tissue^[162].

Klotho is an anti-senescence protein^[6]. It exists in 2 forms: The transmembrane and the soluble secreted form^[163]. Klotho is detected as a soluble protein in body fluids including blood, CSF and urine^[164]. The highest expression of Klotho is in the kidney and the brain^[6], but it is also expressed in parathyroid gland^[165] and heart^[166] with less abundance. Klotho protein is a β -glucuronidase. Reduced klotho expression in chronically diseased kidneys is associated with chronic inflammatory cell infiltrate, sclerosis of intrarenal small sized arteries, interstitial fibrosis and renal tubular atrophy^[16]. Decreased klotho expression underlies excessive fibroblast emergence as a consequence of EMT following acute insults posed on renal tubular epithelium^[12]. The kidney produces and releases Klotho into the circulation and clears Klotho from the blood into the urine^[167]. Exogenous Klotho prevents senescence of endothelial cells induced by uremic milieu^[168]. In different models of mouse CKD (5/6 nephrectomy, Adriamycin nephropathy and unilateral ureteric ligation) exogenous Klotho abolished the induction of the different RAS proteins, including angiotensinogen, renin, angiotensin-converting enzyme, and angiotensin II type 1 receptor, and normalized BP. Klotho also ameliorated renal fibrotic lesions^[169].

Endothelin receptor antagonists, avosentan, and atrasentan, have a significant anti-proteinuric effect when added to RAS blockers. However, dose-dependent peripheral edema is a major obstacle limiting their routine use in CKD patients^[170].

CKD is associated with inflammation and oxidative stress which contribute to CKD progression^[171]. A positive correlation was encountered between the rate of rise in serum creatinine and 2 markers of inflammation, namely, hs-CRP and malondialdehyde^[172]. Uremic status is incriminated in the pathogenesis of chronic inflammation; however, the exact mechanisms are not fully understood. Inflammation can result from multiple co-morbid conditions activating inflammation (like infections and autoimmune systemic diseases)^[173]. Impaired activity of the nuclear 1 factor (erythroid-derived 2)-related factor 2 (Nrf2) transcription factor was associated with inflammation and impaired antioxidant activity in CKD animals^[174]. Bardoxolone methyl is a potent activator of the Nrf2. When patients with type 2 diabetes mellitus and G4 CKD (GFR 15 to < 30 mL/min) were treated with bardoxolone methyl, at a daily dose of 20 mg, there was a significant increase in GFR. However, the treatment group had a significant increase in urine albumin excretion, BP and in the incidence of congestive heart failure and cardiovascular mortality. The last 2 adverse events forced the steering committee to prematurely stop the trial 7 mo after its onset^[175].

The gut has recently emerged as a major instigator of systemic inflammation in CKD. Postmortem examination of gut wall disclosed inflammatory changes throughout the digestive tract in patients on regular dialysis^[15]. The human intestine is now recognized

as an important metabolic organ powered by gut microbiota^[176]. Altered gut microbiome might affect the integrity of the intestinal barrier leading to facilitated blood translocation of bacteria and uremic toxins^[15]. In this context, the intestinal barrier function has not yet been carefully studied. However, recent studies have demonstrated marked disintegration of the colonic epithelial barrier structure and significant alteration of the colonic bacterial flora in humans and animals with advanced CKD^[171]. The fact that circulating lipopolysaccharides (LPS) levels and bacteria-derived uremic retention solutes (indoxyl sulfate, p-cresol, and trimethylamine n-oxide) increase with CKD stages suggests a link between the intestinal barrier and renal dysfunction^[177]. Many uremic toxins are derived from gut microbes. The imbalance of gut microbiota (dysbiosis) is provoked by dietary restrictions in CKD. Prescribed diet is poor in plant fibers and symbiotic organisms (to avoid potassium and phosphorus). Gut bacterial DNA and endotoxin were detected in the CKD serum. Endotoxin levels increase with the CKD stage and correlate with the severity of systemic inflammation^[15]. When lubiprostone (a laxative) was used in uremic mice, reduction in the elevated BUN and protection against tubulointerstitial damage, renal fibrosis, and inflammation were observed. Change in the intestinal microbial composition in favor of Lactobacilli and Prevotella genus was also encountered beside a significant decrease in serum level of indoxyl sulfate, hippurate, and trans-aconitate. All these uremic toxins are of intestinal bacterial origin. These results indicate the possible value of change of gut microbiota in improving the rate of progression of CKD^[178]. Thus, by targeting of the gut microbiome in a trial to restore symbiosis may prove as a potent strategy in reducing inflammation and disease progression in CKD. The efficacy of probiotics to decrease uremic toxin production and to improve renal function has been investigated in some human CKD studies^[177]. However, none of the clinical studies, so far, looked for the impact of probiotics on inflammation and CKD progression in pre-dialysis population. We would like to emphasize that probiotic treatment might decrease serum urea and creatinine by direct degradation. The use of estimated GFR in the assessment will obviously give erroneous results. GFR should be measured using iothexol in such trials. Another critical issue concerning the use of probiotics is the possible production of urease enzyme. Bacterial urease would increase ammonia production. This later product can attack the tight junctions in between intestinal epithelium rendering the intestinal mucosal barrier looser allowing excess translocation of bacterial products and uremic toxins to the intestinal wall and then into circulation. We are still looking for randomized prospective trials targeting the colonic microenvironment in CKD aiming at modulation of gut microbiota, to block LPS absorption to attenuate inflammation, or to target rate of production and adsorption of uremic toxins^[179].

Intestinal alkaline phosphatase (IAP) displays anti-inflammatory properties. This property may be related to detoxification of LPS, resulting in amelioration of intestinal and systemic inflammation; and to the regulation of gut microbial communities and their translocation. Enteral and systemic administration of exogenous IAP attenuates systemic inflammation. Dietary intervention can stimulate IAP and minimize low-grade systemic inflammation^[180]. Intravenous administration of IAP improved kidney function and systemic inflammation in cases of sepsis^[181]. Various spices (e.g., black pepper, red pepper, and ginger) increase IAP activity in the small intestine^[182]. Curcumin; the active ingredient in the herbal remedy and dietary spice turmeric (*Curcuma longa*) increases the expression of IAP and tight junction proteins and corrects gut permeability. These effects would explain the anti-inflammatory effect of dietary curcumin in spite of its' poor bioavailability^[183]. It seems clear from this discussion; that a Mediterranean diet rich in indigestible fibers and in saccharolytic bacterial species fortified by spices like black pepper, red pepper, ginger or curcumin represents an innovative approach in CKD, potentially restoring microbiota balance, ameliorating CKD symptoms and slowing down CKD progression^[184]. Dietary calcium and bound phosphate stimulate IAP^[185,186]. In contrast, free unbound phosphorus in food inhibits IAP^[187]. Vitamin K stimulates IAP^[188].

The superoxide dismutase-mimetic drug, Tempol, improved elevation on serum creatinine, blood urea nitrogen, urine albumin, segmental sclerosis and tubulointerstitial damage that were induced by 5/6 nephrectomy. These results indicate the value of the increased oxidative stress commonly encountered in CKD on the progression of the renal disease. They also highlight the possible value of antioxidant treatment to delay CKD progression^[189].

Sarpogrelate is a serotonin (5-hydroxy tryptamine) receptor antagonist. It inhibits the production of thromboxane A₂ and is used as anti-platelet agent instead of aspirin^[190]. Experimental studies showed Sarpogrelate effect on mesangial type IV collagen production, on albuminuria in DKD, on antibody-mediated glomerular injury and on nephrotoxin-induced kidney fibrosis^[191]. A clinical trial showed a significant decrease of urine albumin excretion in diabetic kidney disease after addition of Sarpogrelate^[192].

ADPKD is the most common inherited disease that leads to dialysis or kidney transplantation. ADPKD is the fourth leading cause of ESRD^[193]. The disease manifests by one or more cysts in each kidney usually during the 3rd decade of life. The number and size of the cysts steadily progress to interfere with the structure and function of individual nephrons. This distraction in the structure and function leads finally to ESRD usually between the 4th and 7th decades of life^[194]. Many clinical trials were planned using different agents to stop the growth in number and size of cysts. All these trials failed to show significant results^[195]. On the other hand, animal studies highlighted the role of the antidiuretic

hormone arginine vasopressin and its second messenger adenosine-3', 5'-cyclic monophosphate (cAMP) as promoters of kidney cyst development and accumulation of secretions within existent cysts. These studies also showed that suppression of vasopressin by either increase of water intake, posterior pituitary ablation or using the vasopressin receptor antagonists inhibit cyst development and growth and hence preserve kidney function^[196]. The first phase 3 prospective double-blinded clinical study of tolvaptan (vasopressin receptor antagonist, V2-receptor antagonist) demonstrated a significant slowing in the rate of increase in total kidney volume and the decline in kidney function over a 3-year period compared to placebo in patients with ADPKD^[19]. These results beside the more recent trial on BP, HALT-PKD^[92], open a big hope to ADPKD patients, especially if their disease is checked in early stages.

The kidney is the most frequent site of amyloid fibril deposition in AL, AA, and several of the hereditary amyloidoses. Amyloid fibrils are a group of soluble proteins that aggregate and deposit extracellularly in tissues as insoluble fibrils, causing progressive organ dysfunction. Substantial progress in understanding the process of amyloid fibril formation and the mechanisms underlying disease manifestations have led to important advances in treatment^[197]. In cases of systemic amyloidosis, the amyloid fibril deposits always contain the non-fibrillar serum amyloid P component (SAP). SAP binds avidly but reversibly to all types of amyloid fibrils and is thus specifically concentrated in all amyloid deposits^[198]. The binding of monoclonal anti-SAP antibodies to the SAP in amyloid deposits activates complement and triggers the rapid clearance of amyloid by macrophage-derived multinucleated giant cells^[20]. The drug (R)-1-[6-[(R)-2-carboxy-pyrrolidin-1-yl]-6-oxo-hexanoyl] pyrrolidine-2-carboxylic acid (CPHPC) efficiently depletes SAP from the plasma but leaves SAP in tissue amyloid deposits. Therapeutic IgG anti-SAP antibodies can subsequently target tissue SAP. An open-label, single-dose-escalation, phase 1 trial was conducted in patient with systemic amyloidosis mainly affecting the liver. One patient had renal involvement. A reduction in kidney amyloid load was observed. The authors are planning a next trial phase, in which patients with clinically significant renal amyloidosis will be included and will receive larger and, if necessary, repeated doses of anti-SAP antibody, with the aim of achieving effective exposure in tissues that do not have the highly permeable sinusoidal endothelium of the liver and spleen^[20].

Micro RNA (miRNA) are non-coding short RNA molecules (average 22 nucleotides) found in plants, animals, some viruses, and human being. Their main function is RNA silencing and post-transcriptional regulation of gene expression. A number of miRNAs are dysregulated in response to acute kidney injury and in CKD. This dysregulation probably contributes to maintenance and progression of CKD of different pathologic entities^[199]. One of such miRNAs is miR-21,

probably involved in regulating kidney tissue response after injury. MiR-21 is expressed in many cell types in the kidney and is upregulated in CKD of different underlying etiology. MiR-21 knockout mice showed far less interstitial fibrosis in response to kidney injury. Similar results were demonstrated in wild-type mice treated with anti-miR-21 oligonucleotides^[200]. These oligonucleotides are administered subcutaneously and have high affinity to renal tissues. When a murine model of Alport syndrome was treated with anti-miR-21 oligonucleotides, no adverse effects were encountered after miR-21 silencing. The treated mice showed substantially milder renal disease compared to vehicle treated mice. The treated Alport mice had improved survival and reduced pathological end points including glomerulosclerosis, interstitial fibrosis, tubular injury, and inflammation^[22]. These results demonstrate that inhibition of miR-21 is a potential therapeutic modality for CKDs in general and Alport nephropathy in specific. Currently, RG-012; the potent inhibitor of miR-21 is being evaluated in a first-in-human Phase I clinical study to evaluate the safety, tolerability and pharmacokinetics of subcutaneous dosing in healthy volunteers. This will be followed by a clinical multicenter study in cases of Alport syndrome.

During September 2015, a new hope was created to diabetic patients. Treatment with low doses of IL-17A succeeded to reverse diabetic nephropathy in genetic models of diabetes in mice. Administration of low doses of IL-17A significantly decreased urine albumin excretion, kidney size, mesangial matrix expansion, urine IP10, TNF α , IL-6, MCP1 and serum urea level in comparison to vehicle^[201].

CONCLUSION

Today, clinical nephrologists appreciate the impact of BP and blood sugar control, the value of RAS blockers and VDR agonists on the outcome of diabetic kidney disease. Chemokine ligand or receptor blockers are about to make the progression of diabetic nephropathy very slow or even completely suppressed. In the time being, CKD patients are irreversibly driven to renal replacement therapy. The question answered in this review is: "Are we approaching the time to change the pessimistic concept of (inevitable progression)?"

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Inflammation and nutrition in children with chronic kidney disease

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Abstract

Chronic inflammation and nutritional imbalance are important comorbid conditions that correlate with poor clinical outcomes in children with chronic kidney disease (CKD). Nutritional disorders such as cachexia/protein energy wasting, obesity and growth retardation negatively impact the quality of life and disease progression in children with CKD. Inadequate nutrition has been associated with growth disturbances in children with CKD. On the other hand, over-nutrition and obesity are associated with poor outcomes in children with CKD. The exact mechanisms leading to these unfavorable conditions are not fully elucidated and are most likely multifactorial. In this review, we focus on the pathophysiology of nutrition disorders and inflammation and their impact on clinical outcomes in children with CKD.

Key words: Nutrition; Inflammation; Chronic kidney disease; Protein energy wasting; Cachexia; Obesity; Growth failure; Maternal nutrition

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Core tip: Nutritional imbalances, such as protein energy wasting, cachexia, obesity and growth retardation, have been associated with poor clinical outcomes in children with chronic kidney disease (CKD). Chronic inflammation may lead to further deterioration of nutritional imbalance in advanced CKD patients. Results of recent studies have increased awareness of the importance of chronic inflammation and nutritional imbalance in children with CKD.

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NUTRITION IMBALANCE IN CHRONIC KIDNEY DISEASE

Nutritional imbalance is prevalent in children with chronic kidney disease (CKD) and may influence clinical outcomes. Wasting, defined as low weight proportion to height, is the consequence of inadequate nutrition intake, and highly prevalent in children with CKD. The term cachexia or wasting syndrome has been defined as the pathological combination of a dramatic decrease in appetite and increase in the metabolism of fat and lean body mass^[1]. The International Society of Renal Nutrition and Metabolism expert panel defined the term protein energy wasting (PEW) as a state of decreased body stores of protein and energy fuels (body protein and fat masses)^[2]. PEW/cachexia, a complex condition of metabolic and nutritional derangement, has been associated with not only malnutrition, but also maladaptive responses, such as anorexia, increased metabolic rate, decreased protein store, reduced body weight and muscle mass. PEW/cachexia cannot be reversed nutritionally. In contrast to PEW/cachexia, malnutrition is the consequence of insufficiency of energy intake and is accompanied by adaptive responses, including hunger, a protective decrease in energy expenditure, preferential use of fat stores for energy and preservation of lean body mass. Nutrition supplementation cannot reverse nutritional deficiency in malnourished patients. Thus, PEW/cachexia and malnutrition are not identical. In addition to PEW/cachexia, two common features of nutritional imbalance in children with CKD are obesity and growth failure^[3-7]. This review focuses on these nutrition disorders and the pathophysiological role of chronic inflammation in children with CKD.

PEW and cachexia in CKD

PEW and cachexia is highly prevalent in CKD patients. PEW was evident in 20% to 75% adult dialysis patients. Studies of CKD in children (CKiD), a multi-center prospective cohort study of children aged 1 to 16 in United States, revealed the prevalence of PEW estimates ranged from 6% to 65%. The wide range of prevalence of PEW in this cohort is likely due to difference in diagnostic criteria^[5]. To better define PEW in children with CKD, we evaluated prevalence of PEW using different diagnostic criteria. The incidence of PEW in CKiD ranged from 7% to 20% by applying 3 different diagnostic criteria, namely, a minimal, a standard and a modified PEW definition (Figure 1). Our results suggested that only the modified PEW diagnostic criteria, which included growth retardation as a criterion, showed modest significance. Our modified PEW diagnostic criteria for children with CKD is defined as the standard ≥ 3 of the 4 criteria as described in adults PEW (biochemical parameters, body and muscle mass assessments and anorexia) with the additional incorporation of growth retardation as

a diagnostic criteria. The etiology of CKD-associated PEW is complex. Common risk factors for PEW in CKD, such as poor nutrition, systemic inflammation, endocrine disorder, comorbid condition, fluid overload and metabolic acidosis have been listed (Figure 2)^[1,3,5]. Of the many complications of CKD-associated PEW/cachexia, CKD patients are prone to muscle weakness and as a result, have difficulties performing their daily routine of activities. Other systemic consequences of PEW in children with CKD comprise increased risk of cardiovascular disease, infection, depression, prolonged hospitalization and mortality, and growth retardation^[3,5]. The incidence rates of hospitalization were almost 2-fold higher for CKD children with PEW^[3]. Mortality rate in patients with CKD is 100-200 times higher than the general population^[8] and represents a major burden to health systems. Importantly, high mortality in patients with CKD has been associated with components of risk factor of PEW/cachexia as listed in Figure 2.

Obesity

Anorexia is prevalent and has contributed to the nutritional imbalance and growth failure in children with CKD^[5]. Ironically, another nutritional disorder - over-nutrition and obesity, is also prevalent in children with CKD^[9,10]. Prevalence of overweight or obesity (34%) exceeds the prevalence of PEW in CKiD cohort. Prevalence of overweight or obesity in children with glomerular and non-glomerular CKD was 46% and 32%, respectively^[11]. In a large cohort of European pediatric renal replacement therapy (RRT) population, the prevalence of overweight and obesity far exceeded the prevalence of underweight (20.8%, 12.5% vs 3.5%, respectively)^[12]. There was a significant increase in body mass index (BMI) after the initiation of RRT in this study cohort. Short stature and glucocorticoid treatment were further associated with an increased risk of overweight and obesity in this transplanted population. Other risk factors strongly associated with increased BMI in patients with RRT were lower initial BMI and higher age at the initiation of RRT, longer duration of dialysis as well as a longer time with a functioning graft^[12].

The obesity paradox or reverse epidemiology is a controversial hypothesis^[13]. It proposes that obesity may, contrary to conventional wisdom, be related to decreased morbidity and mortality in some populations. This hypothesis has been reported in patients with heart failure, myocardial infarction, and acute coronary syndrome^[13,14]. Nevertheless, it was not consistently supported by data in end-stage renal disease (ESRD) patients. Indeed, initial analysis of epidemiologic studies have shown a strong survival advantage of obesity in dialysis patients with the primary outcomes of all-cause and cardiovascular mortality^[14]; and low BMI values are associated with increased mortality rate. However, there is a fundamental flaw in the study design as those investigators compared short-term mortality rate in dialysis patients vs long-term mortality

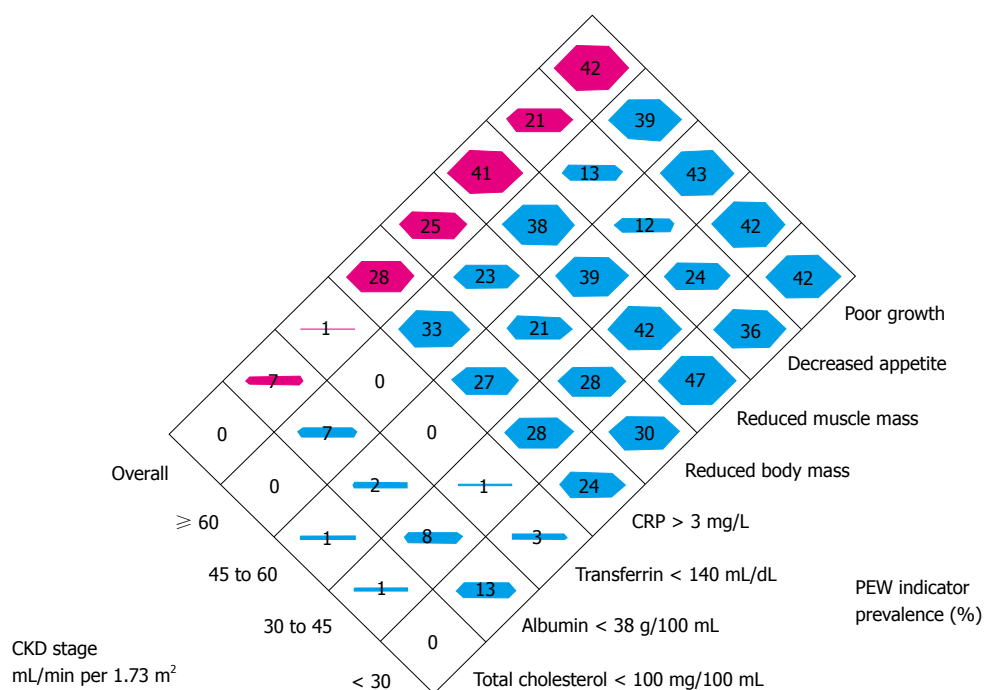


Figure 1 The prevalence of indicators of protein-energy wasting used to form the three definitions. PEW: Protein energy wasting; CKD: Chronic kidney disease; CRP: C-reactive protein.

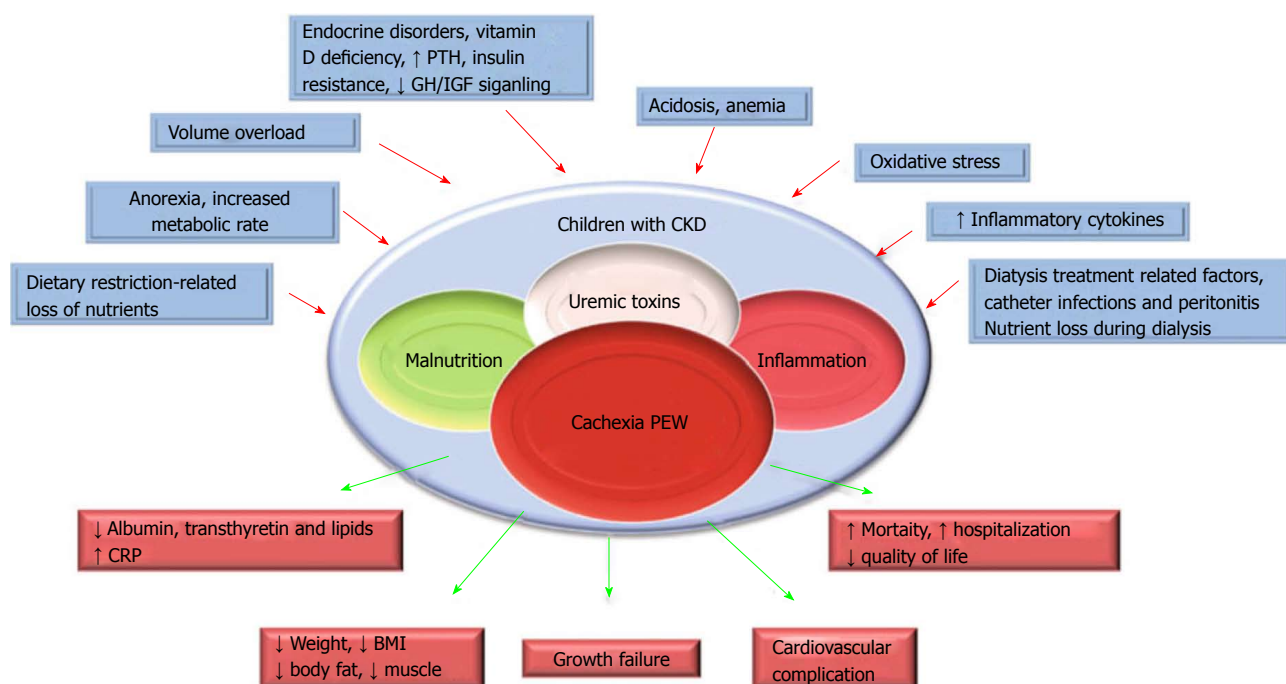


Figure 2 Schematic representation of the causes and manifestations of the protein-energy wasting syndrome in chronic kidney disease. CKD: Chronic kidney disease; GH: Growth hormone; IGF: Insulin-like growth factor; PTH: Parathyroid hormone; BMI: Body mass index; PEW: Protein-energy wasting; CRP: C-reactive protein.

rate in the general population. No evidence of reverse epidemiology of BMI and survival advantage was found in dialysis patients when both patients and general population were analyzed with the same time frame for outcomes, even with multivariable adjustments for age and race^[15]. More recently, association of BMI values with all-cause of mortality rate and disease progression

was analyzed in a large cohort of adult predialysis CKD patients. BMI showed a U-shaped relationship with clinical outcomes, with the best outcomes observed in overweight and mildly obese patients^[16]. Similar findings were observed in children with ESRD, the showing of a U-shaped relationship between BMI values and the risk of all-cause mortality rate (Figure 3). Higher mortality

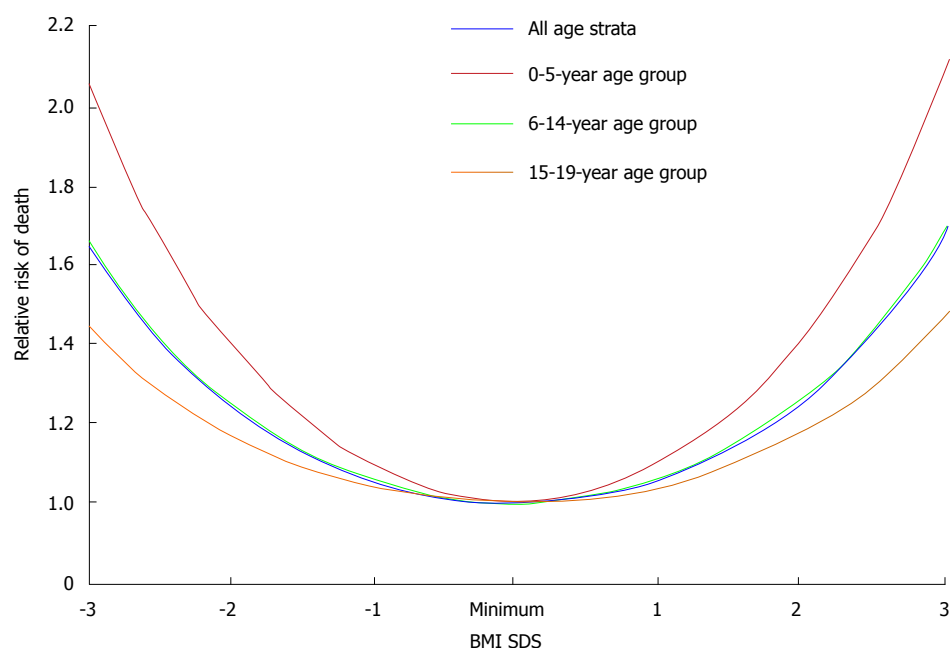


Figure 3 The relative risk of death and confidence intervals for body mass index standard deviation score among children with stage 5 chronic kidney disease. BMI: Body mass index; SDS: Standard deviation score.

rate was observed in obese children relative to non-obese children with CKD after renal transplantation (27% vs 17%, respectively)^[17]. Furthermore, childhood and adolescent obesity have negative impacts on the cardiovascular health. Obesity in adolescence was positively associated with death rate in future decades^[18]. Obesity *per se* is a strong and independent risk factor for the progression of CKD. Obesity hastens the deterioration of renal function among patients with IgA nephropathy and unilateral renal agenesis^[19-21]. In another study, progression of CKD is increased by 1.23 fold for each standard deviation increment of BMI values^[22].

Growth failure

Poor nutrition contributes to the high prevalence of growth retardation in children with CKD but growth retardation may still persist despite improvement of nutritional status in this population. Recent data from International Pediatric Peritoneal Dialysis Network registry suggested that enteral feeding by nasogastric or gastrostomy tube improved nutritional status, as indicated by an increment of BMI values in pediatric patients with stage 5 CKD. Nevertheless, nutritional supplementation did not attenuate growth failure in this population^[23]. Growth failure has been associated with poor clinical outcomes of increased morbidity and mortality rate in children with CKD. About one third of children enrolled in the North American Pediatric Renal Trials and Collaborative Studies (NAPRTCS) registry in 2005 had severe short status^[24]. Similar findings were observed in a recent report from the same registry in 2011^[25]. Prevalence of growth retardation was 29.3% for children enrolled in the Serbian Pediatric Registry of CKD^[26].

The etiology of poor growth in CKD is multifactorial and can be associated with poor nutritional status as well as other comorbidities such as metabolic acidosis, anemia, bone and mineral disorders, genetic factors and perturbations in growth hormone (GH) and insulin-like growth factor (IGF)- I axis signaling pathways. Data from NAPRTCS showed that the greatest height deficits were observed in youngest CKD patients prior to entering RRT programs. After renal transplantation, the greatest height improvement was observed for those youngest patients with the greatest height deficits prior to their RRT^[9]. Growth retardation is associated with poor clinical outcomes in children with ESRD. Five year mortality rate for children on hemodialysis with severe growth failure, moderate growth failure and normal growth was 16.2%, 11.5% and 5.6%, respectively. Moreover, higher hospitalization rate was observed in ESRD children with severe and moderate growth failure relative to those with normal growth^[27].

IMPACT OF INFLAMMATION ON NUTRITIONAL DISORDER IN CHILDREN WITH CKD

Levels of serum inflammatory markers such as C-reactive protein (CRP), IL-6 and TNF- α were elevated in CKD patients^[28]. The etiology of CKD-associated inflammation is multifactorial. Important factors include decreased glomerular filtration rate, underlying disorders and other complications of CKD^[29].

PEW/cachexia

Chronic inflammation is important for the pathogenesis of PEW/cachexia in patients with CKD through various

mechanisms including leptin and melanocortin signaling modulation, inflammatory cytokines and nuclear factor kappa B (NF κ B) signaling.

Aberrant leptin/melanocortin signaling and PEW/cachexia in CKD

Leptin is an anorexigenic hormone. Leptin is mainly secreted by adipose tissues and modulates energy homeostasis through melanocortin signaling. Leptin signaling in the hypothalamus nuclei is enabled by inhibiting neuropeptide Y (NPY) and agouti related peptide (AgRP) neurons and by stimulating pro-opiomelanocortin neurons, which in turn activates the release of α -melanocyte-stimulating hormone and stimulates the type 4 melanocortin receptor signaling (MC4R)^[30,31]. Transgenic mice over-expressing leptin had reduced energy consumption relative to controls^[32]. Leptin is degraded from the circulation in the renal tubules. Serum levels of leptin were elevated in CKD patients with the decline in renal glomerular filtration function^[33,34]. We have shown that leptin/melanocortin signaling is an important mechanism underlying CKD-associated cachexia. Transgenic mice with deletion of leptin receptor (*db/db*) and MC4-R knockout attenuated aberrant metabolic effects of CKD-associated cachexia^[35]. Administration of AgRP, a natural MC4R antagonist, normalized food intake, total weight gain, improved lean mass content as well as basal metabolic rate in CKD mice relative to control mice^[36]. We also evaluated the effects of leptin receptor antagonism in CKD mice. Administration of pegylated leptin receptor antagonist (PLA) attenuated food intake, weight gain, improved lean mass and *in vivo* muscle function as well as normalized basal metabolic rate in mice with CKD. In addition, the administration of PLA significantly decreased expression of uncoupling proteins and corrected aberrant muscle mass signaling pathway as well as normalized muscle protein levels of IL-1 α , IL-1 β , IL-6, and TNF- α in CKD mice^[37]. Thus, inhibition of the leptin/melanocortin signal pathway may represent a novel therapeutic approach for CKD-associated cachexia. Elevated serum levels of leptin were associated with higher prevalence of PEW/cachexia in patients. Malnourished patients had higher serum levels of leptin than those without malnutrition^[38]. Increases in serum leptin levels have been associated with inflammation and a decrease in lean mass content in dialysis patients^[39].

Pro-inflammatory cytokine and PEW/cachexia in CKD

Increased levels of serum inflammatory cytokines were associated with poor clinical outcomes in patients with CKD^[40]. Loss of kidney function, uremia and dialysis treatment *per se* are important causes of inflammation in this population. In addition, gene polymorphisms of inflammatory cytokines have been implicated in CKD patients^[41]. Polymorphisms of TNF- α gene predisposed malnutrition and inflammation in patients with ESRD^[42]. Robust evidence supports a direct pathologic role of

IL-1 α , IL-6, and TNF- α in the development of PEW. Muscle wasting is a cardinal feature of CKD. Elevation of pro-inflammatory cytokines stimulates muscle catabolism. In animal models of CKD, IL-1, IL-6 and TNF- α stimulate inflammation in animal models of CKD. Increased serum levels of IL-6 correlated with increased muscle catabolism while the antagonist of IL-6 receptor attenuated CKD-associated muscle wasting^[43].

PI3K-Akt signal transduction pathway mediates muscle metabolism in response to various extracellular signals. Aberrant PI3K/Akt pathway has been implicated in the etiology of muscle wasting. In skeletal muscle, Akt signaling mediates muscle fast/glycolytic fiber metabolism and muscle atrophy in CKD is associated with reduced Akt signaling in skeletal muscle tissue. In a mouse model of CKD, reduced Akt signaling was associated with skeletal muscle wasting. In contrast, skeletal muscle-specific Akt1 transgenic mice promoted skeletal muscle growth^[44]. Akt1 transgenic mice attenuated renal fibrosis, apoptosis, and inflammation in unilateral ureteral obstruction-induced CKD mice. Importantly, maintenance of muscle mass is associated with favorable clinical outcomes while muscle wasting is related to deterioration of renal function in patients with CKD^[45].

Pro-inflammatory cytokines signal through the central nervous system and induce anorexia^[46]. A meta-analysis of 22 studies with 924 participants (anorexia nervosa = 512, health controls = 412) has shown that compared to controls, the serum level of TNF- α , IL-1 β , IL-6 and TNF-receptor- II were elevated in anorexia nervosa^[47]. An animal study demonstrated that anorectic effects were observed following acute administration of exogenous TNF- α and IL-1 β to mice^[48]. Cytokines regulates energy expenditure. Infusion of IL-1 increased resting energy expenditure in rats and administration of recombinant TNF- α increased energy expenditures in patients with disseminated cancer^[49,50].

NF κ B pathway and PEW/cachexia in CKD

Activation of intracellular NF κ B system has been correlated with PEW/cachexia in CKD^[51]. Several recent articles provide comprehensive reviews for the NF κ B family of transcription factors and its regulation^[52]. Cytokines induce muscle wasting *via* activation of NF κ B while blockade of NF κ B signaling attenuates muscle atrophy. Denervation-induced muscle atrophy was significantly improved in muscle specific IKK knockout mice^[53]. What are the underlying mechanisms by which activation of NF κ B induce significant muscle atrophy? First, ubiquitin-proteasome system (UPS) promoted muscle protein degradation and activation of NF κ B stimulated expression of protein levels of several components of UPS. Second, NF κ B increased the expression of several NF κ B-regulated molecules, especially pro-inflammatory cytokines. This positive feedback loop resulted in the over-stimulation of NF κ B and the subsequent muscle atrophy. Third, NF κ B

suppressed myogenic differentiation likely through the activation of transcription factor YY1^[54]. And fourth, NF κ B may suppress energy intake likely through the suppression of NPY. Phenylpropanolamin (PPA), a synthetic sympathomimetic amine, suppressed food intake likely *via* the signaling of hypothalamic NPY. Cerebral NF κ B knockdown attenuated the anorexic effects in PPA-treated rats by decreasing the expression of NPY and antioxidants^[55].

Obesity

Adipose tissue is an important energy reservoir and an active metabolic organ secreting numerous hormones. The adipokines are cell signaling proteins secreted by adipose tissue, including leptin, adiponectin, IL-6, TNF- α , and monocyte chemotactic protein-1^[56]. Adipose tissue is an important source of inflammation in CKD patients. Adipokines mediate inflammation and accelerate the progression of vascular disease in patients with CKD^[57]. Chronic inflammation may accelerate the progression of renal dysfunction in CKD patients. Elevated expression of adipokines was associated with increased numbers of infiltrated immunocompetent cells in adipose tissue in obese CKD patients^[58]. Elevated serum inflammatory markers such as IL-6, TNF- α and CRP are correlated with thickness of carotid intima media and associated with high mortality rate in CKD patients. Increased expression of inflammatory cytokines in adipose tissue may accelerate atherosclerosis and induce deterioration of renal function in obese CKD patients^[9,59].

Growth failure

Perturbation in the GH/IGF- I axis is an important cause of growth failure in CKD children. GH/IGF- I mediated postnatal growth, body composition and renal function. GH binds to its receptor (GHR) and subsequently regulates the expression of GH-regulated genes, including the *IGF- I* gene. GH insensitivity is commonly observed in growth retarded CKD children, as serum levels of GH were normal or even elevated in this population. Pharmacological or endogenous GH treatments have diminished growth-promoting effects in children with CKD. CKD caused a post-receptor defect in GH pathway *via* the JAK/STAT signaling which in turn, resulted in reduced expression of IGF- I^[60]. GH induces the expression of suppressors of cytokine signaling (SOCS) *via* the JAK-STAT signaling pathway. SOCS proteins, in turn, inactivates GHR/JAK2 complex, thus establishing a feedback loop for GH activity. CKD-induced GH insensitivity was mediated by activation of GH-JAK2 *via* STAT transduction and the overexpression of SOCS proteins^[61].

IGF- I stimulates longitudinal growth at the growth plate. Circulating IGF- I complex constitutes of IGF- I, IGF binding protein (IGFBP) and acid labile subunit. Decline in renal function in CKD patients is associated with elevated serum IGFBP1 levels and the concomitant diminished IGF- I bioactivity. Increased

IGFBPs levels have been associated with decreased longitudinal growth in CKD children. A recent study further exploited the underlying mechanism of CKD-induced GH insensitivity. In CKD rats with acute inflammation, endotoxin aggregates GH resistance and reduced *IGF- I* gene expression, and this effect is related to the increased production of pro-inflammatory cytokines^[62,63].

IMPACT OF MATERNAL NUTRITION

Maternal malnutrition negatively influences the fetal and early life development. This critical period of pre- and early postnatal development exerts long-term effects on body weight and growth. An inadequate or excess maternal nutritional environment may activate multiple fetal responses which persist postnatally and have been correlated with the development of chronic diseases, including CKD and nutritional disorders^[64]. Low birth weight (LBW) was associated with impaired renal reserve (a reduction in the number of nephrons) and structure *per se* (smaller renal size)^[65-67]. Results from animal studies strongly support the notion that maternal malnutrition caused intrauterine growth retardation and a nephron deficit^[66]. LBW was correlated with increased prevalence of early-onset CKD. The odds ratio for ESRD was 1.4 in adults who were born underweight^[68]. LBW was correlated with deterioration of renal function in CKD patients^[69]. Low nephron numbers was a risk factor for hypertension, likely due to the effect of compensatory hypertrophy in the setting of a low nephron number.

Intrauterine and early-life environment substantially impact the development of obesity in childhood and in adulthood. Animal and human studies suggested that an adverse *in utero* environment such as intrauterine growth restriction (IUGR) was closely associated with postnatal development of obesity. Studies also showed that IUGR fetuses exhibited increased body fat accumulation, reduced serum levels of leptin and aberrant epigenomic properties, which subsequently promoted obesity in adult life. Food restriction during rat pregnancy produced hypoglycemic IUGR pups. Subsequently, for those IUGR pups permitted rapid catch-up growth, they exhibited aberrant metabolic responses including hypertriglyceridemia and adult obesity with insulin-resistance. The concept of developmental origins of health and disease has been generally recognized. Infants born to obese, overweight, and diabetic mothers as well as infants born to malnourished mothers are associated with a higher risk of chronic illnesses in adult life. High birth weight enhances the risk of developing obesity and CKD in adult life^[64]. On the other hand, LBW accompanied by an accelerated catch-up growth has also correlated with an increased risk of obesity and CKD in adulthood. In an observational study, LBW and small gestational age in infants were associated with poor growth outcomes in children with mild to moderate CKD^[70].

CONCLUSION

Nutritional disorders, including PEW, cachexia, obesity and growth failure, have major impacts on clinical outcomes in children with CKD. Chronic inflammation is important for the pathogenesis of nutritional disorders in CKD. Increased awareness of nutritional status is needed for CKD children. Further research into the pathophysiology may yield novel therapies for CKD-associated nutritional disorders.

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Kidney function outcomes following thermal ablation of small renal masses

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therapeutic intervention. Renal thermal ablation presents one approach for management of SRMs whereby tumors are treated *in situ* without need for global renal ischemia. These treatment characteristics contribute to favorable renal function outcomes following kidney tumor ablation particularly in patients with an anatomic or functional solitary renal unit.

Key words: Radiofrequency ablation; Cryoablation; Modification of diet in renal disease equation; Kidney function; Dialysis

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Core tip: Because of increased abdominal imaging, an increasing number of incidental small kidney masses are being detected. Renal thermal ablation is one treatment strategy used for the management of these tumors. Oncologic outcomes in published series appear favorable. Thermal ablation allows treatment of kidney masses *in situ* without the need for complete ipsilateral renal ischemia. As a consequence, ablation may be an attractive alternative for patients with baseline kidney dysfunction owing to medical comorbidities who would be at risk for declining kidney function following surgery.

Raman JD, Jafri SM, Qi D. Kidney function outcomes following thermal ablation of small renal masses. *World J Nephrol* 2016; 5(3): 283-287 Available from: URL: <http://www.wjgnet.com/2220-6124/full/v5/i3/283.htm> DOI: <http://dx.doi.org/10.5527/wjn.v5.i3.283>

Abstract

The diagnosis of small renal masses (SRMs) continues to increase likely attributable to widespread use of axial cross-sectional imaging. Many of these SRMs present in elderly patients with abnormal baseline renal function. Such patients are at risk for further decline following

INTRODUCTION

The incidence of small renal masses (SRMs) has continued to increase over the past twenty years^[1]. While several factors may contribute to this observation, the most significant has been the increasing use of

abdominal cross-sectional imaging^[2]. Specifically, routine and widespread use of imaging modalities including (but not limited to) ultrasound, computerized tomography, and magnetic resonance imaging has led to a 2.3% to 4.3% annual increase in renal cell carcinoma with incidental detection of small renal tumors increasing by 60%^[3].

Surgical extirpation in the form of radical (RN) or partial (PN) nephrectomy has served as the mainstay for management of enhancing renal masses^[4]. Over time, however, the utilization of RN to manage SRMs has waned. The loss of normal renal parenchyma with RN for clinical T1 disease is substantial, and RN has been implicated as an independent risk factor for chronic kidney disease (CKD)^[5]. Furthermore, recent data underscores an association between CKD and cardiovascular morbidity and all-cause mortality^[6,7]. Therefore, PN is now more broadly accepted as a treatment alternative with equivalent oncologic results and superior renal function outcomes compared to RN for appropriately selected patients^[8-10].

The majority of incidentally detected renal tumors are relatively small (defined as < 4 cm, clinically stage T1a), low Fuhrman grade, with slow growth kinetics (< 0.35 cm/year), and low potential for metastasis^[11-13]. Furthermore, many of these tumors are detected in older individuals with pre-existing comorbidities. In these individuals, surgical complications may pose a higher risk than the small renal tumor itself. Concerns regarding over diagnosis and overtreatment of patients with relatively low-risk, indolent small renal tumors have led to an increased interest in minimally invasive, ablative therapies as an alternative to extirpative surgical intervention for select patients^[14,15].

THERMAL ABLATION

Thermal ablative techniques include radiofrequency ablation (RFA) and cryoablation (CA) which can be accomplished by open, percutaneous, or laparoscopic approaches^[3]. The underlying concept of RFA involves transfer of electrical current from a generator through needle probes (electrodes) into target tissue. The generator produces high-frequency, alternating electrical current which promotes ionic agitation of cells and subsequent molecular friction. Collectively, these effects contribute to intense heat production and thermal damage. In contrast, CA involves freezing and thawing target tissues through use of a cryoprobe. The freezing action mediates cellular death by creating a direct cytotoxic effect through intracellular ice crystallization. The thaw cycle promotes delayed microcirculatory failure and resultant ischemia. The thermal effect of CA is based on both of these cellular processes.

The American Urological Association SRM guidelines indicate that thermal ablation is an accepted alternative to extirpative techniques in patients with kidney tumors who are poor surgical candidates^[12]. Long-term oncological outcomes appear to be durable for

both RFA and CA in appropriately selected clinical T1a lesions^[3,16-19].

RENAL FUNCTION FOLLOWING SURGICAL EXTIRPATION

Despite its many benefits when compared to RN, deterioration of renal function does occur in a significant percentage of patients following PN. In 2015, Mir *et al*^[20] published a comprehensive literature review with the PRISMA criteria and highlighted that decline in renal function in the operated kidney averaged approximately 20%. This occurrence is attributable to a host of different factors, including baseline kidney function, volume of preserved renal parenchyma, and duration of ischemia time^[21,22]. Specifically, lower baseline eGFR has widely been reported as a significant risk factor for both short-term and long-term decline in renal function^[23]. Recent data from Mukkamala *et al*^[24] of a cohort of 358 patients undergoing minimally invasive partial nephrectomy (PN) revealed that lower pre-operative eGFR, longer ischemia time, and larger tumor size were all significantly associated with progression to lower CKD classes.

RENAL FUNCTION CHANGES FOLLOWING THERMAL ABLATION

Kidney ablation has been described as a treatment alternative in comorbid patients who are poor candidates for major surgery. This cohort of patients includes those with baseline renal insufficiency suffering from CKD. An advantage of ablation is that it does not require clamping of the renal hilar vessels and therefore avoids the need for total ipsilateral kidney ischemia. Thus, it is quite attractive in patients with baseline kidney function disease. At present, several different groups have attempted to better define quantify the magnitude of impact of ablation on global renal function. It is important to note that at present there are no randomized control trials investigating kidney function comparing ablative strategies vs extirpative modalities. Therefore, the subsequent data are all based on single or multicenter observational experiences.

Initially, in 2006, Hegarty *et al*^[25] published a study comparing oncologic and perioperative outcomes of RFA vs CA. While not a primary endpoint of this study, the authors noted no significant difference in serum creatinine for either approach when comparing baseline to post-treatment (RFA: 1.35 mg/dL vs 1.70 mg/dL; CA: 1.35 mg/dL vs 1.3 mg/dL; *P* for both > 0.05).

Subsequently, in 2008, Lucas *et al*^[26] reported on kidney function outcomes for patients with SRMs (< 4 cm) who underwent RFA, PN, or radical nephrectomy (RN). In all cases included in this study, the index patient had a normal appearing contralateral kidney on preoperative imaging and the Modification of Diet in Renal Disease (MDRD) equation

was to estimate glomerular filtration rate (GFR). At a baseline, approximately 25% of each cohort had stage 3 CKD (GFR < 60 mL/min per 1.73 m²) with the mean pretreatment GFR being 73.4, 70.9, and 74.8 mL/min per 1.73 m² for the RFA, PN, and RN groups, respectively. Following the index intervention, the authors specifically reported on stage 3 CKD. In particular, they noted that the 3-year freedom from stage 3 CKD was 95.2% for RFA, 70.7% for PN, and 39.9% for RN. Additionally, patients undergoing RN were 34-times more likely and those undergoing PN were 11-fold more likely to develop stage 3 CKD compared to their RFA counterparts. This study highlighted that even in patients with an anatomically appearing normal contralateral kidney, thermal ablation may be more “renoprotective” compared to surgical extirpation.

Stern *et al.*^[27] similarly presented GFR and cancer outcomes in a series of patients with clinical T1a renal tumors managed by RFA. In this study of 63 patients who were ASA I or II, the average tumor size was 2.1 cm (range, 1.0–4.0). At the time of initial diagnosis, 20% of the cohort had evidence of baseline CKD. The median eGFR is before (76.3 mL/min per 1.73 m²) and after (74.3 mL/min per 1.73 m²) thermal ablation remained stable. The authors suggested that RFA might be a reasonable alternative for the healthy renal tumor patient with intermediate outcomes suggesting preservation of renal function.

More recently, in 2012, Wehrenberg-Klee *et al.*^[28] examined the impact of percutaneous renal thermal ablation on kidney function amongst patients with baseline CKD. In this study of 48 patients with a baseline eGFR of less than 60 mL/min per 1.73 m², 22 underwent CA and 26 were managed by RFA. The mean tumor diameter was 3.4 cm. Overall, in the entire cohort, the mean overall eGFRs did not change significantly between baseline (39.8 mL/min per 1.73 m²) and at 1 mo post-ablation (39.7 mL/min per 1.73 m²) ($P = 0.85$). Thirty-eight patients had eGFR measurements available 1-year following ablation with the mean eGFR being 40.9 mL/min per 1.73 m² compared with a pre-ablation eGFR of 41.2 mL/min per 1.73 m² ($P = 0.79$). The authors further provided data on the subgroup of patients undergoing CA and RFA. For CA, the mean eGFRs at 1 mo and 1 year following treatment were 41.4 mL/min per 1.73 m² and 44.4 mL/min per 1.73 m² compared with respective baseline GFRs of 41.1 mL/min per 1.73 m² and 42.1 mL/min per 1.73 m² ($P = 0.75$ and $P = 0.19$, respectively). Similarly, in the RFA cohort, mean eGFRs at 1 mo and 1 year post-treatment were 38.2 mL/min per 1.73 m² and 37.8 mL/min per 1.73 m², compared with respective baseline GFRs of 38.7 mL/min per 1.73 m² and 40.4 mL/min per 1.73 m² ($P = 0.58$ and $P = 0.09$, respectively). Based on these data, the authors concluded that percutaneous renal ablation (either RFA or CA) did not appear to significantly negatively impact renal function among patients with significant baseline

kidney dysfunction.

In 2014, Ma *et al.*^[29] reported long-term oncologic and renal function outcomes in healthy patients managed by RFA for SRMs. In this series, the Cockcroft-Gault formula was used to the estimated GFRs before and after RFA. Within the cohort of 52 patients (58 renal tumors), paired analysis at a median follow-up of 40 mo demonstrated no significant difference in eGFR before and after RFA (106.3 mL/min vs 99.2 mL/min, $P = 0.06$). Also, in 2014, Wah *et al.*^[30] reviewed outcomes of 200 renal tumors ablated in 165 patients with a focus on oncologic and kidney function outcomes (measured by the MDRD equation). Estimated GFR before and after renal RFA was 54.7 mL/min per 1.73 m² vs 52.7 mL/min per 1.73 m² with a mean percentage change from baseline of 3.1 mL/min per 1.73 m². Within this cohort of patients, only four patients developed significant renal function deterioration (> 25% decrease in eGFR). In all, 161 (98%) of the 165 patients had preservation of renal function. Finally, in a multivariate model querying potential risks for declining kidney function, the authors no association between the percentage of eGFR change with tumor size, polar position, tumor location, and size of tumor.

Collectively, studies described above noted that in general there were no significant changes from baseline renal function following probe ablative therapy.

KIDNEY FUNCTION CHANGES IN A SOLITARY KIDNEY MODEL

Perhaps the most interesting population to examine when considering renal function outcomes following therapy is patients with kidney tumors in a solitary kidney. This has long been a treatment challenge for urologists, as this population not only exhibits a baseline deficiency in renal function but also susceptibility to further decrement in function following interventional therapy. In this regard, in 2008 Raman *et al.*^[31] reported on a small series of 16 patients with 21 renal masses (cT1a, ≤ 4 cm) in solitary kidneys managed by RFA. The mean pre-treatment GFR using the modified MDRD equation was 54.2 mL/min per 1.73 m² consistent with stage 3 CKD. Mean follow-up was just over 30 mo. At last follow-up, the mean eGFR declined by 11.8% to 47.5 mL/min per 1.73 m². Additionally, for those patients with multiple early serum Cr values, it was apparent that following an initial 7.5% decline 6 wk following RFA, eGFR remained relatively stable up to 18 mo and later. These authors concluded that RFA adequately preserves renal function in patients with small renal tumors in a solitary kidney.

To further this analysis, several groups have specifically compared kidney function outcomes of renal ablation vs PN in a solitary kidney model. In 2010, in a multi-institutional study, Raman *et al.*^[32] reported on 89 patients with 98 renal tumors in a solitary kidney managed by RFA or open PN (OPN) with cold ischemia.

Renal function was calculated using the modified MDRD equation. When comparing the two groups, the median tumor size was greater for those managed by OPN (3.9 cm vs 2.8 cm, $P = 0.001$), while the median preoperative eGFR was lower in the RFA group (46.5 mL/min per 1.73 m² vs 55.9 mL/min per 1.73 m², $P = 0.04$). Compared to RFA, patients treated with OPN had a greater decline in eGFR at all times evaluated, including early after the procedure (15.8% vs 7.1%), 12 mo after surgery (24.5% vs 10.4%) and at the last follow-up (28.6% vs 11.4%, P for all < 0.001). Additionally, for patients with a pretreatment eGFR of > 60, there was a new onset decline < 60 in 7% of RFA patients vs 35% of OPN patients. Similarly, for patients with pre-ablation eGFR of > 30 mL/min per 1.73 m², there was a new onset of decline in < 30 7% of patients after RFA and 17% after OPN. Based on these findings, the authors suggested that further emphasized the potential benefit of ablative techniques for managing tumors in solitary renal units.

Similar observations were noted by Krambeck *et al*^[33] who reported a single institution series of percutaneous or open RFA in 30 patients with 55 total tumors in a solitary kidney system. In contrast to the above mentioned studies, the Cockcroft-Gault formula was used for calculation of renal function changes. No difference in preoperative and postoperative calculated creatinine clearance was noted (61.5 mL/min vs 58.4 mL/min, $P = 0.072$). Additionally, there was no difference in systolic ($P = 0.102$) and diastolic ($P = 0.790$) blood pressure before and after ablation. This group summarized that RFA of renal masses in the solitary kidney appears to be relatively safe with no adverse effects on renal function and blood pressure.

Finally, in 2009, Tuma *et al*^[34] reviewed their experience with laparoscopic PN, CA, and RFA for tumors in solitary renal units focusing on oncologic and kidney function outcomes. This study patients who underwent laparoscopic PN ($n = 36$), CA ($n = 36$) and RFA ($n = 29$), respectively. These investigators observed a mean decrease in eGFR calculated one month post-treatment by 18% (PN), 3% (CA), and 7% (RFA). Furthermore, 5 of 36 patients undergoing laparoscopic PN required some form of hemodialysis, in contrast to 0 patients in the CA and RFA arms. The authors concluded that although oncological outcomes are superior for laparoscopic PN, there appears to be somewhat poorer renal function outcomes than those patients managed by CA and RFA.

Collectively, these data comparing experience in solitary kidney systems underscore a renal function benefit when considering thermal ablation vs PN.

CONCLUSION

Thermal ablation is an increasingly utilized treatment option for comorbid patients presenting with SRMs. Studies to date highlight that renal preservation is superior when compared to partial or RN. Such

considerations may be more significant when evaluating anatomic or functional solitary renal units at particular risk for post-treatment kidney injury. Prospective studies are requisite to better define the role of probe ablative therapy in managing small kidney tumors.

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Basic Study

C5b-9 does not mediate tubulointerstitial injury in experimental acute glomerular disease characterized by selective proteinuria

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Abstract

AIM: To determine whether complement membrane attack complex (C5b-9) has a pathogenic role in tubulointerstitial injury in a renal disease model characterized by acute highly selective proteinuria.

METHODS: Protein-overload nephropathy (PON) was induced in adult female Piebald-Viral-Glaxo rats with or without complement C6 deficiency (C6⁻ and C6⁺) by daily intraperitoneal injections of bovine serum albumin (BSA, 2 g/d), and examined on days 2, 4 and 8.

RESULTS: Groups with PON developed equivalent levels of heavy proteinuria within 24 h of BSA injection. In C6⁺ rats with PON, the tubulointerstitial expression of C5b-9 was increased and localized predominantly to the basolateral surface of tubular epithelial cells (TECs), whereas it was undetectable in C6⁻ animals. TEC proliferation (as assessed by the number of BrdU+

cells) increased by more than 50-fold in PON, peaking on day 2 and declining on days 4 to 8. There was a trend for a reduction in the number of BrdU+ TECs on day 4 in the C6⁻ PON group ($P = 0.10$ compared to C6⁺) but not at any other time-point. Kidney enlargement, TEC apoptosis (TUNEL⁺ cells) and markers of tubular injury (tubule dilatation, loss of TEC height, protein cast formation) were not altered by C6 deficiency in PON. Interstitial monocyte (ED-1+ cell) accumulation was partially reduced in C6⁻ animals with PON on day 4 ($P = 0.01$) but there was no change in myofibroblast accumulation.

CONCLUSION: These data suggest that C5b-9 does not mediate tubulointerstitial injury in acute glomerular diseases characterized by selective proteinuria.

Key words: Apoptosis; Proliferation; Tubulointerstitial; Proteinuria; C5b-9; Complement; Rats

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Core tip: The intra-renal assembly of the complement membrane attack complex (C5b-9) in the tubular lumen may be one of the principal mediators of chronic tubulointerstitial damage in nephrotic glomerular disease. This study shows that in an acute glomerular disease model (protein overload nephropathy) characterized by the rapid onset of highly selective proteinuria, C5b-9 does not mediate early tubulointerstitial injury. This may be due to the low luminal formation of C5b-9 in this model, and suggests that other factors, such as the filtration of albumin, growth factors and/or microtubular protein-cast obstruction, are more important in the pathogenesis of tubulointerstitial injury under these circumstances.

Rangan GK. C5b-9 does not mediate tubulointerstitial injury in experimental acute glomerular disease characterized by selective proteinuria. *World J Nephrol* 2016; 5(3): 288-299 Available from: URL: <http://www.wjgnet.com/2220-6124/full/v5/i3/288.htm> DOI: <http://dx.doi.org/10.5527/wjn.v5.i3.288>

INTRODUCTION

In humans with chronic glomerular disease, the degree of tubular atrophy and interstitial disease are the strongest histological parameters which correlate with renal function and predict progression^[1]. Irreversible damage to the glomerular capillary wall and ultrafiltration of serum-derived proteins into the tubular lumen is one of the non-immunologic mechanisms that evokes secondary tubulointerstitial disease, a process that is independent of the original inciter of glomerular injury^[2,3]. Clinical^[4-11] and experimental^[12-21] evidence suggests that the abnormal presence of serum-derived complement components in the tubular lumen

during proteinuric states, leads to the assembly of the complement membrane attack complex (C5b-9) (via the alternative pathway) on the apical brush border of tubular epithelial cells (TECs), and that this is an important factor in the causation of tubulointerstitial damage in proteinuric renal diseases^[22-26]. Under these conditions, the binding of serum-derived properdin (the only known positive regulator of the alternative pathway) to tubular heparin-sulfate is a pivotal facilitator of C5b-9 formation on the apical surface of the tubular lumen^[27-30].

In previous studies, the progression of tubulointerstitial damage has been compared in various models of non-immune mediated chronic kidney disease using rats unable to generate C5b-9 (due to the genetic absence of the C6 complement component)^[16,17,31,32]. In chronic proteinuric models (puromycin aminonucleoside, PAN; remnant kidney, RK; adriamycin nephropathy, AN) C5b-9 was localised to the tubular lumen and brush border of TECs^[16,17,32], and tubulointerstitial injury was attenuated in C6 deficient rats compared to the C6 replete group, despite equivalent proteinuria and renal function. Conversely, in non-proteinuric models, only peritubular (but not luminal) C5b-9 formation occurred, and C6 deficiency did not alter the progression of tubulointerstitial damage under these circumstances^[31]. Thus, these data emphasise that intraluminal C5b-9 formation may be an important determinant of whether it has a pathological role in chronic kidney diseases.

In humans, the urinary excretion of C5b-9 (a marker of intraluminal complement formation) is dependent on the type of glomerular pathology, being highest in diseases characterized by non-selective proteinuria (such as focal segmental glomerulosclerosis, diabetic nephropathy and membranous nephropathy) and absent in minimal change disease (a disease characterized by highly selective proteinuria)^[6,33]. These observations suggest that proteinuria *per se* is not a prerequisite for intraluminal C5b-9 formation, and that it may not mediate tubulointerstitial damage in glomerular disease (as in minimal change disease) characterized by highly selective proteinuria^[34,35]. To test this hypothesis in the preclinical setting, protein-overload nephropathy (PON) was induced in rats deficient or sufficient in complement C6. In contrast to PAN and AN, the proteinuria in PON is almost immediate in onset and highly selective in composition^[36]. During the first week, PON is characterised by marked renal enlargement^[37], TEC proliferation^[38] and mild interstitial inflammation^[14].

MATERIALS AND METHODS

Animals

Female Piebald-Viral Glaxo (PVG) rats with ($n = 28$) or without ($n = 25$) C6 deficiency were obtained from the breeding colony at the University of Washington, Seattle, WA, United States (Body weight 173 ± 3 g, mean \pm SEM)^[39]. The original source for the breeding pairs with normal complement activity was Harlan

Sprague-Dawley (Cambridge, United Kingdom) whereas for the C6 deficient animals it was Bantin and Kingman Universal (Edmonds, WA, United States)^[40]. Before the study, the haemolytic activity in serum from each rat was measured by a standard CH₅₀ assay^[39]. All rats were housed in groups of three to four per cage under standard laboratory conditions and allowed free access to commercial rat pellets and tap water. Experimental protocols were approved by the Animal Use Care Committee of Westmead Hospital (Protocol No. 135.02-08) and the Animal Use Committee at the University of Washington. The study was conducted in accordance with the Australian Code for the care and use of animals for scientific purposes and the National Institutes of Health Guide for the Use and Care of Laboratory animals.

Experimental model of PON

PON was induced in groups of animals by daily intraperitoneal injections of bovine serum albumin (2 g, BSA, A4503, Sigma-Aldrich, St Louis, United States), as previously described^[38], from day 1 until day 8 (i.e., total of eight consecutive injections) under ether anaesthesia, and groups of animals were sacrificed on days 2, 4 and 8. A separate group of control animals received saline only and were sacrificed at the same timepoints ($n = 2$ C6⁺, $n = 1$ C6⁻ per timepoint). Four animals with PON ($n = 2$ C6⁺ and $n = 2$ C6⁻) died during the study and were excluded from all subsequent analyses. Three were due to respiratory arrest from ether anaesthesia and another was euthanased on day 3 due to weight loss and physical signs of distress.

On the day prior to sacrifice, rats were placed in metabolic cages for 16 h to assess proteinuria. To assess the effects of C6 deficiency on TEC proliferation, 3 h prior to sacrifice, animals received a single intraperitoneal injection of bromodeoxyuridine (BrdU, 50 mg/kg, Amersham Life Science). At the time of sacrifice, animals were anaesthetised by an intraperitoneal injection of ketamine: Xylazine, a mid-line laparotomy was performed, the inferior vena cava and aorta were transected and both kidneys were removed and weighed.

Renal function and proteinuria

Proteinuria was assessed by the sulfosalicylic acid method^[32]. Urinary creatinine, serum creatinine, urea, albumin and total protein were assessed by the Institute of Clinical Pathology and Medical Research, Westmead Hospital using an auto-analyzer^[32].

Histology

Coronal sections of the kidney were immersion-fixed in methyl Carnoy's solution or neutral-buffered formalin and embedded in paraffin^[32]. Arbitrary coronal sections, 4 μ m in thickness, were stained with periodic acid-schiff (PAS). Tissue for immunofluorescence was embedded in OCT compound (Lab-Tek products, Miles Laboratories, Naperville, IL, United States) and snap-frozen in liquid nitrogen^[32].

C5b-9 immunohistochemistry

The presence of rat C5b-9 was determined using biotinylated anti-rat C5b-9 monoclonal antibody 2A1 followed by fluorescein isothiocyanate streptavidin, as previously described^[16,17,31,32].

Assessment of TEC proliferation

TEC proliferation was assessed by immunohistochemistry using antibodies against proliferating cell nuclear antigen (PCNA) and BrdU. Tissue sections were deparaffinized with Histoclear^R (National Diagnostics, Atlanta, GE, United States) and rehydrated. Endogenous peroxidase activity was quenched with 3% hydrogen peroxide for 10 min, followed by incubation with a blocking agent (Background Buster, Accurate Chemical and Scientific Corporation, Westbury, NY, United States). The kidney sections were then incubated with either of the following primary and secondary antibodies: A mouse antibody reactive against anti-BrdU (Amersham Biosciences, United Kingdom), followed by a biotinylated rabbit anti-mouse IgG_{2a} antibody. Immunoreactivity of the tissue sections was visualized with Vectastain Elite ABC reagent (Vector Laboratories, Burlingame, CA, United States) and DAB. As a negative control for non-specific immunoreactivity, pre-immune serum (from the same animal species as the primary antibody) was substituted in place of the primary antibody, with each staining procedure. The slides were counterstained with 2% methyl-green or PAS. In some slides, double immunohistochemistry for proximal TEC brush border using anti-rabbit Fx1A and PCNA was performed, counterstained with PAS.

Assessment of TEC apoptosis

Apoptosis was evaluated by the *in situ* cell death detection terminal deoxynucleotidyl transferase-mediated nick end-labeling (TUNEL) method using a commercial kit (Roche Diagnostics, Sydney, Australia). The TUNEL method was performed on formalin-fixed slides according to the manufacturer's instruction. Permeabilisation was achieved with proteinase K (20 g/mL) treatment. TUNEL positive cells were visualised with DAB. Positive and negative controls were prepared as recommended by the manufacturer. TUNEL positive cells were defined according to strict criteria as DAB positive cells with morphological features of apoptosis.

Assessment of tubular injury and interstitial inflammation

Tubular injury was assessed by immunohistochemistry for antibodies against vimentin (marker of TEC dedifferentiation), ED-1 (marker of monocytes and macrophages) and α -smooth muscle actin (SMA, marker of myofibroblasts), as previously described^[31,32].

Quantification of immunohistology

Random selection methods were used to determine the microscopic fields for evaluation. For PCNA, percentage area of positive staining in the renal cortex was

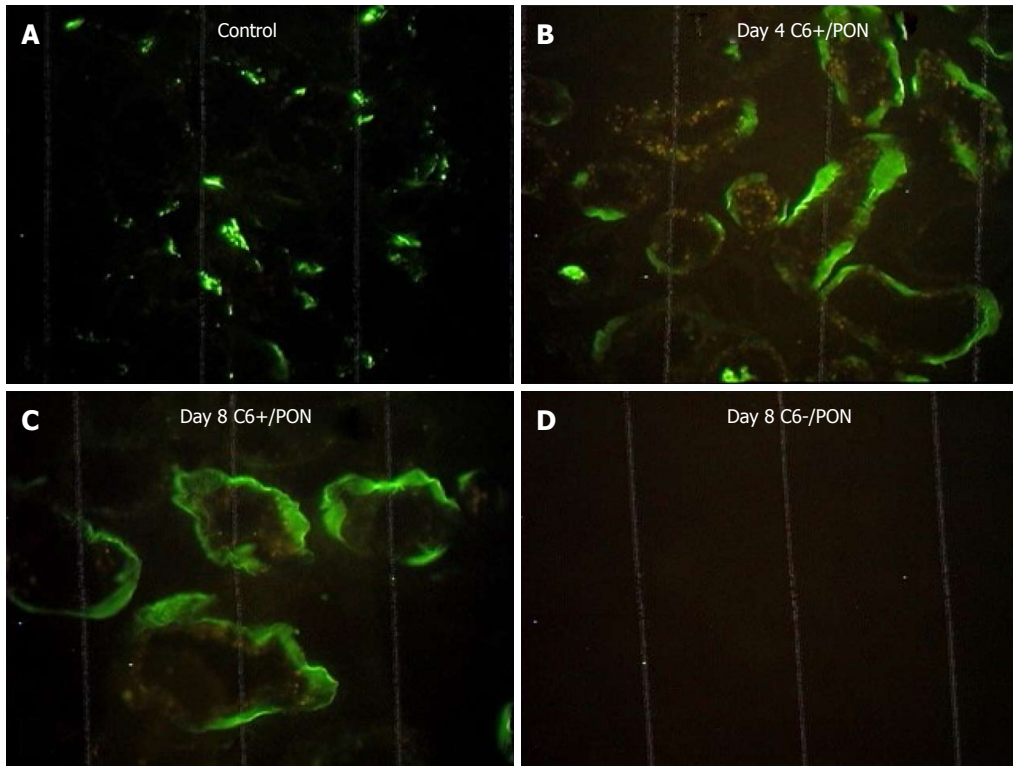


Figure 1 The tubulointerstitial deposition of C5b-9 is increased in C6⁺ rats with protein-overload nephropathy. Representative sections are shown. A: Animal injected with saline (control); B: C6⁺ animal with PON on day 4; C: C6⁺ animal with PON on day 8; D: C6⁻ animal with PON on day 8 ($\times 400$ magnification). Predominant basolateral deposition of C5b-9 was present in PON. PON: Protein-overload nephropathy.

assessed by quantitative image analysis in 10 cortical fields, as previously described, using Optimas Image analysis software^[32]. For BrdU, TUNEL and ED-1, the number of positive cells/nuclei were counted in 10 non-overlapping cortical fields ($\times 200$). In addition, previous studies in PON^[38] showed that TEC TUNEL positivity in this model increases from the outer cortex (subcapsular region, defined as Field 1) to the inner cortex/outer medulla (defined as Field 4), and therefore ten separate fields for each region was evaluated separately in each slide stained for TUNEL.

To determine mean tubule diameter and TEC height, images were digitized and viewed on a computer and analysed with public-domain image analysis software (Image J version 1.33, NIH software). To assess tubule diameter, the shortest cross-sectional diameter of a tubule was measured by line morphometry, as previously described^[31,32]. At least five non-overlapping but contiguous cortical fields ($\times 400$ magnification) and three tubules (the largest three) were assessed per field. The tubule cell height was the perpendicular length of a TEC.

Statistics analysis

The data were analyzed with JMP statistical software package (version 4.04, SAS institute, Carey, NC, United States). Data are expressed as mean \pm SE, median and interquartile range. Comparisons between control group and the complement sufficient group with PON were performed the Kruskal-Wallis test followed by

the Tukey-Kramer honest significance difference. To determine the effect of complement deficiency in PON, comparisons between C6⁺ and C6⁻ groups with PON at each timepoint were performed using the Wilcoxon test. A *P* value of less than 0.05 indicated statistical significance.

RESULTS

Tubulointerstitial deposition of C5b-9 is increased in C6⁺ rats with PON

By immunohistochemistry of the kidney, in control C6⁺ animals, occasional focal areas of C5b-9 were present in the peritubular region of the tubulointerstitium (Figure 1A). In C6 sufficient animals with PON, C5b-9 deposition was increased compared to control animals but localized to the basolateral membrane of TECs (Figure 1B). This pattern of C5b-9 immunoreactivity increased throughout the time-course, peaking on day 8 (Figure 1C). Only occasional and rare areas of C5b-9 could be detected on the luminal brush border in PON. C5b-9 was completely absent in C6⁻ deficient animals with PON (Figure 1D).

Serum protein and urinary protein excretion are similar in C6⁺ and C6⁻ rats with PON

Both total protein and albumin increased in the serum of C6⁺ rats with PON at all time-points (Table 1). This was paralleled by the development of marked proteinuria. Severe proteinuria was detected by urine

Table 1 Biochemical data in the experimental groups

Group	n	Serum protein (g/dL)	Serum albumin (g/dL)	Proteinuria (mg/24 h)	Serum creatinine (mmol/L)	Serum urea (mmol/L)
Control	9	65 ± 1 (65, 63-66)	39 ± 1 (39, 37-42)	2 ± 1 (2, 1-2)	43 ± 3 (40, 36-48)	7 ± 0 (7, 6-8)
PON d 2 C6 ⁺	6	98 ± 3 ¹ (97, 92-102)	74 ± 1 ¹ (74, 73-76)	857 ± 176 ¹ (972, 679-1080)	97 ± 17 ¹ (86, 67-117)	27 ± 5 ¹ (23, 17-38)
PON d 2 C6 ⁻	5	97 ± 5 ¹ (99, 93-104)	74 ± 4 ¹ (76, 71-79)	789 ± 214 ¹ (788, 11-1145)	117 ± 31 ¹ (97, 64-177)	35 ± 11 ¹ (21, 17-58)
PON d 4 C6 ⁺	6	101 ± 2 ¹ (102, 97-109)	73 ± 1 ¹ (75, 72-76)	1214 ± 131 ¹ (1094, 1013-1197)	93 ± 9 ¹ (94, 80-125)	24 ± 2 ¹ (25, 20-30)
PON d 4 C6 ⁻	6	96 ± 2 ¹ (95, 89-98)	70 ± 1 ¹ (69, 67-73)	1026 ± 267 ¹ (965, 724-1719)	76 ± 5 ¹ (73, 65-85)	19 ± 1 ¹ (18, 17-21)
PON d 8 C6 ⁺	8	97 ± 3 ¹ (97, 88-101)	68 ± 3 ¹ (70, 64-72)	1177 ± 70 ¹ (1165, 709-1321)	74 ± 6 ¹ (65, 53-81)	24 ± 3 ¹ (20, 15-29)
PON d 8 C6 ⁻	9	97 ± 2 ¹ (99, 95-101)	70 ± 3 ¹ (71, 70-73)	1227 ± 116 ¹ (1228, 952-1529)	69 ± 6 ¹ (73, 52-86)	22 ± 3 ¹ (19, 17-28)

Data are expressed as mean ± SEM (median, Inter-quartile range); ¹P < 0.05 compared to control group. PON: Protein-overload nephropathy.

Table 2 Body and kidney weight in the experimental groups

Group	n	Day 1 BW (g)	Final BW (g)	KW (g)	KW:BW
Control	9	186 ± 21 (196, 158-200)	182 ± 5 (192, 168-195)	0.60 ± 0.02 (0.62, 0.53-0.66)	0.33 ± 0.01 (0.33, 0.31-0.34)
PON d 2 C6 ⁺	6	179 ± 2 (168, 164-173)	176 ± 4 (174, 168-184)	1.11 ± 0.05 ¹ (1.06, 1.03-1.22)	0.63 ± 0.03 ¹ (0.62, 0.55-0.72)
PON d 2 C6 ⁻	5	174 ± 3 (167, 159-170)	169 ± 2 (171, 166-176)	1.07 ± 0.11 ¹ (1.20, 0.81-1.26)	0.63 ± 0.07 ¹ (0.67, 0.47-0.75)
PON d 4 C6 ⁺	6	172 ± 2 (181, 174-187)	167 ± 3 (169, 161-173)	1.20 ± 0.07 ¹ (1.29, 1.19-1.35)	0.72 ± 0.05 ¹ (0.76, 0.74-0.84)
PON d 4 C6 ⁻	6	177 ± 9 (165, 176-190)	175 ± 3 (177, 167-183)	1.10 ± 0.05 ¹ (1.09, 0.95-1.11)	0.62 ± 0.02 ¹ (0.60, 0.56-0.67)
PON d 8 C6 ⁺	8	167 ± 4 (165, 159-176)	163 ± 4 (160, 157-173)	1.33 ± 0.08 ¹ (1.30, 1.24-1.38)	0.82 ± 0.05 ¹ (0.80, 0.70-0.88)
PON d 8 C6 ⁻	9	167 ± 3 (165, 160-172)	158 ± 2 (158, 152-166)	1.32 ± 0.22 ¹ (1.30, 1.11-1.57)	0.83 ± 0.04 ¹ (0.78, 0.72-0.95)

Data are expressed as mean ± SEM (median, inter-quartile range); ¹P < 0.05 compared to control group. PON: Protein-overload nephropathy; BW: Body weight; KW: Kidney weight.

dipstick analysis within 24 h of BSA injection, and by quantitation, peaked between days 4 and 8 (Table 1). There were no differences, in either the serum total protein and albumin or proteinuria, between the C6⁺ and C6⁻ groups with PON at any time-point.

Renal dysfunction is similar in C6⁻ and C6⁺ rats with PON

Both the serum urea and creatinine increased in C6 sufficient rats with PON, peaking between days 2 and 4, and beginning to decline by day 8. These changes were not altered by C6 deficiency (Table 1).

Kidney enlargement and TEC proliferation are similar in C6⁻ and C6⁺ rats with PON

Kidney weight increased by more than two-fold in PON, peaking on day 8, and was not altered by C6 deficiency (Table 2). In PON, TEC proliferation increased in both proximal and distal tubules, as assessed by either PCNA or BrdU immunohistochemistry (Table 3, Figures 2 and 3). In the C6 sufficient group, quantitative analysis

showed that the renal cortical expression of PCNA peaked on day 4 and declined on day 8 in PON. The number of BrdU positive cells increased by more than 50-fold, peaking on day 2 (earlier than PCNA) and declining on days 4 and 8. The number of BrdU positive TECs and cortical PCNA expression were both strongly correlated with the serum creatinine (Spearman Rho 0.75 and 0.85 respectively, both *P* < 0.001). There was a trend for a reduction in PCNA and BrdU staining in the C6⁻ group compared to the C6⁺ on day 4, but this did not reach statistical significance (*P* = 0.09 and 0.10 respectively). There were no differences in PCNA or BrdU between C6⁺ and C6⁻ PON groups at any other time-point.

TEC apoptosis is similar in C6⁻ and C6⁺ rats with PON

In PON, the number TUNEL positive TECs increased and this was statistically significant in Field 4 (or the inner cortex/outer medulla) (*P* = 0.03 compared to the control group) whereas there was a trend for an increase in Field 1 (subcapsular cortex) (*P* = 0.15). These data

Table 3 Markers of proliferation and apoptosis in the experimental groups

Group	n	Cortical PCNA (%)	BrdU + TECs (cells/mm ²)	TUNEL + TECs (Field 1)	TUNEL + TECs (Field 4)
Control	9	0 ± 0 (0, 0-0)	0.2 ± 0.0 (0.2, 0-0.2)	6.9 ± 2.5 (8, 0-11.2)	2.7 ± 2.1 (0, 0-5.6)
PON d 2 C6 ⁺	6	0.24 ± 0.06 ¹ (0.28, 0.13-0.36)	8.8 ± 2.7 ¹ (8.9, 2.7-15.3)	-	-
PON d 2 C6 ⁻	5	0.21 ± 0.07 ¹ (0.18, 0.14-0.30)	6.8 ± 3.1 ¹ (6.4, 0.6-13.2)	-	-
PON d 4 C6 ⁺	6	0.34 ± 0.08 ¹ (0.30, 0.09-0.48)	4.6 ± 0.8 ¹ (5.2, 3.4-7.4)	-	-
PON d 4 C6 ⁻	6	0.19 ± 0.04 ¹ (0.18, 0.14-0.30)	3.2 ± 0.5 ¹ (1.2, 1.7-3.7)	-	-
PON d 8 C6 ⁺	8	0.15 ± 0.05 ¹ (0.11, 0.03-0.16)	2.4 ± 0.7 ¹ (3.1, 0-4.1)	13.4 ± 3.3 (12.8, 5.6-16.8)	10.0 ± 2.6 ¹ (9.6, 5.6-17.6)
PON d 8 C6 ⁻	9	0.09 ± 0.03 ¹ (0.08, 0.02-0.11)	2.0 ± 0.7 ¹ (1.2, 0.1-3.7)	11.4 ± 3.7 (9.6, 0-17.6)	7.8 ± 2.8 (6.4, 0-16)

Data are expressed as mean ± SEM (median, Inter-quartile range); ¹P < 0.05 compared to control group; Field 1 was the sub-capsular cortex; Field 4 was the inner cortex/outer medulla. PCNA: Proliferating cell nuclear antigen; TUNEL: Transferase-mediated nick end-labeling; PON: Protein-overload nephropathy; TEC: Tubular epithelial cells.

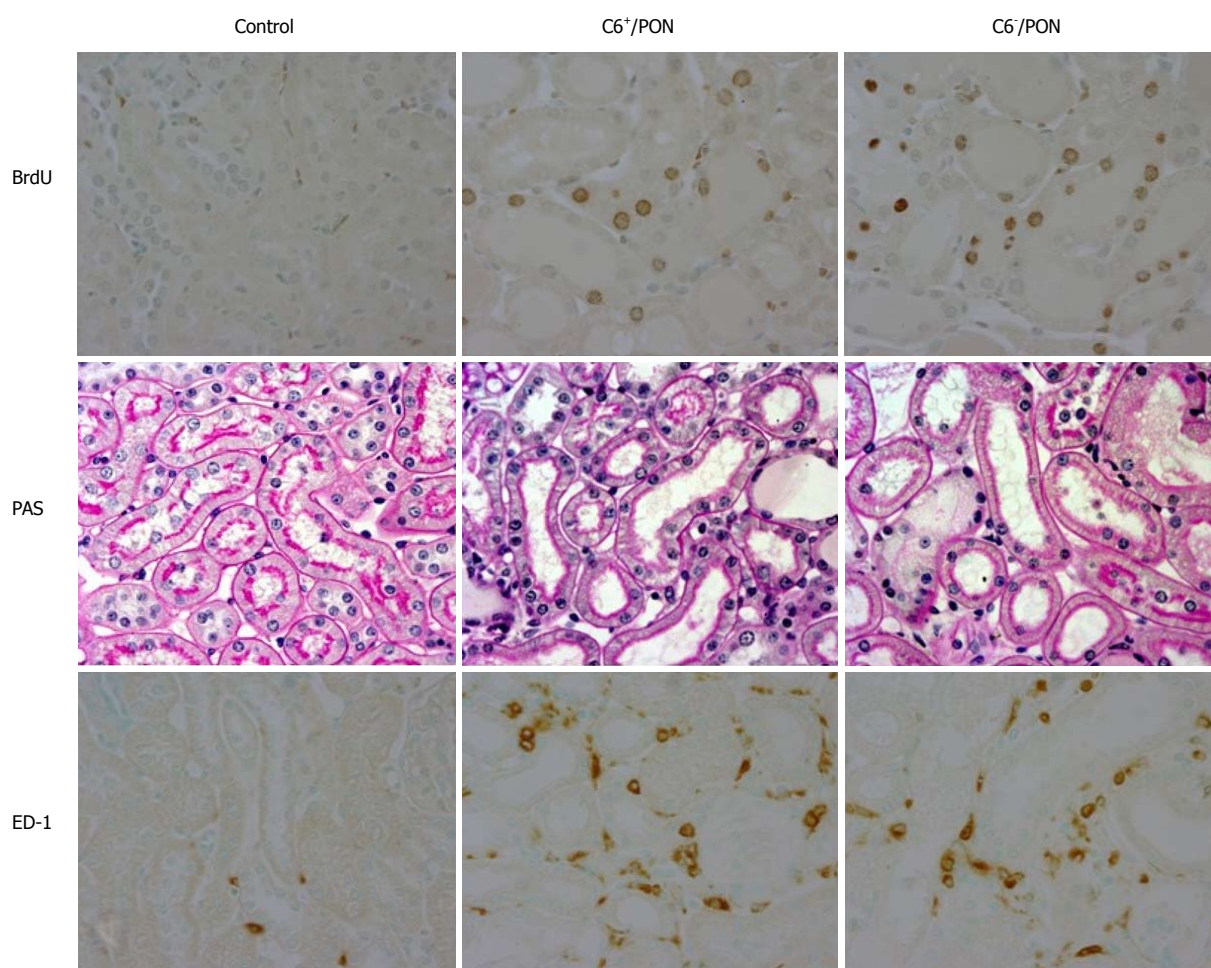


Figure 2 Cortical tubulointerstitial injury in control and protein-overload nephropathy groups on day 8. Representative renal cortical sections (magnification × 400) show immunostaining for BrdU, periodic acid-schiff and ED-1.

are consistent with previous studies on the changes in TEC apoptosis in this model^[38]. However, the increase in TUNEL positivity in TECs in Field 4 was not altered by C6⁻ deficiency (Table 3).

Tubulointerstitial injury is similar in C6⁻ and C6⁺ rats with PON

TEC injury and atrophy, tubular dilatation and distal protein cast formation are the histopathological features

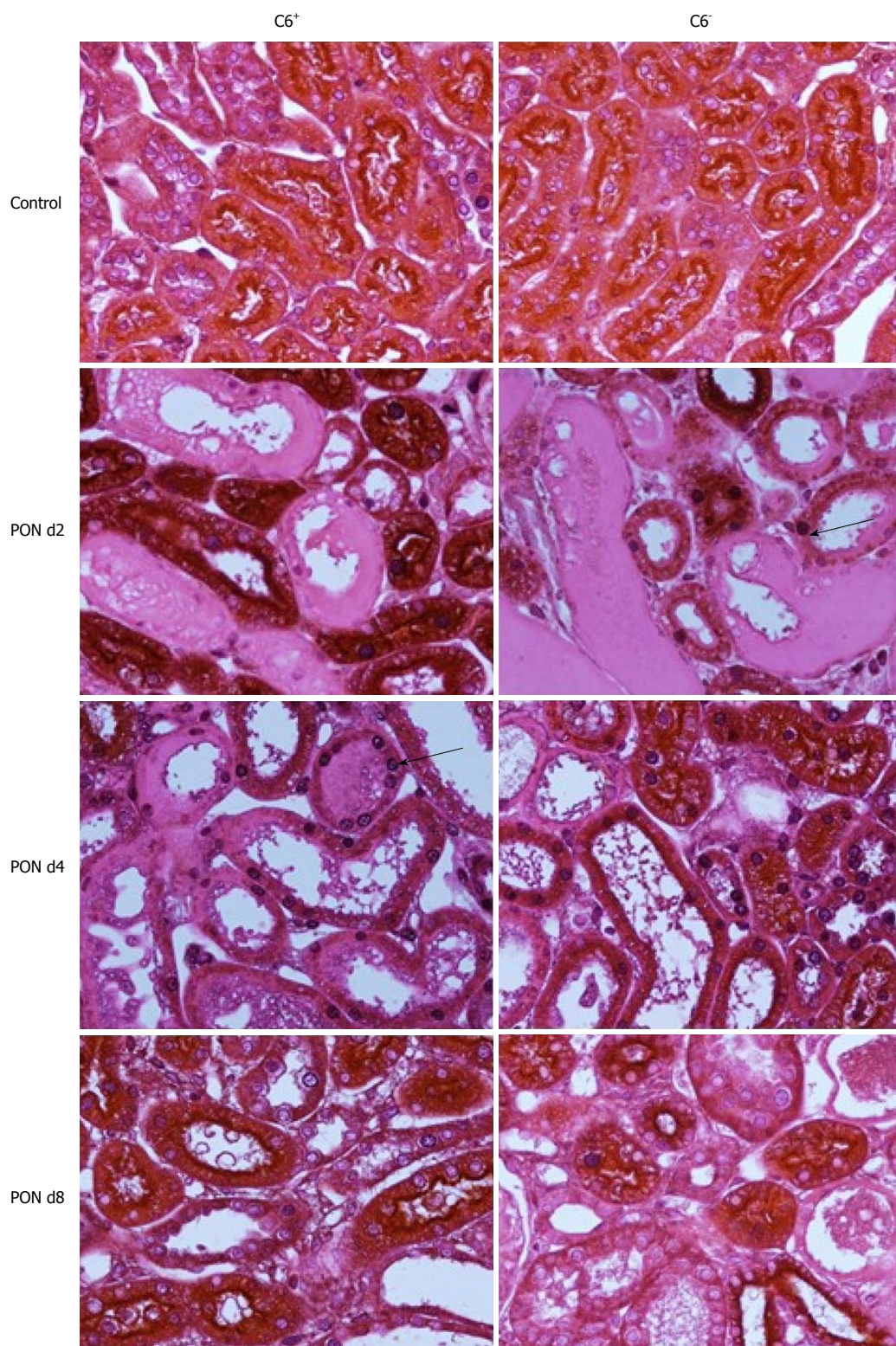


Figure 3 The renal cortical expression of proliferating cell nuclear antigen is increased in protein-overload nephropathy. Representative sections ($\times 200$ magnification) are shown. PCNA positive TEC nuclei are labeled purple (Nickel-enhanced DAB-positive, arrows). Brush border are labeled with anti-Fx1A (brown) and counterstained with periodic acid-schiff. PCNA: Proliferating cell nuclear antigen; TEC: Tubular epithelial cells.

of PON^[41]. Quantitative morphometric analysis was undertaken to precisely determine whether these parameters were altered by C6 deficiency. TEC injury and atrophy was assessed by the cross-sectional height of TECs and vimentin staining. In PON, the mean TEC

height decreased on days 2 and 4, and increased on day 8 (Table 4). On day 8, tubules were noted to have more brush border, accounting for the rise in the TEC height. TEC injury was also assessed by vimentin staining. In control animals, vimentin was present in

Table 4 Assessment of tubulointerstitial injury in the experimental groups

Group	n	TEC height	TEC diameter	Protein cast/field	Interstitial ED-1/field
Control	9	82.3 ± 1.7 (83.3, 78.0-87.0)	253.0 ± 3.7 (256.6, 241.7-259.0)	0	9.4 ± 0.5 (9.3, 8.4-10.6)
PON d 2 C6 ⁺	6	70.9 ± 5.2 ¹ (69.4, 59.5-79.6)	312.1 ± 13.9 ¹ (311.8, 279.4-343.4)	-	20.9 ± 1.6 ¹ (20.1, 17.3-25.5)
PON d 2 C6 ⁻	5	76.6 ± 2.8 ¹ (76.1, 66.6-80.6)	300.8 ± 8.2 ¹ (308.7, 292.7-316.6)	-	14.5 ± 4.0 (11.3, 9.3-17.6)
PON d 4 C6 ⁺	6	68.0 ± 2.3 ¹ (68.3, 60.8-71.5)	309.0 ± 11.1 ¹ (296.8, 291.1-341.1)	2.9 ± 0.5 (3.4, 2.8-3.7)	29.6 ± 2.3 ^{1,2} (28.3, 25.4-38.2)
PON d 4 C6 ⁻	6	67.8 ± 3.8 ¹ (69.3, 61.1-75.4)	303.2 ± 12.6 ¹ (313.0, 275.7-324.2)	2.2 ± 0.6 (1.5, 1.1-3.4)	21.0 ± 1.8 ¹ (20.9, 16.7-23.9)
PON d 8 C6 ⁺	8	74.7 ± 2.7 ^{1,2} (76.2, 71.0-78.9)	304.0 ± 9.2 ¹ (295.5, 280.4-319.1)	1.1 ± 0.3 ³ (0.7, 0.5-1.4)	73.5 ± 6.7 ^{1,2} (78.0, 59.2-85.7)
PON d 8 C6 ⁻	9	70.0 ± 2.9 ¹ (67.7, 63.3-75.3)	298.0 ± 3.8 ¹ (298.6, 289.8-308.1)	1.5 ± 0.5 (1.2, 0.5-2.3)	56.1 ± 7.8 ¹ (56.7, 38.9-80.1)

Data are expressed as mean ± SEM. The units for TEC height and TEC diameter are in arbitrary units. ¹*P* < 0.05 compared to control (median, Inter-quartile range); ²*P* < 0.05 when compared to PON d 2, C6⁺; ³*P* < 0.05 compared to PON d 4, C6⁺. PON: Protein-overload nephropathy; TEC: Tubular epithelial cell.

endothelial cells of glomeruli and peritubular capillaries. In PON, this distribution was preserved, but in addition, there was more prominent peritubular capillary staining and rarely vimentin-positive spindle-shaped cells were noted around tubules on day 8. However, vimentin-positive tubules were not detected during the time-points of the study in PON. In addition, there were no qualitative differences in vimentin staining between the C6⁺ and C6⁻ groups with PON (data not shown).

The mean cross-section tubule diameter increased in a time-dependent manner, peaking on days 2 to 4 and decreasing on day 8 (Table 4). There was a strong relationship between tubular diameter and serum creatinine (Spearman Rho 0.85, *P* < 0.001), as well as the number of BrdU positive TECs and renal cortical PCNA expression (Spearman Rho 0.83 and 0.80 respectively, both *P* < 0.001). In addition, the number of proteinaceous casts in distal tubules increased in PON, peaking on day 4 and declining slightly by day 8. However, neither tubule diameter or cast formation was affected by complement deficiency in PON (Table 4).

Interstitial monocyte accumulation increased progressively in PON in a time-dependent manner peaking on day 8, when it more than 7-fold higher than the control group. There was a partial reduction in interstitial ED-1 accumulation in C6⁻ animals with PON which reached statistical significance only on day 4 (*P* = 0.01 compared to C6⁺ PON) but not on days 2 (*P* = 0.10 compared to C6⁺ PON) and 8 (*P* = 0.15, compared to C6⁺ PON).

In control animals, α-SMA was constitutively present in capillaries and this distribution was not altered in PON. Rarely, on day 8, some α-SMA positive cells were present around dilated collecting tubules, representing early foci of fibrosis. However, there were no qualitative differences in complement sufficient animals (data not shown).

DISCUSSION

The results of this study provide further important

refinements about the role of the complement membrane attack complex (C5b-9) in non-immunological mediated chronic kidney disease models characterized by nephrotic-range proteinuria. First, in contrast to chronic proteinuric models (PAN, RK and AN)^[16,17,32], C5b-9 deposition in acute and short-term PON was localized predominantly to the basolateral membrane of TECs and virtually undetectable on the lumen of TECs. The reasons for this difference have not been investigated in the present study but it presumably relates to the pattern of glomerular injury and selectivity of the proteinuria in PON, given that properdin remains detectable on the apical membrane of TECs in this model^[28]. Similar to minimal change disease in humans (in which urinary and renal C5b-9 is not increased)^[6,33,35], the early stage of PON is characterized by highly selective proteinuria, predominantly albuminuria (consisting of both heterologous and autologous albumin)^[36,42] with marginal changes in glomerular size permselectivity^[43]. For example, in PAN, albumin constitutes 57% of the protein excreted in the urine whereas in PON, this is significantly higher at 90%^[36]. In addition, it is also possible that large amounts of albumin in the tubular lumen may also, in some way, minimize the density of complement proteins needed to assemble C5b-9^[6].

Moreover, in divergence to previous studies using other models of chronic proteinuria (PAN, RK, AN)^[16,17,32], tubulointerstitial injury was not altered by C6 deficiency in PON. In the present study, tubulointerstitial injury was assessed by several methods, including TEC proliferation and apoptosis, morphometric assessment and interstitial ED-1 accumulation. There was a trend for a reduction in TEC proliferation (as assessed by BrdU and PCNA) on day 4 and no significant changes were seen at any other time-point. In addition there were no differences detected in TEC injury, as objectively assessed by morphometric assessment. Although a reduction in interstitial monocyte accumulation was detected on day 4, this was insufficient to alter either TEC injury or renal dysfunction. The predominant basolateral deposition of

C5b-9 could explain the neutral effects of C6 deficiency on tubulointerstitial injury in PON in the present study. This is supported by previous studies in which C6 deficiency does not alter the progression of non-proteinuric models of chronic kidney disease^[31], and the observation that CD59 (a membrane bound inhibitor of C5b-9) is located on the basolateral region of TECs and absent on the lumen^[44-46].

The PVG C6 deficient rat strain was a chance discovery made by Leenaerts *et al*^[40] who first described the specific defect in the complement system in these animals. The key abnormality is a partial and isolated defect of C6 (unstable mRNA or point mutation) due to a spontaneous autosomal recessive genetic defect^[47]. The abnormality was restricted to PVG rats obtained from Bantin and Kingman (Fremont, CA, United States) and not present in rats from other animal vendors (Harlan SD, Harlan Olac, others)^[40]. In the C6 deficient strain the activation of the complement system proceeds normally to the level of C5, and thus the generation of opsonic C3b and chemotactic C5a are not known to be affected^[40]. The C6 deficient strain are not susceptible to immunocompromised infections^[40], have normal T-cell responses^[48] and their tissue antigenic expression and immunity are identical to the C6 sufficient PVG strain^[40,48]. The exact mechanisms underlying the tubulointerstitial localisation of C5b-9 to the basolateral region in C6⁺ rats with PON in the present study, are not clear but previous evidence would suggest that it is a consequence of non-immunological mediated TEC injury, intra-renal complement synthesis and presumably a sequela of interstitial oedema and inflammation in this model^[49-51]. The TEC injury and interstitial inflammatory response in PON is postulated to be secondary to "proteinuria-induced TEC injury" rather than immunologic factors^[14]. The PON model does not show evidence of tubulointerstitial rat IgG or circulating antibodies to BSA^[14].

Abbate and colleagues reported that C3 mediates both glomerular as well as tubulointerstitial injury in mice with PON^[52]. In the latter study, C3 deficiency attenuated both proteinuria and tubulointerstitial injury in PON^[52]. Furthermore, the latter study also demonstrated that PON-mediated renal injury was only partially attenuated in C3^{-/-} kidneys transplanted into wild-type recipients, suggesting that circulating C3 may be more critical to the pathogenesis of PON than the local generation of C3 within kidney^[52] (though this finding may be specific to PON as it was not the situation in AN^[53]). In any case, the results of the current study suggest that C6 is not likely to be a critical down-stream mediator of C3-induced renal injury in PON, and emphasise potential hierarchical roles for other complement factors, such as C3b and/or C5a^[46,52,53] in this setting.

In contrast to the findings of the current study, Eddy and colleagues previously detected C5b-9 in the tubular lumen in PON^[14]. The different results obtained in our study could be due to the lower dose of BSA and proteinuria (two-fold less than the current study) as well

as the use of uninephrectomy in the model described by Eddy *et al*^[14]. These factors may alter the pattern of the glomerular injury and selectivity of proteinuria^[54], and influence the formation of C5b-9 in the tubular lumen^[17].

In this study, the time-dependant changes of tubulointerstitial injury in PON were quantitated precisely, for the first time, by morphometric analysis. These data highlight some important pathophysiological insights into the nephron response to excess luminal proteins. Initially, we found that the TEC height decreased on day 2 but increased on day 8. The increase in TEC height on day 8 was temporally associated with a focal increase in the number of proximal TECs expressing brush border and a reduction in the mean serum creatinine. These changes were preceded by a dramatic increase in kidney weight and TEC proliferation, which is a consistent feature of nephrotic glomerular disease in human biopsy studies^[55,56]. Taken together, these data suggest that the transient increase in TEC proliferation in acute nephrotic glomerular disease may be a compensatory mechanism that leads to nephron hypertrophy and attempts to restore renal function as well as provide an increased surface area to absorb the excess luminal proteins. This hypothesis is also supported by data showing that inhibition of TEC proliferation by rapamycin causes cast nephropathy and acute renal failure in PON^[41]. The current study also highlights non-complement dependant mechanisms of tubulointerstitial injury in glomerular diseases, particularly the adverse effects of intratubular obstruction due to protein-cast nephropathy^[37], as detected by the increase in cross-section tubule diameter in PON.

Bearing in mind the limitations of PON as an animal model, the results of the current preclinical study have the strongest implications for pathogenesis of early renal injury associated with human minimal change disease. So far, only a few studies in humans have compared the pattern of complement expression in minimal change disease with other types of glomerulonephritides^[6]. Based on clinical experience from renal biopsy samples minimal change disease is not believed to be mediated by C5b-9^[34]. Furthermore, despite the detection of properdin on the apical brush border of proximal tubules in rats with PON^[28], the urinary excretion of C5b-9 in human minimal change disease is low^[6,33]. Taken together, these findings could provide a potential explanation for the lower incidence of end-stage kidney disease in minimal change nephropathy in comparison to other types of nephrotic glomerular diseases^[57].

The results of this study have a number of important limitations. First, the animal model of PON does not exactly replicate human glomerular disease, in that injections of heterologous albumin are required to induce selective proteinuria, and this has systemic effects^[58] which are not present in the human counterpart. Second, further studies are required to confirm and define the mechanisms underlying the absence of luminal C5b-9 formation in acute PON. In this regard, the urinary excretion of C5b-9 as well as other

complement regulatory proteins (particularly properdin and Factor H)^[29,30,59] in PON could be compared to other chronic proteinuric and non-proteinuric models of chronic kidney disease as well as different types of proteinuric diseases in humans, in future studies. Finally, the present study has only examined the acute stages of PON, and different mechanisms of C5b-9-mediated injury could be involved if it is combined with renal mass reduction and/or if the injections of BSA are continued for a longer duration^[14].

In conclusion, the results of the present study shows that C5b-9 does not mediate tubulointerstitial injury in acute short-term PON, and this may be due to the low level of luminal C5b-9 formation. Taken together with the results of previous studies using C6 deficient animals in chronic proteinuric renal disease models^[16,17,32], these data suggest that the selectivity of proteinuria may be an important factor in the causation of tubulointerstitial damage in nephrotic glomerular diseases. Specifically, in the case of acute PON, the pathogenesis of tubulointerstitial injury is C5b-9-independent and the tubular filtration of excess albumin, growth factors and microtubular protein-cast obstruction are likely to be more critical^[37]. Further studies to understand the role of complement system will be helpful in defining new therapies for the generic treatment of kidney diseases characterized by chronic proteinuria^[29].

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COMMENTS

Background

In humans with chronic glomerular disease, the degree of tubular atrophy and interstitial disease are the strongest histological parameters which correlate with renal function and predict progression.

Research frontiers

The proteinuria in protein-overload nephropathy (PON) is almost immediate in onset and highly selective in composition.

Innovations and breakthroughs

Interstitial monocyte (ED-1⁺ cell) accumulation was partially reduced in C6⁻ animals with PON on day 4 ($P = 0.01$) but there was no change in myofibroblast accumulation.

Applications

The results of this study provide further important refinements about the role of the complement membrane attack complex (C5b-9) in non-immunological

mediated chronic kidney disease models characterized by nephrotic-range proteinuria.

Terminology

PON was induced in adult female Piebald-Viral-Glaxo rats with or without complement C6 deficiency (C6⁻ and C6⁺) by daily intraperitoneal injections of bovine serum albumin (2 g/d), and examined on days 2, 4 and 8.

Peer-review

This paper is well written and has interesting findings.

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Update on kidney transplantation in human immunodeficiency virus infected recipients

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Abstract

Improved survival of human immunodeficiency virus (HIV) infected patients with chronic kidney disease following the introduction of antiretroviral therapy resulted in the need to revisit the topic of kidney transplantation in

these patients. Large cohort studies have demonstrated favorable outcomes and proved that transplantation is a viable therapeutic option. However, HIV-infected recipients had higher rates of rejection. Immunosuppressive therapy did not negatively impact the course of HIV infection. Some of the immunosuppressive drugs used following transplantation exhibit antiretroviral effects. A close collaboration between infectious disease specialists and transplant professionals is mandatory in order to optimize transplantation outcomes in these patients. Transplantation from HIV⁺ donors to HIV⁺ recipients has been a subject of intense debate. The HIV Organ Policy Equity act provided a platform to research this area further and to develop guidelines. The first HIV⁺ to HIV⁺ kidney transplant in the United States and the first HIV⁺ to HIV⁺ liver transplant in the world were recently performed at the Johns Hopkins University Medical Center.

Key words: End-stage kidney disease; Human immunodeficiency virus; Antiretroviral therapy; Kidney transplantation

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Core tip: Experience with kidney transplantation in human immunodeficiency virus (HIV) positive patients is evolving. With appropriate selection of candidates, the outcomes appear similar to that in HIV negative population. There are challenges with kidney transplantation in HIV positive patients including increased risk for acute rejection and drug-drug interactions. Optimal immunosuppressive regimen is unknown. This article discusses the recent advances in kidney transplantation among HIV positive patients.

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INTRODUCTION

Human immunodeficiency virus (HIV) infection continues to be a healthcare problem worldwide. According to the Centers for Disease Control and Prevention, it is estimated that roughly 50000 people get infected with HIV each year in the United States. At the end of 2012, around 1.2 million people were living with HIV infection in the United States^[1]. HIV infection used to be lethal in the past. The advent of highly active antiretroviral therapy resulted in a paradigm shift in that chronic illnesses now surpass opportunistic infections as causes of death in patients infected with HIV. Kidney disease continues to cause significant morbidity and mortality among the HIV infected population^[2-4]. Currently, HIV-related nephropathies are considered as the third leading cause of end stage renal disease (ESRD) among African Americans^[5,6].

There are several known etiologies for chronic kidney disease (CKD) in HIV infected patients. There is paucity of accurate epidemiological data due to the lack of renal biopsies performed in suspected cases and due to the inconsistent reporting of the disease. In the pre anti-retroviral therapy (ART) era, HIV associated nephropathy (HIVAN) used to be the most common cause of CKD in HIV infected patients affecting primarily African Americans. However more recently, hypertension, diabetes mellitus and cardiovascular disease evolved as significant causes of renal dysfunction in this patient population. HIV associated immune complex-mediated disease, IgA nephropathy, HIV-associated thrombotic microangiopathy and antiretroviral medication related toxicities are also important etiologies for CKD. Furthermore, most HIV sero-positive patients are co-infected with hepatitis C virus which can also cause CKD^[7]. Progression to ESRD in CKD patients who are also HIV infected is more rapid than those without HIV infection^[8]. Kidney diseases associated with HIV infection are summarized in Table 1.

According to Medicare claims data, the number of prevalent HIV positive ESRD patients has increased more than 14-fold from 1999 to 2010^[9]. Outcomes of HIV infected dialysis patients have improved dramatically^[8,9]. Actual number of HIV positive patients who received kidney transplants or who are on the waiting list is unknown. This is due to the fact that the Organ Procurement and Transplantation Network (OPTN) does not collect data on HIV infection among wait-listed candidates, and some states prohibit the reporting of HIV status.

HIV was once an absolute contraindication for kidney transplantation; however recent studies highlight the safety of kidney transplantation in HIV positive patients who are well-controlled on ART. Several challenges continue to exist in this area including choice of immunosuppression drugs, drug-drug interaction and heightened

risk of infections. Furthermore, the possibility of considering HIV positive donors has been a topic of discussion in the recent years in order to allow an increase in the donor pool as discussed later.

ACCESS TO TRANSPLANTATION IN HIV INFECTED PATIENTS

Historically, HIV infected patients were excluded from the consideration for kidney transplantation due to the concern for worsening infections and rejection. Hemodialysis and peritoneal dialysis were the only forms of treatment available for these patients^[10]. In a survey of 148 United States transplant centers published in 1998, the majority of responding centers would not transplant kidney from deceased (88%) or living (91%) donors into HIV-infected patients. Most centers feared that transplantation in such patients would be harmful to the recipient, and some believed that it would be a waste of scarce donor organs^[11]. However, recent studies have demonstrated that kidney transplantation in HIV positive patients with ESRD who are receiving ART is safe and effective. Outcomes were comparable to recipients without HIV infection. Furthermore, HIV positive individuals have higher waitlist mortality rates than their HIV negative counterparts. This along with an understanding of the role of immune activation in HIV disease pathogenesis and how immunosuppressant drugs exert antiviral effects contributed to a renewed interest in studying the outcomes of transplantation in these patients^[8].

OUTCOMES OF KIDNEY TRANSPLANTATION IN HIV INFECTED PATIENTS

Early experience with kidney transplantation in HIV positive patients before the rollout of ART was disappointing. This experience was based on case reports and small series of patients with short follow up^[12,13]. Among 39 kidney transplants in HIV positive patients between 1980 and 1996, outcomes were suboptimal with 21 deaths after a mean follow up of 48 mo^[14]. These included cases where HIV was transmitted during kidney transplant. Retrospective analysis of the United States Renal Data System database from 1987 to 1997 demonstrated inferior three and five-year graft and five-year patient survivals in HIV positive deceased donor kidney transplant recipients as compared to HIV negative patients^[15].

Following the introduction of ART, several small studies showed encouraging patient and allograft survivals. The largest prospective trial of kidney transplantation in HIV-infected patients was conducted by Stock *et al*^[16] and included 150 patients who were followed for up to five years at 19 United States transplant centers. This study showed one and three-year patient survival rates of 94.6% and 88.2% respectively. Corresponding graft survival rates were 90.4% and 73.7% respectively.

Table 1 Causes of kidney disease in human immunodeficiency virus infected patients

Cause	Characteristics
HIVAN	Collapsing glomerulopathy in the setting of high grade HIV viremia Affects almost exclusively African Americans Manifests with high-grade proteinuria in the absence of hypertension Treated with antiretroviral therapy
HIV-immune complex	Manifests with hematuria and sub-nephrotic range proteinuria Variable presentation with AKI Poorly understood
Diabetic nephropathy	Similar presentation to patients without HIV. Proteinuria followed by decreased GFR
Hypertension	Similar presentation to patients without HIV
Thrombotic microangiopathy	Typically presents with AKI, subnephrotic range proteinuria with hematuria along with features of microangiopathic hemolytic anemia
IgA nephropathy	Hematuria with variable degree of proteinuria and decreased GFR
Tenofovir toxicity	Variable degree of decreased GFR with features of proximal tubular injury
Immune-complex membranoproliferative glomerulonephritis and cryoglobulinemia in the setting of HCV co-infection	Nephritic syndrome picture with positive cryoglobulin and hypocomplementemia

HIV: Human immunodeficiency virus; HIVAN: HIV-associated nephropathy; AKI: Acute kidney injury; HCV: Hepatitis C virus.

These rates were generally between those reported in the Scientific Registry of Transplantation Recipients (SRTR) database for kidney transplant recipients ≥ 65 years and all kidney transplant recipients during a similar time frame. However, there were higher rates of acute rejection at one year (31%) and three years (41%)^[16]. In a study that included 40 HIV positive patients, Kumar *et al*^[6] reported one and two year patient survival rates of 85% and 82% respectively. Corresponding graft survival rates were 75% and 71% with a 22% acute rejection rate. HIV viral load remained undetectable and CD4 T-cell counts were > 400 cells/mm³. No opportunistic infections or progression to AIDS up to 2 years were observed in these patients^[6]. Patient and graft survival rates were similar to HIV negative patients in the study by Roland *et al*^[17] involving 18 HIV positive kidney transplant recipients with median follow up of 3.4 years. In a retrospective review of the UNOS database from 2004 to 2006, no differences in patient survival were observed between 100 HIV positive and 36492 HIV negative kidney transplant recipients (95.4% vs 96.2%, $P = 0.32$). However, death-censored graft survival was significantly lower in the HIV positive patients (87.9% vs 94.6%, $P = 0.03$). Donor age, cold ischemia time of at least 16 h and delayed graft function were associated with a greater than four-fold increase in allograft loss among the HIV positive patients^[18]. A recent study

reported 10 year outcomes of kidney transplantation in HIV positive patients from 2002 to 2012 using the SRTR database. When risk stratified by hepatitis C virus (HCV) infection status, monoinfected HIV positive recipients had similar five-year (75.0% vs 75.8%, $P = 0.58$) and 10-year (55.9% vs 56.0%, $P = 0.49$) graft survivals when compared to matched controls who were negative for both HIV and HCV. On the contrary, patients coinfecting with HIV and HCV had inferior five-year (52.0% vs 64.0%, $P = 0.02$) and 10-year (27.0% vs 36.2%, $P = 0.004$) graft survival rates when compared to HIV negative but HCV positive matched controls. Coinfection with HCV, panel reactive antibodies $> 80\%$, acute rejection episodes and cold ischemia time > 10 h were independent risk factors for graft loss. Patient survivals were higher in monoinfected HIV positive recipients at five-years (88.7%) and 10-years (63.5%). On the other hand, patient survivals were inferior among coinfecting HIV positive recipients (HV⁺/HCV⁺) at 5-year (66.3%) and 10-year (29.3%)^[19]. Mate kidney analyses using SRTR database from 2000 to 2013 showed similar long term outcomes of kidney transplantation in HIV positive patients relative to noninfected recipients. HIV and HCV coinfecting patients had inferior outcomes in this analysis^[20].

European transplant centers have similar experience to that in the United States. In a series of 27 HIV infected patients who received kidney transplant, two-year patient and graft survival rates were 98% and 96% respectively. Acute rejection rate was at 15% which is lower than what was reported in the United States. Most patients in this study received basiliximab induction followed by maintenance with tacrolimus, mycophenolate mofetil (MMF) and steroids^[21]. A more recent study from the United Kingdom included 33 HIV infected patients, 50% of whom received living donor kidneys and underwent induction with interleukin-2 (IL-2) receptor antibody and were maintained on triple immunosuppression. Three year patient and allograft survival rates were 91.3% and 87.4% respectively. Acute rejection rate was 44% and 2 patients developed BK nephropathy^[22].

LISTING CRITERIA FOR HIV POSITIVE PATIENTS

Data regarding the evaluation of HIV infected patients for kidney transplant is limited. It is believed that, compared to HIV negative patients, only a smaller percentage of HIV infected patients evaluated for kidney transplantation are actually placed on the list. Barriers to listing for transplant were discussed in a retrospective study of 309 HIV infected patients evaluated for renal transplantation in one United States center between 2000 and 2009. Only 20% were listed for transplant compared with 73% in HIV negative patients evaluated during the same period ($P < 0.00001$). The most common reason for not advancing the evaluation process was the lack of documentation of HIV control. CD4 T-cell count and viral load data were not

Table 2 Inclusion criteria for kidney transplant listing in human immunodeficiency virus positive patients

Meet standard criteria for placement on transplant waiting list for kidney transplantation plus the following
Well-controlled HIV disease with viral load < 50 copies/mL and CD4 count > 200 cells/mm ³
Absence of opportunistic infections or neoplasms
Stable antiretroviral regimen
Psycho-social clearance with demonstration of no active history of drug and/or alcohol use. Patients on stable methadone maintenance program can be considered

HIV: Human immunodeficiency virus.

available in 35% of patients and in 21%, CD4 T-cell count and viral load did not meet the eligibility criteria. Other factors associated with incomplete evaluation process were Black race and history of illicit drug use^[23].

The European experience was slightly different, and data from the EuroSIDA cohort study included 88 HIV infected ESRD patients. Inappropriate levels of CD4 T cell count and viral load were reported in 30% of cases and two-thirds of patients were excluded because of cardiovascular diseases or diabetes^[24]. Generally accepted criteria for listing HIV positive patients for kidney transplantation are shown in Table 2^[25,26]. An exception is usually given to certain treatable and preventable infections such as tuberculosis, esophageal candidiasis, and *Pneumocystis jiroveci* pneumonia.

SPECIAL CONSIDERATIONS AND CHALLENGES FOR KIDNEY TRANSPLANTATION IN HIV-INFECTED PATIENTS

Donor factors

In the past, most kidney transplants done for HIV infected patients were from deceased donors. However a report of 48 living donor transplants showed improved outcomes and less rejection rates^[16]. Therefore, it is possible to proceed with kidney transplantation from living donors; however, donors need to be informed with the challenges associated with transplanting HIV positive recipients.

Infections

It appears that the degree of immunosuppression from drug therapy and HIV itself does not necessarily lead to increased risk of infectious complications following transplantation in appropriately selected HIV positive candidates. Studies did not show increased incidence of opportunistic infections in HIV infected patients who underwent kidney transplantation^[17].

Rejection

Most studies reported higher rates of acute rejection

compared to HIV negative recipients. In a retrospective analysis of the SRTR database, 516 HIV infected kidney transplants performed between 2003 and 2011 were compared to uninfected counterparts within the same period. Rates of acute rejection within the first year were 15% compared to 8% in the control group^[27]. Although this did not affect short-term graft survival in these studies, it merits further studying as it may impact long term graft function. The two variables in clinical studies that were frequently associated with increased risk for acute rejection were deceased donor organs and the use of cyclosporine. One hypothesis was that perhaps the use of ART with potential interaction with calcineurin inhibitors (CNIs) may have resulted in subtherapeutic blood levels of CNIs. It is also possible that intense immunosuppression was deliberately avoided in these patients to prevent infectious complications as noted in the multicenter study reported by Stock *et al.*^[16]. HIV contains host HLA molecules which can increase the risk for allosensitization. HIV infected recipients may also have increased memory cell phenotype. However, a report by Canaud *et al.*^[28] may provide a better explanation of the high rates of rejection. In this study, authors performed protocol renal transplant biopsies on 19 recipients with HIV infection who had undetectable plasma level of HIV-1 RNA. It was found that HIV-1 infected the allograft in 68% of these patients. In 62% of instances, infection was located in the podocytes while remaining 38% of the infection was located in tubular cells. Podocyte infection was associated with faster deterioration of allograft function and nephrotic range proteinuria. It was suggested that perhaps this infection may stimulate the immune system *via* recruitment of inflammatory cells and cause cross reactivity with alloantigen and therefore be partially responsible for acute rejection^[28]. The authors also developed a non-invasive test for HIV infection of the allograft by performing quantitative PCR of HIV RNA and DNA in the urine. Results correlated well with biopsy findings.

Kidney infection with HIV

HIV-associated nephropathy is a well-described aggressive form of focal segmental glomerulosclerosis where the HIV directly infects the kidney cells. Specialized immunocytochemistry studies demonstrate the presence of the HIV core protein (p24) and the envelope glycoprotein (gp120) implicating infection of renal cells by HIV^[29]. Past studies using in situ hybridization and PCR have demonstrated that HIV-1 can directly infect renal epithelial cells which act as a reservoir for HIV^[29]. In the transplanted kidney, reinfection with HIV can occur early on after transplant and in the absence of HIV viremia. The mechanism is not well understood, however it is hypothesized that the virus is translocated from the recipient T-cells to the donor kidney cells. Unlike native kidney HIVAN, transplanted kidney did not demonstrate similar pathological appearance. Podocyte infection and tubular reinfection were the two

salient features of HIV infection of the allograft^[28].

IMMUNOSUPPRESSANT DRUGS

The early studies of kidney transplantation in HIV positive patients used no induction immunosuppression and maintenance therapy with cyclosporine and MMF. More than half of the patients developed acute rejection requiring treatment with anti-thymocyte globulin^[6]. As mentioned earlier, some immunosuppressive drugs including CNIs, MMF and rapamycin, have shown efficacy against HIV with reduced viral replication. There are no studies comparing tacrolimus vs cyclosporine in this setting. Retrospective analysis showed that cyclosporine was associated with a higher incidence of rejection. On the other hand, some centers prefer cyclosporine over tacrolimus due to the diabetogenic effect of tacrolimus which can be enhanced by protease inhibitors (PIs).

Immunosuppressive drugs may exert antiviral effects, either by reducing cellular targets for the virus, or *via* direct antiviral effects^[30]. For instance, cyclosporine can interfere with HIV gag processing. MMF interacts with nucleoside reverse transcriptase inhibitors (NRTIs) like abacavir, didanosine and tenofovir thus potentiating their anti-viral effects^[31-33]. It is also thought that sirolimus may be associated with downregulation of the CCR5 receptor which may decrease HIV infectivity^[34]. Sirolimus is less nephrotoxic than CNIs and is an effective anti-proliferative agent that could be beneficial against Kaposi's sarcoma^[35]. Glucocorticoids are inducers of CYP 450 system. They can also increase CD4⁺ T cell population, suppress HIV viral load and inhibit cytokine CCL2. As steroids are tapered following kidney transplantation, CD4 count may decrease and CNI level may go up. This may result in enhance CNI toxicity and possibility of infections. Close monitoring is therefore recommended^[36,37].

In terms of induction therapies, monoclonal anti-interleukin-2 receptor antibodies have been shown to enhance CD4 T-cell counts. No negative outcomes associated with their use have been reported. On the other hand, several issues were reported with the use of antilymphocyte polyclonal antibodies. Increased risk of infections and hospitalizations was reported with the use of Thymoglobulin in 11 HIV infected patients when it was used to treat rejection^[38]. In the multicenter United States study that included 150 patients, administration of Thymoglobulin as induction therapy was associated with twice as many serious infections per follow up year compared to patients who did not receive this therapy^[16].

Until further evidence becomes available, we recommend induction therapy using anti-IL-2 receptor monoclonal antibodies such as basiliximab. We recommend using tacrolimus plus MMF with or without steroids depending on immune risk. The use of Thymoglobulin is not contraindicated but it should be used with caution due to severe depletion of lymphocytes and the potential for severe thrombocytopenia.

USE OF ART FOLLOWING TRANSPLANTATION

There are six classes of ART drugs currently available in the United States. These include nucleoside and non-nucleoside reverse transcriptase inhibitors (NNRTI), PIs, integrase strand-transfer inhibitors, CCR5 antagonists such as maraviroc and fusion inhibitors^[39]. There is no consensus on the ideal ART regimen for kidney transplant recipients. It is generally recommended that patients continue the same ART regimen prescribed pre-transplant. Goal is the maintenance of HIV suppression while minimizing interaction with immunosuppressive drugs and their side effects. Multiple drug interactions exist between ART and immunosuppressive drugs. This is discussed in length below. Integrase strand transfer inhibitors such as raltegravir and dolutegravir have no interaction with immunosuppressive drugs at the CYP 450 level. It is recommended that they be used in combination with abacavir and lamivudine/emtricitabine. Renal dosing of medications is recommended as most kidney transplant recipients will have some degree of CKD. PIs and NNRTI are metabolized through liver and therefore do not require any dose adjustments. Raltegravir does not require renal dose adjustment either. ART that usually require renal dosing include nucleosides and nucleotides. Tenofovir can cause renal toxicity and should be avoided or used with caution in patients with kidney transplant. CCR5 chemokine receptor is used by R5 tropic virus for cell entry. Maraviroc blocks this receptor and can also impair lymphocyte chemotaxis with a theoretical reduction in organ transplant rejection^[40]. Collaboration between infectious disease and transplant professionals with HIV viral load monitoring is essential in these cases^[14].

IMMUNOSUPPRESSION AND ART: DRUG-DRUG INTERACTIONS

Complex pharmacokinetic interactions between therapies used for immunosuppression and antiretroviral drugs can happen. MMF inhibits inosine monophosphate dehydrogenase which blocks purine synthesis. It is metabolized mainly by glucuronidation in the liver. Atazanavir, an inhibitor of UDP-glucuronosyl transferase may lead to increased mycophenolic acid (MPA) levels^[14]. Ritonavir on the other hand, may reduce MPA levels by inducing glucuronidation. Drugs that affect cytochrome P-450 may also influence the levels of CNIs and sirolimus. For example, PIs inhibit CYP 450 and p-glycoprotein efflux system resulting in increased serum levels of CNIs. Patients on PIs may require only small doses of CNI given less frequently. Special attention should be given when stopping PIs in these patients as this may result in acute rejection^[41-43]. On the other hand drugs in NNRTI group can reduce CNI serum levels due CYP 450 induction. Stopping NNRTIs may result in CNI toxicity^[44]. Maraviroc, is a P-450 3A4 substrate, but does not inhibit or induce the enzyme and hence, it is not expected to interact with CNIs. Integrase

Table 3 Key points

Kidney transplantation in patients with HIV infection is a viable therapeutic option
 Ideal immunosuppressive regimen remains uncertain
 Higher rates of rejection are reported in clinical trials
 Immunosuppressive therapy does not seem to negatively impact the course of HIV infection
 Some immunosuppressive drugs may exert antiretroviral actions
 Special attention should be paid to the potential interaction between ART and immunosuppressive drugs
 A close collaboration between infectious disease specialists and transplant professionals is mandatory in order to optimize transplantation outcomes in these patients
 Transplantation from HIV+ donors to HIV+ is currently being researched

HIV: Human immunodeficiency virus.

strand transfer inhibitors such as raltegravir, has excellent anti-retroviral effects without affecting CYP system and hence no significant interaction with CNIs. This was studied by Tricot *et al.*^[45] in 5 patients who did not suffer any acute rejection. However lower barrier to resistance in this group of drugs may increase chances for virologic failure.

In addition to ART and immunosuppressive drug-drug interactions, several antibiotics and antifungal drugs used for treatment and prevention of infections in HIV patients can inhibit cytochrome P450 system and hence affect the CNI levels.

The complexity of drug-drug interactions highlights the importance of team approach that includes transplant nephrology, infectious disease and specialized pharmacy.

PATIENTS COINFECTED WITH HIV AND HCV

As mentioned, outcomes were inferior with kidney transplantation in patients coinfecting with HIV and HCV when compared to HIV monoinfected transplant recipients^[19,20]. Factors contributing to this may include HCV infection related increased risk for the development of post-transplant diabetes mellitus, liver damage, cardiovascular disease and infections. Coinfected patients may represent a social and biological high risk group. For instance, these patients generally are younger with lower income, have longer HIV disease duration and dialysis vintage prior to transplantation with greater likelihood of drug addiction history^[20]. This raises the question whether HCV coinfection should be a relative contraindication for kidney transplantation in HIV positive patients. However over the last couple of years, there have been significant advances in the treatment of HCV infection with the introduction of directly acting antiviral agents (DAA) into the clinical arena^[46]. These agents can achieve a sustained virologic response in the range of 90%-95% with minimal side effects. Moreover, unlike interferon based therapy, DAA are safe to use after organ transplantation. These therapeutic advances are likely

to improve long-term outcomes in HCV infected organ transplant recipients.

HIV TO HIV TRANSPLANTATION

A study from South Africa by Muller *et al.*^[47,48] reported the outcomes in 27 HIV positive patients who received deceased donor kidneys from HIV positive donors. All donors had normal kidney function and all kidneys were biopsied. At one, three and five years after transplant, patient survival rates were 84%, 84% and 74% respectively with corresponding death-censored graft survival rates of 93%, 84% and 84%. HIV viral loads remained suppressed without evidence for opportunistic infections during the follow-up in all patients. Three patients developed HIVAN in the transplanted kidneys on protocol biopsies despite the lack of HIV viremia^[47,48]. Whether the South African experience can be applied to the United States is not fully clear. In addition to the ethical dilemmas, concerns include possibility of superinfection with more virulent strains and development of drug resistance^[49,50]. Viral tropism is another concern with theoretical risk for super infection with a more aggressive strain such as X4 tropic virus compared to R5 tropic virus. Tropism studies are available but may take up to a week to complete making it less useful for decision making during the narrow time window available to make transplant decisions^[40]. Quality of donor organs and the risk for recurrence of HIVAN are also potential issues in HIV to HIV transplantation.

On November 21, 2013, President Obama signed the HIV Organ Policy Equity (HOPE) Act into law. This law reversed the federal ban on considering HIV positive donors and authorized clinical research in the area of transplantation from HIV positive organ donors^[51]. As a result, a work group from the OPTN was charged with the development of policies that permit safe recovery of such organs. OPTN granted permission to Johns Hopkins University Hospital, Baltimore, MD to perform organ transplantation between HIV positive donors and recipients as of February 9, 2016. The transplant team at this center now has performed the first HIV⁺ to HIV⁺ kidney transplant in the United States and the first HIV⁺ to HIV⁺ liver transplant in the world. Experts estimate that using HIV infected donors will make available an additional 500 solid organ donors a year^[52,53]. Moreover, this may reduce the discard of organs due to false positive results from nucleic acid testing currently being used which has false positive rates between 0.1% and 0.85%.

CONCLUSION

Key point regarding kidney transplantation in HIV infected patients are summarized in Table 3. Evidence thus far supports the viability of kidney transplantation in appropriately selected HIV positive patients with acceptable outcomes. Ideal immunosuppressive regimen is not yet defined in this population. Special attention should be paid

to potential drug interactions between some of the ART medications and immunosuppressive drugs. Studies have shown increased incidence of acute rejection episodes and achieving therapeutic CNI levels can be challenging especially if the patient is on ART regimens which include PIs and NNRTIs. ART regimens containing integrase strand transfer inhibitors such as raltegravir may be preferred due to minimal drug interactions. Patients coinfecting with HIV and HCV have inferior outcomes with kidney transplantation. However, outcomes are likely to improve in these patients in the coming years corresponding with the availability and use of DAA to treat HCV infection. The option for HIV positive donor to HIV positive recipient organ transplantation is actively researched in the United States and could further expand donor pool for HIV infected patients.

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Reclassification of membranoproliferative glomerulonephritis: Identification of a new GN: C3GN

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Abstract

This review revises the reclassification of the membranoproliferative glomerulonephritis (MPGN) after the consensus conference that by 2015 reclassified all the

glomerulonephritis basing on etiology and pathogenesis, instead of the histomorphological aspects. After reclassification, two types of MPGN are to date recognized: The immunocomplexes mediated MPGN and the complement mediated MPGN. The latter type is more extensively described in the review either because several of these entities are completely new or because the improved knowledge of the complement cascade allowed for new diagnostic and therapeutic approaches. Overall the complement mediated MPGN are related to acquired or genetic cause. The presence of circulating auto antibodies is the principal acquired cause. Genetic wide association studies and family studies allowed to recognize genetic mutations of different types as causes of the complement dysregulation. The complement cascade is a complex phenomenon and activating factors and regulating factors should be distinguished. Genetic mutations causing abnormalities either in activating or in regulating factors have been described. The diagnosis of the complement mediated MPGN requires a complete study of all these different complement factors. As a consequence, new therapeutic approaches are becoming available. Indeed, in addition to a nonspecific treatment and to the immunosuppression that has the aim to block the auto antibodies production, the specific inhibition of complement activation is relatively new and may act either blocking the C5 convertase or the C3 convertase. The drugs acting on C3 convertase are still in different phases of clinical development and might represent drugs for the future. Overall the authors consider that one of the principal problems in finding new types of drugs are both the rarity of the disease and the consequent poor interest in the marketing and the lack of large international cooperative studies.

Key words: Glomerulonephritis reclassification; Dense deposit disease; Membranoproliferative glomerulonephritis; C3 glomerulopathies; Targeting complement pathways; Complement dysregulation

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Core tip: The complement pathway dysregulation has been recognized as the main cause of some membranoproliferative glomerulonephritis (MPGNs). This fact is at the basis of the new classification of the disease and of the findings of new entities as the complement factor H related protein nephropathy. Genetic studies as well as improvement in proteomics allowed recognizing the complement dysregulation as the cause of some renal diseases as the MPGN and the atypical hemolytic uremic syndrome that may be considered as strictly related diseases. The anti-complement drugs represent a new approach in the treatment of these diseases and their use in larger evidence based randomized trials is required.

Salvadori M, Rosso G. Reclassification of membranoproliferative glomerulonephritis: Identification of a new GN: C3GN. *World J Nephrol* 2016; 5(4): 308-320 Available from: URL: <http://www.wjgnet.com/2220-6124/full/v5/i4/308.htm> DOI: <http://dx.doi.org/10.5527/wjn.v5.i4.308>

INTRODUCTION

By 2015, nephrologists and renal pathologists held a consensus meeting to formulate a new etiology/pathogenesis-based system to classify glomerulonephritis (GN)^[1]. According to the consensus report, GNs have been classified into five etiology/pathogenesis-based categories (Table 1).

According to the new classification, membranoproliferative GNs (MPGN) have been reclassified and divided into different chapters on the basis of pathophysiology. In addition, new entities have been found. This review will discuss the new classification of MPGNs and will principally describe the complement-dysregulation dependent C3 glomerulopathies (C3G).

MPGN

Until recently, the MPGNs have been distinguished according the histological and ultra structural findings and were classified as MPGN type I, type II and type III. The glomerular lesions include mesangial hypercellularity, endocapillary proliferation and duplication of glomerular basement membrane (GBM) lesions^[2]. Sub-endothelial and mesangial deposits are predominant in MPGN type I^[3]. Highly osmiophilic electron-dense intramembranous deposits characterize type II GN^[4], which is also known as dense deposits disease (DDD). In type III MPGN deposits may be found in the sub-endothelial and sub-epithelial spaces^[5].

With the discovery of the complement role in generating glomerular diseases^[6], a new classification of MPGN was developed, based on pathophysiology and considering whether immunoglobulins accompany the complement using immunofluorescence on biopsy specimens^[7,8] (Figure 1).

This new classification resulted in three principal consequences: (1) to identify new entities, which until now were unknown or misdiagnosed; (2) to highlight new diagnostic approaches. Indeed in the case of Ig-mediated MPGN, a work-up for infections, autoimmune diseases and monoclonal gammopathies should be adopted. In the case of complement-mediated GN, a complete study of the complement alternative pathway (AP) should be performed; and (3) to differentiate the therapeutic approach according to the type of MPGN. In summary, the three different forms of MPGN are now recognized as follows: (1) Immunocomplexes-associated MPGN with complement over activation (old MPGN type I); (2) MPGN with intramembranous dense deposits (old MPGN type II); and (3) C3GN, a new entity complement-mediated GN. DDD and C3GN are both related to complement dysregulation and are “*de facto*” included in the same chapter.

IMMUNOCOMPLEXES ASSOCIATED MPGN

Immune-complexes mediated MPGN is caused by the deposition of immunocomplexes in the glomeruli. The immunocomplexes activate the classical pathway (CP) of complement and cause the deposition of complement factors or of the membrane attack complex (MAC) in the mesangium and capillary loops^[9].

The MPGN is an uncommon cause of nephropathy (approximately 5 per million persons per year) and is more often secondary to infections, autoimmune disease and monoclonal gammopathy^[10].

Pathophysiology

MPGN and infections: Hepatitis C and B, which are often accompanied by circulating cryoglobulins, are a frequent cause of MPGN^[11-14]. In addition, chronic bacterial infections, fungal and parasitic infections may also cause MPGN^[15-17].

Immunocomplexes depositions are the first step. Consequently, CP is activated and in addition to the direct damage cause by MAC, C3a and C5a are generated that favor leukocyte accumulation, cytokine release and a further glomerular damage.

MPGN and autoimmune diseases: Mixed cryoglobulinemia is frequently associated with hepatitis C infection, systemic lupus erythematosus, scleroderma, Sjögren syndrome and rheumatoid arthritis. These are the autoimmune diseases that more frequently cause MPGN due to the persistence of circulating immunocomplexes^[18-21]. Under these conditions, circulating immunocomplexes may also activate the complement CP with the abovementioned subsequent events described for MPGN due to infections.

MPGN and monoclonal gammopathy: The renal deposition of monoclonal immunoglobulins (MIg) may determine a wide spectrum of renal lesions as recently

Table 1 Classification of glomerulonephritis

Pathogenetic type	Specific disease entity	Pattern of injury: Focal or diffuse	Score or class
Immune-complex GN	IgA nephropathy, IgA vasculitis, lupus nephritis, infection-related GN, fibrillary GN with polyclonal Ig deposits	Mesangial, endocapillary, exudative, membranoproliferative, necrotizing, crescentic, sclerosing or multiple	Oxford/MEST scores for IgA nephropathy ISN/RPS class for lupus nephritis
Pauci-immune GN	MPO-ANCA GN, proteinase 3-ANCA GN, ANCA-negative GN	Necrotizing, crescentic, sclerosing, or multiple	Focal, crescentic, mixed, or sclerosing class (Berdens/EUVAS class)
Anti-GBM GN Monoclonal Ig GN	Anti-GBM GN Monoclonal Ig deposition disease, proliferative GN with monoclonal Ig deposits, immunotactoid glomerulopathy, fibrillary GN with monoclonal Ig deposits	Necrotizing, crescentic, sclerosing, or mixed Mesangial, endocapillary, exudative, membranoproliferative, necrotizing, crescentic, sclerosing or multiple	
C3 glomerulopathy	C3 GN, dense deposit disease	Mesangial, endocapillary, exudative, membranoproliferative, necrotizing, crescentic, sclerosing or multiple	

GN: Glomerulonephritis; MEST: Mesangial hypercellularity, endocapillary hypercellularity, segmental sclerosis, interstitial fibrosis/tubular atrophy; ISN/RPS: International Society of Nephrology/Renal Pathology Society; MPO: Myeloperoxidase antibodies; ANCA: Antineutrophil cytoplasmic antibodies; EUVAS: European vasculitis study group; GBM: Glomerular basement membrane.

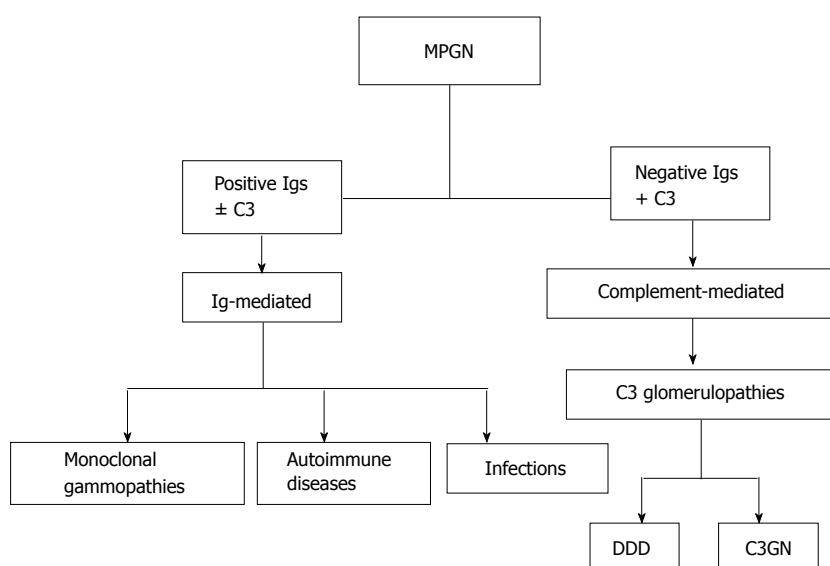


Figure 1 Proposed classification for membranoproliferative glomerulonephritis based on the presence or absence of Igs and the presence of C3 by immunofluorescence. MPGN: Membranoproliferative glomerulonephritis; Igs: Immunoglobulins; DDD: Dense deposit disease.

described^[22]. Monoclonal gammopathy as well as light chain and heavy chain diseases may result in MPGN^[23]. These lesions may hide a variety of severe hematological diseases ranging from low-grade B cell lymphoma, chronic lymphocyte leukemia to multiple myeloma^[9]. In a recent monocenter study of MIg-associated MPGN after excluding infections and autoimmune disease, 26 out of 28 patients were serum electrophoresis-positive and 27 out of 28 patients were urine electrophoresis-positive^[24]. In this monocentric study, out of 126 patients affected by MPGN, 41% were urine- or serum-positive for monoclonal gammopathy.

Monoclonal gammopathies are associated with complement activation; indeed, the abnormal immunoglobulin might activate the AP^[10].

Recently, cases of C3 glomerulopathies, including C3GN and DDD (see below) associated with MIgs have been described^[25]. In these patients the monoclonal

immunoglobulin causes a complement dysregulation by interfering with the function of complement-regulating proteins, such as factor H or acting as an autoantibody against factor H or factor B^[26-28].

Clinical and therapy

Immunocomplex-mediated MPGNs principally affect children and young adults. Its clinical presentation may range from nephrotic syndrome and acute nephritic syndrome, to asymptomatic proteinuria and hematuria. Renal dysfunction frequently occurs, and 40% of the patients progress to end stage renal disease (ESRD) in approximately 10 years.

The efficacy of the different therapeutic approach is difficult to evaluate due to the small number of patients and because several trials include the three different types of MPGN^[29,30]. The therapy most widely used is based on anti-cell proliferation agents^[31]. Over-

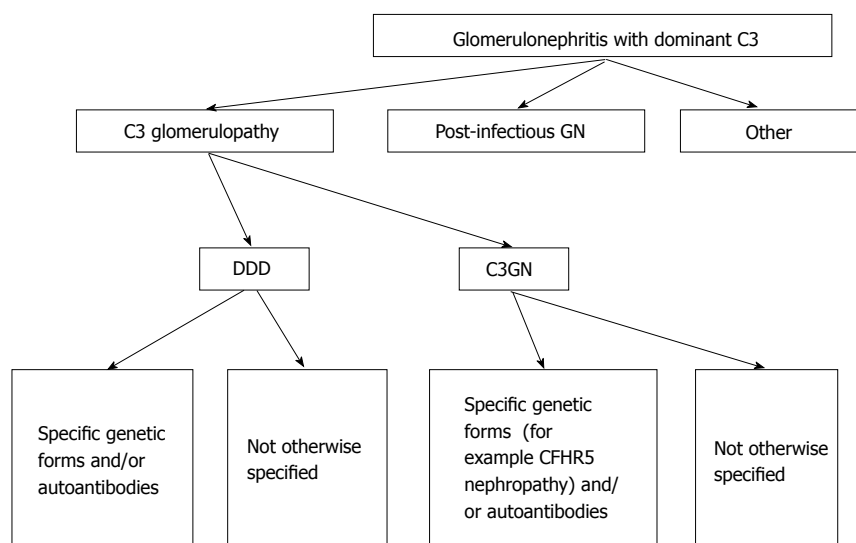


Figure 2 Approach to the classification of glomerulonephritis with dominant C3. DDD: Dense deposit disease; CFHR5: Complement factor H related protein.

activation of complement is often present, but whether anticomplement drugs might be useful in this context remains to be elucidated.

According to different studies, renal transplantation is a viable option in patients with ESRD, even if the disease recurs after transplantation with a frequency ranging from 27% to 65%^[32-34]. In a recent study, after the exclusion of patients with DDD, a recurrence rate of 41% has been reported^[34]. Such a high recurrence rate has been confirmed by a study published in 2016, which evaluated the recurrence rate using the new classification^[35]. In another study the recurrence of Ig-mediated MPGN was lower (23.5%) and after a follow-up of 15 years, the graft survival rate of MPGN patients was similar to those of controls affected by different diseases^[36].

COMPLEMENT MEDIATED MPGN

MPGN patients that have on renal biopsy clear glomerular C3 staining with few or no immunoglobulin deposition are referred to as complement-mediated MPGN and are defined as C3G. C3Gs are less common than immune-complex-mediated MPGNs and are further divided into two groups according to the presence or absence of highly electron-dense deposits into the GBM.

The disease with intramembranous deposits corresponds to the DDD (previously called MPGN type II). The disease without dense deposits, with C3 prevalence and no Igs on the glomeruli and with MPGN aspect on normal histology, is referred to a recently recognized entity: the C3GN. The distinction between the two diseases often requires the use of electron microscopy. C3GN was initially described by Servais *et al.*^[37] who described a series of 19 patients and proposed the term C3GN to highlight a disease that is characterized by C3 prevalence on the glomeruli, without intramembranous deposits. In addition, Servais *et al.*^[37] observed that this new entity often shares common genetic risk factors with atypical hemolytic uremic syndrome (aHUS).

Overall the term C3G was introduced to define all MPGNs that are characterized by the prevalence of C3 in the glomeruli^[38], including DDD.

The term C3G has also been introduced because C3 isolated accumulation was recognized to include several heterogeneous entities and due to our improvement in the understanding of complement-mediated kidney injuries. Consequently, several complement factor abnormalities resulting in glomerular lesions have been identified. In 2013, a first consensus meeting on C3G was held to better clarify the pathogenic aspects and terminology^[39]. The consensus conference resulted in an improved classification (Figure 2) that also documented that need of future work.

C3G are all caused by dysregulation of the complement AP and of the terminal complement complex (TCC)^[40] (Figure 3).

Clinical features

DDD has an estimated prevalence of 2 to 3 per million populations^[41] and prevails in childhood and in young adults^[42]. C3GN prevalence is difficult to be evaluated, as this disease is new and as time progresses, more patients are identified with family studies and with an improvement in the Genetic wide association studies.

Overall, patients affected by DDD are younger with respect to patients affected by C3GN^[43]. Both diseases affect males and females with the same frequency^[44-46]. Renal manifestations are similar in DDD and C3GN^[43] and include hypertension, hematuria and proteinuria more often in the nephrotic range. Non renal manifestations of DDD include ocular lipoproteinaceous deposition and acquired lipodystrophy^[47,48].

MIg in the serum may also be associated with both DDD and C3GN^[25,49-51]. These patients often have a poor renal prognosis.

Progression to ESRD is common in both DDD and C3GN. Renal transplantation is feasible but with a high rate of disease recurrence^[52].

Complement factor H related protein (CFHR5)

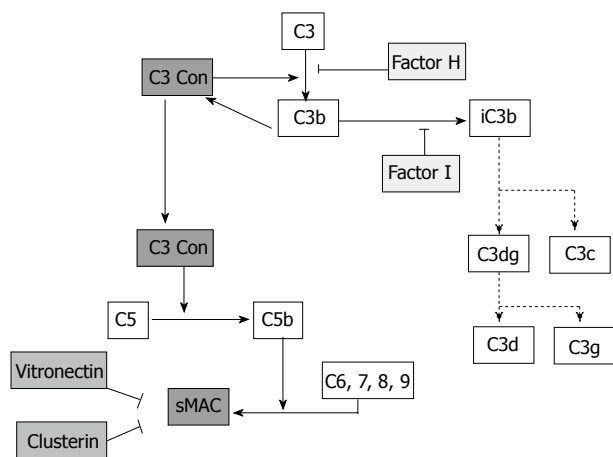


Figure 3 Pathway of complement and complement regulator factors. C3 Con: C3 convertase; sMAC: Serum membrane attack complex; C3dg, C3c, C3d, C3g: Complement degradation products.

nephropathy is a subtype that is well identified in C3GN caused by the presence of an abnormal CFHR5 protein. The disease is inherited and was first identified in Cypriot families^[53]. The disease may often occur with clinical manifestations that are similar to IgA nephropathy with microscopic hematuria or macroscopic hematuria after an acute upper respiratory tract disease^[54]. Progression to ESRD is common. Interestingly, ten patients affected by CFHR5 nephropathy received a successful renal transplantation^[54].

Pathophysiology

Dysregulation of the complement AP may occur principally due to acquired or genetic abnormalities^[9] (Figure 4).

The auto-antibodies are the most frequently acquired abnormality. Auto-antibodies may be directed against the complement-regulating factors, such as factor H, factor I, factor B as well as against C3 convertase itself^[55,56].

The first described autoantibody was the C3 nephritic factor (C3 NeF), which binds and stabilizes C3 convertase^[57]. A second type of C3 NeF properdin dependent has also been described^[58]. C3 NeFs are principally present in patients affected by DDD but are less frequently found in C3GN and absent in CFHR5 nephropathy^[43].

In DDD, auto-antibodies that bind factor B and target C3b have been described in patients affected by MPGN type II^[56,59]. Anti CFH auto-antibodies have also been found in patients affected by DDD and C3GN^[60,61].

Anti CFH auto antibodies are also frequently present in aHUS. A recent study^[62] highlights that anti-factor H antibodies are equally present in C3G and aHUS, but that the auto-antibody structure is different in the two diseases. Indeed, in C3G, the auto-antibody principally binds to the amino terminal domains, while in aHUS, it binds to the carboxyterminal domain^[10]. As previously mentioned, the two diseases are strictly related, but several differences are present.

The discovery of familial cases of C3G highlights that

in several cases, a familial genetic basis of the disease occurs.

In 2010, Martínez-Barricarte *et al.*^[63] described a family in which some members were affected by a mutant form of C3 resistant to cleavage by C3 convertase. Consequently, this caused an AP dysregulation restricted to the fluid phase and these patients continuously produced and consumed C3 produced by the normal C3 allele. These patients were affected by the classic DDD. Complement factor H-related (CFHR) genes are often involved. There are five CFH-related proteins (CFHR1-5 and genetic abnormalities of these proteins have been recognized and may cause disease. Recently, Chen *et al.*^[64] described two patients from the same family affected by DDD and with an abnormal deletion in the complement factor H-related (CFHR) gene cluster. This resulted in a hybrid CFHR protein that inhibited the complement decay-factor H-mediated.

Another genetic cause of C3G has been reported by Gale^[53]. Gale *et al.*^[53] described two families of Cypriot origin whose members were affected by a mutation in CFHR protein 5. These patients were affected by a C3G that was defined as CFHR5 nephropathy. Indeed, genome-wide linked analysis (GWLA) allowed localization of a genetic abnormality in chromosome 1q31-32. In these patients, a larger CFHR5 protein is generated that is less effective in associating with surface-bound C3b. The resulting disease was known as CFHR5 nephropathy.

Recently, Malik *et al.*^[65] described an autosomal dominant complement-mediated C3G associated with abnormal copies in the CFHR3 and CFHR1 loci.

Finally, Habbig *et al.*^[66] described two siblings affected by renal disease. Both children had a homozygous deletion of 224 lysine of CFH. This deletion led to a defective complement control^[67]. The renal disease was compatible with C3G. The authors proposed the name of C3 deposition glomerulopathy (C3DG) due to the absence of DDD.

Overall, these families highlight the genetic origin of several C3Gs related to a dysregulation of the AP and TCC.

Summarizing, the disease mechanisms in C3G caused by genetic defects identified in family studies may be classified into three categories: (1) homozygous deficiency dysfunction of CFH resulting in excessive C3 activation; (2) hyperfunctional C3 producing excessive C3 activation despite normal CFH activity; and (3) abnormal CFHR protein that enhances CFH dysregulation and consequent excessive C3 activation.

Diagnosis

The diagnosis of C3G and differential diagnosis between DDD and C3GN should include a comprehensive pathological analysis and a complete work-up on the genetic and biochemical aspects of complement pathways, with particular regard to the AP.

Using light microscopy, in the case of C3 prevailing without Ig on glomeruli, only a suspicious diagnosis of

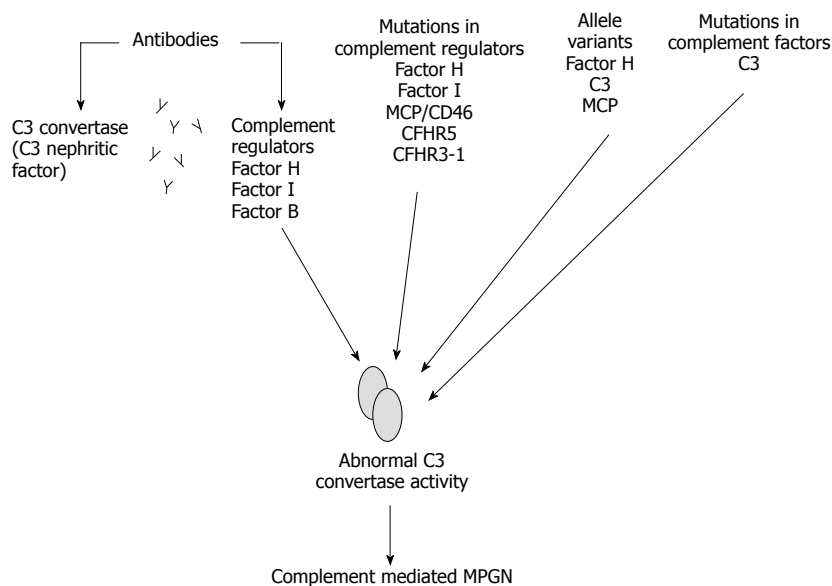


Figure 4 Acquired and genetic abnormalities associated with complement-mediated membranoproliferative glomerulonephritis. MCP: Membrane cofactor protein; CHFR: Complement factor H related proteins; MPGN: Membranoproliferative glomerulonephritis.

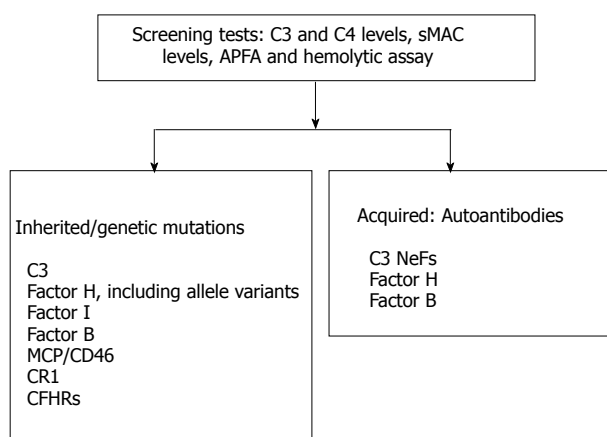


Figure 5 Proposed work-up of complement mediated membranoproliferative glomerulonephritis. APFA: Alternative pathway functional assay; CFHR: Complement factor H related proteins; CR1: Complement receptor 1; MCP: Membrane cofactor protein; sMAC: Serum membrane attack complex; MPGN: Membranoproliferative glomerulonephritis.

C3G may be formulated. The definitive diagnosis might only rely on ultra-structural basis.

Overall DDD, is characterized by dense osmiophilic band-like deposits within the GBM. C3GN may be characterized by sub endothelial and mesangial deposits, though intramembranous and sub epithelial deposits may also be present^[68]. Several patients may present an overlap in the ultra-structural findings and are difficult to be classified. Proteomic studies may be useful for their identification^[50,69].

The evaluation of the complement AP is essential for an improved diagnosis. The evaluation may be performed in several ways: (1) evaluating the total hemolytic complement assay^[70]; (2) evaluating the complement alternative pathway assay^[71]; and (3) evaluating the complement factor H functional assay^[72].

In addition, the C3, C4 and serum MAC (sMAC) levels should be determined. In the case of positivity of these

tests, genetic and enzyme-linked immunosorbent assays for complement abnormalities should be performed^[8] (Figure 5).

Mutations in the *CFH*, *CFI* and *CD46* genes have been reported in some patients affected by DDD^[39,43]. Changes in factor *B* and *C3* genes may also be present^[56,63]. In *CFHR5* nephropathy, an internal duplication in the *CFHR5* gene is present^[53]. Other rearrangements of the *CFHR2-CFHR5* hybrid gene and other abnormalities in *CFHR1* and *CFHR5* have been reported^[73-75].

An interpretation of identified variants may be difficult to be understood for several reasons^[76]. The pathogenic variants accounts for only 25% of patients affected by DDD and C3GN^[43,46]. In addition, mutations in other genes, such as thrombomodulin (*THBD*), diacylglycerol kinase-epsilon (*DGKE*), and the *CFHR* gene family have been recently found to be implicated to contribute to these diseases^[77,78].

Moreover, further studies did not confirm a pathogenic role for several missense variants that were originally thought to be at the basis of the disease. Consequently, several amino acid changes in the gene structure are not “*de facto*” related to the disease^[79].

Finally, most variants have a low penetrance and combined variants have been reported in 3% to 12% of patients^[80].

The non-genetic causes of C3G are principally auto-antibodies: (1) C3 NeF: It binds directly to C3 convertase prolonging its survival. C3NeFs are found in 80% of patients affected by DDD and in 50% of patients affected by C3GN^[43,59]. A C3NeF can stabilize C5 convertase in addition to C3 convertase, which has been previously described^[81]. The detection of C3 NeF may be performed in several ways^[82]. Further studies are needed to better correlate the presence of C3 NeF with the cause of the diseases and with treatment efficacy; (2) C4 NeF: The role of C4 NeF is still unclear, even if this auto-antibody has been found in some patients affected by MPGN^[83]; (3) Anti-factor H auto-antibodies: Have been described

Table 2 Complement testing in patients with C3 glomerulopathy

Test	Interpretation	Limitations
C3 and C4 levels	C3 frequently depressed and support diagnosis; Normal C4 suggests an alternative pathway process	Non-specific
Soluble C5b-9	May be indicator of active disease; May identify patients who will benefit from C5 blockade	Test not widely available
C3 nephritic factor	Associated with C3 glomerulopathy; May identify patients who will benefit from B cell targeted therapies	Levels do not correlate with disease activity; also seen in MPGN type I
Factor H protein levels	May identify underlying mechanism of alternative pathway activity; May identify patients who will benefit from plasma infusion/exchange	Test not widely available
Autoantibodies to factor H and factor B	May identify underlying mechanism of alternative pathway activity; May identify patients who will benefit from B cell targeted therapies	Test not widely available
Genetic mutation screening	May identify underlying mechanism of alternative pathway activity	Not widely available; Clinical implications unknown
Factor H		
CFHR1, 2, and 5		
Factor I		
C3		
Factor B		

MPGN: Membranoproliferative glomerulonephritis; CFHR: Complement factor H related proteins.

Table 3 Possible treatment of C3 glomerulopathies

Nonspecific treatment
Replace deficient gene products
Plasma infusion
Liver Transplantation
Eliminate autoantibodies and/or mutant proteins
Plasma exchange
Immunosuppression
Treatment of plasma cell dyscrasia
Inhibition of complement activation
Eculizumab (anti C5)
Inhibition of the C3 Convertase
Renal transplantation
New trials ongoing

in patients affected by DDD^[41] and C3GN^[46]. They may be detected using an enzyme-linked immunosorbent assay. If an anti-factor H is found, then monoclonal gammopathy should be excluded principally in older people^[28,50]; and (4) Anti-factor B auto-antibodies are not frequently found and their research by enzyme-like immunosorbent assay is not easy and is often not available^[56]. Overall, the suggested complement investigations in C3G are indicated in Table 2 as suggested by the previously cited consensus report^[39].

Treatment

Several treatments for C3Gs may be attempted. According to evidence-based medicine, to date, most of the treatments have not yet been proven to be effective in C3G (Table 3).

Non-specific or supportive measures: By extrapolating from the treatment of other chronic renal diseases, blood pressure control, reduction of proteinuria and the lowering serum lipid levels should have a beneficial effect in patients affected by C3G, and principally in those affected by a low disease progression^[46].

In the previously mentioned French study^[43], the renin-angiotensin-aldosterone system (RAAS) blockade was associated with prolonged renal survival, but these findings have not been confirmed by a United States study^[49]. In the latter study, the RAAS blockade had beneficial effects only when associated with steroids. In another study, Maisch *et al.*^[84] documented the efficacy of a lipid-lowering strategy by statins.

Replacement of deficient gene products: Due to the unavailability of purified complement regulating factors, often a functioning factor may be administered by plasma infusion. The limitation is the need of lifelong substitution therapy.

Plasma infusion is not beneficial in patients affected by a mutation in the membrane cofactor protein because the factor is membrane-bound and not circulating^[85]. Plasma infusion is similarly ineffective or even contraindicated in patients affected by gain-of-function mutations or in patients affected by a C3 convertase resistant to factor H^[63]. Because CFH, CFI, CFB and C3 are produced by the liver, a simultaneous liver-kidney transplantation may be effective and therapeutically useful^[86].

In consideration of frequent short-term complications, of the mortality rate of 15% and of the growing experience with eculizumab, an anti-complement drug, a combined liver-kidney transplantation will lose indication^[87,88].

Elimination of the auto-antibodies and/or mutant protein: The use of plasma exchange has a strong rationale^[89], but to date, its efficacy has only been confirmed by single case reports. Three patients with DDD had a beneficial effect from plasma exchange, but they were also treated with immunosuppression^[90-92]. However, McCaughan *et al.*^[93] reported the lack of efficacy of plasma exchange, despite the complete removal of C3NeF. Moreover, in the eculizumab era, the plasma exchange will continue to be used after evaluation of individual patients.

Efficacy of immunosuppression is not yet established.

Treatment with steroids led to a clinical improvement in children affected by C3G treated on the basis of a renal biopsy, revealing signs of acute glomerular inflammation with crescents, but a similar improvement was similarly observed in non-treated patients^[94]. The combination of steroids with other immunosuppressants has been reported to have a higher beneficial effect^[95-97]. These effects have been principally documented in the aHUS. Treatment with an anti-CD20 monoclonal antibody has been effective in one patient affected by DDD with documented anti-CFB auto-antibodies^[59].

Very recently, the beneficial effect of mycophenolate mofetil (MMF) in C3G has been reported in a randomized Spanish study^[98]. However, due to the lack of controlled trials, treatment with immunosuppressants should be restricted to patients with proteinuria, progressive loss of glomerular filtration rate (GFR) and those with signs of severe inflammation on renal biopsy^[89].

An immunosuppressant-based strategy should also be attempted in patients with C3G associated with monoclonal gammopathy, even if the result of such a treatment differed according to different authors^[50,51].

Inhibition of complement activation: The most adequate approach to the treatment should be the complement cascade blockade. Eculizumab is a recombinant, fully humanized monoclonal antibody that binds to the C5 complement protein and blocks C5 cleavage^[89]. In recent years, eculizumab was highly effective in several kidney diseases, including aHUS and antibody-mediated rejection (ABMR) after renal transplantation^[99]. The efficacy of eculizumab in C3Gs to date is only based on the report of single patients, on an open label proof of concept study in 6 patients, and on one ongoing randomized clinical trial (RCT) whose results are unknown to date^[100]. Overall, 14 patients affected either by DDD or C3GN treated with eculizumab have been reported. Eight of these patients were described in single case reports and the treatment was successful in seven patients^[93,101-107]. In addition to the clinical response, an improvement in renal histology has been observed in patients who underwent a repeated renal biopsy. However, such good results were not confirmed by the proof-of-concept study^[108,109]. In this study, a clinical response to eculizumab has been observed in only three patients.

Furthermore, in a recent study, three more patients affected by rapidly progressive C3G have been reported^[110]. All these patients responded to eculizumab with an improvement in renal function, a regression of proteinuria and an improvement of glomerular lesions. The phenotypic expression of C3G (DDD vs C3GN) does not predict the response to treatment, even if in biomarkers studies, a higher terminal pathway activity in C3GN has been found^[111].

Overall, these results revealed disparate results to the treatment and highlight the possibility that complement dysregulation is not always the same in these patients

and that in some of the patients, a resistance to C5 cleavage blockade might exist. The unresponsiveness to eculizumab may have different explanations.

Recently, Nishimura *et al.*^[112] documented that some patients affected by paroxysmal nocturnal hemoglobinuria (PNH) had a missense mutation at arginine 885 at the level of the C5 gene. This mutation caused a resistance to C5 cleavage by eculizumab.

In addition, patients affected by C3G, after eculizumab administration, may have a persistent fluid phase C3 convertase activity in the absence of terminal complement activity, which has been documented in a patient with C3G caused by a hybrid CFHR2/CFHR5 protein^[64]. In this patient, after eculizumab administration, a block of C5 cleavage and sMAC generation has been obtained, but the hyperfunctioning C3 convertase remained active. Consequently, patients with a C3 convertase dysregulation greater than C5 dysregulation should not be treated with C5 blockade^[113]. Moreover, has been documented that this block might aggravate the C3 convertase activity *via* a feed-back mechanism. Consequently, patients affected by C3G with a prevailing C3 convertase activity should be treated with drugs inhibiting C3 convertase. Blocking the complement AP at the C3 level might be essential in several patients affected by C3G, but the usefulness of such a blockade should be weighed against potential drawbacks as the block of C3b with its critical role in innate immunity.

To date, there are essentially 3 drugs aimed to exert a blockade at the C3 level. The compstatin analog Cp40 was documented to be effective in inhibiting complement dysregulation *in vitro* in C3G^[114]. Compstatin binds to C3 and C3b, preventing the complement dysregulation caused by genetic mutations or by auto-antibodies. To date, compstatin is used in trials for macular degeneration and PNH. Similarly, a monoclonal antibody, which inhibits C3 convertase induced by C3NeF by binding to C3b is currently in the preclinical phase^[115]. The most advanced drug among the C3 inhibitors is CDX1135, which is also known as TP10 and the soluble complement receptor 1 (sCR1). CR1 is a cell surface glycoprotein expressed on several cells, including immune cells. sCR1 is a protein that can regulate C3 convertase. Under normal conditions, only small quantities of sCR1 are in circulation. Administration of a high quantity of sCR1 in patients undergoing cardiac surgery revealed that this protein is able to exert a complement inhibition effective and safe^[116,117]. Recently, at Iowa University, the efficacy of sCR1 has been documented *in vitro* and in mice affected by C3G^[118].

Renal transplantation: C3G has a frequent evolution towards ESRD. Renal transplantation has been proposed for ESRD patients affected by C3G. Renal transplantation in such patients has two principal challenges: (1) whether to perform a dual liver-kidney transplantation; and (2) The high recurrence rates of the disease and its treatment.

The question of liver-kidney transplantation has

been previously mentioned above^[86-88] and has been documented that in the eculizumab era, the liver-kidney transplantation will lose its relevance.

In the case of the kidney transplant alone the principal challenge is the high recurrence rate. In the case of DDD, the risk of recurrence is over 70%^[119], with a high risk of graft loss^[120,121]. These data confirmed a retrospective United States study including 75 children affected by DDD^[122] and a more recent Irish cohort, including 33 patients affected by DDD^[123]. Fewer data have been reported on the recurrence risk of C3GN. The most relevant study has been published by Zand *et al.*^[124] from the Mayo clinic. They report 21 renal transplant patients affected by C3GN. The recurrence rate was as high as 70% and the graft failure occurred in 50% of the patients. Importantly, all these reports with high recurrence rates also include patients transplanted in the pre anti-complement era. Transplants in patients affected by CFHR nephropathy has been reported in 11 subjects. All transplants were successful, despite the histological recurrence in three patients^[125].

The treatment of recurrent disease has not yet been the object of clinical trials. Close monitoring is mandatory following renal transplantation to promptly detect the clinical signs of recurrence. Patients with circulating auto-antibodies might be treated by agents targeting T and B cells, but we should remember that the transplanted patients are already on immunosuppressant drugs.

Anti-complement drugs are promising. McCaughan *et al.*^[93] described the first report of a transplanted patient affected by recurrent DDD and who was successfully treated by eculizumab. However, the long-term dependence on eculizumab and the long-term safety of the drug remain open questions and the object of future RCTs. Another interesting approach is the use of sCR1, but its use to date has been limited to the native disease and not to its recurrence after transplantation.

Clinical trials ongoing: C3G is a rare disease and it is not surprising that ongoing RCTs are scarce. From one perspective the market interest is poor due to the few numbers of patients. However, a wide comprehensive multinational network among centers should be developed to include a significant number of patients for a RCT.

To date, four clinical trials are ongoing on C3G. Two trials aimed to evaluate eculizumab therapy in DDD and C3GN^[100,126]. Two other RCTs are evaluating the effect of two different formulations of sCR1 on C3G^[127,128].

Other drugs, such as compstatin and monoclonal antibody against C3 convertase, are still in the pre-clinical phase.

CONCLUSION

The Mayo Clinic/Renal Pathology Society Consensus Conference held in 2015 allowed the elaboration of a new etiology-pathology based classification of the GN, which substitutes for the old morphologic-based classification (1). In addition, before the Consensus Conference, the

MPGNs had been the object of new classifications for several years. To date, it is clear that the MPGNs should be distinguished into two principal categories: The immune-complex-mediated MPGN and the complement-dysregulation-mediated MPGN. This finding is principally relevant, not only from a taxonomic perspective, but also from a diagnostic and therapeutic approach. New findings in the complement related pathways and in genetics allowed for an improved understanding and definition of complement related MPGN, in addition to the discovery of new entities, such as C3GN and CFHR5 GN. To date, the MPGNs have a new diagnostic approach with a new network that applies to the immune-complexes related MPGN and complement-related MPGN.

Currently, fewer new drugs are available for the treatment of immune-complexes MPGN.

With the discovery of complement-inhibitor drugs, there has been more progress for the complement related MPGN. However, due to the rarity of the disease, well conducted RCTs are scarce. This finding supports the need to perform more multinational cooperative studies to identify an evidence-based medicine therapeutic approach.

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Renal biopsy: Still a landmark for the nephrologist

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Abstract

Renal biopsy was performed for the first time more than one century ago, but its clinical use was routinely introduced in the 1950s. It is still an essential tool for diagnosis and choice of treatment of several primary

or secondary kidney diseases. Moreover, it may help to know the expected time of end stage renal disease. The indications are represented by nephritic and/or nephrotic syndrome and rapidly progressive acute renal failure of unknown origin. Nowadays, it is performed mainly by nephrologists and radiologists using a 14-18 gauges needle with automated spring-loaded biopsy device, under real-time ultrasound guidance. Bleeding is the major primary complication that in rare cases may lead to retroperitoneal haemorrhage and need for surgical intervention and/or death. For this reason, careful evaluation of risks and benefits must be taken into account, and all procedures to minimize the risk of complications must be observed. After biopsy, an observation time of 12-24 h is necessary, whilst a prolonged observation may be needed rarely. In some cases it could be safer to use different techniques to reduce the risk of complications, such as laparoscopic or transjugular renal biopsy in patients with coagulopathy or alternative approaches in obese patients. Despite progress in medicine over the years with the introduction of more advanced molecular biology techniques, renal biopsy is still an irreplaceable tool for nephrologists.

Key words: Renal biopsy; Acute kidney injury; Bleeding; Haematuria; Hematoma; Chronic renal failure

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Core tip: Percutaneous renal biopsy is an irreplaceable tool in the clinical practice of nephrologists to determine diagnosis, prognosis and treatment of several kidney diseases. This procedure is considered safe if it is performed in well-trained centers. Main indications are acute glomerulonephritis and nephrotic syndrome. Since bleeding is the major primary complication, careful evaluation of risks and benefits must be considered. The risk of complications in patients with coagulopathy may be reduced by using laparoscopic or transjugular renal biopsy or alternative approaches in obese patients. Despite progress in medicine over the years, renal biopsy is still an irreplaceable tool for nephrologists.

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INTRODUCTION

Percutaneous renal biopsy (PRB) is still considered an irreplaceable tool for diagnosis, prognosis and choice of treatment of several primary or secondary kidney diseases. The indications uniformly recognized by most nephrologists are represented by nephritic and/or nephrotic syndrome and unexplained acute or rapidly progressive renal failure^[1]. Primary glomerulonephritis are the more common renal disease in renal biopsy registries. Among them IgA nephropathy (IgAN) is the most frequent renal diagnosis. Regarding systemic diseases, systemic lupus erythematosus (SLE) is the most frequent indication for PRB, because this last determines the level of activity and/or chronicity of the lesions and the reversibility of renal lesion as a result of therapy. PRB can also be helpful in vasculitis to assess the severity of the damage and the potential reversibility after therapy. In diabetes the use of PRB is motivated by a relatively recent or very late appearance of proteinuria > 1 g and/or a rapid decline in GFR and/or active urinary sediment, in the absence of other signs of microangiopathy (retinopathy and neuropathy); in fact, in these patients primitive forms of glomerular diseases are frequently reported, superimposed or not to the typical lesions of diabetes. In advanced chronic renal failure, PRB is useful to assess a rescue therapy or to know the causal nephropathy in view of renal transplantation^[2].

PRB is also an informative procedure in renal transplantation, both in the postoperative, for the differential diagnosis of acute rejection vs other diseases, and in follow-up of organ transplantation for differential diagnosis between recurrence of primary renal disease, development of glomerulonephritis *ex novo*, and acute or chronic rejection (Table 1).

HISTORY

The first renal biopsy of native kidney was performed in 1901 in a surgical procedure for renal decapsulation in the treatment of a Bright's syndrome^[3]. The PRB was born in 1944 when Nils Alwall adapted a technique for percutaneous liver biopsy in the kidney, using an aspiration needle technique^[4] with a radiographic procedure for the localization of the right kidney and keeping the patient in a sitting position. With this innovative method, he obtained adequate tissue in ten of the thirteen patients^[5]. However, this procedure has been for the first time described in the literature by Iversen and Brun^[6] in 1951, which also used an aspiration needle and the sitting position but, in contrast to Nils Alwall,

Table 1 List of Indications for renal biopsy

Nephrotic syndrome
Acute kidney injury (when rule out obstruction, and pre-renal causes)
Systemic disease with renal dysfunction (in diabetic patients only if it presents with atypical features)
Non-nephrotic proteinuria, and in some circumstances isolated microscopic hematuria
Unexplained chronic kidney disease
Familial renal disease (may avoid biopsy in other family members affected)
Renal transplant dysfunction

they used intravenous pyelography for localization of the right kidney; unfortunately they obtained adequate tissue only in 53% of patients^[6]. Given the poor results of this technique, Kark *et al*^[7] in 1954 made significant changes including the prone position of the patients with a sandbag placed under the abdomen to reduce the mobility of the kidney and the introduction of a new type of needle, the Franklin-modified Vim-Silverman needle, which trapped the tissue in the needle and then sheared it off, achieving adequate tissue in 96% of patients and no major complications. To localize the lower pole of the kidney they used as landmark the distances between the vertebral spinous processes and the 11th and 12th ribs, and the movement of a finder needle following a deep inspiration^[7]. Over the years the technique has been improved more and more, increasing the adequacy of the sample and reducing the risk of complications.

In 1962 the use of radiological images was introduced for the localization of the kidney, later replaced by the ultrasound real-time imaging. Since then this procedure, which was initially performed by nephrologists, has gradually become a prerogative of radiologists. In fact, between 1964 and 1974 the PRB was performed in 95% of cases by nephrologists^[8], while in 1980s the number of nephrologists who performed the PRB was gradually reduced in favour of radiologists and in 2011, Lane *et al*^[9] showed that radiologists were the main performers of this technique (Figure 1)^[10].

A recent european survey stated that in 60% of the centers renal biopsy is performed by nephrologists, in 30% by radiologists and in 5% by nephrologists and radiologists^[11]. Today, the standard procedure for PRB involves the use of real-time ultrasound and automated spring-loaded biopsy device^[12].

NEEDLE TYPES AND SIZE

There are different types of biopsy needles and the first used was an aspiration needle, subsequently replaced by the cutting Vim-Silverman needle, which trapped the tissue in the needle and then sheared it off. The evolution of the latter is the Tru-Cut needle, which is a manually operated sheathed needle designed for manual capture of high-quality tissue samples with minimal trauma to the patient. Today it is replaced by automatic spring-loaded biopsy guns and semi-automatic biopsy guns with

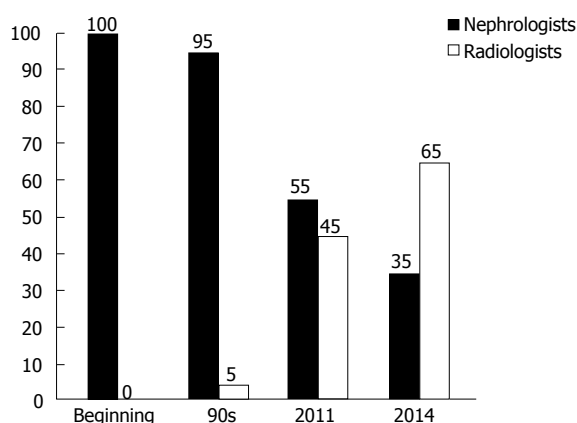


Figure 1 Rate of performers (nephrologists and radiologists) of renal biopsy along the course of the years^[10].

better and safer performance.

The optimal needle size for native renal biopsies has not been established, but the most used are three: 18 gauge (internal diameter 300-400 μm), 16 gauge (internal diameter 600-700 μm) and 14 gauge (internal diameter 900-1000 μm). The first one is reserved to paediatric patients because the internal diameter of the needle is barely bigger than an adult glomerulus (200-250 μm), while the other two are more appropriate for the adult patients^[13,14]. On the other hand, the length of this device is almost the same and is around 20 cm.

SAMPLE ADEQUACY

The number of glomeruli is the main determinant of the biopsy adequacy but it varies based on the type of glomerular disease. For example in focal disease, such as focal segmental glomerulosclerosis, the diagnosis can be made by identifying even one glomerulus that presents the typical lesions but the probability to make diagnoses is directly proportional to the number of glomeruli^[15]. Therefore, in a kidney in which 20% of glomeruli are sclerotic, if a bioptic sample includes five glomeruli the probability to miss affected glomeruli is about 35%. This percentage falls down to 10% if the bioptic sample includes ten glomeruli and to 1% if it includes twenty glomeruli^[16,17]. Therefore, the minimum number of glomeruli required to define an adequate bioptic sample is ten, and usually, to get this target at least two different cores are taken which are divided for light microscopy (LM) (placed in formalin or another fixative), immunofluorescence (IF) (placed in transport solution-saline solution- and quickly frozen), and electron microscopy (EM) (fixed in 2%-3% glutaraldehyde or 1%-4% paraformaldehyde)^[18].

Actually, the latter is not frequently and widespread performed in the practice of renal biopsy since it is possible to get a diagnosis in most cases with the contribution of the LM and the IF. However, due to the relevance of EM in some specific glomerular diseases, it has been recommended that renal tissue for EM be set aside in

each case if EM cannot be performed routinely^[19]. As an alternative, IF may be also performed on paraffin sample, using only one core for LM and IF and further reducing the risk of complications resulting from biopsy. The technique is certainly more complicated and needs more time for preparation but provides comparable results with the classic procedure with the exception of complement factors; consequently, it may be used in selected cases and/or in patients with greater bleeding risk.

About the optimal needle size for native renal biopsies, there is not a general consensus to achieve a good compromise between sample adequacy and lower number of complications. In adult patients a 14 or 16 gauge needle seems to be appropriate^[20], while in paediatric patients it is better to use 18 gauge needles^[21].

COMPLICATIONS

Even if PRB is considered a safe procedure, it is not without complications (Table 2) that, in very rare cases, may also cause death or require extreme procedures such as nephrectomy^[22-24]. For this reason it is always necessary to evaluate the risk/benefit for the patient, inform him/her and obtain a signed consent. Furthermore, complications are divided into major complications that need a treatment or an intervention to stop the problem, and minor complications that spontaneously resolve without intervention or further treatment; in both cases, bleeding is the main consequence of PRB and can occur at different levels: (1) in the collecting duct system, causing micro - gross haematuria which may result in clots formation in the urine (ureter or bladder) with risk of obstructive renal failure; (2) below the kidney capsule, causing subcapsular hematoma formation that in rare cases may lead to the Page kidney, which consists in renal ischemia caused by prolonged compression of the kidney from haemorrhage with resulting arterial hypertension characterized by high renin levels^[25]; and (3) in the perinephric space, causing hematoma formation which may be asymptomatic, in the majority of cases, or result into a clinically relevant complication, such as lumbar pain, significant drop in haemoglobin concentration, or need for a blood transfusion.

However, the risk of complications after renal biopsy is not high (Table 3). In fact, in a systematic review and meta-analysis of 34 retrospective and prospective studies including 9474 adult patients who underwent biopsy of the native kidney, using ultrasound real-time imaging and automatic biopsy device, the overall incidence of bleeding complications were: Transient gross haematuria 3.5%, request for transfusion therapy 0.9%, demand on angiographic control of bleeding 0.6%, request for nephrectomy for control of bleeding 0.01% and death 0.02%^[26]. Thus, the risk of using invasive procedures to stop bleeding is very rare^[27,28]. More frequently we can treat this complication with medical treatment such as administration of endovenous fluid and/or blood products^[29]. Moreover in some cases of persistent hemorrhage, before

Table 2 Types of complications after renal biopsy

Minor complications	Major complications
Bleeding	Bleeding
Asymptomatic haematoma	Hematoma requiring blood transfusion or invasive procedure to stop bleeding
Microscopic and gross haematuria	Urinary tract obstruction with or without AKI
Anaemia (drop in haemoglobin concentration ≥ 1 g/dL)	Hypotension related to bleeding
Pain (> 12 h)	Nephrectomy
Pyelonephritis	Sepsis
Perinephric infection	Other organs and/or blood vessels perforation
Arteriovenous fistula	Death

AKI: Acute kidney injury.

Table 3 List of main studies (> 500 biopsies) reporting minor, major complications and mortality rate after renal biopsy

Ref.	Year of publication	No. of biopsies	% Minor complications	% Major complications	% Mortality
Fenerberg <i>et al</i> ^[24]	1998	1081	9.6	1.11	0.09
Prasad <i>et al</i> ^[28]	1998	1090	3	0.36	0
Preda <i>et al</i> ^[20]	2003	515	9.5	2.7	0
Whittier <i>et al</i> ^[51]	2004	750	6.7	6.4	0.13
Atwell <i>et al</i> ^[44]	2010	5832	-	0.7	0
Stratta <i>et al</i> ^[29]	2007	1137	24.2	0.36	0
Korbet <i>et al</i> ^[23]	2014	1055	8.1	6.6	0.09
Mai <i>et al</i> ^[21]	2013	934	5.9	0.86	0
Tøndel <i>et al</i> ^[13]	2012	9288	1.9	0.9	0
Prasad <i>et al</i> ^[28]	2015	2138	5.4	5.1	0

performing embolization of a pseudoaneurysm or surgery to stop the bleeding, we can resort to off-label drug use such as recombinant activated factor VII^[30].

Specific symptoms and signs post-biopsy

Lumbar pain: The pain is an extremely common consequence of PRB and usually occurs at the end of anaesthesia. If necessary it is possible to administer a mild analgesic. Otherwise, the onset of greater pain suggests the development of a major complication and further diagnostic tests must be performed.

Microscopic haematuria: It is the most common consequence of this procedure; it is usually asymptomatic^[31] and resolves spontaneously over a few days.

Gross haematuria: It occurs in 3% of renal biopsies and typically disappears in few hours or days. Occasionally gross haematuria may cause a significant drop in haemoglobin concentration requiring a blood transfusion or, in rare cases, it may result in clots formation with or without obstructive renal failure. On the contrary, persistent haematuria after three days suggests the onset of major complications such as arteriovenous fistula (AVF)^[32].

Acute anaemia: A decrease of haemoglobin concentration ≥ 1 g/dL occurs in more than 50% of uncomplicated renal biopsies^[33], whereas a fall ≥ 2 g/dL occurs in 10% of

uncomplicated cases and is consequently associated with increased risk of complications^[34].

Perinephric hematoma: The presence of asymptomatic hematoma is frequently detected during a renal ultrasound after biopsy and does not constitute *per se* a complication. Prospective studies showed that perinephric hematoma is detectable in 90% of patients 24-72 h after the procedure, while this percentage drops to 15% immediately after the biopsy. Most of the perinephric hematomas are small, asymptomatic and they resolve spontaneously in few months; only in 2% of cases they may cause a clinically relevant complication such as lumbar pain, a decrease in haemoglobin concentration, or the need for blood transfusion. However, the absence of hematoma at 1 h was highly predictive of an uncomplicated course^[35].

Waldo *et al*^[36] showed that patients which did not present perinephric hematoma one hour after biopsy did not develop major complications in 95% of cases, while the presence of hematoma was predictive for major complications in 43%. Therefore, the routine use of ultrasound at 1 h after PRB may have a role in determining an uncomplicated course^[36].

AVF: It is not a frequent complication and is due to trauma of the wall of blood vessels; it is clinically asymptomatic and resolves spontaneously in most cases^[37]. In rare cases AVF can cause the development of an aneurysm, which may manifest clinically with high

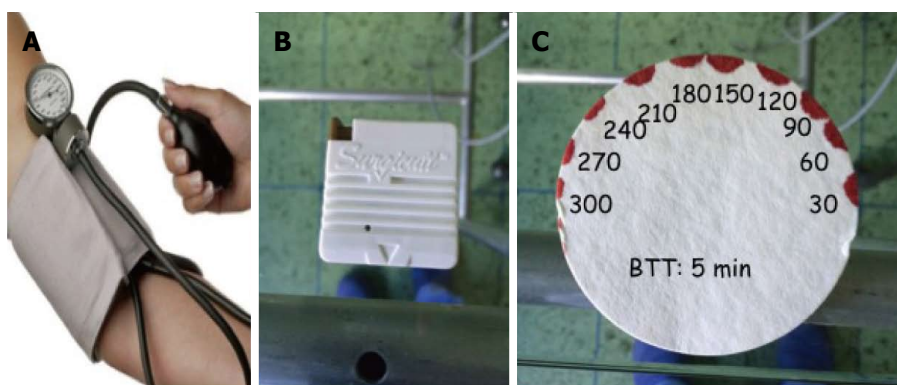


Figure 2 Bleeding time procedure. A: Place the sphygmomanometer on the upper arm and inflate to 40 mmHg; B: Make a small cut on the lower arm with automatic standard device; C: Blotting paper is used to draw off the blood every 30 s (normal range 3-7 min).

blood pressure, heart failure, and kidney failure. Important signs that suggest this complication are the persistence of gross haematuria, the presence of abdominal bruit and palpable thrill^[38,39] but diagnosis confirmation requires Doppler ultrasound or magnetic resonance imaging, or angiography. The treatment of symptomatic cases is based on superselective transcatheter arterial embolization or, in rare cases, surgery^[40].

CONTRAINDICATIONS AND RISK FACTORS

Contraindications to renal biopsy and risk factors must be taken into account to minimize the risk of complications.

The presence of intravascular coagulopathy, polycystic kidneys, obstruction of the urinary tract, hydronephrosis, infections of the upper urinary tract are regarded as absolute contraindications. Otherwise, there are some conditions, which require caution, considered as relative contraindications, such as compromised cardiopulmonary function or hemodynamic instability, severe obesity, inability of the patient to cooperate, solitary kidney, advanced age, severe hypertension (> 160/95 mmHg), and renal failure^[41]. The last one causes functional alterations of coagulation factors as the von Willebrand factor (vWF) and the Factor VIII, abnormalities in platelet membrane, accumulation of uremic toxins that inhibit platelet aggregation, high levels of prostacyclin and nitric oxide which are factors that reduce platelet aggregation. Another element that often contributes to increase the risk of bleeding in renal failure is the presence of anaemia. Other diseases associated with greater risk of bleeding are those with arteriolar involvement as SLE, vasculitis, scleroderma, amyloidosis and advanced diabetic nephropathy because they interfere with the first mechanism of haemostasis, known as the vascular phase, reducing the arteriolar contraction.

PROCEDURES PRE-BIOPSY

Before performing the PRB it is very important to follow some recommendations to minimize the risk of complications. Renal ultrasound is essential to evaluate the presence of anatomical abnormalities of the kidney (presence of multiple cysts, hydronephrosis, solitary kidney)

that may represent a risk factor for the development of complications.

Laboratory tests may reveal the potential presence of coagulopathy. To totally assess the steps of haemostasis it is useful to use the bleeding time that evaluates the time of platelet aggregation (Figure 2). In case of advanced renal failure and/or prolonged bleeding time, the administration of desmopressin acetate - DDAVP (0.3 µg/kg), estrogen and cryoprecipitate has shown a reduction of the bleeding risk^[42,43].

Antiplatelet agents and oral anticoagulants have to be withdrawn at least one week before renal biopsy^[44], the last ones until normalization of INR, and replaced with low molecular weight heparin (LMWH). Other drugs that may cause alterations in coagulation are the non-steroidal anti-inflammatory drugs (NSAIDs), which should be not taken for at least 5 d before PRB.

ALTERNATIVE APPROACHES FOR RENAL BIOPSY

In some cases, PRB may be contraindicated because of bleeding diatheses or habitus of the patients such as obesity. In these circumstances we can perform renal biopsy with alternative methods such as under CT guidance^[45] or with laparoscopic^[46] and transjugular approach^[47]. These techniques may have some limits. CT guidance, for example, does not assess any possible movements of the kidney related to breathing, laparoscopic biopsy requires general anaesthesia and transjugular biopsy seems to be associated with a lower diagnostic power due to the need to pass through the medulla first^[48].

In obese patients a new approach of PRB under real-time ultrasound guidance has been proposed with the patient in supine antero-lateral position (SALP). Gesualdo *et al.*^[49] reported a case series of 110 patients undergoing PRB, divided into two groups: Low risk group (90 patients) if the body mass index (BMI) was ≤ 30 in the absence of respiratory disorders and high risk group (20 patients) if BMI was > 30 with breathing problems. The first group underwent classical PRB in prone position and the other group in SALP, demonstrating, at the end of the study, that there were no substantial differences about adequacy samples and patients safety^[49]. Moreover, an open renal biopsy may be performed when uncorrectable

contraindications are present. Nomoto *et al*^[50] reported 931 cases of open kidney biopsies concluding that this is a safe procedure with 100% of sample adequacy but an important limitation of this technique is the use of general anesthesia.

PERIOD OF OBSERVATION

After biopsy, the patient must be at rest for at least 6-8 h in the supine position. Blood pressure should be monitored frequently, and urine must be checked to evaluate the presence of gross haematuria. If there are no signs of bleeding within 6 h, the patient may sit up, because most of complications occur within 6-8 h. However, since some complications may also occur later, the ideal observation time should be continued for 24 h. In a case series of 750 biopsies of native kidney it was reported that 67% of major complications appeared within the first 8 h, suggesting that observation for 24 h is safer in renal biopsy^[51].

CONCLUSION

PRB is a safe procedure and the risk of development of major complications is very rare. Instead, the minor consequences due to the procedure occur more frequently. These are micro- and/or gross haematuria, drop in hemoglobin concentration > 1 g/dL, development of asymptomatic perinephric hematoma. All these minor adverse events can be more safely managed and do not bring particular complications to the patient. It is mandatory to identify risk factors for bleeding such as anaemia, prolonged bleeding time or advanced renal failure, severe arterial hypertension and correct them when possible; where this is not possible, it is recommended to postpone the procedure.

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Management of nocturnal enuresis - myths and facts

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Abstract

Nocturnal enuresis often causes considerable distress or functional impairment to patient and their parents necessitating a multidisciplinary approach from paediatrician, paediatric nephrologist, urologists and psychiatrist.

Mechanisms of monosymptomatic nocturnal enuresis are mainly nocturnal polyuria, bladder overactivity and failure to awaken from sleep in response to bladder sensations. Goal oriented and etiology wise treatment includes simple behavioral intervention, conditioning alarm regimen and pharmacotherapy with desmopressin, imipramine and anticholinergic drugs. Symptoms often recurs requiring change over or combination of different modes of treatment.

Key words: Nocturnal enuresis; Monosymptomatic; Conditioning alarm; Desmopressin; Imipramine

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Core tip: Nocturnal enuresis often causes considerable distress to patient and their parents' lifestyle necessitating a multidisciplinary management. Simple behavioral interventions, conditioning alarm regimen and pharmacotherapy as desmopressin, imipramine and anticholinergic drugs are the mainstay of therapy used as per underlying etiology or parents' concern. Therapy should be structured and goal directed to reduce recurrence.

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INTRODUCTION

Enuresis though often conceived as a simple problem can have multiple hidden etiologies necessitating a multidisciplinary approach involving paediatrician, paediatric nephrologist, urologists and child and adolescent psychiatrist. The complexity in both assessment and treatment underscores the need for practice parameters

Table 1 Lower urinary tract symptoms

Consistently increased (≥ 8 times/d) or decreased (≤ 3 times/d) voiding frequency
Daytime incontinence
Urgency
Hesitancy
Straining (application of abdominal pressure to initiate and maintain voiding)
A weak stream
Intermittency (micturition occurs in several discrete spurts)
Holding maneuvers (strategies used to postpone voiding)
A feeling of incomplete emptying
Post-micturition dribbling
Genital or lower urinary tract pain

for clinicians confronting this problem.

DEFINITIONS

As per DSM-IV-TR, enuresis is defined as repeated voiding of urine into the bed or clothes at least twice per week for at least three consecutive months in a child who is ≥ 5 years of age^[1]. A child may also be considered to be enuretic if the frequency or duration is less, but there is associated distress or functional impairment. As per International Children's Continence Society (ICCS), enuresis can be defined as urinary incontinence while asleep in a child aged at least 5 years^[2]. The DSM-III and ICD-10 define a bed-wetting frequency of twice per month in the past 3 mo for children ages 5 and 6 years and once per month in the past 3 mo for children ages 7 years or older. The DSM-IV-TR includes voluntary as well as involuntary voiding, although most studies exclude children who voluntarily or intentionally wet their bed or clothes. Nocturnal enuresis refers to voiding during sleep; diurnal enuresis defines wetting while awake.

TYPES

Enuresis may be of monosymptomatic or non-monosymptomatic forms.

Monosymptomatic enuresis (MNE) denotes enuresis in children without any other lower urinary tract symptoms and without a history of bladder dysfunction^[2]. Non-monosymptomatic (NMNE) enuresis is defined as enuresis in children with other lower urinary tract symptoms (Table 1). It can also be classified as primary enuresis occurring in children who have never been consistently dry throughout the night, or secondary enuresis which refers to the resumption of wetting after at least 6 mo of dryness^[3].

EPIDEMIOLOGY

The reported prevalence of enuresis at different ages varies considerably because of inconsistencies in its definition as stated earlier, differences in the method of

data collection, and differences in the characteristics of the population sampled. Nocturnal incontinence occurs in 12% to 25% of 4-year-old children, 7% to 10% of 8-year-old children, and 2% to 3% of 12-year-old children^[4]. It may be problematic even in late teenage years (1% to 3%)^[5] and if untreated enuresis (especially if severe) can persist indefinitely with prevalence rates of 2%-3% in adulthood^[6,7]. Primary enuresis is twice as common as secondary enuresis. Enuresis seems to be more common among boys (2:1) in whom the problem is often more difficult to treat^[8,9]. Enuresis is more common at all ages in lower socioeconomic groups and in institutionalized children. Majority of children have primary nocturnal enuresis whereas children with secondary enuresis may have precipitating factor such as an unusually stressful event (*e.g.*, parental divorce, birth of a sibling, school trauma and sexual abuse). The spontaneous cure rate of night time enuresis is 14% to 16% annually^[10].

ETIOLOGY

Factors that are believed to contribute to enuresis include genetics, sleep disturbances, maturational delay and abnormal secretion of antidiuretic hormone (ADH, vasopressin). Psychological and behavioral abnormalities although common are likely to be a result of enuresis rather than the cause.

Genetics

Bakwin showed that compared with a 15% incidence of enuresis in children from non-enuretic families, 44% and 77% of children were enuretic when one or both parents, respectively, were themselves enuretic. Scandinavian linkage studies depicted a locus for enuresis on chromosome 13 (ENUR 1) and another (ENUR 2) on chromosome 12^[11,12].

Sleep aspects

Whether sleep disturbances are a result of the enuresis or contributes to the pathogenesis of enuresis is still debatable. Attempts at arousal were more often successful in control subjects than in boys with enuresis (40% vs 9%)^[13]. In contrast another sleep study found that children with severe enuresis were actually "light sleeper" but they did not wake before voiding^[14]. The arousal centre may be suppressed in these children. Persistently overactive bladder may lead to the abnormal arousal response just like the analogy of someone constantly knocking at the door leading to one either ignoring the knock or even installing an extra lock. Enuresis has been associated with snoring or sleep apneas due to adenotonsillar hypertrophy. This may be due to paradoxical rising of the arousal threshold due to constant stimuli from the obstructed airways or polyuria secondary to increased anti natriuretic peptide due to persistent negative intra-thoracic pressure found in sleep apnea syndrome.

Maturational delay

Since most cases of MNE resolves spontaneously a delayed maturation of a normal developmental process has been explored. Increased incidence of delayed language and slowed motor performances has been identified in some studies among children with enuresis^[15]. Urodynamic and EEG findings have shown progressive maturation in bladder stability along with EEG changes suggesting increased central nervous system recognition of bladder fullness and the ultimate ability to suppress the onset of bladder contraction. Bladder capacity at birth is only around 60 mL and thereafter increases with age^[16]. Children with nocturnal enuresis have been noted to have a smaller bladder capacity (functional rather than anatomical) even when there are no day time concerns^[17]. There are reports of lower average height and lower mean bone age and late sexual maturation in enuretic than in non-enuretic children and adolescent. There is a greater incidence of enuresis in children who were delayed in the attainment of motor and language milestones as well.

Nocturnal polyuria

Increased urinary output overnight might also play an important role in MNE^[18]. The cause may include increased fluid intake before bedtime, reduced response to antidiuretic hormone, and or decreased secretion of ADH.

Role of ADH

Despite the utility of desmopressin in the treatment of MNE the relationship between ADH secretion and night time urinary output remains controversial.

Initial studies did suggest presence of a blunted response to vasopressin in enuretic children compared with age-matched controls but subsequent studies failed to reproduce this observation^[19].

Some studies have also demonstrated decreased nocturnal secretion of ADH but whether this is primary or secondary to the small bladder capacity (ADH secretion is thought to be stimulated with bladder distension) is not clear^[20].

Additionally it needs to be emphasized that abnormalities in ADH secretion does not explain as to why these children do not wake to void.

Psychosocial factors

Psychiatric disorders in children with enuresis are higher than the rate found in non-enuretic groups but the relationship may be of etiologic relevance or it may be coincidental or occurring in response to the symptom of enuresis^[9]. Children with enuresis had 2.88 times increased odds (95%CI: 1.26-6.57) of having attention deficit hyperactivity disorder (ADHD) as compared with those without enuresis^[21]. It has been suggested that both enuresis and ADHD might be related to delays in central nervous system maturation^[22]. Enuresis has sometimes been described as a masturbatory equivalent,

an expression of bisexuality, or the somatic expression of a defect in body image.

Adverse event to medications

Enuresis may rarely results as a side effect of a medication such as lithium, valproic acid, clozapine and theophylline (secondary enuresis).

MECHANISM

The pathophysiology of enuresis is complex, involving the central nervous system (several neurotransmitters and receptors), circadian rhythm (sleep and diuresis), and bladder function derangements. Urinary continence is obtained in three sequential steps: Enlargement of the bladder capacity, voluntary control of the sphincter muscles, and voluntary control of the micturition reflex.

There are three commonly proposed mechanisms to bedwetting (Figure 1)^[23,24].

The locus coeruleus (LC), a noradrenergic neuron group in the upper pons is crucial for arousal from sleep and overlaps both functionally and anatomically with the pontine micturition centre, which coordinates the micturition reflex. The LC also has axonal connections with the hypothalamic cells that produce vasopressin^[25-27]. Hence disturbances in this region of brainstem might be the missing link to a unifying pathogenic mechanism.

EVALUATION

Evaluation of a child with enuresis consists of detailed history, focused examination and appropriate investigations.

History

Detailed history is the key to the treatment success of a child suffering from enuresis. In every instance, both the parents and the child should be interviewed, and sensitivity to the emotional consequences of the symptoms should be high. Special focus in history should include: (1) Daytime wetting/urgency/holding maneuvers/weak or interrupted urinary stream including dribbling or straining; (2) Primary or secondary enuresis; (3) Frequency and pattern of nocturnal enuresis (including number of wet nights per week or month, number of episodes per night, time of episodes, approximate volume of each episode); (4) Daily fluid intake and urine output diary. (This will identify whether the child drinks adequately as well as whether the majority of fluid intake happens in late afternoon/evening, daytime urinary frequency, presence of polyuria - which might indicate other underlying cause such as diabetes, kidney disease or psychogenic polydipsia); (5) Stool history (including history of constipation/fecal soiling/encopresis); (6) Any relevant medical history (e.g., review of history of sleep apnea, sickle cell disease or trait, diabetes, recurrent urinary tract infection, gait/neurological abnormalities); (7) Details of any previous interventions for enuresis; (8) Family history of nocturnal enuresis; (9) Social history (may be important in secondary enuresis);

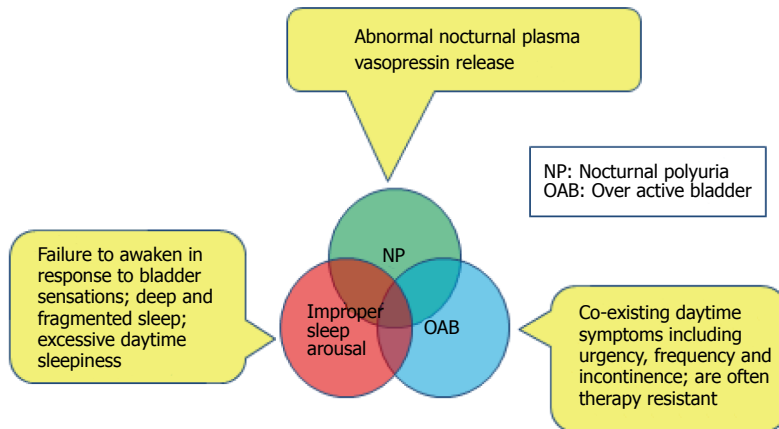


Figure 1 Mechanisms to bedwetting: Three commonly proposed mechanisms often overlap each other.

(10) Importantly effort should be made to understand how the problem has affected the child and family and the degree of motivation in both the child and family; (11) Behavioral history including behavior screening questionnaire; and (12) The sleeping arrangements for the child at home should be explored.

A voiding diary is helpful in not only identifying any underlying bladder dysfunction such as day time frequency but also in establishing a baseline record of the enuresis pattern. This may serve as standard against which the success of subsequent interventions can be gauged. Not infrequently, this baseline monitoring itself is associated with a dramatic improvement.

Physical examination

Although in most of the cases (particularly in children with MNE) physical examination is usually normal, detailed physical examination is still important to ensure any other underlying etiology is not being missed. A quick but focused physical examination in children with enuresis should include: (1) Growth: Poor growth may indicate an underlying renal problem and should prompt further examination attempting to identify any other renal disorder related signs such as hypertension; (2) Adenotonsillar hypertrophy or other signs of sleep apnea: Rarely they may be the underlying cause for enuresis; (3) Abdominal palpation: Will help in identifying fecal mass (severe constipation/encopresis) or distended bladder (bladder outlet obstruction); (4) Perianal excoriation or vulvovaginitis which may indicate pinworm infection; (5) Detailed examination of lumbosacral spine as well as neurologic examination of perineum and lower limbs will aid in identifying any occult spinal cord abnormalities; and (6) Detection of wetness in the undergarments may be a sign of daytime incontinence.

Appropriate investigations

Investigations are usually minimally required in children with MNE. Cayan reported that the findings of ultrasonography and uroflowmetry were no different in children with nocturnal enuresis than in children without the condition^[28]. So performing more than a urinalysis and culture for children with nocturnal enuresis would neither be cost effective nor helpful to the child. More invasive

tests are indicated only in NMNE.

Urinalysis: This can aid in ruling out ketoacidosis, diabetes insipidus, water intoxication, and/or occult urinary tract infection^[29]. First-morning specific gravity may be helpful in predicting who will respond to desmopressin treatment.

Imaging: Ultrasonography may be useful in NMNE for estimating bladder capacity, post-void residual volume, and bladder wall thickness. Voiding cystourethrogram can be useful in children with significant daytime complaints or history of recurrent urinary tract infection. Abdominal radiograph although rarely used for determining the presence and/or extent of stool retention is also helpful in convincing the parents about the severity of the constipation^[30]. Neuroimaging such as magnetic resonance imaging of the spine will be needed if the lumbosacral/perianal/lower limb neurological examination demonstrates any abnormality^[31].

Urodynamic studies: Urodynamic studies are limited to children with suspected bladder dysfunction as per history/examination and or ultrasound or voiding cystourethrogram results.

Frequently, the psychological and developmental damage may actually be more significant and devastating to the child than the symptom of enuresis itself so many a times psychological evaluation may be needed.

DIFFERENTIAL DIAGNOSIS

Although with detailed history and examination the diagnosis is not very difficult but following underlying conditions should not be overlooked: (1) Underlying medical conditions resulting in polyuria such as sickle cell disease, diabetes mellitus, diabetes insipidus, etc.; (2) Severe constipation/encopresis; (3) Bladder bowel dysfunction; (4) Spinal dysraphism; (5) Chronic kidney disease such as nephronophthisis; (6) Pinworms; (7) Psychogenic polydipsia; and (8) Upper airway tract obstruction.

TREATMENT

Bed wetting while asleep is considered normal at least

till 5 years. Even subsequently need for intervention is often not a medical decision being influenced primarily by the family and the child's perception towards enuresis.

Evaluating the impact on the child and family

In children aged ≥ 5 years, enuresis is considered abnormal. Reasons for proactive management can be manifold including the distress caused to child and family, difficulty of "sleeping over" on holiday or at friends' houses, social withdrawal, reduced self-esteem, and potential disturbance of the child's and the parents' sleep architecture that may have an impact on daytime functioning and health^[32]. Additional reasons include the risk that some parents may be intolerant of their child's wetting and the significant inconvenience and costs associated with frequent laundering of bed-sheets and clothing^[33]. In primary care, "trial and error" treatment for enuresis is often the rule rather than the exception; this approach is a waste of time and money and increases frustration among families and doctors. It may also have an adverse psychological effect on the child.

Prior to initiation of any management it is important to understand the prime concern of the family as well as their expectation. The age at which enuresis is considered to be a "problem" varies depending upon the family. If both parents wet the bed until late childhood, they may not be concerned that their seven-year-old wets the bed. In contrast, parents may be concerned about a four-year-old who wets if he has a three-year-old sibling who is already dry. Often the family may not want any active intervention once they understand the self resolving nature of the problem as well as the usual absence of any identifiable underlying physical anomaly. Sometimes the family wants a quick solution (maybe for a planned travel or sleep over) or often is aiming for a long term cure. Therapy should be goal-oriented, and follow-up should be consistent.

Goals of treatment

The goals of interventions for nocturnal enuresis include^[34]: (1) To stay dry on particular occasions (e.g., sleep over); (2) To reduce the number of wet nights; (3) To reduce the impact of enuresis on the child and family; and (4) To avoid recurrence.

Historically nocturnal enuresis management as per Glicklich's review explored fascinating "treatments" as cauterization of sacral nerves, penile ligation, inflated vaginal balloons to compress the bladder neck, and electric shocks to the genitalia. Structured approach has been shown to be beneficial and is professed to be the approach of choice. History, physical examination and/or laboratory tests give clues as to the management plans. Daytime wetting, abnormal voiding (unusual posturing, discomfort, straining, or a poor urine stream), a history of urinary tract infections or evidence of infection on urinalysis or culture, and genital abnormalities are

indications for nephro-urologic referral and subsequent treatment plan is influenced by any identified underlying aetiology. In case of coexisting constipation-disimpaction and establishment of a healthy bowel regimen leads to better control of enuresis. Snoring and enlarged tonsils or adenoids may signal sleep apnea and surgical correction of upper airway obstruction may result in improvement or cure of enuresis.

Step 1

Simple interventions: Initial interventions are usually restricted to educational and simple behavioral interventions. A number of common sense approaches (Table 2) to enuresis have evolved over time and despite lack of evidence they can be considered supportive for uncomplicated MNE.

Behavioral interventions: Behavioral interventions for treating bedwetting are defined as interventions that require a behavior or action by the child that promotes night dryness and includes strategies which reward that behavior. These include: (1) Simple behavioral interventions - behaviors or actions that can be achieved by the child without great effort; (2) Complex behavioural interventions - multiple behavioural interventions which require greater effort by the child and parents to achieve, including enuresis alarm therapy.

Simple behavioral interventions are often used as a first attempt to improve nocturnal enuresis and include reward systems such as star charts given for dry nights, lifting or waking the children at night to urinate, retention control training to enlarge bladder capacity (bladder training) and fluid restriction.

Awakening the child to void during the night (to preempt the symptom). Generally, given the enuretic child's sound sleeping ability, this does not lead to significant sleep disruption.

Lifting: Involves taking the child to the toilet during the night usually before the time that bedwetting is expected, without necessarily waking the child.

Waking: Involves waking the child to allow him/her to get up and urinate.

Neither waking nor lifting children and young people will promote long-term dryness but can be used in the short-term management of nocturnal enuresis.

Reward systems (e.g., star charts): The child might receive a star for every dry night, and a reward after a preset number of stars have been earned^[35].

Bladder-stretching exercises to increase functional bladder capacity have been used without consistent evidence of effectiveness. The effort not to void despite considerable urgency is unpleasant for both the child and the family.

Retention control training: Attempting to increase the functional bladder capacity by delaying urination for extended periods of time during the day.

Stop-start training: Teaching children to interrupt their stream of urine in order to strengthen their pelvic

Table 2 Common sense approaches for the management of uncomplicated monosymptomatic nocturnal enuresis^[39,40]

What to do	How to do	How it works
Educate parents about	High prevalence of enuresis Relatively high spontaneous cure rate Non-volitional nature of the symptom	Reduce their guilt Encourage hope Avoid a punitive response or the development of a control struggle
Encourage child	Keeping of a journal Keeping a dry bed chart Changing the wet bed	Raises awareness in the child
Maintain voiding diary record	Daytime diary used to: Measurement of maximum voiding volume (excluding the first morning void); over a minimum of 3-4 d for accuracy; measurement on weekends or school holidays are ideal Bedwetting diary completed for seven consecutive days/nights	Assess the child's bladder capacity Assess for the presence of nocturnal polyuria
Fluid intake regulation	Decrease fluids especially caffeinated beverages, before bedtime. Ensuring adequate fluid consumption in the morning and afternoon and avoiding excessive fluid during evening	Decrease nocturnal urine production

floor muscles.

The impact of bedwetting can be reduced by using bed protection and washable/disposable products; using room deodorizers; thoroughly washing the child before dressing; and using emollients to prevent chafing.

Urotherapy is a commonly used terminology and usually includes education on normal bladder function, regular voiding habits and voiding posture, life-style advice regarding fluid intake and prevention of constipation and instruction on the use of bladder diaries or frequency-volume charts^[36]. It encompasses various methods of pelvic floor muscle training, behavioral modification, neuromodulation and catheterization. The first-line treatment of daytime incontinence in childhood is basic urotherapy, *i.e.*, advice regarding fluid intake and regular voiding habits. The same advice is routinely given to enuretic children as well. This is not illogical, given the role of detrusor over activity in enuresis, but to date evidence for the efficacy of this approach is weak. However, urotherapy is certainly not harmful and alleviates concomitant daytime symptoms.

Simple behavioural methods in twelve randomised controlled trials including a Cochrane review found it to be superior to no active treatment but appear to be inferior to enuresis alarm therapy and some drug therapy (such as imipramine and amitriptyline).

Despite anecdotal reports, there is no empirical evidence to suggest efficacy of hypnotherapy, dietary manipulation, acupuncture, chiropractic treatment and psychotherapy and desensitization to allergens^[37].

Step 2

Active interventions (enuresis alarms/pharmacotherapy): Active interventions are usually planned if simple strategies as discussed above fail to yield positive results even after 3 to 6 mo. These interventions are usually based on recommendations of ICCS standardization document on MNE which have been reviewed and endorsed by committees representing the American Academy of Pediatrics, European Society for Paediatric Urology, European Society for Paediatric Nephrology, and the ICCS. Two first-line treatment options are suggested

- desmopressin and enuresis alarm^[38]. The choice of initial treatment may be based on the parents' and child's preference, their motivations, the physician's experience and local resources.

Information from diaries may identify one of four subtypes of MNE and allow further fine-tuning of treatment (Figure 2).

Indications for referral: MNE usually can be managed effectively by the primary care provider. However, children with refractory nocturnal enuresis may benefit from referral to a healthcare professional who specializes in the management of recurrent or refractory enuresis (*e.g.*, developmental-behavioral pediatrician or urologist if structural or anatomic abnormalities are suspected). Additional indications for referral include non-monosymptomatic enuresis; developmental, attention or learning difficulties; behavioral or emotional problems; and known or suspected physical or neurologic problems.

Conditioning regime: Conditioning awakening to the sensation of a full bladder is the most benign and successful of the generic treatments of enuresis since its description in 1938. A careful meta-analysis of decades of conditioning studies has shown an initial success rate (defined as a reduction to less than one wet night per month) of approximately 66%, with more than half the subjects experiencing long-term success^[39]. The few existing studies that compare conditioning with pharmacologic treatments have generally shown conditioning to be significantly more effective than imipramine^[40] and desmopressin (DDAVP)^[41].

Enuresis alarm

Enuresis alarms is the most commonly prescribed conditioning regime which has a level 1, grade A International Consultation on Incontinence (ICI) recommendation. Portable transistorized alarms that the child wears on the body have replaced the old bell-and-pad type, but the principle is the same. The first drops of urine moisten the fabric separating two electrodes, thereby completing the circuit and setting off the alarm that the child is wearing.

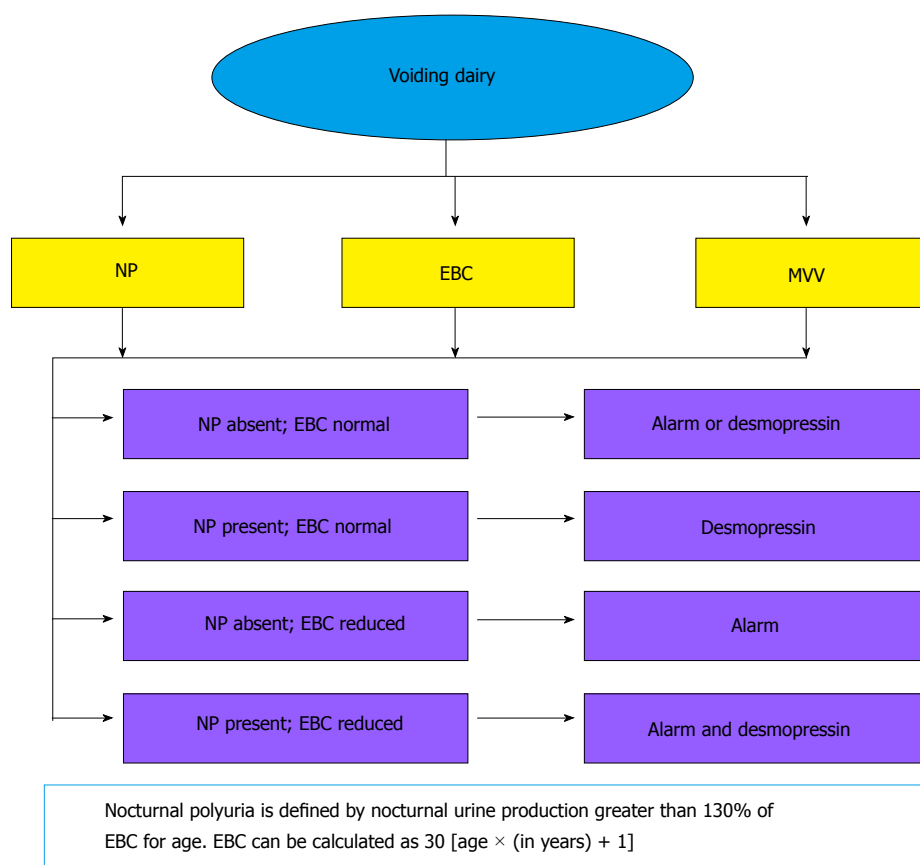


Figure 2 First line management of monosymptomatic nocturnal enuresis as per voiding diary^[45]. NP: Nocturnal polyuria; EBC: Expected bladder capacity; MVV: Maximum voiding volume.

Initially if children do not wake with the noise or vibration, it is important for their parents to wake them. Gradually the child awakens earlier and earlier in the course of the enuretic episode and the wet spot diminishes in size until the sensation of bladder fullness causes the child to awaken before wetting. Response is not immediate and treatment should be continued for 2-3 mo or until the child is dry for 14 consecutive nights (whichever comes first). Success is followed by over-learning (e.g., extra drinks are given at bedtime to cause additional stress to the detrusor muscles in the bladder. Alarm treatment is then continued until 14 consecutive dry nights are once again achieved) and intermittent reinforcement in which the child uses the alarm every other day before discontinuing it. Lack of parental help to awaken the child to finish voiding in the toilet is a major reason for failure of the conditioning treatment.

Enuresis alarm should not be tried if: (1) The child wets the bed only once or twice per week; (2) The child or parents do not seem to be enthusiastic about the enuresis alarm; (3) Rapid or short-term improvement seems to be the goal for the parents; and (4) The parents seem to express negative feelings/blame their child for wetting the bed.

Lack of success with the approach in the past or a relapse after previous success does not preclude successful subsequent treatment with a conditioning device. Throughout the behavioral treatment, rewarding the success with a sticker chart and reinforcing positive

change is critical to maintaining the child's investment in the process. Enuresis alarms are by far the most effective means of long term control as well as preventing relapses. In a meta-analysis of 56 randomized trials (3257 children), sixty-six percent of children became dry for 14 consecutive nights during alarm use vs only 4% in the no-treatment control group [relative risk (RR) for treatment failure 0.38, 95%CI: 0.33-0.45]. Additionally nearly a half of children remained dry even after stopping the treatment, compared with almost none in the no-treatment group (45% vs 1%, RR for relapse 0.56, 95%CI: 0.46-0.680)^[42].

Pharmacotherapy: Two medications, DDAVP and imipramine have proven efficacy in the treatment of enuresis.

Desmopressin is a synthetic analogue of the ADH vasopressin which has been used to treat central diabetes insipidus, bleeding disorders such as von Willebrand disease and primary nocturnal enuresis. It decreases urine production at night when taken at bedtime. Desmopressin has a level 1, grade A recommendation from the ICI in 2009^[43]. It is administered orally in 0.2 mg tablets in doses of 0.2 to 0.6 mg nightly or, less commonly, intra-nasally as a spray in doses of 10 to 40 µg (one to four sprays) nightly; the lowest effective dose is determined empirically with each child. Due to variable absorption and risk of over dosage nasal sprays are

usually not advocated. Desmopressin is also available as a fast-melting oral lyophilisate (melt; dosage, 120-360 µg). As this form does not require extra water to take this medication, it has become popular particularly for children under 12 years medication should be taken 1 h before the last void before bedtime to allow timely enhanced concentration of urine to occur. Fluid intake should be reduced from 1 h before desmopressin administration and for 8 h subsequently to encourage optimal concentrating capacity and treatment response, as well as to reduce the risk of hyponatremia/water intoxication. Desmopressin is primarily utilized as an alternative to enuresis alarms for children and families who seek rapid or short-term improvement of enuresis; where enuresis alarms have failed or have been refused by family or are unlikely to succeed because of family dynamics. The initial duration of treatment should be for 2-6 wk, to ascertain its anti-enuretic effect. If a sufficient degree of improvement is experienced, then treatment can be continued for an additional 3 mo - where appropriate. Structured withdrawal of medication may reduce relapse rates^[44]. If a second voiding diary indicates that nocturnal urinary production is not reduced, consider a dose increase. As a rule of thumb, one third of unselected enuretic children are reliably dry as long as they take the drug, one third has a partial response and one third is not helped at all. Fluid overload (water intoxication) is potentially the most serious complication with desmopressin. It is associated with overdrinking at bedtime and its symptoms include headache, nausea, hyponatraemia, cerebral oedema, and convulsions. Overall desmopressin has an excellent safety profile with very few significant adverse events reported^[45]. The reported success rates of DDAVP treatment for enuresis have ranged from 10% to 65%, but as many as 80% of patients relapse after treatment^[46]. Depression of endogenous ADH secretion is not a concern as children who have used DDAVP for as long as 1 year have demonstrated the ability to concentrate their urine appropriately in response to a water deprivation challenge. It seems reasonable at least to consider a trial of withdrawal of DDAVP at 3- to 6-mo intervals.

In comparison to arousal alarms, treatment effects were not sustained after discontinuation of therapy (the rate of failure or relapse was 65% and 46% with desmopressin and alarms, respectively; relative risk of failure 1.42, 95%CI: 1.05-1.91). Comparison with some tricyclic drugs (e.g., amitriptyline) suggests that they might be as effective as desmopressin although in two trials, children were less likely to achieve 14 dry nights with imipramine than desmopressin (RR 0.44, 95%CI: 0.27-0.73) but there was not enough information about subsequent relapse^[47]. There were more side effects with the tricyclics.

The British National Formulary currently suggests that drug therapy is not usually appropriate for children under 7 years of age and should be reserved for children in whom alternative measures have failed.

Failure to therapy

Inability to achieve > 50% improvement in symptoms is defined as resistant to therapy. If this happens despite an adequate trial of treatment with an enuresis alarm (i.e., three months) and/or desmopressin (at a dose of 0.4 mg) and in absence of any concern regarding the family/child's motivation then referral to a healthcare professional who specializes in the management of bedwetting (e.g., developmental behavioral pediatrician, pediatric urologist) may be warranted.

Possible reasons for lack of response include: (1) Overactive bladder; (2) Underlying disease (e.g., diabetes mellitus/diabetes insipidus); (3) Occult constipation; (4) Sleep apnea; (4) Incorrect use of alarm; and (5) Social and emotional factors.

If on additional evaluation (which may include repeat bladder diary, ultrasound scan (if not done before), rectal examination/abdominal X ray for constipation) no underlying aetiology is found then combination therapy or switch to imipramine, may be considered. For children with suspected day and night detrusor over activity/small functional bladder capacity, a combination of oxybutynin and desmopressin may be indicated (level 2, grade B).

Imipramine (a tri-cyclic anti-depressant) in a single bedtime dose of 1.0-2.5 mg/kg had been used for many years as a third line agent for enuresis. Tricyclic antidepressants (TCAs, e.g., imipramine, amitriptyline and desipramine) decrease the amount of time spent in REM sleep, stimulate vasopressin secretion, and relax the detrusor muscle although the exact mechanism of action in treating enuresis is unknown. Major rare significant adverse effects include cardiotoxicity and hepatotoxicity. Minor side-effects are related to their anti-cholinergic actions and include postural hypotension, dry mouth, constipation, perspiration, tachycardia, nausea, lethargy and insomnia. A pretreatment electrocardiogram may be obtained to detect any underlying rhythm disorder. Treatment can be continued for 4-6 mo. In a systematic review, compared with placebo, treatment with TCA was associated with reduction of approximately one wet night per week^[48].

Other drugs

Anti-cholinergic drug tolterodine, oxybutynin and propiverine is in fact useful as an add-on therapy in enuretic children who have not responded to desmopressin alone^[49]. They carry a risk for constipation and for UTI due to the accumulation of residual urine. Other drugs, including indomethacin, phenmetrazine, amphetamine sulfate, ephedrine, atropine, furosemide, diclofenac, and chlorprothixene have been tried in the treatment of nocturnal enuresis. A 2012 systematic review of randomized trials of drugs other than tricyclic antidepressants and desmopressin found that although indomethacin, diclofenac, and diazepam were better than placebo in reducing the number of wet nights, none of the drugs was better than desmopressin^[50]. Atomoxetine used in ADHD has been

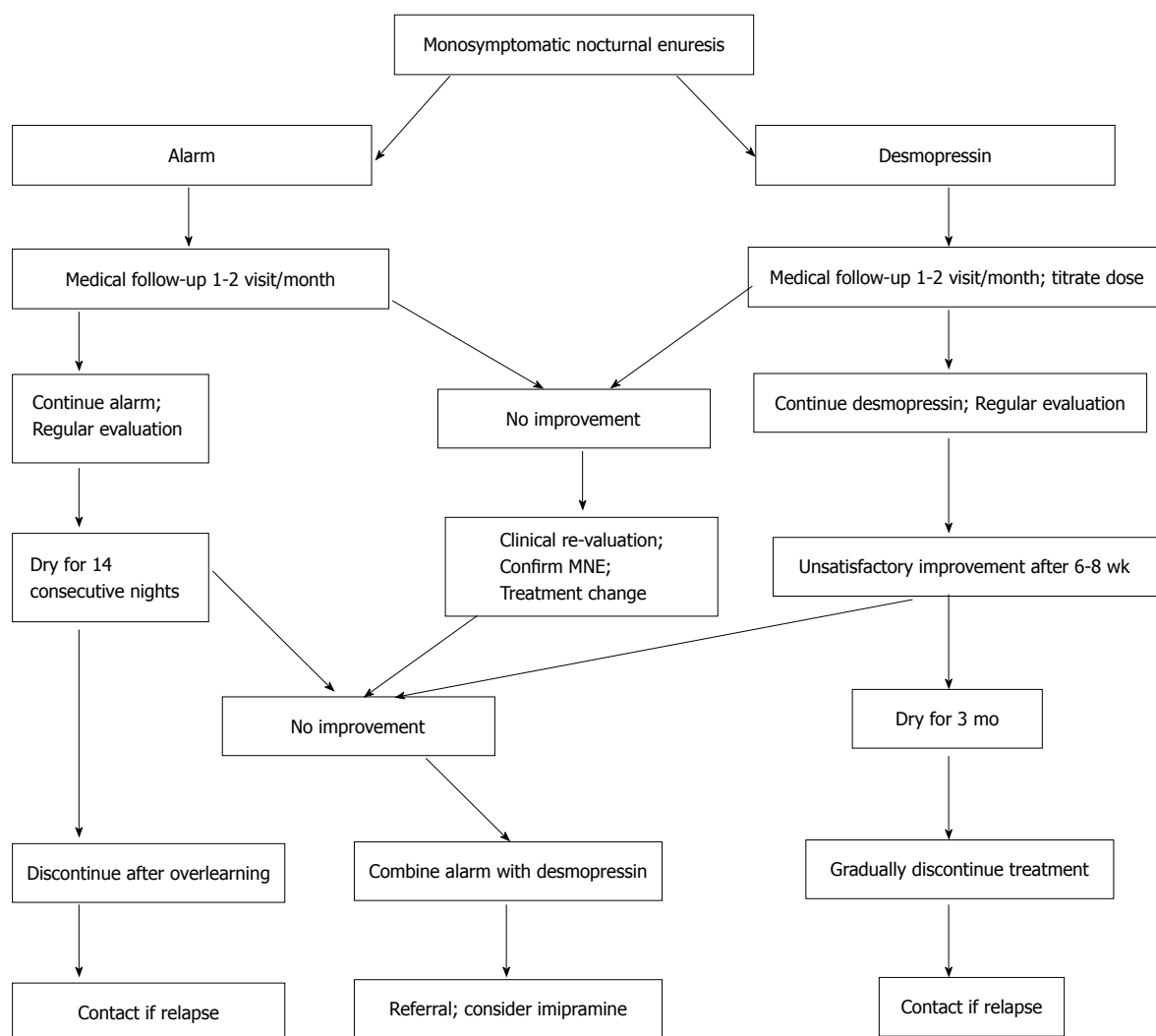


Figure 3 Treatment flowchart for monosymptomatic nocturnal enuresis. MNE: Monosymptomatic enuresis.

found to decrease frequency of bedwetting among children with enuresis with or without^[51].

In summary: Differentiating between MNE and NMNE forms the cornerstone in the management of these children. Once MNE is identified and initial steps like counseling, bladder diaries, *etc.*, have failed the first treatment for the family who is well-motivated and well informed is the enuresis alarm. Desmopressin is the first line treatment for families who are not sufficiently motivated to use the alarm, who have recently used the alarm (correctly) without success or who are considered unlikely to comply with alarm treatment. Anti-cholinergic therapy has the greatest chance of success in the child with signs of detrusor over activity, *i.e.*, low daytime voided volumes. If desmopressin, the alarm and the anti-cholinergic treatment have all been tried without success, or have been judged unsuitable, the cautious use of imipramine may be considered (Figure 3).

Follow-up: Following successful treatment with either the alarm or desmopressin, patients should be advised

to contact the clinic if relapse is experienced after discontinuation of therapy. If relapse occurs, desmopressin, alarm, or combined therapy should be re-considered. The most likely fundamental reason for not responding to alarm or desmopressin therapy is that the actual diagnosis is NMNE and not MNE. When a detailed history is obtained, the majority of these children have at least subtle daytime symptoms. If a patient is treatment-resistant and a bladder diary has not been completed, it is imperative this is undertaken or to refer the child to a specialty center as OAB and dysfunctional voiding may be present.

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Basic Study

Constitutive renal Rel/nuclear factor- κ B expression in Lewis polycystic kidney disease rats

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Author contributions: Schwensen KG conducted the animal study, performed sample collection and histological staining; Ta MHT performed the immunofluorescence, Western blotting, and qPCR experiments, analyzed data and drafted the manuscript; Huso DL and Watnick T developed the Pkd2 knockout mouse model and provided paraffin embedded sections that were utilized for staining; Liuwantara D assisted with data interpretation and provided technical guidance with experimental methods; Rangan GK conceived of the study, conducted the animal study, performed sample collection and histological staining and reviewed and edited the manuscript; all authors read and approved of the final manuscript.

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Abstract

AIM: To determine the temporal expression and pattern of Rel/nuclear factor (NF)- κ B proteins in renal tissue in polycystic kidney disease (PKD).

METHODS: The renal expression of Rel/NF- κ B proteins was determined by immunohistochemistry, immunofluorescence and immunoblot analysis in Lewis polycystic kidney rats

(LPK, a genetic ortholog of human nephronophthisis-9) from postnatal weeks 3 to 20. At each timepoint, renal disease progression and the mRNA expression of NF- κ B-dependent genes (TNF α and CCL2) were determined. NF- κ B was also histologically assessed in human PKD tissue.

RESULTS: Progressive kidney enlargement in LPK rats was accompanied by increased renal cell proliferation and interstitial monocyte accumulation (peaking at weeks 3 and 10 respectively), and progressive interstitial fibrosis (with α smooth muscle actin and Sirius Red deposition significantly increased compared to Lewis kidneys from weeks 3 to 6 onwards). Rel/NF- κ B proteins (phosphorylated-p105, p65, p50, c-Rel and RelB) were expressed in cystic epithelial cells (CECs) of LPK kidneys as early as postnatal week 3 and sustained until late-stage disease at week 20. From weeks 10 to 20, nuclear p65, p50, RelB and cytoplasmic I κ B α protein levels, and TNF α and CCL2 expression, were upregulated in LPK compared to Lewis kidneys. NF- κ B proteins were consistently expressed in CECs of human PKD. The DNA damage marker γ -H2AX was also identified in the CECs of LPK and human polycystic kidneys.

CONCLUSION: Several NF- κ B proteins are consistently expressed in CECs in human and experimental PKD. These data suggest that the upregulation of both the canonical and non-canonical pathways of NF- κ B signaling may be a constitutive and early pathological feature of cystic renal diseases.

Key words: Inflammation; Nuclear factor- κ B; Polycystic kidney disease; Tumour necrosis factor alpha; Chemokine CCL2

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Core tip: Until now, there has been limited information regarding the specific nuclear factor (NF)- κ B proteins involved in polycystic kidney disease (PKD) and their expression throughout disease progression. Our study demonstrated that a diverse array of NF- κ B proteins is expressed in the renal cyst-lining cells of a chronic rodent model of PKD, and that NF- κ B expression is constitutive over time. NF- κ B was also identified in human PKD, suggesting that NF- κ B upregulation is common to renal cystic disease models. Our data suggest that components of both the canonical and non-canonical NF- κ B pathway are upregulated in PKD. Future studies should be directed at verifying whether specific NF- κ B inhibition can attenuate interstitial inflammation and cyst growth, and slow the decline in renal function in *in vivo* models of PKD.

Ta MHT, Schwensen KG, Liuwantara D, Huso DL, Watnick T, Rangan GK. Constitutive renal Rel/nuclear factor- κ B expression in Lewis polycystic kidney disease rats. *World J Nephrol* 2016; 5(4): 339-357 Available from: URL: <http://www.wjgnet.com/2220-6124/full/v5/i4/339.htm> DOI: <http://dx.doi.org/10.5527/wjn.v5.i4.339>

INTRODUCTION

Polycystic kidney diseases (PKD) are a group of genetic disorders characterized by the formation of multiple renal cysts and an increased life-time risk for kidney failure^[1]. The two most common forms, autosomal dominant and recessive PKD (ADPKD and ARPKD), are due to mutations in *PKD1/2* and *PKHD1* respectively^[2-4]. These genes encode proteins that are localized to the cilia of most cells within the body, including renal tubule epithelia^[5,6]. Interstitial inflammation is a universal histological feature associated with renal cyst growth and formation^[7-9], and is possibly mediated by the release of pro-inflammatory cytokines from cyst-lining epithelial cells (CECs)^[8]. Abnormalities in apoptosis and increased proliferation of CECs^[7,10], interstitial fibrosis^[11] and hypertension^[12] are also typical features of PKD.

The nuclear factor (NF)- κ B system is a key regulator of pro-inflammatory and pro-apoptotic gene transcription^[13,14]. The Rel proteins, namely p65 (RelA), RelB and c-rel, contain transcription-binding domains (TADs) that allow them to bind DNA^[13,14]. In contrast, p50 (NF- κ B1) and p52 (NF- κ B2), which are derived from the breakdown of p105 and p100 respectively, do not possess TADs and therefore must bind to Rel proteins in order to modulate transcription^[13,15]. In the inactive state, NF- κ B proteins normally exist as dimers, and are bound to inhibitor of κ B (I κ B) proteins in the cytoplasm^[13]. Upon activation by certain stimuli, I κ B kinase (IKK) proteins are activated, phosphorylating the I κ B proteins and leading to their degradation, thus freeing the NF- κ B proteins to translocate to the nucleus and regulate transcription^[13,14]. NF- κ B signaling can be broadly classified as canonical or non-canonical^[13,16]. The canonical pathway is activated by a wide range of stimuli including tumor necrosis factor (TNF) α and lipopolysaccharide^[13,17], and typically involves the p65:p50 dimer and I κ B α ^[13,16,18]. The non-canonical pathway is known to be stimulated by fewer stimuli (e.g., CD40 ligand and lymphotoxin- β 2^[16]) and usually implicates the RelB:p52 dimer in mediating lymphoid organ development^[13,16].

There is an overlap between NF- κ B-regulated genes and the pathophysiological features of PKD, such as inflammation (e.g., TNF α , CCL2), cell growth (e.g., *cyclin D1*), apoptosis (e.g., *Bcl-xL*) and hypertension (e.g., *ANGII*)^[8,9,12,17,19]. These commonalities provide a theoretical basis for the involvement of NF- κ B in PKD. Recent studies have provided preliminary evidence for NF- κ B involvement in the pathogenesis of cystic renal disease. For example, the use of small interfering RNA (siRNA) to over-express or delete ciliary proteins *in vitro*, leads to upregulation of NF- κ B signaling^[20,21]. Park *et al*^[22] demonstrated that *PKD2* transgenic mice have higher levels of renal NF- κ B proteins and phosphorylated-IKK α / β compared to wild type controls. Moreover, upregulated NF- κ B expression has been identified in the CEC nuclei in *Pkd1*^{-/-} and *PKD2* transgenic mice^[22], and in human ADPKD^[22].

Despite the complexity of the NF- κ B system, the previous studies of NF- κ B in PKD mainly or solely focused on p65, and in some cases did not specify the

particular NF- κ B subunits that were investigated^[22,23]. Therefore, the first aim of the current study was to examine the expression and localization of a spectrum of NF- κ B proteins, in a chronic disease model of PKD and in human cystic renal disease. We investigated the expression of p65, p50, RelB and c-rel, as well as I κ B α and phosphorylated p105 (P-p105, which is synthesized prior to p50 production and accordingly is a marker of NF- κ B upregulation^[24]). In addition, cyst development in ADPKD is progressive, beginning *in utero* and continuing through to the later decades of life^[9], but no studies have examined whether the NF- κ B system changes throughout disease progression in PKD. Thus, the second aim of this study was to characterize NF- κ B expression over the time-course of disease in the Lewis polycystic kidney (LPK) rat (a chronic model of ARPKD^[25]). Based on data showing that the expression of a NF- κ B-dependent gene, *TNF α* , is upregulated in *cpk* mice and increases further over time^[26], and previous correlations between *TNF α* and NF- κ B signaling in *in vitro* models of PKD^[27], we hypothesized that NF- κ B protein expression is elevated in LPK kidneys compared to control kidneys in the early stages of PKD, and increases incrementally throughout the course of the disease.

MATERIALS AND METHODS

Lewis polycystic kidney disease model of PKD

The LPK rat is a genetic ortholog of *NPHP9* (human nephronophthisis) due to a point mutation in *Nek8*^[28]. It is characterized by post-natal distal nephron and collecting duct ectasia which progress over approximately 20 wk, and is associated with hypertension and renal failure^[25]. In this study, LPK rats and Lewis/SSN rats were obtained from the breeding colony at Westmead Hospital. This colony was established in 2008 from a single founder homozygous breeding pair from the Animal Resources Centre (Perth, Western Australia). The colony was maintained by mating homozygous male and female breeder pairs. Animals were housed under standard conditions and allowed food and water *ad libitum* at the Animal Care Department at Westmead Hospital. All protocols and procedures were approved by the Western Sydney Local Health District Animal Ethics Committee, (Protocol number 4100). Since disease is more severe in male than in female LPK rats^[25], for this study, male LPK rats were sacrificed at postnatal weeks 1, 2, 3, 4, 6, 10, 16 and 20 ($n = 6-9$ per timepoint) and were compared to male Lewis animals at the same timepoints ($n = 3-6$ per timepoint).

Assessment of kidney enlargement and renal function in LPK rats

Kidney enlargement was determined using the kidney to body weight ratio (KW:BW) at the time of tissue collection. Renal function was assessed in blood at the time of sacrifice, and was analyzed by the Institute of Clinical Pathology and Medical Research. To assess proteinuria and creatinine clearance (CrCl), rats were placed in metabolic

cages for 16 h. Creatinine clearance was calculated using $\text{CrCl} = [\text{Urine creatinine } (\mu\text{mol/L}) \times \text{Urine volume (mL/min)}] / \text{Serum creatinine } (\mu\text{mol/L})$, and was corrected for body weight. Rats were euthanized as previously described^[29].

Immunohistochemistry

Coronal kidney slices were fixed in either 37 g/L formaldehyde or methyl Carnoy's solution for 24 h, then paraffin embedded. For immunohistochemistry, 4 μm sections were deparaffinized, blocked with 0.03 g/mL hydrogen peroxide, and antigen retrieval was performed by microwave oven heating (for formalin slides only, 100% power, 10 min in 1 \times Antigen Decloaker, Biocare Medical, CA). Sections were blocked with 100 mL/L goat serum, and incubated with primary antibodies for 1 h overnight at 4 $^{\circ}\text{C}$. The primary antibodies used were: (1) anti-Ki67 to assess proliferation (1:100, ab16667, Abcam, Cambridge, United Kingdom); (2) anti-ED-1 for CD68⁺ monocytes to assess inflammation (1:400, MCA341R, Serotec, United Kingdom); (3) anti- α smooth muscle actin (α -SMA) to assess myofibroblast accumulation (and also a marker of vascular smooth muscle cells, 1:4000, A2547, Sigma-Aldrich, St. Louis, MO); and (4) anti-phosphorylated-p105 (P-p105) to assess NF- κ B expression (1:50, #4808 Cell Signaling Technology, Danvers, MA). Secondary biotinylated antibodies were applied for 30 min at room temperature (anti-mouse, 1:200, 65-6440; anti-rabbit, 1:200, 65-6140, Life Technologies, Carlsbad, CA). Vectastain ABC reagent (Vector Laboratories, Burlingame, CA) was applied for 20 min, followed by diaminobenzidine. Sections were counterstained with methyl green (Sigma-Aldrich) then dehydrated. To assess interstitial fibrosis, Sirius Red staining was performed on methyl Carnoy's fixed sections with 1 g/L Direct Red 80 and 1 g/L Fast Green FCF (Sigma-Aldrich) for 24 h. To quantify immunohistology, whole slide digital images (20 \times magnification) were acquired using a scanner (ScanScope CS2, Aperio, CA). Percentage cyst volume was assessed in Periodic Acid Schiff (PAS) stained sections by whole-slide digital analysis in Aperio ImageScope (v11.2.0.780).

Immunofluorescence

For immunofluorescence, formalin-fixed slides were deparaffinized and antigen retrieval was performed by microwave oven heating as described above. Sections were soaked in Tris Buffered Saline (TBS), 4 mL/L TritonX, for 30 min then blocked with 30 g/L BSA in TBS, 2 g/L Tween20 for 1 h. Slides were incubated with primary antibodies for 1 h overnight at 4 $^{\circ}\text{C}$: (1) p65 (1:100, #8242, Cell Signaling); (2) p50/p105 (1:100, ab7971, Abcam); (3) RelB (1:100, bs-3562R, Bioss Antibodies, Woburn, MA); (4) c-rel (1:100, orb5913, Biorbyt, Cambridge, United Kingdom); and (5) γ -H2AX (1:100, ab2893, Abcam). Secondary antibody (1:200, Alexa Fluor 546 goat anti-rabbit IgG, A-11010, Life Technologies) was applied for 30 min at room temperature, following by DAPI for 5 min. Slides were mounted using Fluorescence Mounting Medium (Dako, Glostrup, Den-

mark). Immunofluorescence was assessed using an Olympus BX53/DP80 microscope (Olympus Corporation, Shinjuku, Japan) and images were taken at 20 \times and 40 \times magnification using the software (cellSens, v1.6, Olympus).

Western blot

Nuclear and cytosolic extracts were obtained from 100 mg of kidney cortex from Lewis and LPK animals at weeks 3, 10 and 20, using a previously described method^[30] and stored at -80 $^{\circ}$ C. Protein concentration of the extracts was assessed using the DC Protein Assay (Bio-Rad). Western blot was performed as previously described^[31]. Primary antibodies used were: p50/105 (1:1000, ab7971, Abcam), p65 (1:1000, #8242, Cell Signaling Technology, Danvers, MA), RelB (1:1000, #4922, Cell Signaling), I κ B α (1:1000, #4814, Cell Signaling), β -actin (1:1000, #4970, Cell Signaling), and GAPDH (1:1000, #5174, Cell Signaling). Densitometry was quantified using ImageJ (v1.47, National Institutes of Health, United States) and normalized using β -actin (for nuclear extracts) or GAPDH (for cytoplasmic extracts).

Quantitative real-time polymerase chain reaction

Quantitative real-time polymerase chain reaction (qPCR) was performed to assess the mRNA expression of two pro-inflammatory genes, *TNF α* and chemokine (C-C motif) ligand 2 (*CCL2*). Renal tissue was snap-frozen in liquid nitrogen and stored at -80 $^{\circ}$ C. RNA was extracted using the RNeasy Mini Kit (Qiagen, Venlo, Limburg, Netherlands). RNA was reverse-transcribed into cDNA (SuperScript III First-Strand Synthesis System, Thermo Fisher Scientific, Waltham, MA) using oligo(dT) and dNTP, and using the cDNA Synthesis Mastermix according to manufacturer's instructions. Real-time quantitative PCR was performed using Platinum SYBR Green qPCR SuperMix-UDG (Thermo Fisher) on a Bio-Rad CFX96 machine (Bio-Rad Laboratories, Hercules, CA). The PCR primers were: *TNF α* (forward: 5' GTC GTA GCA AAC CAC CAA GC 3', reverse: 5' TGT GGG TGA GGA GCA CAT AG 3')^[32], *CCL2* (forward: 5' AGC CCA GAA ACC AGC CAA CTC 3', reverse: 5' GCC GAC TCA TTG GGA TCA TCT T 3')^[33], and *GAPDH* (forward: 5' GAA CAT CAT CCC TGC ATC CA 3', reverse: 5' CCA GTG AGC TTC CCG TTC A 3')^[34]. PCR parameters were 95 $^{\circ}$ C for 2 min and 95 $^{\circ}$ C for 15 s, 60 $^{\circ}$ C for 30 s, and 72 $^{\circ}$ C for 30 s for 40 cycles; melting temperature was measured between 65 $^{\circ}$ C and 95 $^{\circ}$ C. Data were analyzed using CFX Manager software (v3.1.1517.0823, 2012 release, Bio-Rad). Gene expression was quantified using the $\Delta\Delta$ CT method. The mRNA quantities of *TNF α* and *CCL2* in each sample were normalized using *GAPDH* mRNA quantity.

Assessment of NF- κ B expression in human PKD

Renal tissue was obtained from two ADPKD patients (ID no. P17 and P18) and one ARPKD patient (ID no. ARPKD1). Kidney tissue was also obtained from two non-PKD patients (ID no. C1 and C2; both kidneys were removed due to renal cancer, and the non-cancerous portions used as controls). All patients provided written informed consent,

and the study was approved by the Human Research Ethics Committee at Westmead Hospital (HREC/09/WMEAD/305; SSA/12/WMEAD/327). Samples were paraffin-embedded, and PAS staining, immunohistochemistry and immunofluorescence were performed as described for LPK kidneys.

Statistical analysis

Results were presented as mean \pm SD. The data were analyzed with the JMP statistical software package (v4.04, SAS institute, Carey, NC, United States) and graphed in GraphPad Prism (v6.04 for Windows, GraphPad Software, San Diego, CA). Comparisons between the experimental groups were performed by ANOVA, followed by a post-hoc analysis with the Tukey-Kramer HSD test. A *P*-value of < 0.05 was interpreted as statistically significant.

RESULTS

Time-course of renal disease progression in LPK rats

Body weight and kidney enlargement: Body weight increased steadily in both experimental groups from weeks 1 to 20, but was lower in LPK animals (Figure 1A). In LPK rats, the KW:BW was increased compared to Lewis rats as early as postnatal week 1 and continued to rise until week 16 (Figure 1B). The rate of increase in KW:BW was greatest between weeks 6 and 10 (239% increase) whereas between weeks 10 and 20 only a further 16% increase was observed (Figure 1B).

Histological pattern of renal disease: Qualitative histological analysis showed that there was progressive dilatation of collecting ducts and distal tubules over time in LPK kidneys (Figure 2). At week 1, cystic renal disease was mild, characterized by focal areas of rounded collecting duct dilatation in the corticomedullary region. From week 2 onwards, the rounded dilatation developed into rectangular elongation. As shown in Table 1, the mean dimensions (width and length) of the cyst tubular dilatation increased progressively from week 1 to 20. These histological changes were accompanied by time-dependent increases in renal interstitial inflammation (ED-1 immunohistochemistry) and fibrosis (α -SMA immunohistochemistry and Sirius Red staining) in LPK rats (Table 1). As previously shown^[35], cell proliferation (Ki-67 positive cells) peaked at week 3 in LPK rats. Interstitial ED-1 and α -SMA were highest at week 10 (Table 1). Collagen deposition (as measured by Sirius Red) was elevated in LPK kidneys from week 6 onwards (Table 1).

Renal function: LPK rats developed an increase in 24 h urine volume compared to Lewis rats from week 10 (Table 2). A decline in renal function was indicated by the elevated levels of serum creatinine in LPK compared to Lewis rats from week 10 onwards (Table 2). Creatinine clearance was decreased in LPK rats compared to Lewis at weeks 10 and 16. Serum urea was elevated in LPK rats at all timepoints (Table 2).

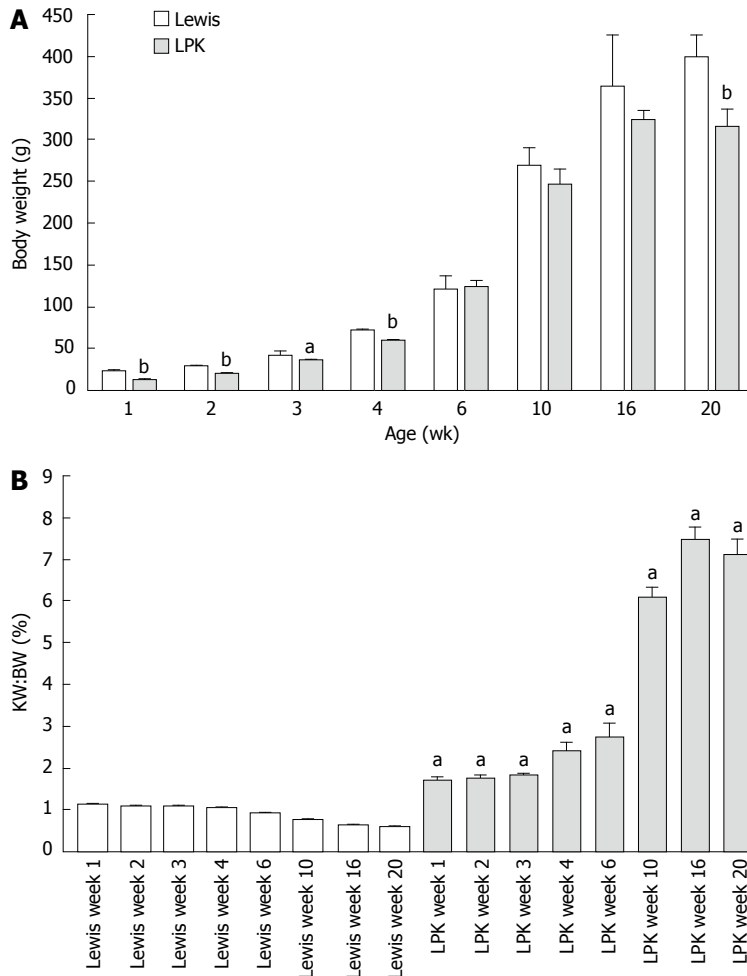


Figure 1 Body weight and kidney weight over time in Lewis and Lewis polycystic kidney rats. A: Body weight of male Lewis and LPK rats from weeks 1 to 20. Data as mean \pm SD. ^a $P < 0.05$ vs age-matched Lewis animals; ^b $P < 0.01$ vs age-matched Lewis animals; B: Percentage two kidney weight to body weight ratio (KW:BW) of male Lewis and LPK rats from weeks 1 to 20. Data as mean \pm SD. ^a $P < 0.05$ vs age-matched Lewis animals. LPK: Lewis polycystic kidney.

Table 1 Histological analysis of Lewis and Lewis polycystic kidney rats from weeks 1 to 20

Parameter		Week 1	Week 3	Week 6	Week 10	Week 16	Week 20
Cyst length (μ m)	Lewis	ND	ND	ND	ND	ND	ND
	LPK	106 \pm 4	347 \pm 88	938 \pm 280	1548 \pm 237	2252 \pm 436	3718 \pm 922
Cyst diameter (μ m)	Lewis	ND	ND	ND	ND	ND	ND
	LPK	69 \pm 18	101 \pm 19	279 \pm 71	442 \pm 81	681 \pm 121	1119 \pm 256
Ki-67 (%)	Lewis	24.3 \pm 8.3	1.9 \pm 0.6	2.4 \pm 1.5	0.2 \pm 0.1	0.3 \pm 0.1	0.2 \pm 0.02
	LPK	7.0 \pm 3.8 ^a	9.9 \pm 2.6 ^a	2.2 \pm 1.5	1.1 \pm 0.4 ^a	1.9 \pm 0.8 ^a	0.8 \pm 0.3 ^a
ED-1 (%)	Lewis	1.87 \pm 0.61	1.26 \pm 0.20	1.32 \pm 0.95	0.56 \pm 0.08	0.22 \pm 0.11	0.16 \pm 0.02
	LPK	1.58 \pm 0.15	3.81 \pm 1.02 ^a	4.87 \pm 1.21 ^a	12.37 \pm 4.10 ^a	0.81 \pm 0.38 ^a	0.84 \pm 0.24 ^a
Sirius Red (%)	Lewis	0.86 \pm 0.38	4.32 \pm 1.58	1.67 \pm 0.52	2.91 \pm 0.77	0.51 \pm 0.43	0.64 \pm 0.40
	LPK	1.96 \pm 1.15	2.88 \pm 1.51 ^a	5.92 \pm 4.20 ^b	16.63 \pm 8.12 ^b	10.34 \pm 5.82 ^b	18.24 \pm 6.00 ^b
α -SMA (%)	Lewis	17.2 \pm 2.12	1.68 \pm 0.42	1.87 \pm 0.32	0.98 \pm 0.86	1.04 \pm 0.06	1.09 \pm 0.13
	LPK	22.4 \pm 2.80	8.70 \pm 3.70 ^a	8.98 \pm 3.41 ^a	20.18 \pm 3.54 ^a	13.75 \pm 0.99 ^a	13.28 \pm 1.68 ^a

Data expressed as mean \pm SD; ^a $P < 0.05$ vs age-matched Lewis male animals; ^b $P < 0.01$ vs age-matched Lewis male animals. α -SMA: Alpha-smooth muscle actin; BrdU: 5-Bromo-2'-deoxyuridine; ND: Not determined.

Time-course of renal Rel/NF- κ B protein localization in LPK rats

p50: In kidneys from Lewis rats, p50 expression was weak and diffuse and localized predominantly to the cytoplasm of tubular cells (Figure 3). In LPK kidneys, this pattern of expression was maintained, but in addition there was strong staining of epithelial cells lining the dilated tubular cystic segments (Figure 3). The staining, which occurred in the majority of cysts, was

detected as early as week 3 and remained persistent at weeks 10 and 20. Populations of intensely staining interstitial cells were also noted in LPK kidneys in the outer medulla at weeks 6 and 20 (data not shown).

P-p105: In Lewis rats, at week 3, P-p105 staining was observed in focal areas of tubules and in selected cortical glomerular cells (Figure 4). This pattern of staining was stronger at weeks 10 and 20. In LPK rats,

Table 2 Renal function for male Lewis and Lewis polycystic kidney rats from weeks 3 to 20

Parameter		Week 3	Week 6	Week 10	Week 16	Week 20
24 h urine volume (mL)	Lewis	ND	6 ± 1	9 ± 2	9 ± 2	ND
	LPK	ND	7 ± 1	25 ± 2 ^b	23 ± 9 ^b	ND
Serum creatinine (μmol/L)	Lewis	24 ± 3	22 ± 5	29 ± 10	24 ± 1	27 ± 5
	LPK	20 ± 2 ^a	28 ± 14	33 ± 3 ^b	63 ± 9	191 ± 38 ^b
Endogenous CrCl (mL/min)	Lewis	ND	0.5 ± 0.2	1.8 ± 0.3	2.5 ± 0.6	ND
	LPK	ND	0.5 ± 0.2	0.8 ± 0.1 ^b	0.6 ± 0.2 ^b	ND
CrCl/BW (fold-change over Lewis)	Lewis	ND	1 ± 0.4	1.6 ± 0.1	1.7 ± 0.3	ND
	LPK	ND	0.9 ± 0.3	0.8 ± 0.1 ^b	0.4 ± 0.1 ^b	ND
Serum urea (mmol/L)	Lewis	5 ± 1	6 ± 1	6 ± 1	6 ± 1	8 ± 1
	LPK	7 ± 0 ^b	8 ± 1 ^b	14 ± 1 ^b	22 ± 3 ^b	44 ± 4 ^b

Data expressed as mean ± SD; ^a*P* < 0.05 *vs* age-matched Lewis male animals; ^b*P* < 0.01 *vs* age-matched Lewis male animals. BW: Body weight; CrCl: Creatinine clearance; ND: Not determined.

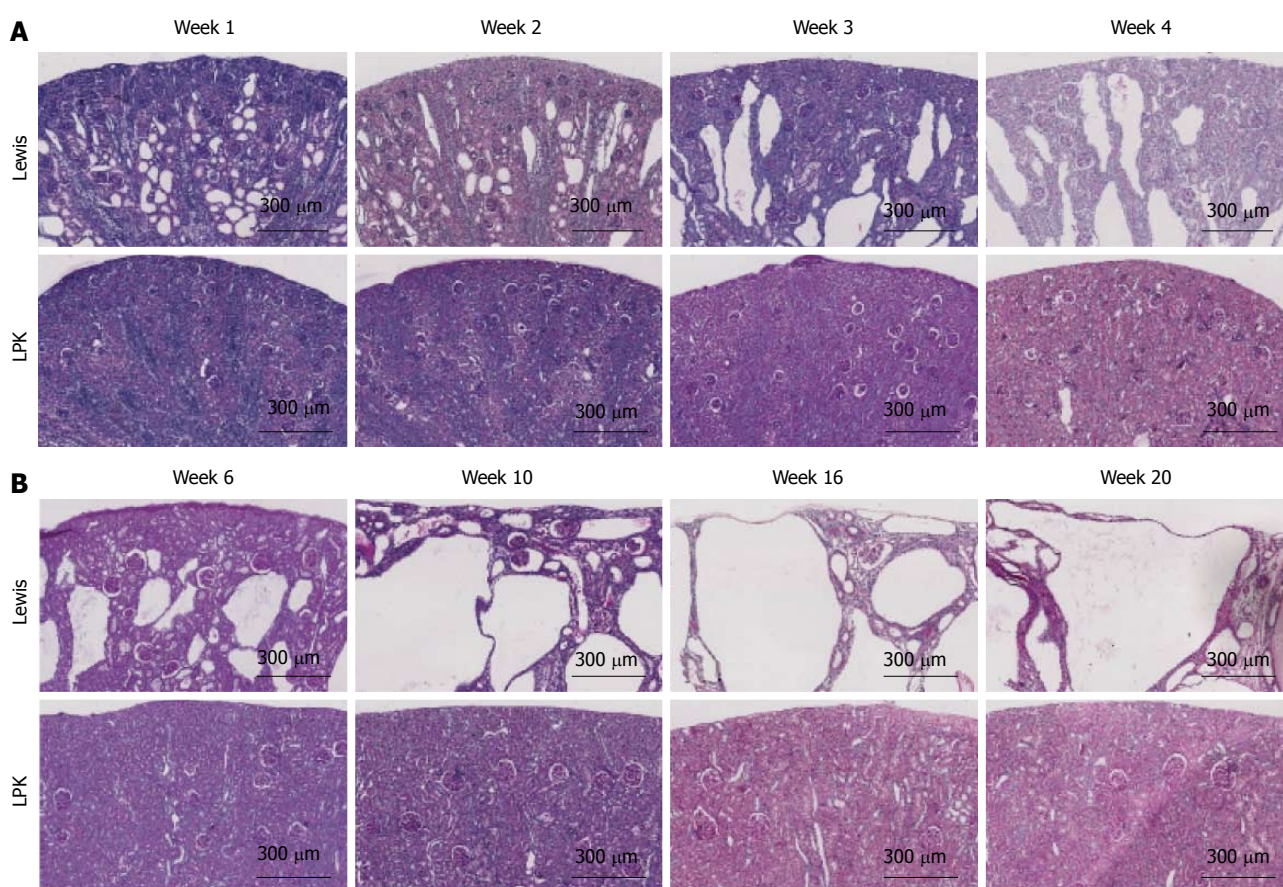


Figure 2 Whole-slide digital images of Periodic Acid Schiff-stained sections of Lewis control and Lewis polycystic kidney rat kidneys, at (A) weeks 1 to 4, and (B) weeks 6 to 20. LPK: Lewis polycystic kidney.

P-p105 was strongly expressed in cystic epithelial cells at week 3, 10 and 20 in the majority of cysts (Figure 4).

p65: In Lewis rats, p65 was localised to the cytoplasm of tubules. Expression was strongly upregulated at week 3, but declined at weeks 10 and 20 (Figure 5). In LPK kidneys, p65 was strongly expressed in the cytoplasm of cortical CECs in the majority of cysts at all timepoints, starting from week 3 (Figure 5).

RelB: In Lewis rats, RelB expression in the kidney was

weak at all timepoints (Figure 6). In contrast, in LPK there was strong staining of cysts at mid to late disease, particularly at weeks 10 and 16 (Figure 6).

c-rel: In Lewis rats, the renal expression of c-rel was localized to the nuclei and cytoplasm of tubules and interstitial cells (Figure 7). In control animals, nuclear c-rel expression was higher at week 3, declining at weeks 10 and 20, but cytoplasmic c-rel expression remained consistent over time. In LPK rats, cystic epithelial cell staining was evident at weeks 3 to 20, in the nuclei and

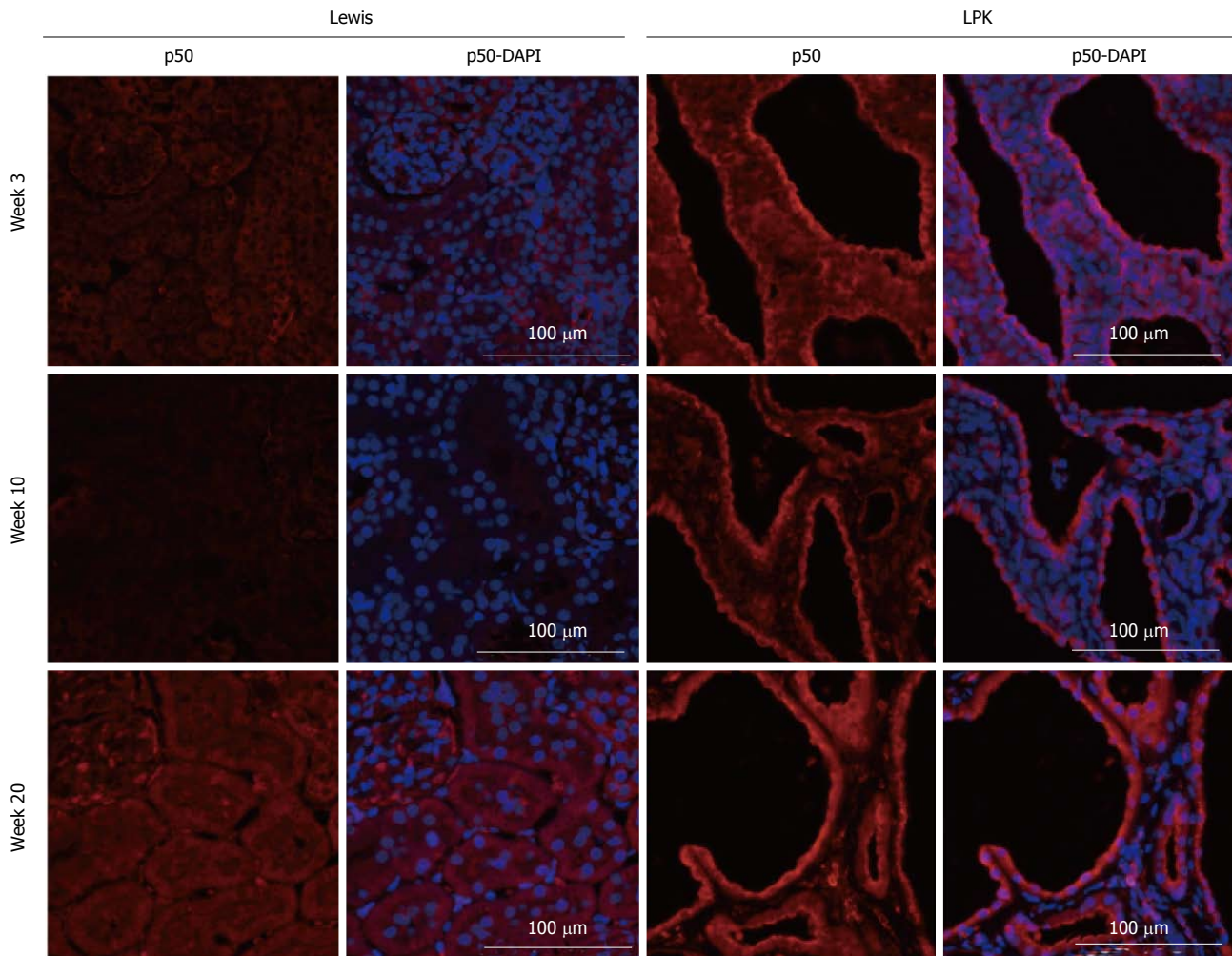


Figure 3 Immunofluorescence staining for p50 (red) at weeks 3, 10 and 20 in Lewis and Lewis polycystic kidney cortex. Also shown are corresponding DAPI-merged images with nuclei labeled using DAPI (blue). LPK: Lewis polycystic kidney.

cytoplasm (Figure 7).

Time-course of nuclear Rel/NF- κ B and cytoplasmic I κ B α expression in the kidney

Western blotting of nuclear kidney extracts found that p65 expression was increased in LPK rats, peaking at week 10, when it was 18-fold higher than in Lewis animals (Figure 8). Similarly, p50 was elevated in LPK rats compared to the Lewis group, peaking at week 20 (Figure 8). For RelB, at week 3, nuclear expression was similar between Lewis and LPK rats (Figure 9). However, at weeks 10 and 20, there was a marked upregulation in nuclear RelB expression in LPK rats. Cytoplasmic I κ B α displayed the same temporal pattern of expression as nuclear RelB in LPK kidneys (Figure 9).

Time-course of renal NF- κ B-dependent gene expression in LPK rats

The upregulation of Rel/NF- κ B proteins in LPK rats was accompanied by time-dependent increases in the mRNA expression of NF- κ B-dependent genes (*CCL2* and *TNF α* mRNA), which were elevated in LPK rats compared to the Lewis group, particularly at the late stages of

disease (Figure 10).

Renal expression of NF- κ B in human ADPKD and ARPKD

Human ADPKD and ARPKD kidneys displayed cystic dilatations of varying sizes, lined by either flattened single-layer, or hyperplastic epithelial cells (Figure 11). Abnormal glomeruli, interstitial fibrosis and large numbers of infiltrating cells were also observed. By immunofluorescence, in control kidneys, p50 staining was intensely expressed in proximal tubule brush borders, but weak in the glomeruli and in renal tubule cytoplasm (Figure 12). In ADPKD tissue, p50 staining was strong in the cytoplasm and moderate in the nuclei of CECs. Strong expression was also detected in intraluminal and interstitial cells. A similar pattern of staining was observed in ARPKD, with strong expression in CEC cytoplasm and moderate expression in nuclei (Figure 12). Among the CECs, p50 expression was notably more intense in areas of hyperplasia than in areas where the epithelial layer was single-cell thick. Staining was also strong and diffuse in interstitial cells (Figure 12). A similar pattern of staining was observed for P-p105, p65 and RelB (Figures 11 and

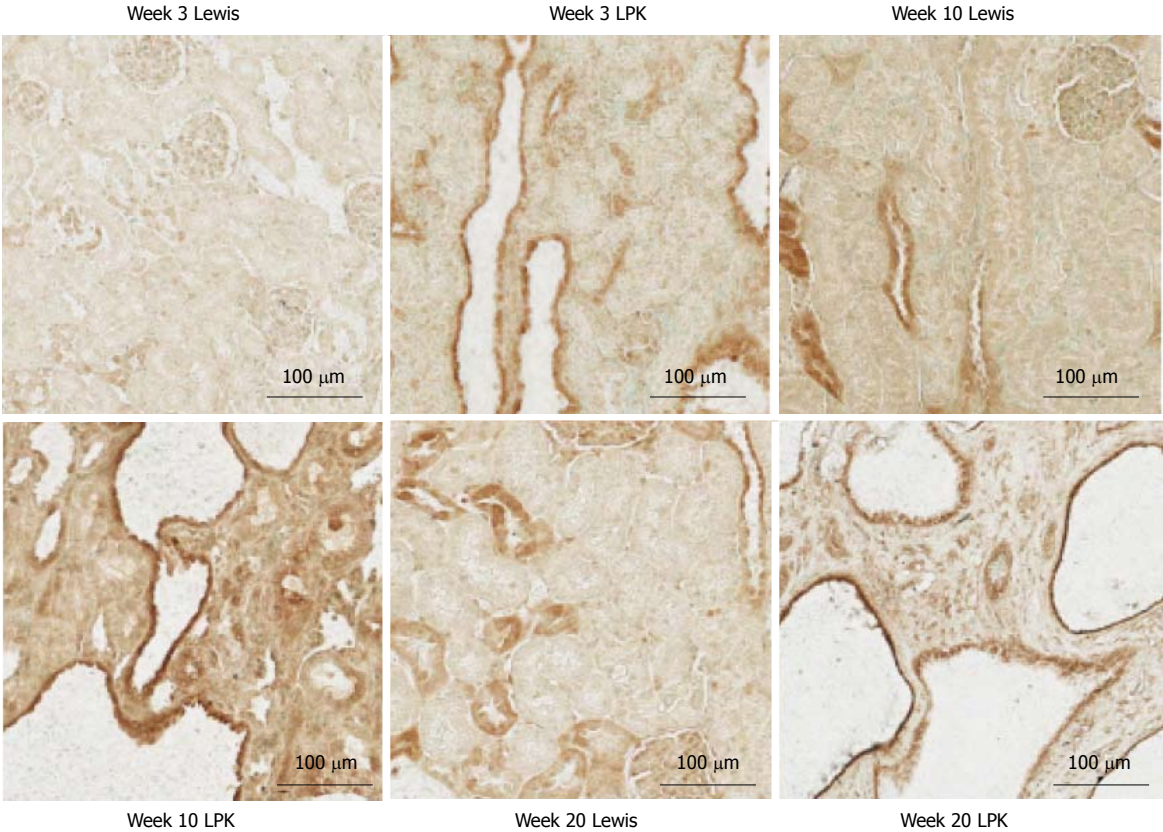
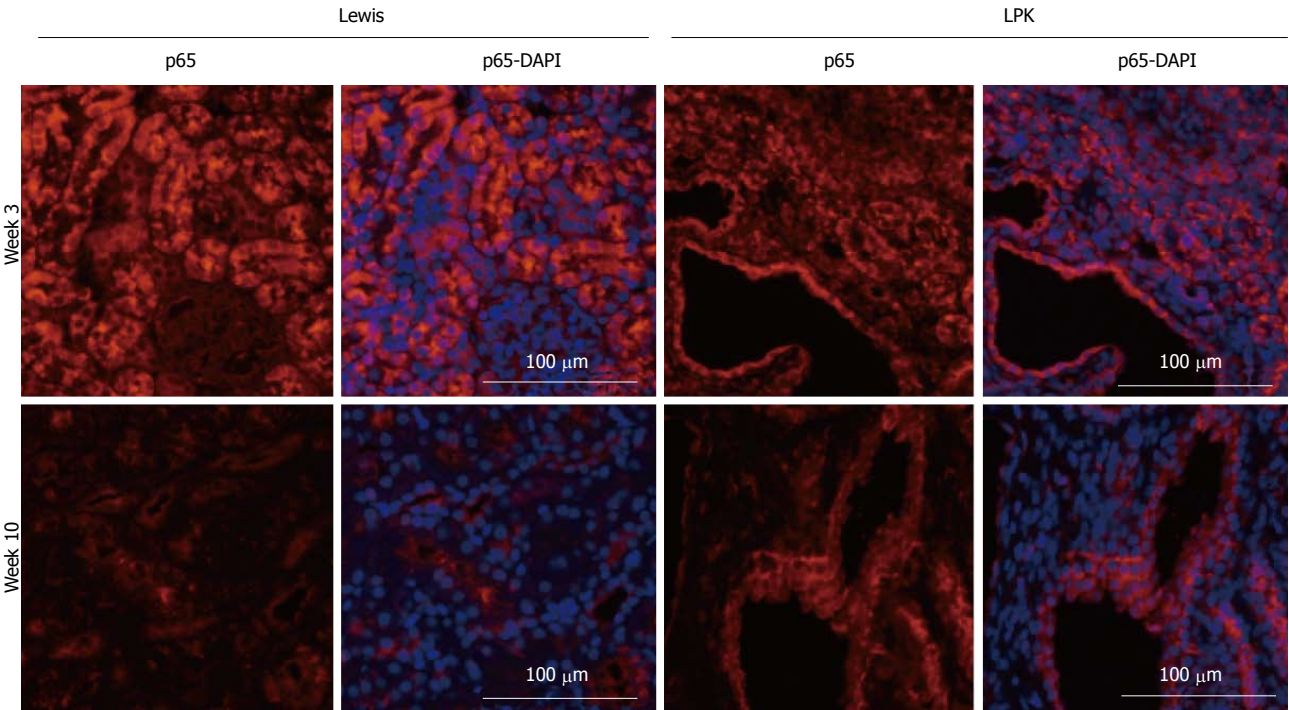


Figure 4 Immunohistochemistry for P-p105 at weeks 3, 10, and 20 in Lewis and Lewis polycystic kidney cortex. LPK: Lewis polycystic kidney.



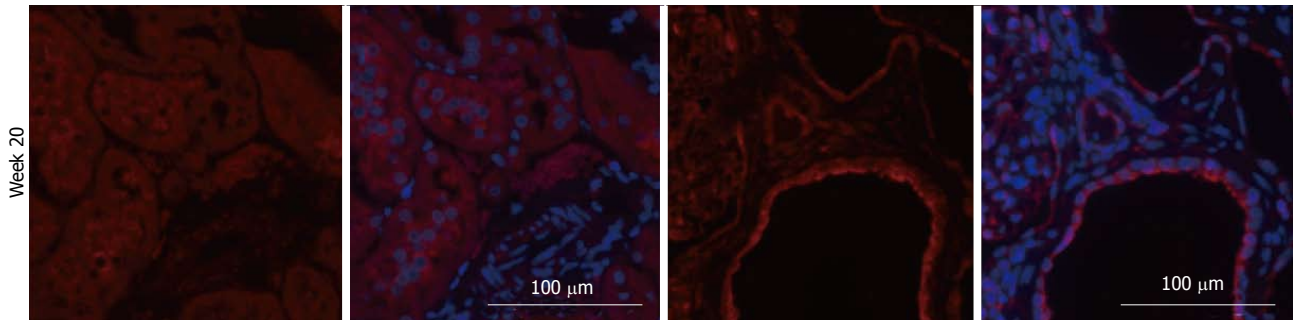


Figure 5 Immunofluorescence staining for p65 (red) at weeks 3, 10, and 20 in Lewis and Lewis polycystic kidney cortex. Also shown are corresponding DAPI-merged images with nuclei labeled using DAPI (blue). LPK: Lewis polycystic kidney.

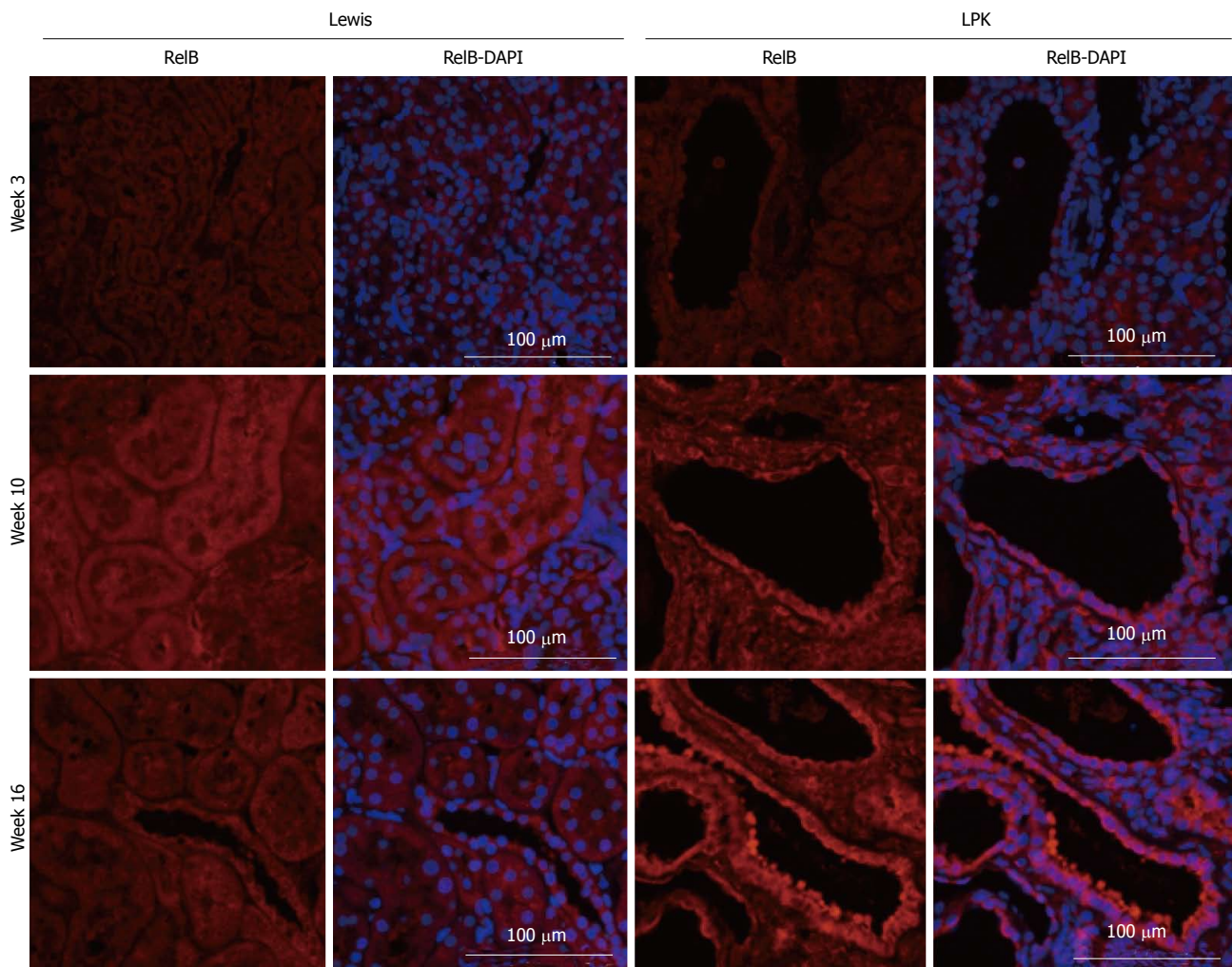


Figure 6 Immunofluorescence staining for RelB (red) at weeks 3, 10 and 16 in Lewis and Lewis polycystic kidney cortex. Also shown are corresponding DAPI-merged images with nuclei labeled using DAPI (blue). LPK: Lewis polycystic kidney.

12).

γ -H2AX expression in experimental and human PKD kidneys

Previous studies in *Nek8* mice revealed that DNA damage is evident early in diseased animals with this mutation^[36]. Therefore, to elucidate whether there is a relationship between NF- κ B activation and DNA damage, we assessed the expression of a phosphorylated histone H2A variant,

γ -H2AX, which is produced during double-strand DNA breakage^[37]. A time-course study of LPK kidneys showed that γ -H2AX was strongly expressed in tubular nuclei of Lewis kidneys at week 3, and weakly expressed in later life (Figure 13). In contrast, γ -H2AX expression in the CECs of LPK kidneys was consistent throughout disease progression (Figure 13). We also found that γ -H2AX was strongly expressed in the cyst-lining cells at postnatal week 1 when NF- κ B/Rel proteins were minimally

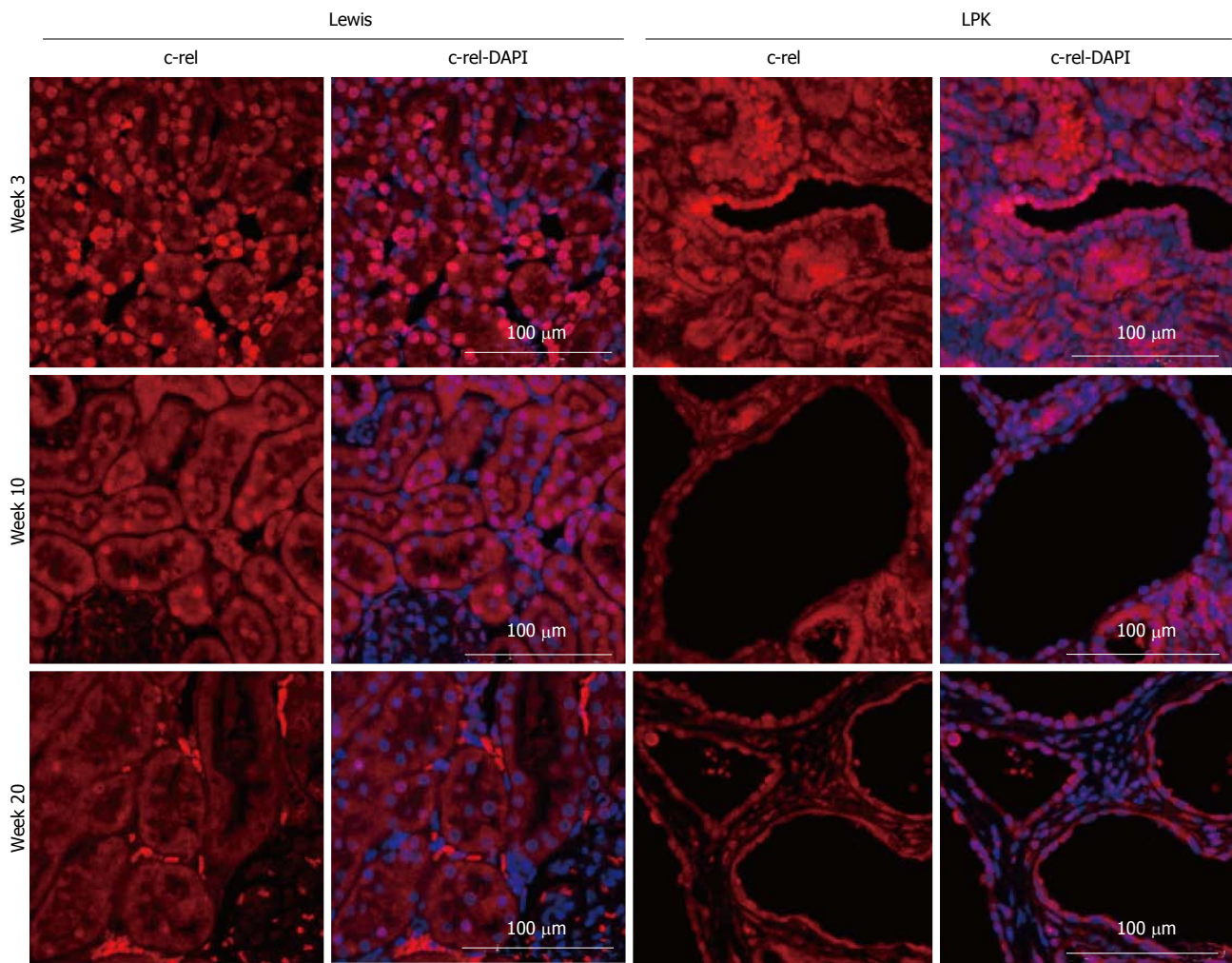
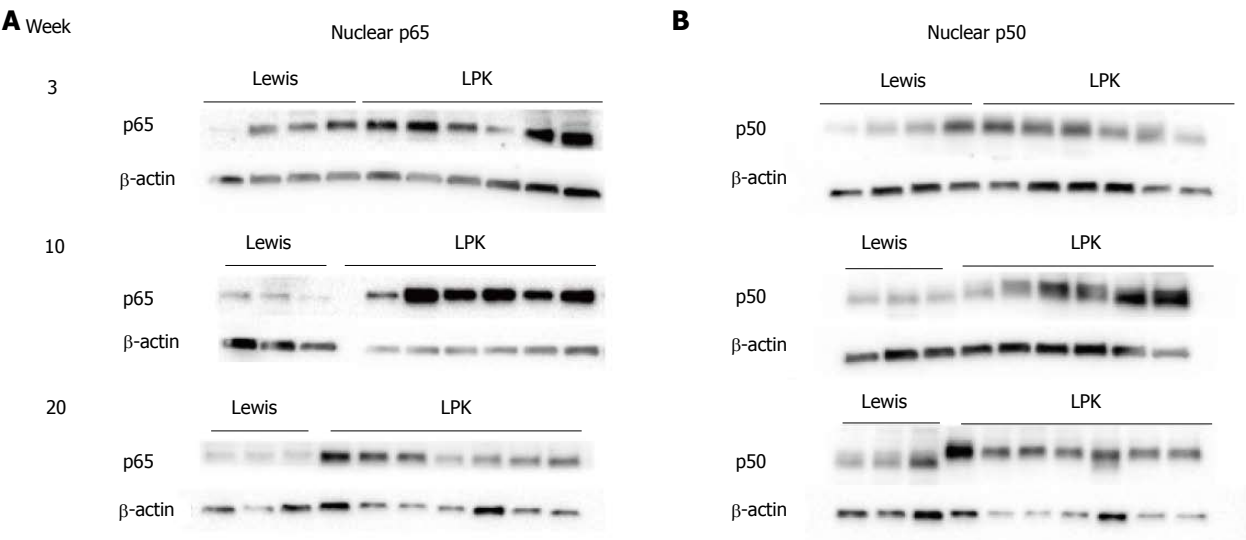


Figure 7 Immunofluorescence staining for c-rel (red) at weeks 3, 10, and 20 in Lewis and Lewis polycystic kidney cortex. Also shown are corresponding DAPI-merged images with nuclei labeled using DAPI (blue). LPK: Lewis polycystic kidney.



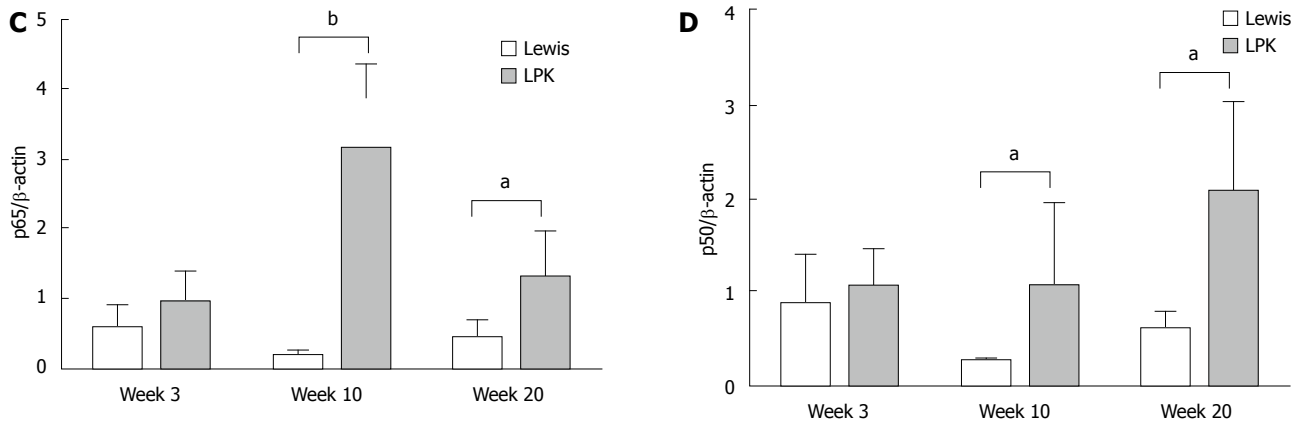


Figure 8 Western blotting for nuclear factor- κ B p65 and p50 proteins in Lewis and Lewis polycystic kidney. Immunoblotting was performed for (A) nuclear p65, and (B) nuclear p50, in Lewis and LPK kidney tissue from weeks 3, 10 and 20. Densitometry of immunoblots was quantified for (C) p65 and (D) p50. ^a $P < 0.05$ vs Lewis for the corresponding timepoint; ^b $P < 0.01$ vs Lewis for the corresponding timepoint. LPK: Lewis polycystic kidney.

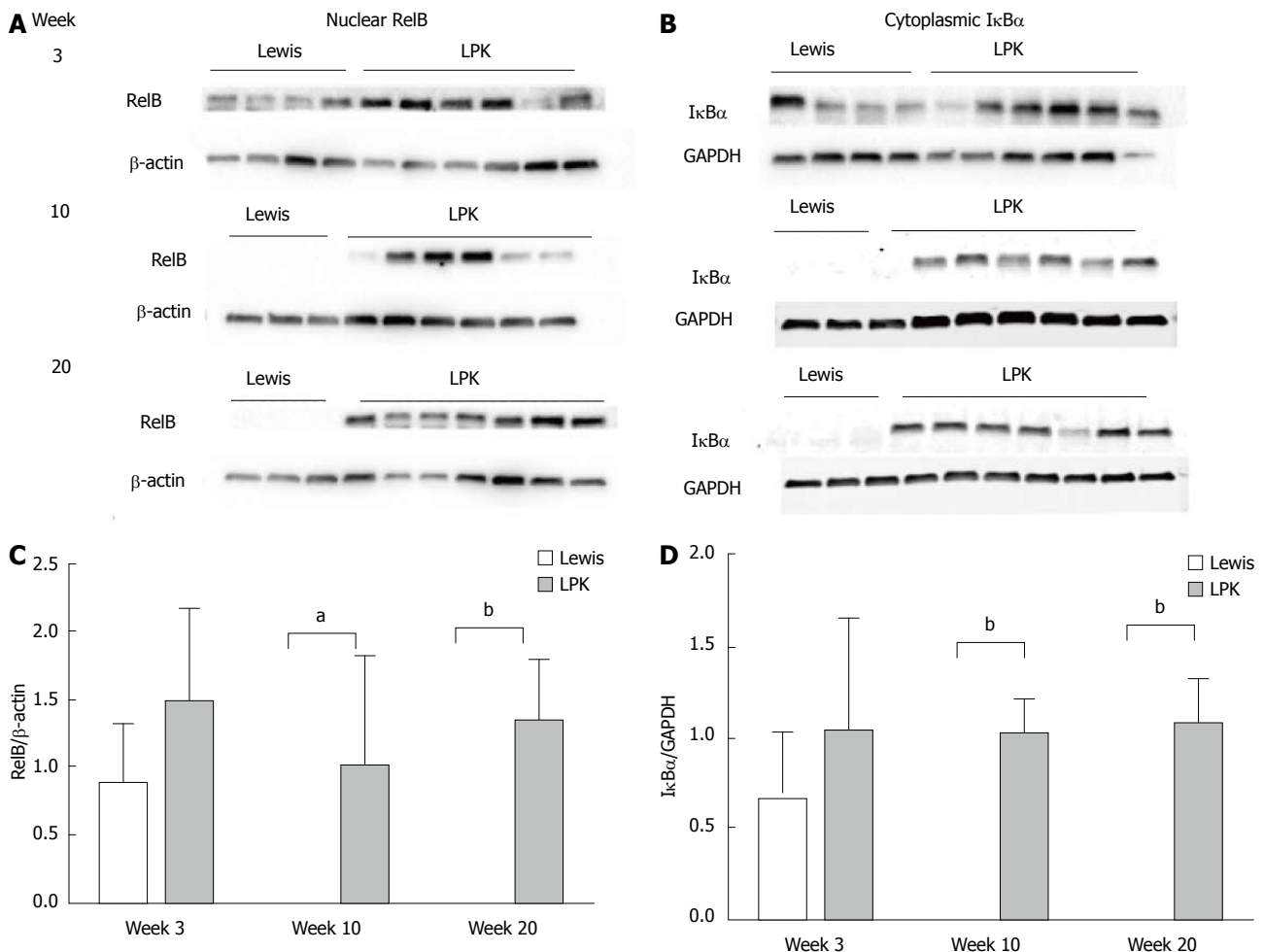


Figure 9 Western blotting for RelB and inhibitor of κ B proteins in Lewis and Lewis polycystic kidney. Immunoblotting was performed for (A) nuclear RelB, and (B) cytoplasmic I κ B α , in Lewis and LPK kidney tissue from weeks 3, 10 and 20. Densitometry of immunoblots was quantified for (C) RelB and (D) I κ B α . ^a $P < 0.05$ vs Lewis for the corresponding timepoint; ^b $P < 0.01$ vs Lewis for the corresponding timepoint. LPK: Lewis polycystic kidney; I κ B α : Inhibitor of kappa B.

expressed (Figure 14). In ADPKD and ARPKD, γ -H2AX was expressed in virtually all CEC nuclei (Figure 15).

DISCUSSION

In the past decade, *in vivo* studies have demonstrated

an upregulation of NF- κ B proteins in PKD, but there has been limited information regarding the specific NF- κ B proteins involved and their expression throughout disease progression^[22,23]. The first main finding of this study is that a diverse array of NF- κ B proteins, including p50, P-p105, p65, RelB and c-rel, is present in LPK

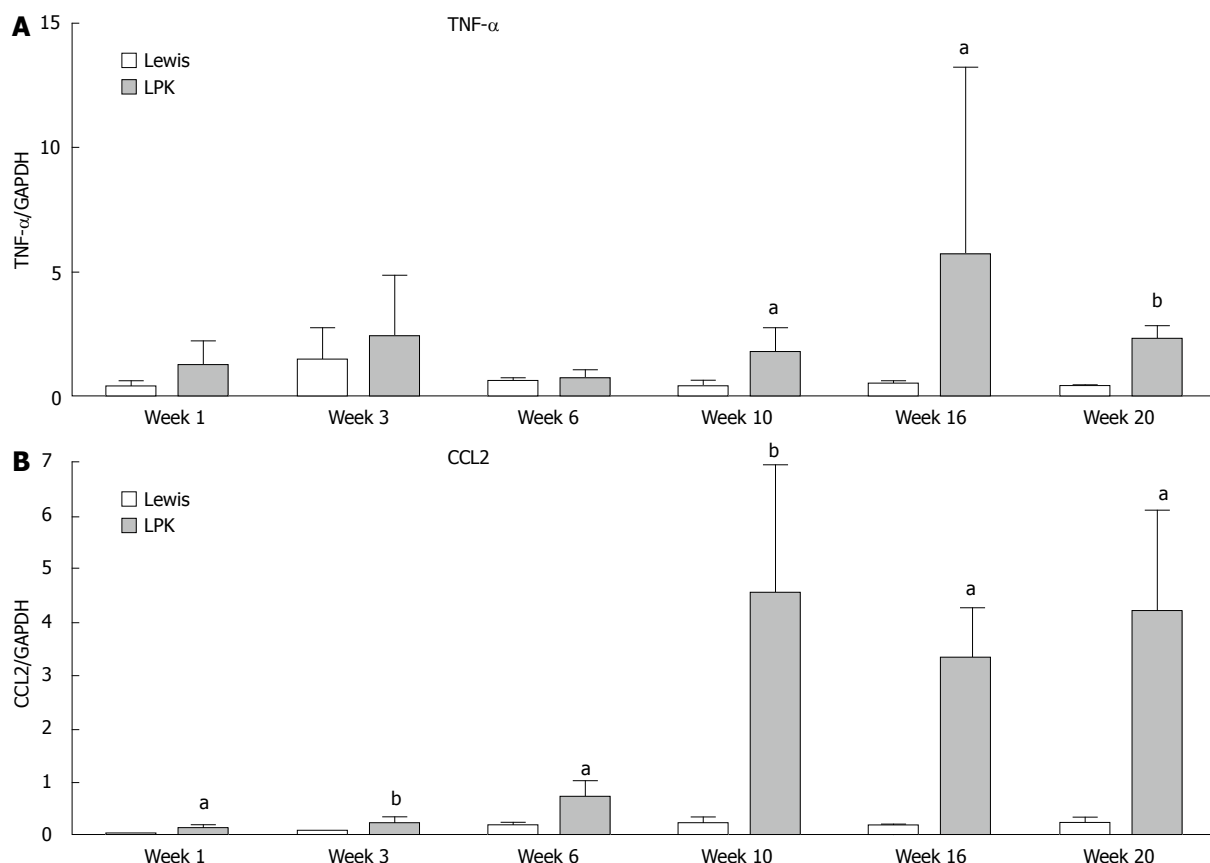


Figure 10 Quantitative polymerase chain reaction data for (A) $TNF\alpha$ and (B) $CCL2$ in Lewis and Lewis polycystic kidney tissue from weeks 1 to 20. The mRNA expression is shown as the target gene corrected for GAPDH. ^a $P < 0.05$ vs Lewis for the corresponding timepoint; ^b $P < 0.01$ vs Lewis for the corresponding timepoint. LPK: Lewis polycystic kidney; TNF: Tumor necrosis factor.

kidneys. The localization of these NF- κ B proteins to the CECs in LPK kidneys concurs with previous *in vivo* studies of $Pkd1^{-/-}$ and $PKD2$ mice^[22,23]. Notably, p65, p50 and RelB were predominantly localized to the cytoplasm rather than to the nuclei of CECs. Since NF- κ B transcription factor activity is critically dependent on the translocation of NF- κ B proteins to the nucleus^[13], western blotting for NF- κ B proteins was also performed in LPK renal nuclear extracts. This confirmed that p65, p50 and RelB proteins were present in LPK nuclei.

The second major finding was that in the LPK rat, renal NF- κ B protein expression occurs early in the disease and is constitutive over time. Expression of p65 was high in both Lewis and LPK kidneys in early life, suggesting that a basal level of NF- κ B activation may be required for development in the normal kidney. Indeed, NF- κ B inhibition in *ex vivo* embryonic kidneys has been shown to impair ureteric bud branching, which is critical for collecting duct development^[38,39]. Whereas in Lewis rats p65 expression was low from week 10 onwards, in LPK rats the high expression of p65 was sustained throughout life. Similarly, P-p105, p50, RelB and c-rel were also identified in CECs of LPK rats at all stages of disease. Western blotting revealed that nuclear p65, p50 and RelB levels were elevated in LPK compared to Lewis kidneys at week 10 and week 20.

We hypothesized that NF- κ B signaling is upregulated

in LPK compared to Lewis kidneys, and therefore predicted that cytoplasmic I κ B α would be absent in LPK kidneys, since degradation of I κ B α is necessary for NF- κ B activation^[13]. However, we found the converse; cytoplasmic I κ B α was consistently identified in all assessed timepoints in LPK kidneys but only present in Lewis kidneys at week 3. Since cytokines can stabilize newly resynthesized I κ B α , decreasing its susceptibility to further degradation^[40,41], it is possible that there is a chronic upregulation of NF- κ B in LPK kidneys that results in the stabilization of I κ B α .

Notably, the histological data showed that NF- κ B proteins are strongly expressed in the cytoplasm of LPK kidneys in early life (week 3), while immunoblotting suggested that nuclear NF- κ B levels are comparable between LPK and Lewis kidneys at this timepoint and do not significantly increase in LPK compared to Lewis until weeks 10 and 20. Overall, this suggests that NF- κ B proteins are present in the CEC cytoplasm at early stages of disease, but that a significant increase in NF- κ B activation does not occur until later in life. However, the persistent nature of NF- κ B protein expression in LPK kidneys indicates that it is a chronic, rather than transient feature of renal cystic disease in this model.

Since NF- κ B regulates the transcription of $TNF\alpha$ and $CCL2$ ^[17], and as the cytokine products of these genes are commonly found in models of PKD^[8], we sought to

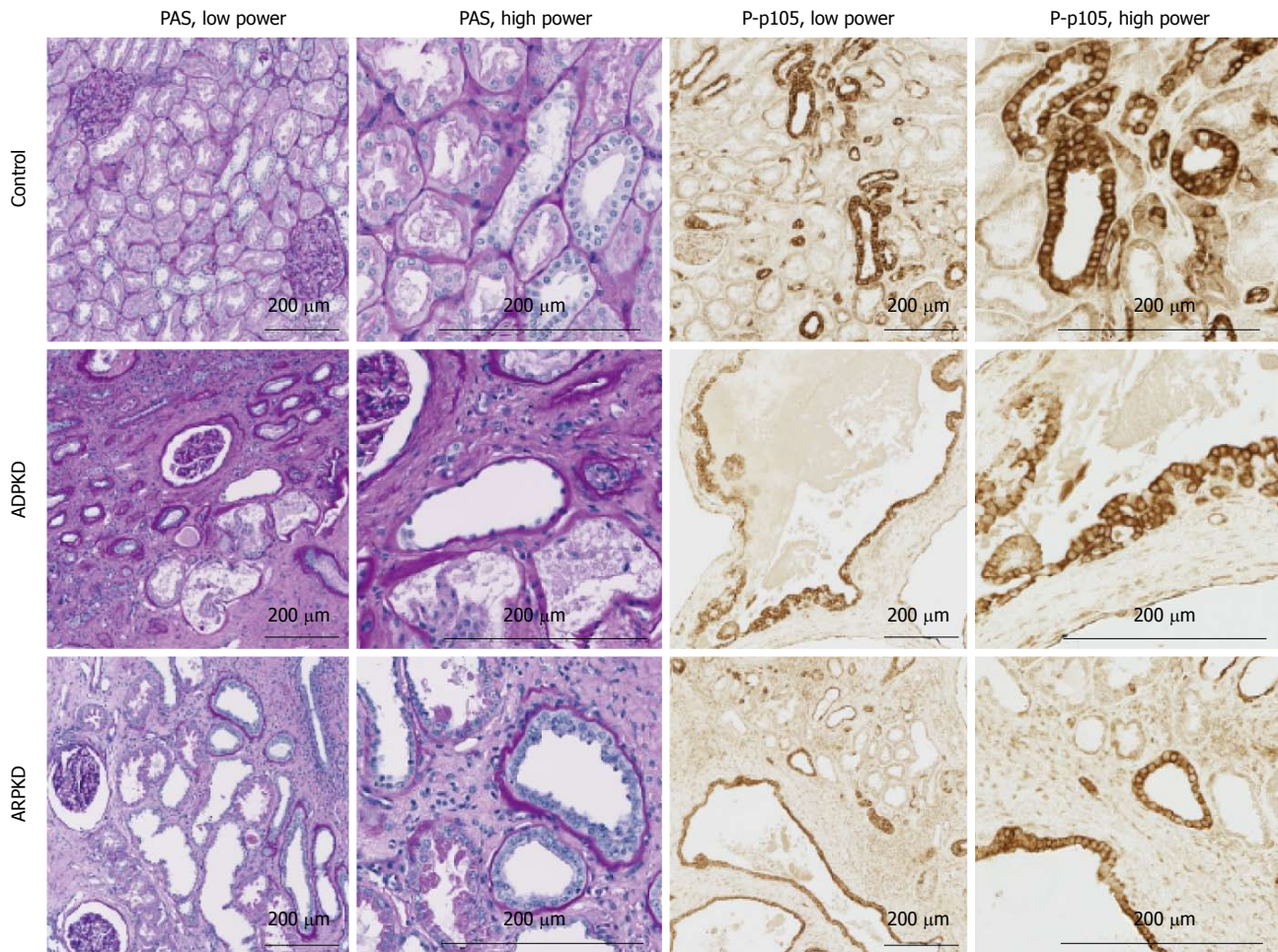


Figure 11 Periodic Acid Schiff staining (left panels) and immunohistochemistry for P-p105 (right panels) in the cortex of human normal kidney, autosomal dominant polycystic kidney disease and autosomal recessive polycystic kidney disease. ADPKD: Autosomal dominant polycystic kidney disease; ARPKD: Autosomal recessive polycystic kidney disease; LPK: Lewis polycystic kidney; PAS: Periodic Acid Schiff.

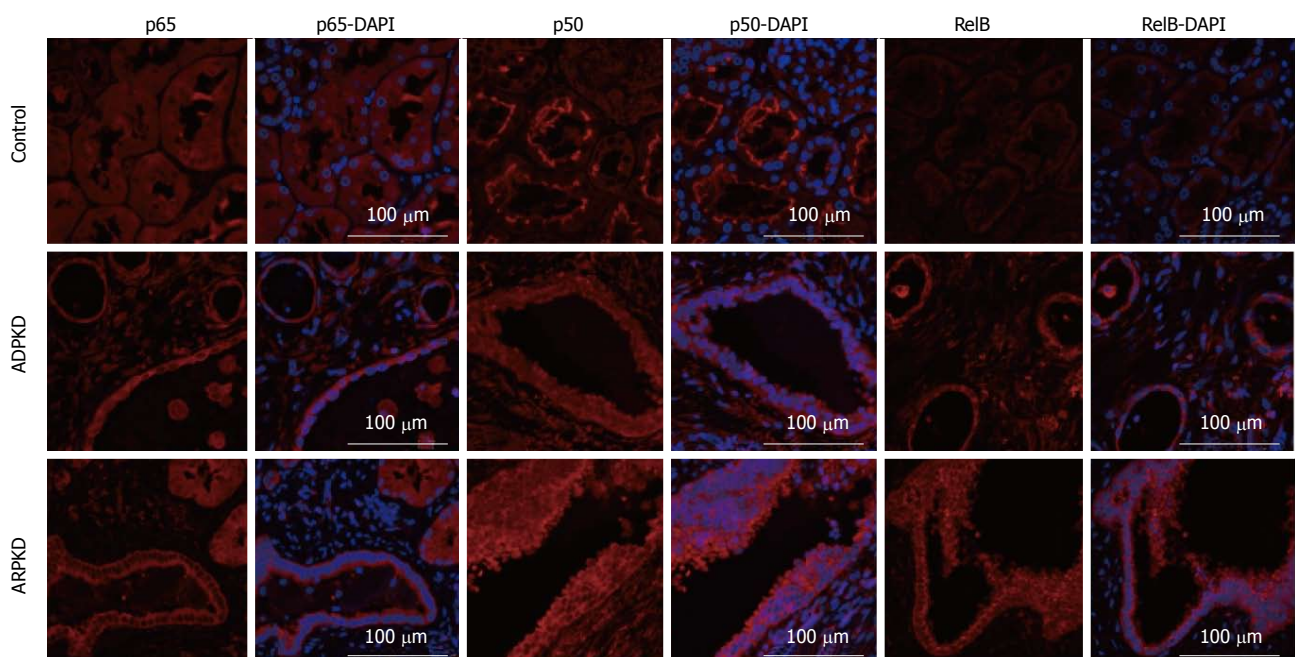


Figure 12 Immunofluorescence staining for p65, p50 and RelB (red) in human normal kidney cortex, and in autosomal dominant polycystic kidney disease and autosomal recessive polycystic kidney disease kidney cortex. Also shown are corresponding DAPI-merged images with nuclei labeled using DAPI (blue). ADPKD: Autosomal dominant polycystic kidney disease; ARPKD: Autosomal recessive polycystic kidney disease; LPK: Lewis polycystic kidney.

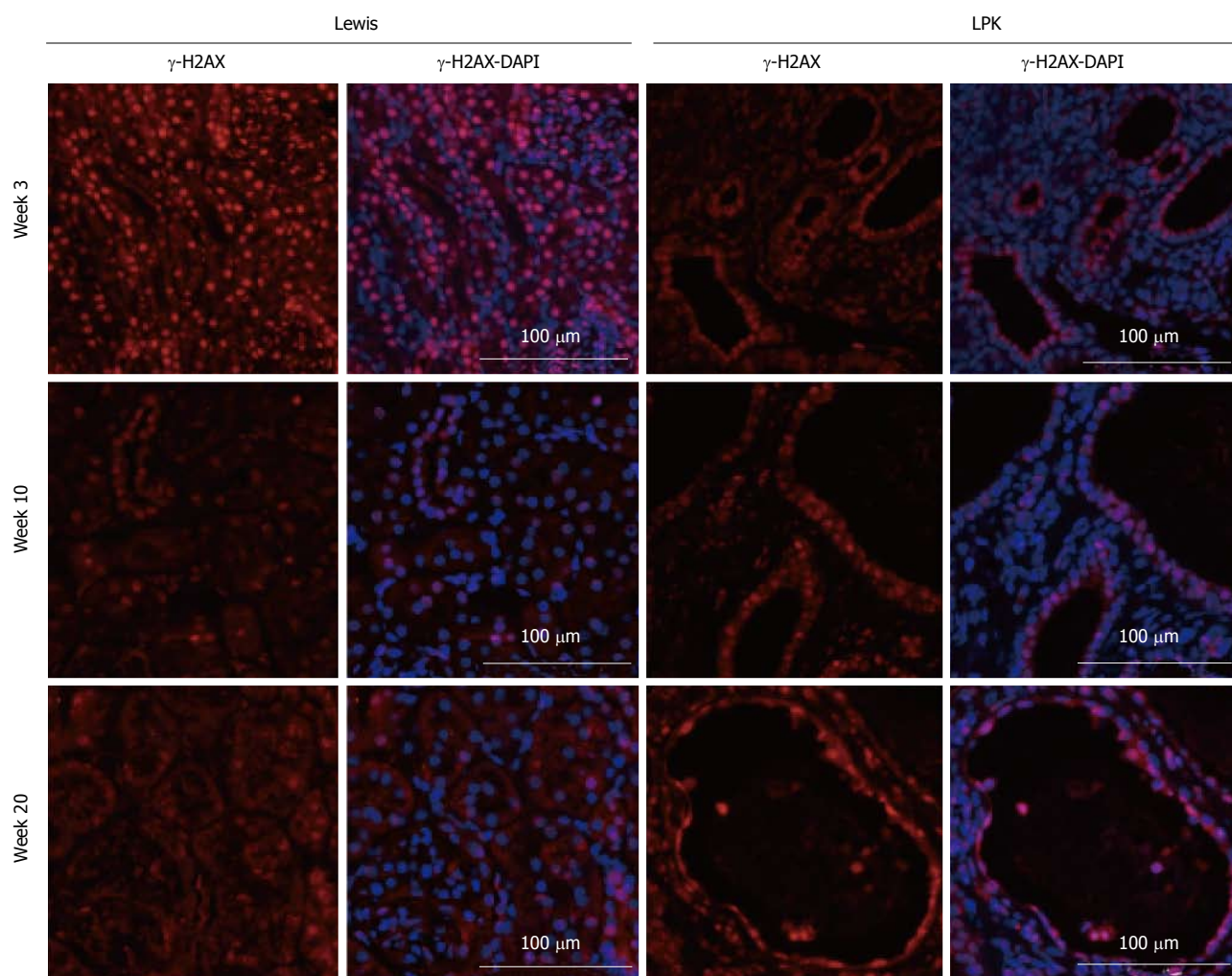


Figure 13 Immunofluorescence staining for γ -H2AX (red) at week 3, 10 and 20 in Lewis and Lewis polycystic kidney cortex. Also shown are corresponding DAPI-merged images with nuclei labeled using DAPI (blue).

determine the expression of these genes throughout the time-course of disease in LPK rats. *TNF α* expression was higher in LPK rats compared to Lewis rats, concurring with work by Nakamura *et al.*^[42] which found elevated *TNF α* mRNA expression in *cpk* mice compared to wild-type controls. Nakamura *et al.*^[42] demonstrated a steady increase in *TNF α* from early to late-stage disease. In contrast, LPK kidneys displayed a biphasic pattern of *TNF α* expression, wherein *TNF α* was upregulated at the early (week 1) and then at the late stages of disease (weeks 10 and 20). We also demonstrated an elevation in *CCL2* expression in LPK compared to Lewis at almost all timepoints, which concurs with previous findings of increased *CCL2* expression in homozygous Han:SPRD rats compared to wild-type controls^[43].

To confirm the results observed in LPK rats, we also examined NF- κ B expression in human cystic renal disease. Similar to LPK rats, NF- κ B was localized to CECs in human ADPKD and ARPKD kidneys, and this paralleled with the findings of Park *et al.*^[22] which demonstrated strong staining for phosphorylated NF- κ B in the CECs of ADPKD tissue. NF- κ B expression was particularly intense in regions of CEC hyperplasia, suggesting that the NF-

κ B system regulates transcription in highly proliferating areas. In a related disorder, acquired cystic disease-associated renal cell carcinoma, phosphorylated NF- κ B was identified in hyperplastic epithelial cyst-lining cells^[44]. While our *in vitro* previous work found no association between the rate of proliferation and degree of NF- κ B activation in human ADPKD cells^[31], siRNA-induced expression of a polycystin-1 cytoplasmic terminal tail in human embryonic kidney cells led to increased NF- κ B activation and proliferation^[20]. Further study is therefore required to verify the relationship between NF- κ B and cell proliferation in PKD. We also performed preliminary western blotting for NF- κ B in normal, ADPKD and ARPKD renal tissue, and immunohistostaining for NF- κ B in *Pkd2* knockout mouse kidneys, but the results were inconclusive due to small sample sizes (data not shown). Overall, the current data in LPK rats and human PKD provide a basis for future studies which may confirm the increase in NF- κ B transcription activity by examining the DNA:protein binding activity of p65, p50 and RelB in human PKD tissue.

It is unclear whether NF- κ B activation directly contributes to the pathogenesis of PKD, or whether it is

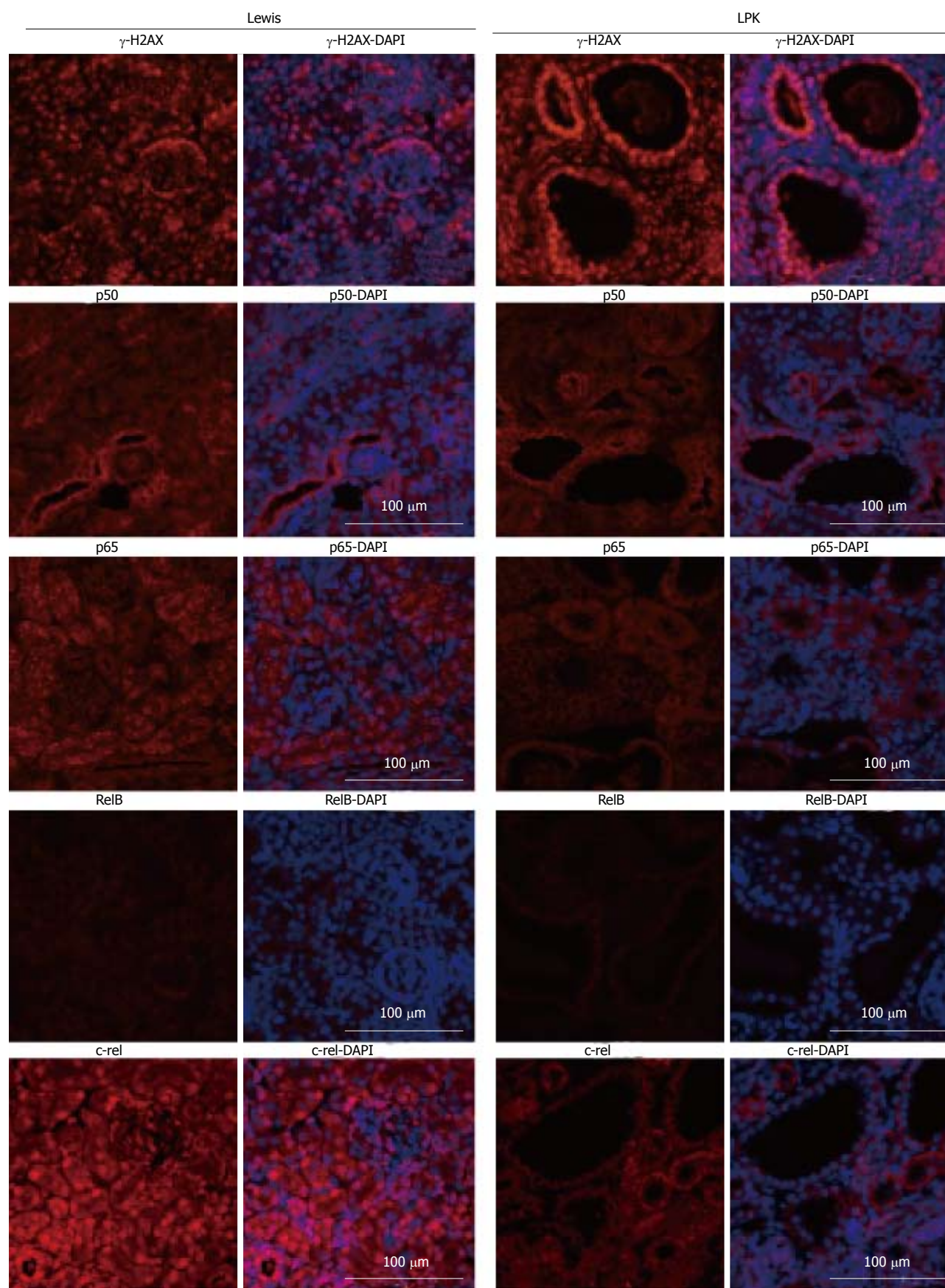


Figure 14 Immunofluorescence staining for γ -H2AX, p50, p65, RelB and c-rel (red) at week 1 in Lewis and Lewis polycystic kidney cortex. Also shown are corresponding DAPI-merged images with nuclei labeled using DAPI (blue). LPK: Lewis polycystic kidney.

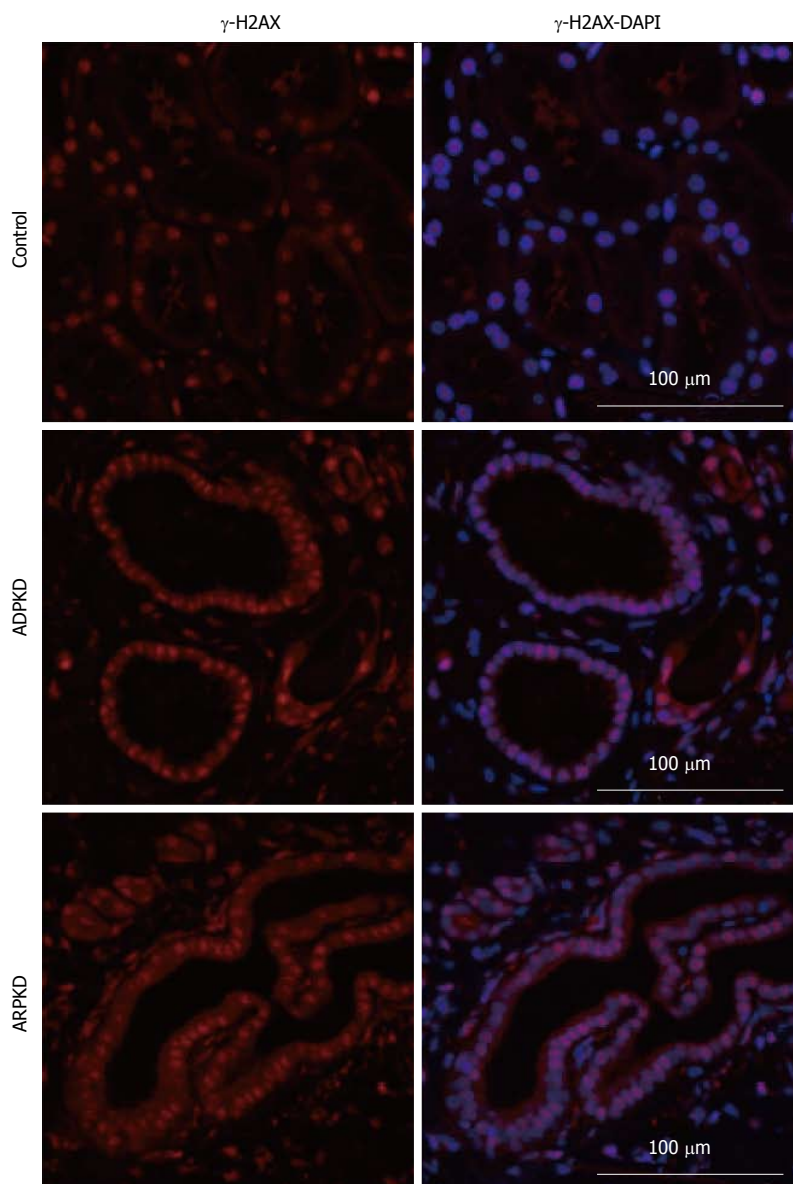


Figure 15 Immunofluorescence staining for γ -H2AX (red) in human normal kidney cortex, and in autosomal dominant polycystic kidney disease and autosomal recessive polycystic kidney disease kidney cortex. Also shown are corresponding DAPI-merged images with nuclei labeled using DAPI (blue). ADPKD: Autosomal dominant polycystic kidney disease; ARPKD: Autosomal recessive polycystic kidney disease.

secondary to the disease, stimulated in response to cytokines and inflammatory cells. Although our study did not directly address this question, it provided some information on the relationship between NF- κ B expression and markers of PKD progression. Firstly, NF- κ B proteins were observed in LPK CECs as early as week 1, and were strongly expressed at week 3, coinciding with cystic expansion and the increases in cell proliferation, inflammation and fibrosis, and continued throughout the disease time-course. The chronological order of these events may suggest that NF- κ B activation contributes to cyst expansion *via* the upregulation of cell proliferation and interstitial inflammation. Qin *et al.*^[23] found that NF- κ B inhibition decreases cyst growth in kidney explants, supporting the theory that cyst growth is NF- κ B-dependent. Secondly, western blotting indicated that NF- κ B protein levels were elevated in mid- to late-stage disease, coinciding with the elevations in *TNF α* and *CCL2* at weeks 10 and 20. Previous work has suggested that in *Pkd1* null mouse embryonic kidney cells, *TNF α* regulates its own

transcription *via* the NF- κ B pathway^[27], and that receptor activator of NF- κ B ligand (RANKL, a cytokine of the TNF family) activates the transcription of *TNF α* ^[45]. Taken together, these results support the notion that *TNF α* may contribute to NF- κ B upregulation in polycystic kidneys, and furthermore suggest there may be a positive-feedback loop in which NF- κ B regulates *TNF α* and *vice versa*. Further study is required to prove or disprove a causal relationship between NF- κ B and cystic renal disease.

Our study provides preliminary data for a role of non-canonical NF- κ B signaling in PKD. At weeks 10 and 20, RelB (a protein typically associated with the non-canonical pathway^[13]) was absent in Lewis but present in LPK kidney extracts, suggesting that while RelB may be involved in NF- κ B signaling in early normal renal development, cystic renal disease involves chronic RelB activity. Thus far, the non-canonical pathway has been associated with fewer physiological functions compared to the canonical pathway, and is mainly known for its role in B- and T-cell organogenesis^[14,15]. However, studies have

suggested that non-canonical NF- κ B signaling may also be involved in acute kidney injury^[46] and IgA nephropathy^[47]. Interestingly, RANKL is a stimulus of the non-canonical NF- κ B pathway and has been implicated in NF- κ B signaling in *Pkd1*^{-/-} murine cells^[45,46]. However since LPK kidneys also displayed elevations in p65 and *TNF α* , which are typically associated with canonical NF- κ B signaling^[13,16], it is likely that PKD involves a combination of canonical and non-canonical NF- κ B upregulation. Future investigation of other NF- κ B family members normally associated with the non-canonical pathway (e.g., p52 and IKK α ^[16]) and NEMO (which is exclusive to the canonical pathway^[14]) would be useful to further characterize NF- κ B signaling in PKD.

Since DNA damage has been proposed to play a role in renal ciliopathies^[48-50], we investigated the DNA damage marker, γ -H2AX, in polycystic kidneys. Our study demonstrated that γ -H2AX was strongly expressed in ADPKD and ARPKD CEC nuclei, suggesting that DNA damage may be a component of human renal cystic disease. Furthermore, γ -H2AX was apparent in CECs of LPK kidneys throughout the time-course of disease. Given that the LPK rat possesses a mutation in *Nek8*^[28], and abnormalities in this gene lead to DNA double-strand breaks (DSBs) and abnormal structuring of epithelial cells in 3D spheroid cultures^[36], our data add credence to the theory that *Nek8*-linked DNA damage is involved in the pathogenesis of renal ciliopathies and renal cystic diseases^[48]. Interestingly, γ -H2AX was strongly expressed at week 1 in LPK kidneys, when the expression of NF- κ B proteins was low (Figure 14). Since Tilstra *et al.*^[51] have demonstrated that NF- κ B inhibition can slow the progression of DNA damage in an animal model of senescence, there is potential for NF- κ B inhibition to be investigated as a strategy to ameliorate DNA injury as well as inflammation in PKD.

One limitation of this study was the small sample size and lack of genotype data for the human ADPKD and ARPKD specimens. Since in ADPKD, the *PKD2* mutation is associated with a slower progression to renal failure compared to the *PKD1* mutation^[52], future studies may determine whether NF- κ B activation differs according to the genotypic form and/or type of mutation (e.g., missense vs nonsense) in ADPKD patients^[53]. Also, although we demonstrated that NF- κ B proteins are constitutively present in CECs of LPK rats throughout the time-course of disease, it remains unknown whether this holds true in human PKD progression. Further characterization of renal NF- κ B signaling in *Pkd1* and/or *Pkd2* knockout mice (which are orthologous to human ADPKD^[54]) may aid to bridge our understanding of NF- κ B in human PKD. Future studies may also employ southwestern histochemistry or electrophoretic mobility shift assays (EMSA) to confirm that DNA:protein binding activity is upregulated in LPK kidneys and human PKD tissue.

In conclusion, this study found that several NF- κ B proteins are expressed in the CECs of kidney tissue of LPK rats and human ADPKD and ARPKD patients. Taking into account previous studies, NF- κ B upregulation has

now been identified in human PKD or animal models that possess mutations in *PKD1*, *PKD2*, *PKHD1*, and *NEK8*, suggesting that it is a shared feature of cystic renal diseases and independent of genotypic variation^[22,23]. Our study demonstrated that in the LPK rat, NF- κ B proteins are expressed in early disease and are constitutively present throughout life. This may suggest that NF- κ B inhibiting drugs need to be commenced in the early stages of PKD, in order to reduce or delay the increases in cell proliferation, interstitial inflammation and cyst volume. Our study also highlighted that RelB and non-canonical NF- κ B signaling may be involved in the late stages of PKD. Although these data provide a basis for the role of NF- κ B throughout disease progression in PKD, a direct causal relationship between NF- κ B and PKD has not yet been proved. Future studies should address this question through cross-breeding studies of *IKK* knockout and *Pkd1*^{-/-} mice, or by testing selective NF- κ B inhibitors (e.g., IKK inhibitors) in *in vivo* models of PKD.

COMMENTS

Background

The nuclear factor (NF)- κ B transcription factor system is a key regulator of genes controlling inflammation and growth. The aim of this study was to determine the temporal expression of Rel/NF- κ B proteins in renal tissue in polycystic kidney disease (PKD).

Research frontiers

To date, there has been limited information regarding the particular NF- κ B subunits involved in PKD and their expression throughout the time-course of disease progression. Further studies are required to elucidate whether NF- κ B signaling directly contributes to cyst growth in PKD.

Innovations and breakthroughs

This study found that NF- κ B protein expression is upregulated in a rodent model of PKD, the LPK rat, and that this expression occurred early, was constitutive, and trended toward an increase over time. NF- κ B upregulation was also identified in the cyst-lining cells of human autosomal dominant and recessive PKD, suggesting that this transcription factor system may regulate inflammation in cystic renal disease.

Applications

Although this study provides promising data regarding NF- κ B as a possible target for PKD, it should be noted that functional data are required to demonstrate that selective NF- κ B inhibition is effective in reducing renal cystic disease in animal models. There are no selective NF- κ B inhibitors approved for human use. Currently approved drugs that possess NF- κ B-inhibiting properties, such as disulfiram, may be potential therapies, but require further investigation in experimental models of PKD.

Terminology

Cystic epithelial cells: Epithelial cells that line the cyst, separating the cyst lumen from the renal interstitium. These cells rapidly proliferate, contributing to cyst expansion, and are thought respond to stimulation by intraluminal cytokines. **Non-canonical NF- κ B signaling:** A pathway of NF- κ B signaling that typically regulates the development of immune organs. **Southwestern histochemistry:** A technique which allows the expression of DNA-binding transcription factors to be assessed *in situ*. **Selective NF- κ B inhibition:** A strategy of NF- κ B inhibition by targeting specific components of NF- κ B.

Peer-review

The paper is interesting and well written. The methods are sound and the conclusions are consistent with the results.

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Case Control Study

Diabetes mellitus increases the prevalence of anemia in patients with chronic kidney disease: A nested case-control study

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Abstract

AIM: To compare anemia prevalence between matched chronic kidney disease (CKD) patients with and without diabetes mellitus (DM) and to assess factors associated with anemia development.

METHODS: This is a nested case-control study of 184 type-2 diabetic and 184 non-diabetic CKD patients from a prospectively assembled database of a Nephrology outpatient clinic, matched for gender, age and estimated glomerular filtration rate (eGFR). Prevalence of anemia (hemoglobin: Men: < 13 g/dL, women: < 12 g/dL and/or use of recombinant erythropoietin) was examined in comparison, in the total population and by CKD Stage. Univariate and multivariate logistic regression analyses were conducted to identify factors associated with anemia.

RESULTS: The total prevalence of anemia was higher

in diabetics (47.8% *vs* 33.2%, $P = 0.004$). Accordingly, prevalence was higher in diabetics in CKD Stage 3 (53.5% *vs* 33.1%, $P < 0.001$) and particularly in Stage 3a (60.4% *vs* 26.4%, $P < 0.001$), whereas it was non-significantly higher in Stage 4 (61.3% *vs* 48.4%; $P = 0.307$). Serum ferritin was higher in diabetics in total and in CKD stages, while serum iron was similar between groups. In multivariate analyses, DM (OR = 2.206, 95%CI: 1.196-4.069), CKD Stages 3a, 3b, 4 (Stage 4: OR = 12.169, 95%CI: 3.783-39.147) and serum iron (OR = 0.976, 95%CI: 0.968-0.985 per mg/dL increase) were independently associated with anemia.

CONCLUSION: Prevalence of anemia progressively increases with advancing stages of CKD and is higher in diabetic than matched non-diabetic CKD patients and diabetes is independently associated with anemia occurrence. Detection and treatment of anemia in diabetic CKD patients should be performed earlier than non-diabetic counterparts.

Key words: Anemia; Diabetes; Chronic kidney disease; Ferritin; Prevalence of anemia

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Core tip: Anemia is an established complication of chronic kidney disease (CKD) and diabetes mellitus is proposed to further increase anemia occurrence through various mechanisms. However, a direct comparison between diabetic and non-diabetic CKD patients with regards to anemia is currently missing. This study evaluates in comparison the prevalence of anemia in carefully matched CKD patients with and without diabetes mellitus.

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INTRODUCTION

Anemia is a major complication of chronic kidney disease (CKD) contributing to the clinical significance and the complex therapeutic approach of the uremic syndrome^[1]. The prevalence of anemia (defined as serum hemoglobin levels < 130 g/L for men and < 120 g/L for women) in the general population is estimated at 7.6%, but among patients with CKD anemia is reported at least twice as prevalent, reaching 15%^[2]. Anemia is generally associated with the severity of renal insufficiency, as serum hemoglobin levels and estimated glomerular filtration rate (eGFR) present an almost linear correlation^[3]. Anemia commonly occurs after CKD

Stage 3, with prevalence increasing from 5% in CKD Stage 1, to 75%-80% in pre-dialysis CKD Stage 5^[4,5]. The main pathogenetic mechanism for the development of anemia in CKD is the impaired production of erythropoietin from kidney^[6]. Iron deficiency or decreased availability, caused mainly by increased levels of hepcidin, due to inflammation accompanying chronic uremia, may constitute another important mechanism^[7]. Additionally, folate and vitamin B₁₂ deficiency, due to malnutrition and chronic inflammation result in increased red blood cells and immature erythroblasts apoptosis^[6]. Results from observational studies in pre-dialysis CKD patients suggest that anemia is associated with poor quality of life, increased hospital admissions, progression of kidney disease, and elevated mortality^[8].

Diabetes mellitus (DM) is the leading cause of CKD and ESRD^[9] and is proposed to elevate the risk of anemia development even in the absence of renal impairment. Anemia has been found in about 10% of patients with DM and normal kidney function^[10]. In a cohort of > 9000 patients without renal disease, DM was an independent determinant of hemoglobin levels^[11]. Many factors have been suggested to contribute in the pathogenesis of anemia in these patients, such as erythropoietin deficiency due to efferent sympathetic denervation of the kidney in the context of diabetic neuropathy, chronic inflammatory reaction leading to functional iron deficiency, non-selective urinary protein excretion leading to transferrin and erythropoietin loss and the use of renin-angiotensin-aldosterone system (RAAS) blockers which are central in the treatment of proteinuric diabetic nephropathy^[12].

Preliminary data suggest that anemia may be more common and occurs at earlier CKD stages in diabetic patients^[13]. An observational study in 1 million CKD patients of all stages indicated that prevalence of anemia in patients with DM was around 30%^[14]. In another study, including patients with type 2 DM and CKD, the prevalence of anemia increased from 15% in Stage 1 to 90% in Stage 5^[15]. However, epidemiologic data from a direct comparison between diabetic and non-diabetic CKD patients with regards to anemia are currently missing. On this context, the aim of this study was to examine in comparison the prevalence of anemia in matched CKD patients with and without DM and to evaluate additional factors that may contribute in anemia development.

MATERIALS AND METHODS

Study design

This is a nested case-control study in a prospectively assembled cohort of CKD patients first visiting the Nephrology Outpatient clinic of the General Hospital of Grevena, Greece between 1/01/2007 and 1/05/2015. Inclusion criteria were diagnosis of CKD and a complete dataset for the present analysis. Exclusion criteria were type 1 DM, Stage 5 CKD (eGFR < 15 mL/min per 1.73 m²) or kidney transplant. In total, 184 patients with type

2 DM were included and represented the cases. After this group was formed an equal number of non-diabetic patients were selected from the same cohort by an investigator blinded to patient data apart from matching parameters to form the control group. Matching was performed for gender, age (± 5 years) and eGFR (± 5 mL/min per 1.73 m^2) with particular care so that both cases and controls belonged to the same CKD stage. All study procedures belonged to the routine clinical practice of the Nephrology Outpatient clinic and all patients provided informed written consent prior to study enrollment. The study protocol was approved by the Institutional Ethics Committee and all investigations were performed according to the Declaration of Helsinki (2013 amendment).

Study data collection

For the purpose of this study, demographic and anthropometric parameters as well as cardiovascular risk factors and co-morbidities were recorded for each patient on their first outpatient visit within the aforementioned period. These included age, gender, height and weight, from which body mass index (BMI) was calculated according to the formula weight divided by height squared, as well as history of hypertension, dyslipidemia, DM, coronary heart disease, stroke, peripheral vascular disease, and cardiac arrhythmias. Moreover, data with regards to drug therapy were collected, such as medications for the treatment of DM (insulin and/or other non-insulin hypoglycaemic agents), use of medications that may affect erythropoiesis, such as oral iron supplements, recombinant erythropoietin, ACEIs or ARBs, cyclosporine, tacrolimus, *etc.*, and use of drugs interfering in the coagulation process (aspirin, clopidogrel, acenocoumarol, ticlopidine, heparin). During this visit blood samples were also acquired for the evaluation of routine hematological and biochemical parameters, including among others, serum urea, creatinine, sodium, potassium, uric acid, glucose, lipid profile and liver function tests. Patients were also instructed to perform a 24-h urine collection immediately before their next visit so that urine protein excretion would be evaluated.

Definitions

Anemia was defined as serum hemoglobin levels < 130 g/L for men and < 120 g/L for women, according to the 2012 Kidney Disease: Improving Global Outcomes (KDIGO) guidelines for anemia in CKD^[11] and/or use of recombinant erythropoietin for known anemia. The diagnosis of DM was based on American Diabetes Association criteria^[16], or on the basis of history of type 2 DM under dietary intervention or use of hypoglycaemic agents. Calculation of eGFR was performed from serum creatinine levels using the Modification of Diet in Renal Disease (MDRD) equation^[17]. Definition and staging of CKD was performed according to the KDIGO 2012 guidelines^[18], *i.e.*, Stage 1 CKD as eGFR ≥ 90 mL/min per 1.73 m^2 plus evidence of kidney injury for more than 3

mo; Stage 2 kidney CKD as eGFR ≥ 60 and < 90 mL/min per 1.73 m^2 and evidence of kidney injury; Stage 3a CKD as eGFR ≥ 45 and < 60 mL/min per 1.73 m^2 , Stage 3b CKD as eGFR ≥ 30 and < 45 mL/min per 1.73 m^2 and Stage CKD 5 as eGFR < 15 mL/min per 1.73 m^2 .

Statistical analysis

Statistical analysis was performed with Statistical Package for Social Sciences 21 (SPSS Inc, Chicago, IL). The Shapiro-Wilk test or Kolmogorov-Smirnov tests were used to examine the normality of distribution for quantitative variables. Continuous variables are presented as mean ± 1 SD or median range (presented in brackets) and categorical variables are described as absolute and relevant frequencies (n , %). χ^2 test or Fisher's exact test for qualitative variables, and Student's *t*-test, Mann-Whitney test or analysis of variance (ANOVA) for quantitative variables were used for between-group comparisons. In addition, multiple logistic regression analysis was performed to evaluate the association of various studied parameters (demographic, clinical and laboratory) with anemia. Variables were tested for interactions and included in the multivariate model if $P < 0.2$ in univariate analysis. Adjusted odd ratios (OR) with 95%CI are reported. Values of $P < 0.05$ (two-tailed) were considered statistically significant.

RESULTS

Baseline characteristics

A total of 368 patients with CKD (Stages 2-4) were included in this study, forming two groups: The first group consisted of 184 patients with DM and CKD and the second group of 184 matched CKD patients without DM. Baseline demographic, clinical and biochemical characteristics are presented in Table 1. In each group 96 patients (52%) were male and 88 (47.8%) were female. The mean age was 75.91 ± 8.38 and 76.00 ± 9.54 years for patients with and without DM accordingly ($P = 0.908$). Patients were stratified in CKD Stages as follows: Stage 2, 14.1%; Stage 3a, 28.8%; Stage 3b, 40.2%; and Stage 4, 16.8%. With regards to the existing risk factors and comorbidities smoking habit (39.7% vs 16.8%; $P < 0.001$) and history of stroke (8.7% vs 0.5%; $P < 0.001$) were more common in diabetics. As expected, results from routine biochemical tests indicated significant differences in serum glucose levels (diabetics 8.61 ± 2.74 mmol/L, non-diabetics 5.46 ± 0.61 mmol/L; $P < 0.001$) and 24-h urine protein [diabetics 527 (59-9, 300)] mg, non-diabetics 320 (65-3, 100) mg; $P < 0.001$].

Prevalence of anemia in total and in two study groups

As Table 2 depicts the mean hematocrit and hemoglobin levels were $39.02\% \pm 4.3\%$ vs $40.07\% \pm 4.0\%$ ($P = 0.015$) and 128.7 ± 15.6 g/L vs 131.9 ± 14.0 g/L ($P = 0.036$) for diabetic and the non-diabetic CKD patients respectively. Figure 1 presents the distribution of patients

Table 1 Demographic, clinical and routine biochemical characteristics of the two study groups (patients with and without diabetes)

Parameter	Diabetic CKD patients	Non-diabetic CKD patients	P
n	184	184	-
Age (yr)	75.91 ± 8.38	76.00 ± 9.54	0.908
Gender n (%)			
Female	88 (47.8)	88 (47.8)	1
Male	96 (52.2)	96 (52.2)	
Weight (kg)	79.78 ± 14.51	78.51 ± 12.58	0.373
Height (m)	1.67 ± 0.09	1.66 ± 0.08	0.121
BMI (kg/m ²)	28.34 ± 4.16	28.33 ± 3.26	0.979
Urea Nitrogen (mmol/L)	10.95 ± 5.06	10.90 ± 4.82	0.927
Creatinine (μmol/L)	136.14 ± 45.97	134.37 ± 46.85	0.826
eGFR (mL/min per 1.73 m ²)	43.3 ± 14.8	43.7 ± 14.9	0.778
Glucose (mmol/L)	8.61 ± 2.74	5.46 ± 0.61	< 0.001
24 h urine protein excretion (mg)	527 (59-9, 300)	320 (65-3, 100)	< 0.001
CKD Stages n (%)			
Stage 2	26 (14.1)	26 (14.1)	1
Stage 3a	53 (28.8)	53 (28.8)	
Stage 3b	74 (40.2)	74 (40.2)	
Stage 4	31 (16.8)	31 (16.8)	
Hypertension n (%)	171 (92.9)	175 (95.1)	0.379
Dyslipidemia n (%)	103 (56)	86 (46.7)	0.076
Coronary heart disease n (%)	65 (35.3)	60 (32.6)	0.582
Heart failure n (%)	30 (16.3)	34 (18.5)	0.583
Arrhythmia n (%)	20 (10.9)	25 (13.6)	0.426
Stroke history n (%)	16 (8.7)	1 (0.5)	< 0.001
Peripheral vascular disease n (%)	17 (9.2)	13 (7.1)	0.446
Smoking n (%)	73 (39.7)	31 (16.8)	< 0.001

CKD: Chronic kidney disease; eGFR: Estimated glomerular filtration rate.

from the two study groups over the continuum of hemoglobin levels; the distribution was in general towards lower values in patients with DM ($P = 0.024$). Anemia was present in 149 patients accounting for 40.5% of the total population studied (Figure 2). A trend of increasing anemia prevalence was found with the progression of CKD from Stage 2 towards Stage 4, *i.e.*, Stage 2, 9.6%; Stage 3, 43.3%; Stage 4, 54.8% ($P < 0.001$).

With regards to between-group differences, anemia was significantly more prevalent in the diabetic patient group in total (diabetics 47.8%, non-diabetics 33.2%; $P = 0.004$). As shown in Figure 3, prevalence of anemia was higher in non-diabetics but statistically not different between the two groups in CKD Stage 2 (3.8% vs 15.4%, $P = 0.350$) and thereafter higher in diabetic patients: Stage 3, 53.5% vs 33.1% ($P = 0.001$); Stage 3a, 60.4% vs 26.4% ($P = 0.001$); Stage 3b, 48.6% vs 37.8% ($P = 0.184$); Stage 4, 61.3% vs 48.4% ($P = 0.307$) for patients with and without DM accordingly.

Anemia-related parameters and medication use

Results for all other anemia-related parameters are presented in Figure 2. In both groups no significant differences were noted with regards to red blood cell indices, such as mean corpuscular volume, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration. However, serum ferritin levels were significantly higher in patients with DM both in total and in all CKD stages, while serum iron levels were equal between groups, with the exception of CKD Stage 2, in

which patients with DM had 15.39 (6.09-23.63) μmol/L and patients without DM 12.35 (2.15-23.09) μmol/L ($P = 0.027$).

Use of recombinant erythropoietin was similar between the two study groups in total (diabetics 8.2%, non-diabetics 8.7%; $P = 0.851$) and in CKD stages separately and the use of oral iron supplementary therapy was similar (diabetics 14.7%, non-diabetics 9.8%, $P = 0.152$). Regarding other medications that may interfere with development of anemia, use of RAAS-blockers did not differ significantly between diabetics (65.8%) and non-diabetics (70.1%, $P = 0.372$) in total and in CKD stages respectively. Finally, the use of drugs interfering in the coagulation process was higher for patients with DM in total (diabetics 46.7%, non-diabetics 35.9%; $P = 0.034$), but differences were not significant between the two groups in CKD stages.

Factors associated with anemia

Univariate and multivariate regression analyses in the total population studied is presented in Table 3. Anemia was the dependent variable, while several demographic, clinical and laboratory factors that can interfere with development of anemia were the independent variables. Diabetes was an independent factor for anemia occurrence in the total population (OR = 2.206, 95%CI: 1.196-4.069). Advancing stage of CKD was associated with progressively increasing risk for anemia development both in univariate and multivariate analysis; *i.e.*, Stage 3a (OR = 6.068, 95%CI: 2.112-17.430), Stage 3b

Table 2 Comparisons between the two study groups for anemia-related parameters in total study population and by chronic kidney disease stages (statistically significant *P* values are indicated in bold)

Parameter	Total study population	<i>P</i>	Stage 2	<i>P</i>	Stage 3	<i>P</i>	Stage 3a	<i>P</i>	Stage 3b	<i>P</i>	Stage 4	<i>P</i>
Hematocrit (%)												
Diabetics	39.02 ± 4.30	0.015	42.27 ± 4.79	0.324	38.69 ± 3.97	0.001	38.92 ± 4.08	< 0.001	38.53 ± 3.91	0.278	37.63 ± 4.02	0.687
Non-diabetics	40.07 ± 4		41.18 ± 2.83		40.34 ± 4.09		41.93 ± 4.10		39.21 ± 3.71		38.04 ± 3.9	
Hemoglobin (g/L)												
Diabetics	128.7 ± 15.6	0.036	141.8 ± 17.4	0.21	127.4 ± 14.1	0.003	128.2 ± 14.8	0.001	126.9 ± 13.6	0.383	122.7 ± 14.1	0.58
Non-diabetics	131.9 ± 14.0		136.7 ± 10.9		132.7 ± 14.1		138.2 ± 14.0		128.8 ± 13.0		124.7 ± 13.5	
MCV (fL)												
Diabetics	87.62 ± 6.99	0.739	87.76 ± 74.2	0.536	87.70 ± 7.49	0.633	87.78 ± 5.88	0.81	87.65 ± 8.5	0.457	87.11 ± 6.86	0.527
Non-diabetics	87.9 ± 6.99		86 ± 13.7		88.20 ± 8.83		87.45 ± 8.34		88.73 ± 9.17		88.29 ± 7.68	
MCH (pg/cell)												
Diabetics	29.91 ± 5.08	0.748	29.53 ± 2.21	0.684	30.28 ± 5.89	0.494	29.52 ± 2.24	0.792	30.82 ± 7.45	0.526	28.76 ± 2.42	0.415
Non-diabetics	29.78 ± 2.64		29.83 ± 3		29.89 ± 2.55		29.4 ± 2.75		30.25 ± 2.35		29.3 ± 2.73	
MCHC (g/L)												
Diabetics	323.8 ± 17.6	0.523	333.6 ± 12.0	0.03	321.6 ± 19.0	0.362	323.0 ± 21.7	0.982	320.6 ± 16.9	0.174	324.8 ± 11.6	0.282
Non-diabetics	322.6 ± 20.1		319.5 ± 29.5		323.7 ± 18.4		322.9 ± 20.9		324.3 ± 16.4		320.3 ± 19.7	
Serum iron (μmol/L)												
Diabetics	12.35 (1.61-35.73)	0.783	2.75 (1.09-4.23)	0.027	12.17 (1.61-35.73)	0.351	12.71 (1.61-28.28)	0.86	11.01 (2.15-35.73)	0.194	10.92 (3.83-20.23)	0.559
Non-diabetics	12.53 (2.69-27.03)		2.21 (0.38-4.13)		12.53 (2.69-27.03)		12.35 (4.47-23.27)		12.71 (2.69-27.03)		11.99 (3.94-23.81)	
Ferritin (ng/mL)												
Diabetics	200 (17.3-1048.7)	< 0.001	230.3 (62.9-570.7)	0.01	175.3 (17.3-1048.7)	0.003	175.3 (23.4-1048.7)	0.013	175.3 (7.7-1015.6)	0.061	220.2 (25.6-867.3)	0.011
Non-diabetics	148.3 (7.2-993.2)		155.1 (78.6-435.9)		148.3 (22.5-993.2)		155.1 (22.5-294.4)		143.8 (26.9-993.2)		143.8 (7.2-441.4)	
24 h urine protein Excretion (mg)												
Diabetics	527 (59-9300)	< 0.001	283 (68-5100)	0.126	530 (59-9300)	< 0.001	545 (129-1700)	< 0.001	525 (59-9300)	< 0.001	670 (95-3800)	0.647
Non-diabetics	320 (65-3100)		245 (110-780)		300 (65-3100)		250 (65-1500)		375 (104-3100)		560 (117-3100)	
Smoking (<i>n</i> , %)												
Diabetics	73 (39.7)	< 0.001	10 (38.5)	0.375	57 (44.9)	< 0.001	32 (60.4)	0.011	25 (33.8)	< 0.001	6 (19.4)	0.255
Non-diabetics	31 (16.8)		7 (26.9)		22 (17.3)		19 (35.8)		3 (4.1)		2 (6.5)	
Use of erythropoietin (<i>n</i> , %)												
Diabetics	15 (8.2)	0.851	0 (0)	n/a	9 (7.1)	0.271	4 (7.5)	0.118	5 (6.8)	1	6 (80.6)	0.155
Non-diabetics	16 (8.7)		0 (0)		5 (3.9)		0 (0)		5 (6.8)		11 (35.5)	
Iron supplements therapy (<i>n</i> , %)												
Diabetics	27 (14.7)	0.152	1 (3.8)	1	19 (15)	0.076	8 (15.1)	0.111	11 (14.9)	0.314	7 (22.6)	1
Non-diabetics	18 (9.8)		1 (3.8)		10 (7.9)		3 (5.7)		7 (9.5)		7 (22.6)	
ACEIs/ARBs (<i>n</i> , %)												
Diabetics	121 (65.8)	0.372	22 (84.6)	1	88 (69.3)	0.784	38 (71.7)	0.831	50 (67.6)	0.592	11 (35.5)	0.075
Non-diabetics	129 (70.1)		21 (80.8)		90 (70.9)		37 (69.8)		53 (71.6)		18 (58.1)	
Antiplatelet/anticoagulant drugs (<i>n</i> , %)												
Diabetics	86 (46.7)	0.034	9 (34.6)	0.199	62 (48.8)	0.165	27 (50.9)	0.171	35 (47.3)	0.508	15 (48.4)	0.303
Non-diabetics	66 (35.9)		4 (15.4)		51 (40.2)		20 (37.7)		31 (41.9)		11 (35.5)	

ACEI: Angiotensin-converting enzyme inhibitors; ARB: Angiotensin receptor blocker; CKD: Chronic kidney disease; MCH: Mean corpuscular hemoglobin; MCV: Mean corpuscular volume; n/a: Not applicable.

(OR = 7.499, 95%CI: 2.604-21.597), Stage 4 (OR = 12.169, 95%CI: 3.783-39.147). Serum iron levels were also associated with occurrence of anemia (OR = 0.976, 95%CI: 0.968-0.985 per mg/dL increase). Interestingly, female gender was associated with decreased risk for anemia occurrence (OR = 0.389, 95%CI: 0.224-0.675), but this may be related to the lower threshold of hemoglobin for females in the definition used. With regards to other existing comorbidities no significant correlations were observed. Similarly, use of RAAS-blockers and antiplatelet or anticoagulant drugs, as well as the degree of 24-h urine protein excretion were not found to be

associated with the development of anemia.

DISCUSSION

This study examined in comparison the prevalence of anemia in matched CKD patients with and without DM and further aimed to evaluate the possible association of demographic, clinical and laboratory factors with the development of anemia. The overall prevalence of anemia in the population studied was high (40.5%), while the prevalence in patients with DM was about 15% higher than that in non-diabetic counterparts

Table 3 Univariate and multivariate regression analysis for occurrence of anemia (defined as serum hemoglobin levels < 130 g/L for men and < 120 g/L for women and/or use of recombinant erythropoietin) in the total studied population

Parameter	Univariate analysis		Multivariate analysis	
	Unadjusted odds ratio (95%CI)	P	Adjusted odds ratio (95%CI)	P
BMI Groups				
Normal (18.5-25)	Reference group		Reference group	
Underweight (< 18.5)	0.545 (0.046-6.443)	0.63		
Overweight (25-30)	0.714 (0.377-1.350)	0.3		
Obese (> 30)	0.708 (0.348-1.442)	0.342		
Age				
< 75 yr	Reference group		Reference group	
≥ 75 yr	1.623 (1.028-2.564)	0.038	1.198 (0.694-2.069)	0.517
Gender				
Male	Reference group		Reference group	
Female	0.546 (0.357-0.833)	0.005	0.389 (0.224-0.675)	0.001
CKD Stages				
Stage 2	Reference group		Reference group	
Stage 3a	7.207 (2.656-19.566)	< 0.001	6.068 (2.112-17.430)	0.001
Stage 3b	7.162 (2.694-19.038)	< 0.001	7.499 (2.604-21.597)	< 0.001
Stage 4	11.414 (3.999-32.582)	< 0.001	12.169 (3.783-39.147)	< 0.001
Diabetes	1.848 (1.212-2.818)	0.004	2.206 (1.196-4.069)	0.011
Hypertension	0.663 (0.280-1.573)	0.351		
Dyslipidemia	0.745 (0.491-1.130)	0.166	0.659 (0.404-1.074)	0.094
Coronary heart disease	1.446 (0.934-2.239)	0.098	1.048 (0.506-1.960)	0.883
Heart failure	1.725 (1.003-2.967)	0.049	1.228 (0.628-2.398)	0.548
Arrhythmia	0.788 (0.412-1.509)	0.472		
Smoking	1.051 (0.662-1.667)	0.834		
Serum glucose levels (per mg/dL increase)	1.006 (1.002 to 1.011)	0.009	0.999 (0.992-1.005)	0.736
Serum iron (per mg/dL increase)	0.978 (0.970-0.986)	< 0.001	0.976 (0.968-0.985)	< 0.001
Ferritin (per ng/mL increase)	0.998 (0.995-1.001)	0.209		
24 h urine protein excretion (per mg increase)	1.000 (1.000-1.003)	0.146	1.000 (1.000-1.001)	0.772
ACEIs/ARBs	0.690 (0.443-1.075)	0.101	0.963 (0.565-1.641)	0.888
Antiplatelet/ anticoagulant drugs	1.413 (0.927-2.156)	0.108	1.161 (0.669-2.015)	0.595

ACEI: Angiotensin-converting enzyme inhibitors; ARB: Angiotensin receptor blocker; CKD: Chronic kidney disease; BMI: Body mass index.

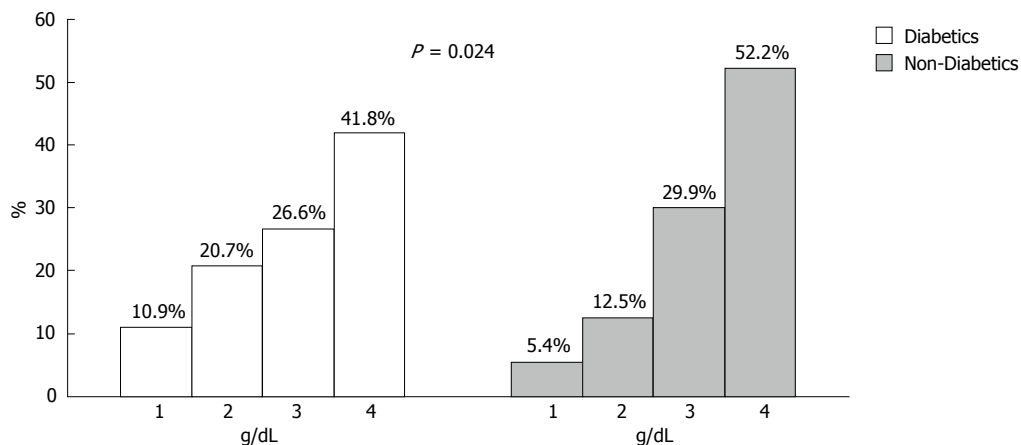


Figure 1 Distribution of serum hemoglobin levels in diabetic and non-diabetic patients. Percentages indicate the % of patients with hemoglobin levels within each depicted category of hemoglobin levels. 1: ≤ 11; 2: > 11-≤ 12; 3: > 12-≤ 13; 4: ≥ 13.

(47.8% vs 33.2%). With the exception of Stage 2, where the overall prevalence was low (9.5%), anemia was more prevalent in the diabetic patients group in the rest CKD stages, with the difference between groups being particularly large at CKD Stage 3a, where diabetic patients had more than two times higher anemia occurrence (60.4% vs 26.4%). Serum ferritin levels, but not iron, was higher in diabetic than in non-diabetic

patients in all stages; as the former also had higher rates of anemia, increased ferritin may mirror its role as an acute phase reactant, signifying higher subclinical inflammation in diabetic patients. In multivariate analyses, among a wide set of demographic, co-morbid, laboratory and medication parameters studied presence of DM, CKD Stages 3a, 3b and 4 and serum iron levels were independently associated with anemia occurrence.

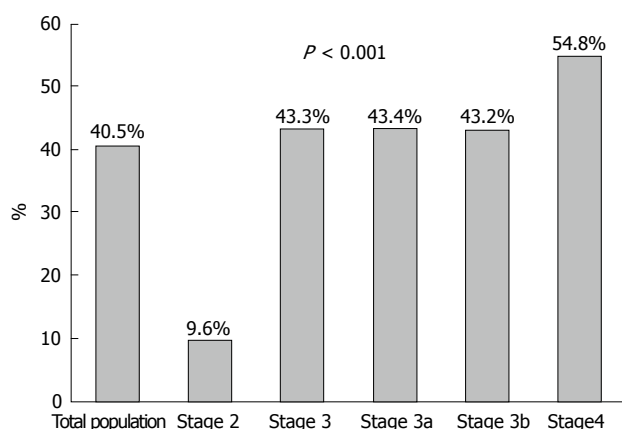


Figure 2 Prevalence anemia in total studied population and in chronic kidney disease Stage 2, 3, 3a, 3b and 4.

Anemia is an established complication of CKD and is per se associated with the severity of renal insufficiency, mostly due to impaired production of endogenous erythropoietin and true deficiency or decreased availability of serum iron^[7,8]. This study further supports this principle, as our results indicated progressing increase in prevalence of anemia with the progression of CKD from Stage 2 (9.6%) to Stage 3 (43.3%) and Stage 4 (54.8%). Moreover, advancing stage of CKD was independently associated with progressively higher OR levels for the development of anemia from CKD Stage 3a (OR = 6.068), CKD Stage 3b (OR = 7.499) and CKD Stage 4 (OR = 12.169). These results are in accordance to the National Health and Nutrition Examination Survey (NHANES) in which prevalence of anemia was 5% in patients with CKD Stage 1 and reached progressively 80% in pre-dialysis Stages 4-5 patients^[4]. Similarly, in another cross-sectional study of 5000 individuals with CKD, prevalence of anemia in overall was 48% and was associated with eGFR deterioration as it increased from 27% to 75% with the progression from CKD Stage 2 to CKD Stage 5^[5].

Previous indirect data suggested that diabetic patients with CKD may exhibit higher rates of anemia in relation to patients without DM. Patients with type 2 DM may experience anemia even in the absence of nephropathy, as indicated by a previous observational study, in which 16% of the individuals who had type 2 DM but no CKD developed anemia in a 7-year follow up^[10]. In a cross-sectional study of > 1 million patients with CKD of Stages 1-5, in which 5% were diabetics, prevalence of anemia was twice as high in diabetics (30% vs 15%) in total, but prevalence in each CKD stage with regards to diabetes presence was not evaluated^[14]. In a cohort study of type-2 diabetic CKD patients, prevalence of anemia was 15% in Stage 1, 25% in Stage 2, 50% in Stage 3 and 90% in Stages 4-5^[15]. Two other studies have associated DM with increased occurrence of anemia in CKD. The first, including almost 5400 individuals with CKD, of whom 27% had DM, indicated an overall prevalence of anemia 11.6% among diabetics, with its frequency

increasing about 45% from CKD Stage 1 to Stage 5^[19]. The second studied 468 unmatched CKD patients of whom 44% were type 1 or type 2 diabetics and prevalence of anemia in patients with DM was 17% in CKD Stages 1-2, 51% in CKD Stage 3 and 59% in CKD Stages 4-5, while DM was associated with a significant fourfold increase in risk of anemia in the regression analysis^[20]. In contrast, results from the Pre-dialysis Survey of Anemia Management Study indicated no significant differences between patients with and without DM regarding the correlation of serum hemoglobin levels and creatinine clearance rate^[21]. Our study further clarifies this issue, showing higher prevalence of anemia in diabetic than carefully matched non-diabetic CKD patients, particularly in Stage 3a, where the majority of individuals with CKD belongs.

As discussed above, several mechanisms promoting anemia in diabetic individuals have been previously described. Erythropoietin deficiency due to efferent sympathetic denervation of the kidney as a result of diabetic neuropathy, subclinical inflammation leading to functional iron deficiency through increased hepcidin levels, increased non-selective proteinuria excretion resulting in transferrin and erythropoietin loss, increased red blood cell destruction because of disorders in the cellular structure caused by DM and advanced glycation end products (AGEs) possibly decreasing erythrocyte lifespan are among them^[11-13,22,23]. Further, increased use of RAAS-blockers in diabetic patients, may promote anemia occurrence through inhibition of the physiologic erythropoietic action of angiotensin II^[24]. In our study, proteinuria was significantly higher in diabetic patients, but it did not display significant associations with anemia in multivariate analysis. Further, the use of RAAS-blockers was practically equal between the two groups, thus it could not significantly affect the results; use of ACEIs or ARBs was also not associated with anemia in multivariate analysis.

A role of chronic inflammation affecting anemia in DM is also proposed. Recent findings suggest that diabetic patients have higher ferritin and hepcidin levels than matched non-diabetic individuals^[25]. Levels of ferritin as a marker of inflammation and hepcidin were shown to correlate strongly in various populations including patients with DM^[26] and CKD of various types^[27]. Increased hepcidin following subclinical inflammation has also been observed in obese individuals^[28]. Hepcidin is the key factor causing functional iron deficiency reducing the efflux of recycled iron from both splenic and hepatic macrophages and also inhibits iron absorption from the gut; the overall reduction of iron available for erythropoiesis leads to anemia^[28]. Our findings support this mechanism of chronic inflammation as ferritin levels were significantly higher in diabetics in overall (200.0 pmol/L vs 148.3 pmol/L; $P < 0.001$) and in almost every CKD stage. In addition, although an increase in serum iron was associated with less anemia occurrence in multivariate analysis, ferritin levels displayed no significant associations, a finding

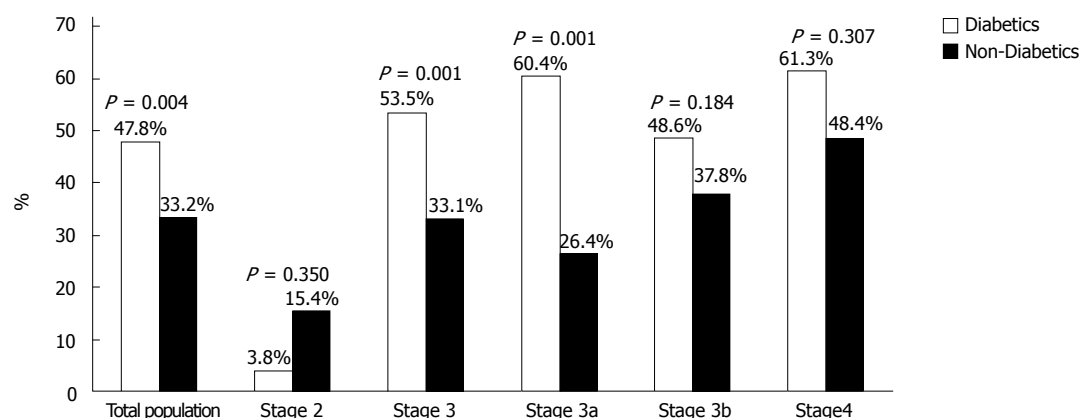


Figure 3 Prevalence of anemia in diabetic and non-diabetic patients in total and in chronic kidney disease Stage 2, 3, 3a, 3b and 4.

suggesting that ferritin could not be considered as a marker of iron stores. Further examination of this pathway including measurement of hepcidin levels could be useful.

This study has methodologic strengths. Although prevalence of anemia in CKD and DM has been examined previously, a direct comparison in patients with and without DM in CKD, to the best of our knowledge, was absent. Apart from the careful matching of individuals to form the two study groups, the capture of several factors that may theoretically affect the development of anemia in DM and a careful multiple logistic regression analysis further strengthen our results. However, there are also some limitations. This is an observational study, thus definite cause and effect associations cannot be established. The use of a unique hemoglobin measurement to determine the diagnosis of anemia may have misclassified some individuals. Finally, observed frequencies and significance levels in some comparisons may have been affected to an extent by the relatively small sample sizes in some of the subgroup analyses.

In conclusion, this study has confirmed that anemia is common in CKD outpatients and increases steadily with advancing Stages of CKD. Furthermore, the prevalence of anemia is higher in diabetic patients with CKD compared to matched non-diabetic counterparts. The difference between diabetic and non-diabetic patients with CKD was more prominent in CKD Stage 3a, where the majority of individuals with CKD belongs. Subclinical inflammation in diabetic patients with moderate CKD may be the most important underlying factor for this association, as indicated by increased ferritin levels in diabetics in our study. As anemia is associated with significant morbidity and mortality, both detection and treatment of anemia in diabetic CKD patients should be performed earlier than in non-diabetic counterparts.

COMMENTS

Background

Anemia is a major complication of chronic kidney disease (CKD) and diabetes mellitus (DM) is proposed to elevate the risk of anemia development. However, epidemiologic data from a direct comparison between diabetic and non-diabetic CKD patients with regards to anemia are currently missing.

Research frontiers

Prevalence of anemia has been extensively studied in patients with CKD. However, current evidence about the role of DM in anemia development in CKD derive only from observational studies in CKD population in which diabetics constitute only a small proportion. DM has been found to further elevate the prevalence of anemia in CKD, but not in all studies. On this context, a study examining in comparison the prevalence of anemia in matched CKD diabetic and non-diabetic patients would further clarify the role of DM in anemia development.

Innovations and breakthroughs

This study is the first to evaluate prevalence of anemia with a case control design in carefully matched diabetic and non-diabetic CKD patients.

Applications

Both detection and treatment of anemia in diabetic CKD patients should be performed earlier than in non-diabetics, in order to prevent anemia-associated complications.

Peer-review

The study deals with a common issue in clinical practice; (*i.e.*, diabetic patients with moderate CKD often appear with low Hb levels for their eGFR levels and have already been investigated for anemia from internists or hematologists for years with no results). Although there are some previous data pointing to the fact that anemia (among many factors studied) is more common in diabetics with CKD, this study adds to current knowledge.

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Retrospective Study

Metformin associated lactic acidosis in Auckland City Hospital 2005 to 2009

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Author contributions: Haloob I conceived the study, performed data collection and wrote the initial manuscript while working at Auckland Hospital; de Zoysa JR helped design the study, reviewed data collection and reviewed and revised the manuscript while working at Auckland Hospital.

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Informed consent statement: Patients were not required to give informed consent to the study because the analysis used anonymous, de-identified clinical data.

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Abstract

AIM: To determine the incidence, clinical characteristics and outcomes of patients with metformin associated lactic acidosis (MALA).

METHODS: Auckland City Hospital drains a population of just over 400000 people. All cases presenting with metabolic acidosis between July 2005 and July 2009 were identified using clinical coding. A retrospective case notes review identified patients with MALA. Prescribing data for metformin was obtained from the national pharmaceutical prescribing scheme.

RESULTS: There were 42 cases of metabolic lactic acidosis over 1718000 patient years. There were 51000 patient years of metformin prescribed to patients over the study period. There were thirty two cases of lactic acidosis due to sepsis, seven in patients treated with metformin. Ten cases of MALA were identified. The incidence of MALA was estimated at 19.46 per 100000 patient year exposure to metformin. The relative risk of lactic acidosis in patients on metformin was 13.53 (95%CI: 7.88-21.66) compared to the general population. The mean age of patients with MALA was 63 years, range 40-83 years. A baseline estimated glomerular filtration rate was obtained in all patients and ranged from 23-130 mL/min per 1.73 m². Only two patients had chronic kidney disease G4.

Three patients required treatment with haemodialysis. Two patients died.

CONCLUSION: Lactic acidosis is an uncommon but significant complication of use of metformin which carries a high risk of morbidity.

Key words: Acute kidney injury; Lactic acidosis; Metformin

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Core tip: Metformin is an effective therapy for type 2 diabetes mellitus. Although few side effects are described in clinical trials, here, we describe observational evidence that suggests that use of metformin is associated with an increased risk of lactic acidosis. We recommend dose reduction in the elderly, withholding the drug if an intercurrent illness occurs and that metformin be halted in patients with chronic kidney disease G4.

Haloob I, de Zoysa JR. Metformin associated lactic acidosis in Auckland City Hospital 2005 to 2009. *World J Nephrol* 2016; 5(4): 367-371 Available from: URL: <http://www.wjgnet.com/2220-6124/full/v5/i4/367.htm> DOI: <http://dx.doi.org/10.5527/wjn.v5.i4.367>

INTRODUCTION

The oral hypoglycaemic agent metformin has been used for close to 50 years^[1]. It has been found to reduce mortality compared to other agents and is recommended as first line therapy for patients with type 2 diabetes mellitus^[2-4]. It is also used for patients with the metabolic syndrome^[5] and overweight women with polycystic ovarian syndrome^[6].

The biguanide, phenformin, clearly caused lactic acidosis^[7]. It has been hypothesized that this severe and significant side-effect is also associated with metformin. Several mechanisms of action have been proposed for the hypoglycaemic effect of the metformin: A reduction in hepatic glucose production, an increase in peripheral glucose uptake, a reduction in gastrointestinal glucose production and a reduction in lipolysis by adipocytes^[8]. The major mechanism is through reduction in hepatic production, mediated by phosphorylation of the transcriptional co-activator cAMP response element-binding protein thus reducing the expression of genes inducing gluconeogenesis^[9].

It is thought that metformin associated lactic acidosis (MALA) may occur through anaerobic stimulation of lactate production by intestinal cells, with impaired elimination of lactate from the liver and contributed to by accumulation of metformin if there is renal failure, overdose or liver failure^[8].

New Zealand has a pharmaceuticals scheme with metformin freely available and fully subsidised. The purpose of this report was to review all cases of lactic

acidosis in patients on metformin at Auckland City Hospital.

MATERIALS AND METHODS

Auckland City Hospital is an adult tertiary referral centre which serves a population of just over 400000 people. Using a health information technology system all cases of metabolic acidosis between July 2005 and July 2009 were identified. Acidosis was defined as a pH \leq 7.35. Lactic acidosis was defined as a lactate of \geq 5 mmol/L, in association with a low bicarbonate and a low PCO₂. Patients with a mixed respiratory and metabolic acidosis were excluded.

The clinical records were available and reviewed for all potential cases. The dose and duration of metformin, other medications, co-morbidities and baseline laboratory data were obtained from the clinical records and primary practice.

Population estimates were obtained from Statistics New Zealand^[10]. Data about metformin use in the Auckland region was obtained from the Pharmaceutical Management Agency of New Zealand (PHARMAC). The incidence of diabetes mellitus was estimated from data from the New Zealand Health Survey^[11].

Poisson regression statistics were used to determine the risk of lactic acidosis, using the general population as the reference.

RESULTS

Eight hundred cases of lactic acidosis were identified by the health information technology system. Two hundred and eighty-eight cases of metabolic acidosis were identified by review of laboratory data. Forty-three cases of metabolic lactic acidosis were identified. One was in a nineteen-year-old female, who had an intentional overdose with ten grams of metformin, and was not included in the analysis, thus leaving forty-two cases. Thirty-two patients had metabolic lactic acidosis which was clearly associated with sepsis (Figure 1). Seven of these cases were in patients also taking metformin (Table 1). Four were in patients with diabetes mellitus, not on metformin. Ten patients were taking metformin and did not have a strong alternate cause for lactic acidosis (Table 2).

The population in Auckland City over this period was estimated at 419000 people and increased to 444000 people over the study period. Eighteen point eight percent of the Auckland population are children and cared for by the regional paediatric institution. Thus, we estimate a total of 1395000 patient years over the study period.

The number of patients receiving metformin between July 2005 and July 2009, in Auckland City, was estimated at 51400 patient years. It was estimated that there were 15600 adult patients with diabetes in Auckland each year.

The incidence of metabolic lactic acidosis was

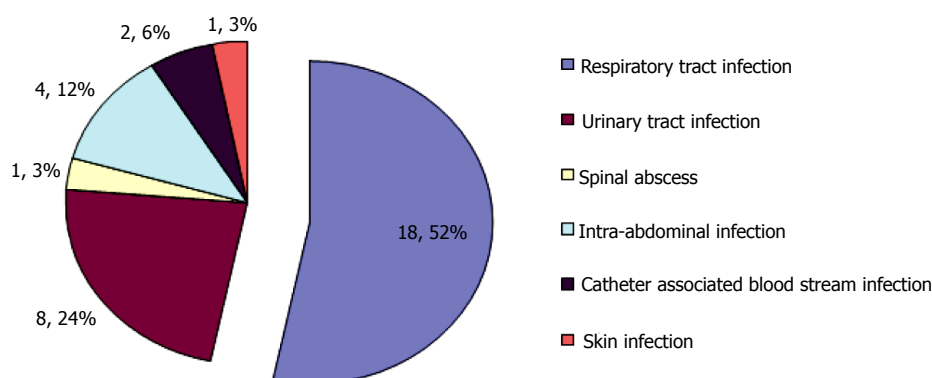


Figure 1 Cause of sepsis in patients with lactic acidosis.

Table 1 Patients on presenting with lactic acidosis and sepsis

	All patients	Patients on metformin
No. of patients	32	7
Age (yr) ¹	22-85	46-81
Sex	19 females	4 female
Ethnicity	19 Europeans, 9 Pacific People, 1 NZ Maori, 1 Indian 2 Chinese	3 Europeans, 3 Pacific People
Baseline Creatinine (μmol/L)	41-200	58-140
² eGFR mL/min per 1.73 m ²	25-90	31-87
Creatinine at presentation (μmol/L)	50-600	103-463
Number who died	15	3
Number receiving acute haemodialysis	Three	Nil
Creatinine at discharge (μmol/L)	53-245	56-60
eGFR mL Tab/min per 1.73 m ²	22-90	77-90

¹The age of the patient is rounded down to the nearest year; ²eGFR: The estimated glomerular filtration rate based on the four variable MDRD formula^[11]. eGFR: Estimated glomerular filtration rate; MDRD: Modification of diet in renal disease.

estimated to be 3.01 per 100000 patient years for the general population. The incidence of metabolic lactic acidosis due to sepsis was estimated as 2.29 per 100000 patient years.

The incidence of metabolic lactic acidosis was 33.07 per 100000 patient years' exposure to metformin. There was a significant increase in the relative risk of lactic acidosis in patients on metformin compared to the general population RR = 13.53 (95%CI: 7.88-21.66).

The incidence of MALA was 19.46 per 100000 patient years exposure to metformin. There were four male and six female patients whose mean age was 63 years, range 40-83 years (Table 2). All patients were prescribed metformin for type 2 diabetes mellitus and were also on either an angiotensin converting enzyme inhibitors or an angiotensin two receptor antagonist. Four patients presented with congestive heart failure, two patients had ischaemic events, three patients had gastroenteritis and one patient had a bradyarrhythmia as the primary

diagnosis. All patients had their renal function tested in the community prior to their presenting illness; the baseline eGFR, as determined using the modified MDRD formula^[12], ranged from 23-90 mL/min per 1.73 m². Only two patients had chronic kidney disease (CKD) 4, five patients had CKD3. In addition to other therapy three patients were treated with emergent haemodialysis (patients 1, 3 and 9, Table 2). Two patients died, one of cardiac ischaemia and one of multi-organ failure (patient 1 and 5 respectively, Table 2).

DISCUSSION

Metformin remains an attractive option in the treatment of type 2 diabetes: It promotes weight loss and has been shown to reduce the complications of and the mortality associated with diabetes^[13]. Monotherapy with metformin appears to carry greater benefits than monotherapy with other hypoglycaemic agents^[14].

The main concern with the use of metformin is the risk of developing lactic acidosis. Although a number of case series exist in the literature it is controversial as to whether MALA actually occurs. In a Cochrane review, with 70490 patient years of exposure to metformin, no cases of MALA were identified^[14]. It was estimated that the hypothetical incidence of lactic acidosis in patients treated with metformin was 4.3 per 100000 patient years and 5.4 per 100000 patient years in non-metformin users^[15]. In our series we report a low rate of lactic acidosis in the general population but that the rate of lactic acidosis in patients on metformin is significantly greater. Other population based studies have also reported a much greater rate of lactic acidosis in patients on metformin: In a recent series Scale and Harvey reported a rate of 120 per 100000 patient years^[16]. There are several potential reasons for the discrepancy between the observational studies and the clinical trial cohorts. Lactic acidosis is uncommon and may occur some time after the initiation of therapy, and thus may be missed in studies with short term follow-up. Lactic acidosis is not commonly listed as a primary discharge diagnosis, and thus may be underdiagnosed. There may be reporting bias in clinical trials. In addition, clinical trials may exclude patients such

Table 2 Demographic and clinical details of patients with metformin associated lactic acidosis

Patients	1	2	3	4	5	6	7	8	9	10
Age (yr) ¹	68	53	68	63	80	40	83	55	67	72
Sex	Male	Male	Male	Female	Male	Female	Female	Female	Female	Female
Ethnicity	Pacific Islander	Indian	European	Pacific Islander	Pacific Islander	Pacific Islander	Pacific Islander	Pacific Islander	European	European
Metformin dose (g/d)	2.5	1.7	2	3	2	2	1	2	1.7	1
Duration of Metformin ²	5 yr	4 yr	5 yr	4 yr	2 yr	1 yr	4 yr	4 yr	4 yr	2 mo
Baseline Creatinine (μmol/L)	114	57	138	123	106	123	180	105	154	139
³ eGFR mL/min per 1.73 m ²	55	130	44	38	58	42	23	47	29	32
Creatinine at Presentation (μmol/L)	449	90	333	612	381	612	376	895	973	304
pH	7.3	7.23	7.14	7.32	6.99	7.21	7.34	6.9	7.35	7.09
Bicarbonate (mmol/L)	15	13	13	16	6	12	12	3	15	15
Lactate (mmol/L)	9.2	6.7	15	6	16	8.4	6	22	6.2	7
Received acute haemodialysis	Yes	No	Yes	No	No	No	No	No	Yes	No
Outcome	Dead	Alive	Alive	Alive	Dead	Alive	Alive	Alive	Alive	Alive
Creatinine at discharge (μmol/L)	-	73	121	95	-	90	167	123	129	96
³ eGFR mL/min per 1.73 m ²	-	97	52	52	-	60	25	39	36	50

¹The age of the patient is rounded down to the nearest year; ²The duration of metformin is rounded down to completed years of therapy, except for patient 10; ³eGFR: The estimated glomerular filtration rate based on the four variable MDRD formula^[11]. eGFR: Estimated glomerular filtration rate; MDRD: Modification of diet in renal disease.

as the elderly or other with co-morbidities that may also contribute to the risk of developing lactic acidosis.

We used data from the New Zealand health survey to estimate the incidence of diabetes in Auckland. This survey estimated the prevalence of diabetes in children as 0.1%-0.4% and the number of diagnosed adult patients at between 3.4% to 6.3%. This is in line with national estimates but does not account potential patients with undiagnosed diabetes. Thus, we are likely to be underestimating the overall incidence of diabetes in the region. We used data from Pharmac to estimate the use of metformin in the region. Pharmac records the subsidised use of metformin. Currently, all New Zealanders enrolled with a general practice are eligible for subsidised metformin and a free health check if they have diabetes. However, this does not extend to non-New Zealand residents in the region. Further, if a number of patients with diabetes are not enrolled with a primary practice, then they are also ineligible for subsidised metformin. Finally, a phased rollout of subsidized medications occurred between 2003 and 2007. All of these factors may also lead to underestimation of the use of metformin in the region.

We describe a series of ten patients with MALA. The mortality in this group of patients is high but not as great as that seen in lactic acidosis associated with sepsis, and all cases were associated with acute kidney injury. Renal replacement therapy is an attractive therapeutic option as it aids in the correction of acidosis and also the removal of lactate and metformin. However, it is not clear that haemodialysis confers any survival benefit^[17]. In our series only three patients received dialysis. Interestingly, the patient who presented with the worst laboratory parameters, case 8, was managed with supportive therapy, did not receive dialysis, and survived with recovery of her renal function.

Clearly metformin is an effective therapy. Here we

describe observational evidence that suggests that use of metformin is associated with an increased risk of lactic acidosis. The standard recommendations are to use metformin cautiously in patients with hepatic impairment and reduce the dose in the elderly. We recommend reducing the dose of metformin in CKD G4 and advise stopping when the eGFR is less than 20 mL/min. We suspect that this later message is well heeded, and may be a reason that only two patients in our series with CKD G4 were found to develop MALA. In addition, we routinely recommend to patients that if they develop an intercurrent illness that metformin be withheld and medical review is sought.

Further investigation of this issue is suggested to confirm the findings in this study, using more robust design and controlling more potential confounding factors, *e.g.*, indication for metformin, co-morbidities and age.

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COMMENTS

Background

The incidence of metformin associated lactic acidosis (MALA) is small when assessed by systematic review, however, randomised controlled trials may underestimate the true incidence by using strict inclusion and exclusion criteria. This retrospective review describes the incidence of MALA and highlights the significant morbidity and mortality that is associated with this condition.

Research frontiers

No randomised clinical trial has been undertaken to assess the safety of metformin in patients with mild to moderate renal impairment. This would be

challenging due to the low incidence of MALA. Use of observational cohort data or national patient registries may better quantify risk and acceptable clinical practice.

Applications

The authors recognise the efficacy of metformin as a therapeutic agent for type 2 diabetes and recommend reducing the dose of metformin in mild to moderate renal impairment, and advise halting metformin when the estimated glomerular filtration rate is less than 20 mL/min. In addition, they recommend to patients that if they develop an intercurrent illness that metformin be withheld and medical review sought.

Peer-review

The paper gives interesting information about the incidence of metformin associated lactic acidosis which is important for clinical practice. It is well written and the analysis has been performed adequately.

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Observational Study

Skin disorders in peritoneal dialysis patients: An underdiagnosed subject

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Abstract

AIM: To examine all skin changes in peritoneal dialysis (PD) patients followed up in our unit.

METHODS: Patients on PD program for at least three months without any known chronic skin disease were included in the study. Patients with already diagnosed skin disease, those who have systemic diseases that may cause skin lesions, patients with malignancies and those who did not give informed consent were excluded from the study. All patients were examined by the same predetermined dermatologist with all findings recorded. The demographic, clinical and laboratory data including measures of dialysis adequacy of patients were recorded also. Statistical Package for Social Sciences (SPSS) for Windows 16.0 standard version was used for statistical analysis.

RESULTS: Among the patients followed up in our PD unit, those without exclusion criteria who gave informed consent, 38 patients were included in the study with male/female ratio and mean age of 26/12 and 50.3 ± 13.7 years, respectively. The duration of CKD was 7.86 ± 4.16 years and the mean PD duration was 47.1 ± 29.6 mo. Primary kidney disease was diabetic nephropathy in 11, nephrosclerosis in six, uropathologies in four, chronic glomerulonephritis in three, chronic pyelonephritis in three, autosomal dominant polycystic kidney disease in three patients while cause was unknown in eight patients. All patients except for one patient had at least one skin lesion. Loss of lunula, onychomycosis and tinea pedis are the most frequent skin disorders recorded in the study group. Diabetic patients had tinea pedis more

frequently ($P = 0.045$). No relationship of skin findings was detected with primary renal diseases, comorbidities and medications that the patients were using.

CONCLUSION: Skin abnormalities are common in PD patients. The most frequent skin pathologies are onychomycosis and tinea pedis which must not be overlooked.

Key words: Skin; Peritoneal dialysis; Onychomycosis; Tinea pedis; Xeroderma

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Core tip: Skin abnormalities are common in peritoneal dialysis patients. We aimed in our study to examine all skin changes in peritoneal dialysis patients followed up in our unit. Among the 38 patients included, all but one patient had at least one skin lesion. Loss of lunula, onychomycosis and tinea pedis are the most frequent skin disorders recorded in the study group. Diabetic patients had tinea pedis more frequently. No relationship of skin findings was detected with primary renal diseases, comorbidities and medications that the patients were using. Skin changes are commonly overlooked and should be sought for timely diagnosis and treatment.

Gursu M, Uzun S, Topcuoğlu D, Koc LK, Yucel L, Sumnu A, Cebeci E, Ozkan O, Behlül A, Koc L, Oztürk S, Kazancıoğlu R. Skin disorders in peritoneal dialysis patients: An underdiagnosed subject. *World J Nephrol* 2016; 5(4): 372-377 Available from: URL: <http://www.wjgnet.com/2220-6124/full/v5/i4/372.htm> DOI: <http://dx.doi.org/10.5527/wjn.v5.i4.372>

INTRODUCTION

The chronic uremic status and the concomitant metabolic disorders may lead to a variety of structural and functional changes in the skin and its appendages. Dermatologic abnormalities are common in chronic kidney disease (CKD) and almost all patients have at least one of the cutaneous involvements^[1]. These abnormalities range from the frequently seen xerosis and pruritus to the more rare disorders like hyperpigmentation, purpuric skin changes, acquired perforating dermatosis, and nail abnormalities^[1-3]. Some of these disorders are described specifically in end stage renal disease (ESRD) like acquired perforating dermatosis, bullous dermatoses, metastatic calcification, and nephrogenic systemic fibrosis, while the others are nonspecific findings that may be associated with various entities. These include pruritus, color changes, xerosis, and half-and-half nails^[4]. Symptoms associated with skin disorders can lead to varying degrees of discomfort, anxiety, depression, sleeping disorders and can affect the quality of life leading to distorted mental and physical health^[5].

The skin changes observed in hemodialysis (HD)

patients have been studied previously^[2,6,7]. But, data about peritoneal dialysis (PD) patients regarding skin changes is lacking in the literature except for a few studies subjecting only skin color changes and xerosis^[8-10] and another study reported in 1992^[1].

We aimed in our study to examine all skin changes in PD patients followed up in our PD unit.

MATERIALS AND METHODS

Patients who gave informed consent among those who were on PD program for at least three months and followed up in our PD unit were included in the study. Patients were using either conventional glucose based solutions or biocompatible solutions as well as icodextrin. Patients with already diagnosed skin disease, those who have systemic diseases that may cause skin lesions, patients with malignancies and those who did not give informed consent were excluded from the study. All patients were examined once by the same predetermined dermatologist with all findings recorded. Baseline data including age, gender, concomitant diseases, duration of CKD and PD therapy, and the medications used by each patient were recorded. Concurrent medications and dose of monthly erythropoietin were also documented. Laboratory investigations in the form of complete blood counts, blood glucose, urea, creatinin, uric acid, aspartate transaminase, alanine transaminase, alkaline phosphatase, gamma glutamyl transferase, total protein, albumin, bilirubin, electrolytes, calcium, phosphorus, parathyroid hormone, ferritin, transferrin saturation, vitamin B12, folic acid, total cholesterol, Low-density lipoprotein cholesterol, and triglyceride levels at the time of physical examination and hepatitis panel collected from the most recent data in the patients' files were recorded. Among PD related parameters, weekly Kt/V urea, peritoneal Kt/V urea, residual renal glomerular filtration rate (GFR) and the transport type of the patients were obtained.

Statistical analysis

Statistical Package for Social Sciences (SPSS) for Windows 16.0 standard version was used for statistical analysis. Numerical parameters were expressed as mean \pm SD. Intergroup comparisons of nonnumeric parameters were done by χ^2 test were used. P values less than 0.05 were accepted as statistically significant.

RESULTS

Among the 52 patients followed up in our PD unit, three patients were already on treatment for a symptomatic skin disorder (one for psoriasis, two for xerosis cutis), one patient had breast cancer and 10 patients rejected to be examined by the dermatologist. The remaining 38 patients were included in the study. Female/male ratio and the mean age were 26/12 and 50.3 ± 13.7 years, respectively. The duration of CKD was 94.3 ± 49.9 mo and the mean PD duration was 47.1 ± 29.6 mo. The PD modality was continuous ambulatory peritoneal dialysis

Table 1 Biochemical and hematological laboratory data of the patients

Parameter	Mean \pm SD	Parameter	Mean \pm SD
Hemoglobin (g/dL)	10.9 \pm 1.3	Phosphorus (mg/dL)	4.7 \pm 1.0
Hematocrit (%)	32 \pm 3	Parathyroid hormone (pg/mL)	600 \pm 502
Ferritin (ng/dL)	288 \pm 294	Alkaline phosphatase (U/L)	180 \pm 342
Transferrin saturation (%)	25 \pm 10	Alanine transaminase (U/L)	12.8 \pm 5.4
Total protein (g/dL)	6.9 \pm 0.5	Aspartate transaminase (U/L)	16.2 \pm 6.6
Albumin (g/dL)	3.5 \pm 0.3	Total bilirubin (mg/dL)	0.5 \pm 0.4
Uric acid (mg/dL)	5.6 \pm 1.0	Direct bilirubin (mg/dL)	0.1 \pm 0.02
Calcium (mg/dL)	8.9 \pm 0.6	Gamma glutamyl transferase (U/L)	24.8 \pm 22.4

Table 2 The medications used by the patients

Drug	n (%)	Drug	n (%)
Calcium-containing phosphorus binders	27 (71)	Alpha blockers	6 (16)
Diuretics	25 (66)	Acetylsalicylic acid	6 (16)
Active vitamin D	21 (55)	RAS blockers	5 (13)
Erythropoiesis stimulating agents	20 (53)	Cinacalcet	5 (13)
Calcium channel blockers	18 (47)	Allopurinol	3 (7)
Beta blockers	13 (34)	Fibrates	1 (2.6)
Statins	14 (37)	Sevalemer	1 (2.6)
Essential amino acid	10 (26)		

RAS: Renin-angiotensin-aldosterone system.

(CAPD) in 31 patients and automated peritoneal dialysis (APD) in seven patients. Diabetes mellitus was the most common cause of ESRD ($n = 11$, 28.9%). Other causes of ESRD were hypertensive nephrosclerosis ($n = 6$, 15.7%), urological disorders ($n = 4$, 10.5%), chronic glomerulonephritis ($n = 3$, 7.8%), chronic pyelonephritis ($n = 3$, 7.8%), autosomal dominant polycystic kidney disease ($n = 3$, 7.8%); while the etiology was not known in the remaining eight patients (21%). Hypertension ($n = 24$, 63.1%), diabetes mellitus ($n = 13$, 34.2%), hyperlipidemia ($n = 11$, 28.9%), hypothyroidism ($n = 8$, 21%), ischemic heart disease ($n = 7$, 18.4%), malignancies ($n = 3$, 7.8%), cerebrovascular disease ($n = 1$, 2.6%) were recorded as comorbidities.

The biochemical and hematological laboratory data of the patients are presented in Table 1. The mean Kt/V urea and weekly creatinine clearance values were 2.46 ± 0.67 and 78 ± 33 L/wk per 1.73 m^2 , respectively. The medications that the patients were using are presented in Table 2.

All patients except for one patient had at least one skin lesion. The skin disorders recorded in patients are presented in Table 3. Loss of lunula, onychomycosis and tinea pedis are the most frequent skin disorders recorded in the study group.

Diabetic and nondiabetic patients were similar regarding skin findings except for tinea pedis which was more common in diabetic patients ($n = 8$, 61% vs $n = 7$, 28%; $P = 0.045$). Patients using erythropoiesis stimulating agents have lower rate of xeroderma cutis compared to those not using them ($n = 11$, 55% vs $n = 3$, 17%; $P = 0.014$) as well as lower rate of onychomycosis ($n = 5$, 25% vs $n = 11$, 61%; $P = 0.024$). Loss of lunula was more rare in patients on statin treatment ($n = 1$, 7% vs $n = 16$, 67%; $P < 0.001$).

Patients using diuretics had higher rate of tinea pedis ($n = 13$, 52% vs $n = 2$, 15%; $P = 0.028$). No relationship of skin findings was detected with primary renal diseases, comorbidities and medications that the patients were using.

DISCUSSION

Skin abnormalities are common in patients with ESRD. Previous studies were mostly about the skin findings in patients on HD treatment. On the other hand, studies about dermatological abnormalities in PD patients are limited to a few studies in which only hyperpigmentation and xerosis were searched for, and an old study in which PD patients were regarded as a separate group^[1,8-10].

It was reported in the study by Picó *et al.*^[1] that patients on different dialytic treatments have different skin abnormalities. The pathologies underlying skin changes in uremic patients are accumulation of uremic toxins, metabolic abnormalities and dryness of the skin^[11-13]. Besides, there are findings supporting the role of the type of dialysis on the profile of skin changes^[2,14]. It has been reported that signs and symptoms related to skin increase after starting HD treatment^[2]. There may also be role of the apparatus used during dialysis and chemical irritation due to dialysis solutions besides dialysis adequacy. In fact, allergic skin reactions have been reported in 10% of patients using icodextrin^[15].

We evaluated in our study the prevalence of skin abnormalities in patients on PD treatment and its relationship with primary renal disorder, comorbidities and the medications.

The most frequent skin finding in our study population was loss of lunula which was observed in 44.7% of our patients. No data was found in the literature about loss

Table 3 The skin findings of the patients

Lesion	n (%)	Lesion	n (%)
Loss of lunula	17 (44.7)	Koilonychia	1 (2.6)
Onychomycosis	16 (42.1)	Pigmented purpuric dermatosis	1 (2.6)
Tinea pedis	15 (39.5)	Neurodermitis	1 (2.6)
Xeroderma cutis	14 (36.8)	Prurigo nodularis	1 (2.6)
Hyperpigmentation	11 (28.9)	Splinter hemorrhage	1 (2.6)
Nevus	6 (15.8)	Subungual hyperkeratosis	1 (2.6)
Acne	4 (10.5)	Verruca vulgaris	1 (2.6)
Uremic pruritus	3 (8.1)	Vitiligo	1 (2.6)
Contact dermatitis	2 (5.3)	Half and half nail	1 (2.6)
Folliculitis	2 (5.3)	Acne rosacea	1 (2.6)
Chronic eczema	1 (2.6)		

of lunula in PD patients. Ozturk *et al*^[16] reported in their study related to nail changes in HD patients, that loss of lunula was present in 58% of HD patients while the rate was 8% in the control group. Renal transplant recipients were compared with healthy subjects regarding nail changes in Egypt^[17]. The rates were similar in both groups (30% vs 26%), and the finding was accepted as a normal variation^[17].

Half and half nail was detected in only one patient in our study, while it was reported at an average rate of 20% in studies reaching even 76%^[4,18,19]. Ozturk *et al*^[16] reported that half and half nail was present in 15% of the HD patients involved in their study. Picó *et al*^[11] also reported increased frequency of this abnormality in HD patients. All these findings lead to the idea that half and half nail may be related with HD specifically.

Hyperpigmentation was observed 28.9% of patients in our study. Increased melanocyte stimulating hormone levels, increased dermal melanin density, dermal accumulation of urochrome pigments and carotenoids are responsible for hyperpigmentation in patients with ESRD^[14,20]. Increased length of time on dialysis and loss of residual renal functions increase the frequency of hyperpigmentation. Hyperpigmentation has been reported to be present in patients with ESRD at rates between 17% and 22%^[1,2,21]. The frequency of splinter hemorrhages and echymoses was higher in relatively old studies, while their rates have decreased in recent studies^[14]. Patients in our study did not have any sign of skin hemorrhage.

Xerosis cutis is one of the most frequent skin lesions in patients with ESRD. Besides decreasing the quality of life, xerosis caused delayed wound healing and propensity to skin infections^[13,22]. The rate of xerosis cutis in the literature is about 50%-85% while the corresponding number in our study is 36.8%^[23]. Morton *et al*^[10] found higher incidence of xerosis and pruritus in PD patients compared to HD patients. They stated that this difference may be related to defects in calcium homeostasis.

We detected onychomycosis and tinea pedis in 42.1% and 39.5% of the patients, respectively in our study. The corresponding rates were 52% and 25% in the study by Picó *et al*^[11] which is the single study in which skin findings of PD patients were evaluated. Moreover, the

authors stated that they were more frequent in diabetic PD patients compared to HD patients and non-diabetic counterparts respectively. Tinea pedis was more frequent in diabetic subjects in our study also ($P = 0.045$). The glucose content of dialysis solutions and the resultant worsening in glucose regulation may cause a propensity for infection.

Patients using diuretics had higher rate of tinea pedis ($P = 0.028$). This may be related with hypervolemia and so edema which necessitates use of diuretics. But it can be just a speculation, because clinical findings of patients were not recorded.

There was no correlation between the frequency of skin lesions and other comorbid diseases, dialysis adequacy parameters and metabolic abnormalities including hyperphosphatemia.

Patients using erythropoiesis stimulating agents have lower rate of xeroderma cutis and lower rate of onychomycosis compared to those not using them.

The other less frequent skin findings detected in our patients are presented in Table 2. It was striking that acquired perforating dermatosis, bullous dermatoses, metastatic calcification and calciphylaxis which are regarded as specific manifestations of HD patients and related to mortality in some cases, were not reported in our study group^[14,24,25]. There was no control group consisting of HD in our study; but when compared with the results of studies carried out with HD patients, PD patients seem to be protected from severe skin lesions. Onychomycosis and tinea pedis comprised the majority of skin pathologies in our study^[11].

There may be several reasons for the difference between these two dialysis modalities regarding patterns of skin pathologies. The ultrafiltration process spread to 24 h protects PD patients from hemodynamic instability; and generally prevents excessive ultrafiltration. So, a more stable hemodynamic status provides better and continuous tissue perfusion. More importantly, HD procedure itself may cause skin hypoxia. Previous studies showed that transdermal oxygen perfusion may decrease by as much as 15-20 mmHg during hemodialysis and dermal microcirculation may be distorted^[26,27]. The type of the HD membrane used may be effective on this effect^[28]. The involvement of skin, which is the most distal organ in

the body, is a predictable result of hypoxemia and tissue hypoxia. PD, with more stable hemodynamic status and better skin oxygenation, may allow lesions easy to cope with to be more frequent. On the other hand, exposure to high amount of glucose for a prolonged time may increase the frequency of fungal skin infections in both diabetic and nondiabetic PD patients.

Skin abnormalities are common in in PD patients. The spectrum of clinical presentation is different from HD patients based on recent reports. The most frequent skin pathologies are onychomycosis and tinea pedis which must not be overlooked.

COMMENTS

Background

Dermatologic abnormalities are common in chronic kidney disease and almost all patients have at least one of the cutaneous involvements. Some of these disorders are described specifically in end stage renal disease while the others are nonspecific findings that may be associated with various entities. Symptoms associated with skin disorders can lead to varying degrees of discomfort, anxiety, depression, sleeping disorders and can affect the quality of life leading to distorted mental and physical health.

Research frontiers

The skin changes observed in hemodialysis patients have been studied previously. But, data about peritoneal dialysis patients regarding skin changes is lacking in the literature except for a few studies subjecting only skin color changes and xerosis. The authors aimed to examine all skin changes in peritoneal dialysis (PD) patients followed up in the authors' PD unit.

Innovations and breakthroughs

Data about peritoneal dialysis patients regarding skin changes is limited in the literature. This study showed that skin abnormalities are common in PD patients. The spectrum of clinical presentation is different from hemodialysis patients based on recent reports. The most frequent skin pathologies are onychomycosis and tinea pedis which must not be overlooked.

Terminology

Peritoneal dialysis is an option for patients with end stage renal disease. The most commonly encountered skin lesion in chronic kidney disease are xerosis and pruritus. Xerosis is abnormal dryness of the skin which also may cause pruritus.

Peer-review

Skin abnormalities are common in in PD patients. The most frequent skin pathologies are onychomycosis and tinea pedis which must not be overlooked.

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Measurement of the intestinal permeability in chronic kidney disease

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Author contributions: Terpstra ML and Singh R performed the electronic search, all co-authors searched their own personal databases; Terpstra ML and Singh R independently screened all the articles for meeting the inclusion criteria; Bemelman FJ was consulted if there was discussion about inclusion; Terpstra ML extracted all data and wrote the paper under supervision of Geerlings SE and Bemelman FJ.

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Abstract

AIM: To evaluate methods measuring the intestinal permeability in chronic kidney disease (CKD) and clarify whether there is an increased intestinal permeability in CKD.

METHODS: We reviewed the literature in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-analysis (PRISMA) protocol and performed a systematic literature search through MEDline and EMBASE. All controlled trials and cohort studies using non-invasive methods to assess intestinal permeability in CKD patients were included. Excluded were: Conference abstracts and studies including patients younger than 18 years or animals. From the included studies we summarized the used methods and their advantages and disadvantages. For the comparison of their results we divided the included studies in two categories based on their included patient population, either assessing the intestinal permeability in mild to moderate CKD patients or in end stage renal disease (ESRD) patients. Results were graphically displayed in two plots, one comparing the intestinal permeability in mild to moderate CKD patients to healthy controls and one comparing the intestinal permeability in ESRD patients to healthy controls.

RESULTS: From the 480 identified reports, 15 met our inclusion criteria. Methods that were used to assess the intestinal permeability varied from markers measured in plasma to methods based on calculating the urinary excretion of an orally administered test substance. None of the applied methods has been validated in CKD patients and the influence of decreased renal function on the different methods remains unclear to a certain extent. Methods that seem the least likely to be influenced by decreased renal function are the quantitative PCR (qPCR) for bacterial DNA in blood and D-lactate. Considering

the results published by the included studies; the studies including patients with mild to moderate CKD conducted conflicting results. Some studies did report an increase in intestinal permeability whilst other did not find a significant increased permeability. However, despite the variety in used methods among the different studies, all studies measuring the intestinal permeability in ESRD point out a significant increased intestinal permeability. Results should nevertheless be interpreted with caution due to the possible influence of a decreased glomerular filtration rate on test results.

CONCLUSION: The intestinal permeability in CKD: (1) could be measured by qPCR for bacterial DNA in blood and D-lactate; and (2) seems to be increased in ESRD.

Key words: Chronic kidney disease; Intestinal barrier function; Intestinal permeability; Markers; Renal failure

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Core tip: Several methods are currently being used to measure the intestinal permeability, there is however no gold standard. In addition to this, most methods are influenced by renal function. We suggest that preferred methods to assess the intestinal permeability in chronic kidney disease patients could be quantitative PCR for bacterial DNA in blood and D-lactate. Independent of the used method, all studies measuring the intestinal permeability in patients with end stage renal disease (ESRD) reported a significantly increased intestinal permeability. Even though these results should be interpreted with caution due to the disadvantages of the applied methods, it seems likely that there is a connection between ESRD and intestinal barrier dysfunction.

Terpstra ML, Singh R, Geerlings SE, Bemelman FJ. Measurement of the intestinal permeability in chronic kidney disease. *World J Nephrol* 2016; 5(4): 378-388 Available from: URL: <http://www.wjgnet.com/2220-6124/full/v5/i4/378.htm> DOI: <http://dx.doi.org/10.5527/wj.v5.i4.378>

INTRODUCTION

Within the last three decades an increasing number of studies highlight the role of chronic systemic inflammation in the progression of chronic kidney disease (CKD) to end stage renal disease (ESRD) and its associated complications, such as cardiovascular disease^[1,2]. Even though the inflammatory status has been pointed out as an important prognostic factor in CKD, the pathophysiology has not been elucidated. Factors that appear to be involved are retained uremic toxins, hypervolemia, hypertension, underlying disease (diabetes, autoimmune disease, *etc.*) and infection^[3,4]. In addition to this, more recent studies have been opposing alterations in the gut as possible source of inflammation^[5-7].

An important aspect of the alterations in the gut is a decreased barrier function causing an increased intestinal permeability, which possibly leads to diffusion of endotoxins and bacterial DNA through the epithelial barrier into the circulation. An interesting finding was reported in uremic rats; gut bacteria and their DNA fragments were found in the intestinal wall and the mesenteric lymph nodes, whilst their non-uremic controls showed no signs of these fragments in the obtained biopsies^[8]. The entry of uremic retention solutes, bacterial DNA, endotoxins and other possibly noxious compounds from the intestinal lumen into the circulation is likely to contribute to the inflammatory status of CKD patients and thus their prognosis.

The suggestion of an increased intestinal permeability as a prognostic factor in CKD has led to an increased interest in non-invasive methods measuring the intestinal permeability in CKD patients. There are numerous approved ways to assess the intestinal permeability, which was outlined in 2010 by Grootjans *et al.*^[9]. There is however no gold standard and each method comes with its own advantages and disadvantages. An important aspect is how renal function interferes with the test results; most studies assessing the intestinal permeability exclude CKD patients to prevent possible bias obtained by a decreased estimated glomerular filtration rate (eGFR).

Presently there is no overview available on the results of studies assessing the intestinal permeability in CKD.

This systematic review provides an overview of the studies assessing the intestinal permeability of the small and large intestine in CKD patients. We will answer two research questions: (1) what is the best available method to determine the intestinal permeability in CKD patients; and (2) what is currently known on intestinal permeability in CKD.

We discuss the methods used to assess the intestinal permeability in CKD patients and their advantages and disadvantages, specifically focusing on the influence of renal function. In addition to this we extracted the data derived from these studies in order to summarize the results of the currently available evidence of what is known about the intestinal permeability in CKD.

MATERIALS AND METHODS

We reviewed the literature in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) protocol^[10]. The study protocol was registered in the PROSPERO international registry; registration number CRD42015025101. PROSPERO is an international database of prospectively registered reviews in health and social care in which key features from the review protocol are recorded and maintained as a permanent record. PROSPERO aims to provide a comprehensive listing of systematic reviews registered at inception to help avoid unplanned duplication and enable comparison of reported review methods with what was planned in the protocol^[11].

Two co-authors (Terpstra ML and Singh R) performed

a systematic literature search through MEDLINE and EMBASE, combined with a search through personal databases of all co-authors. Search terms for each database are described in supplementary tables.

The references obtained through the search were stored within Endnote X7 file. Titles and abstracts of the obtained articles were screened by two co-authors independently, Terpstra ML and Singh R. In case of discussion about inclusion, a third investigator was consulted (Bemelman FJ). All trials and cohort studies using non-invasive methods to assess the permeability of the small and large intestine in CKD patients were included. Only methods directly reflecting the intestinal permeability of a patient at a specific time were included, studies demonstrating the effect of different compounds on the intestinal barrier were excluded. Furthermore studies only assessing the permeability of the stomach were excluded since there are no uremic and other noxious compounds produced here and the environment is almost sterile.

Other exclusion criteria were: Conference abstracts, patients younger than 18 years and animal studies. Studies reporting data that had been previously published were also excluded.

Whilst analyzing the publications possibly meeting the inclusion criteria; articles cited in the included studies were also assessed on their relevance and included when meeting the eligibility criteria.

Each reference was categorized in Endnote according to the inclusion/exclusion criteria.

For each included study the methodological quality assessment was provided by using the Newcastle - Ottawa quality assessment scale for cohort studies^[12]. For this scorings system not all items were applicable to the type of included studies; points were only given for those sections that were relevant. Hence, the maximum amount of stars that could be obtained was 6.

From the included studies the following data were retrieved: Data on CKD etiology and renal function, sample size of the subgroups, description of control group, method(s) used to assess the permeability, part of the intestine that is evaluated by this method, mean or median levels of the used marker per subgroup (if provided) and *P* value of the statistical test that was used to compare the groups. If applicable, the interaction between the measurement outcome and renal function was evaluated; whether or not the measurement outcome was corrected for the renal function.

All data were summarized in two tables: Table 1 summarizes the mechanism of action and (dis)advantages of each method and Table 2 summarizes the results obtained by each study.

In attempt to compare results studies were divided in two categories: Studies comparing the intestinal permeability in mild to moderate CKD patients (eGFR 15-90) to healthy controls and studies comparing intestinal permeability in ESRD [eGFR < 15; both hemodialysis (HD) and non-hemodialysis (non-HD)] patients to healthy controls. For each study providing the mean

and standard deviation the standardized mean difference was calculated through Review Manager 5.3. Biostatistics analysis was performed after consultation of a biomedical epidemiologist. In case of missing data the authors of the studies were contacted in order to obtain the required data. If studies only provided mean and standard deviation values of subgroups, we calculated the mean and standard deviation for the entire group with the following formula: $\text{sqrt}[(6-1)*1.47^2 + (24-1)*2.28^2]/(6-1+24-1)$. $Sp = \sqrt{(n1 - 1) \times S1^2 + (N2 - 1) \times S2^2 / (n1 - 1 + n2 - 1)}$ ^[13].

Results were graphically displayed in two plots, one comparing the intestinal permeability in mild to moderate CKD patients to healthy controls and one comparing the intestinal permeability in ESRD patients to healthy controls. Since different methods were used among the different included studies, results were not pooled and no meta-analysis was performed.

RESULTS

Our search through MEDLINE and EMBASE yielded 646 articles. The personal databases retrieved one more article and the search through the references lists of the relevant studies yielded three more studies. After removing duplicates, 480 articles remained and were screened for meeting the inclusion criteria. In 24 articles the full text was assessed. Reasons for exclusion are summarized in Figure 1. A total number of 15 studies were included in our study.

For each included study the methodological study was assessed through the Newcastle - Ottawa quality assessment scale^[12]. The amount of stars scored by each study is summarized in supplementary tables. The mean amount of stars obtained by each study was 4.7 with a range from 4 to 6 stars. Methods that were used to assess the intestinal permeability varied from markers measured in plasma to methods based on calculating the urinary excretion of an orally administered test substance. The used methods, their mechanisms of action and (dis)advantages are summarized in Table 1. Most commonly used were the sugar absorption test^[14-17], D-lactate (plasma)^[18,19] and chromium-51 labeled ethylenediamine tetra acetic acid (⁵¹Cr-EDTA) (plasma)^[20-23]. More recent studies focused on bacterial DNA^[18,19,24] and endotoxins or LPS^[16,18,25-27] in blood as a projection of intestinal permeability. Few studies used other methods such as polyethylene glycols (PEGs) in urine^[28].

Results provided by each included study are summarized in Table 2. From the 15 included studies, 7 studies provided sufficient data to calculate the mean differences: 4 studies comparing the mild to moderate CKD patients to healthy controls and 3 studies comparing the ESRD patients to the healthy control population (Figures 2 and 3).

Despite the variety in used methods among the different studies, results considering the ESRD patient population are uniform. As displayed in Figure 3, all studies comparing ESRD patients to healthy controls point out a significantly increased intestinal permeability,

Table 1 Characteristics of the used methods assessing the intestinal permeability

Marker for intestinal permeability	Mechanism of action	Advantages	Disadvantages	Influence renal function	Part of the intestine evaluated	Ref.
D-lactate (plasma)	Produced by bacteria in the colon. Present in human blood at very low concentrations as a product of methylglyoxal metabolism. In case of increased intestinal permeability levels will rise due to increased translocation across the intestinal mucosa	Non-invasive Low levels in healthy subjects, high specificity Mainly large intestine; thus focusing on part of the bowel with the highest bacterial load	Possibly increased fermentation of undigested carbohydrates to D-lactate in case of bacterial overgrowth	Influenced by renal function to some extent	Mainly large intestine	[18,19]
Sugar absorption test (urine)	Method based on calculating the urinary excretion of orally administered test substance that reflects the non-mediated diffusion of that probe across the intestinal barrier. Most commonly used combination of sugars is a oligosaccharide or disaccharide (lactulose, cellobiose) combined with a monosaccharide (mannitol). By adding sucralose to the test, which is not degraded by the bacteria of the colon, the colonic permeability can be assessed	Non-invasive Different sugar combinations can assess different parts of the gastrointestinal tract	Relative impractical in use Results could be influenced by decreased bowel motility 32 Used according to different protocols and different combinations of sugars which makes the comparison of studies difficult Relative large inter- and intra-individual variety	Influenced by renal function. Corrected by using the ratio of administered sugars. It is however not clarified whether this correction is sufficient due to possible different renal clearance of the administered sugars	Small intestine, large intestine (only if sucralose, is added)	[14-17]
⁵¹ Cr-EDTA (urine)	Method based on calculating the urinary excretion of orally administered test substance that reflects the non-mediated diffusion of that probe across the intestinal barrier	Not degraded by bacteria in the colon, useful marker for both the small and large intestinal permeability	Radioactivity Not commonly used nowadays due to radioactivity	Influenced by renal function. Corrected in included studies: 24-h Cr-EDTA excretion = 100% of the total oral dose excreted in the urine in 24 h/creatinine	Both small and large intestine	[20-23]
Endotoxin level (blood), LPS (plasma)	Indirect measurement of translocation of bacterial products	High specificity	Not eligible to use among patients with inflammation in the GI tract	Unlikely to be influenced by renal function	Both small and large intestine	[18,25,26]
Bacterial derived DNA (16S rRNA PCR) (blood)	Direct measurement of bacterial products in blood	Optimal tool for detection and identification of bacterial isolates	Not eligible to use among patients with inflammation in the GI tract	Unlikely to be influenced by renal function	Both small and large intestine	[18,19,24]
Polyethylene glycols (PEG) (urine)	Method based on calculating the urinary excretion of orally administered test substance that reflects the non-mediated diffusion of that probe across the intestinal barrier. It is hypothesized that, as saccharides in sugar absorption test, molecular PEG will only cross the intestinal mucosa to the circulation in case of barrier integrity loss. Increased urinary levels of large PEGs therefore reflect an increased intestinal permeability	Biologically inert and not degraded by bacteria, thus providing information of the whole intestinal permeability	High inter- and intra-individual variations have been reported, even in healthy controls ^[34]	Influenced by renal function	Both small and large intestine	[28]

AVF: Arteriovenous fistula; CAPD: Continuous ambulatory peritoneal dialysis; CKD: Chronic kidney disease; CVC: Central venous catheter; ESRD: End stage renal disease; HD: Hemodialysis; IgAN: IgA nephropathy; IgA GN: IgA glomerulonephritis; IC-GN: Immunocomplex glomerulonephritis; INS: Idiopathic nephrotic syndrome; Li: Lithium; LPS: Lipopolysaccharide; PD: Peritoneal dialysis; PEG: Polyethylene glycols; TER: Trans epithelial electrical resistance.

independent of the used method.

Figure 2 shows the results considering the mild to

Table 2 Results considering the intestinal permeability published in the included studies

Ref.	Population	Study size	Marker used to assess intestinal permeability (values provided as mean \pm standard deviation)	Results	Part of the intestine evaluated
Shi <i>et al</i> ^[18]	ESRD (both HD and non-HD) <i>vs</i> healthy controls ESRD group further divided patients with bacterial DNA and without bacterial DNA in their blood samples	ESRD <i>n</i> = 52 (HD <i>n</i> = 22, ND <i>n</i> = 30) Controls <i>n</i> = 10	D-lactate (plasma) Endotoxins (blood) Bacterial DNA (blood)	D-lactate plasma levels higher: ESRD HD <i>vs</i> controls <i>P</i> = 0.039 ESRD non-HD <i>vs</i> controls <i>P</i> = 0.044 HD <i>vs</i> non-HD <i>P</i> > 0.05 ESRD with bacterial DNA <i>vs</i> ESRD without bacterial DNA <i>P</i> < 0.05 ESRD HD with bacterial DNA <i>vs</i> ESRD non-HD with bacterial DNA <i>P</i> > 0.05 Endotoxin significantly higher: ESRD HD <i>vs</i> controls <i>P</i> < 0.05 ESRD non-HD <i>vs</i> controls <i>P</i> < 0.05 ESRD HD 0.95 \pm 0.12 EU/mL ESRD non-HD 0.70 \pm 0.15 EU/mL Controls 0.17 \pm 0.10 EU/mL Presence of bacterial 16S rDNA: ESRD HD 6/22 patients ESRD non-HD 6/30 patients Controls: 0/10 patients	Large intestine Mostly large intestine Mostly large intestine
Wang <i>et al</i> ^[19]	ESRD patients (non-HD) <i>vs</i> healthy controls ESRD group further divided patients with bacterial DNA and without bacterial DNA in their blood samples	ESRD <i>n</i> = 30 Controls <i>n</i> = 10	D-lactate (plasma) Bacterial 16S rDNA (blood)	Plasma D-lactate higher: ESRD with bacterial DNA <i>vs</i> ESRD without bacterial DNA <i>P</i> = 0.0233 ESRD with bacterial DNA <i>vs</i> controls <i>P</i> = 0.067 ESRD with bacterial DNA: 13.53 \pm 1.47 μ g/mL ESRD without bacterial DNA: 5.71 \pm 2.28 μ g/mL Controls: 4.82 \pm 0.93 μ g/mL D-lactate plasma levels both ESRD groups combined: 7.274 \pm 2.16 μ g/mL ¹ ESRD: 6/30 bacterial DNA in blood Controls: no bacterial DNA in blood	Large intestine
Bossola <i>et al</i> ^[24]	HD patients (AVF en CVC) <i>vs</i> healthy controls	HD <i>n</i> = 58 (AVF <i>n</i> = 44, CVC <i>n</i> = 14) Controls <i>n</i> = 30	Bacterial 16S rDNA (blood)	HD patients: 12/58 bacterial DNA in blood (= 20.7%) Healthy controls: No bacterial DNA in blood AVF patients 5/44 (= 15.9%) CVC patients 5/14 (35.7%) <i>P</i> = 0.22	Both small and large intestine
McIntyre <i>et al</i> ^[25]	HD patients, PD patients, CKD patients (stage 3-5) <i>vs</i> healthy controls	HD <i>n</i> = 120 PD <i>n</i> = 25 CKD stage 3-5 <i>n</i> = 90 Controls <i>n</i> = 14	Endotoxin level (blood)	Significant higher endotoxin levels in HD <i>vs</i> PD <i>P</i> < 0.008 Dialysis patients (HD + PD) <i>vs</i> CKD <i>P</i> < 0.001 CKD <i>vs</i> controls <i>P</i> > 0.05 HD patients: 0.64 EU/mL PD patients: 0.56 EU/mL HD + PD patients: 0.62 \pm 0.37 EU/mL CKD patients: 0.11 \pm 0.68 EU/mL Controls: Not provided	Both small and large intestine
Feroze <i>et al</i> ^[26]	HD patients, follow up for 42 mo	HD <i>n</i> = 303	Endotoxin level (blood)	No significant association between elevated circulating endotoxin levels and mortality Mean endotoxin levels: 2.31 \pm 3.10 EU/mL Significant less recovery of Cr-EDTA: CAPD <i>vs</i> controls <i>P</i> < 0.0005	Both small and large intestine
Zuckerman <i>et al</i> ^[20]	No control group CAPD patients <i>vs</i> healthy controls	CAPD patients <i>n</i> = 11 (5 with significant urine output) Controls <i>n</i> = 32	Cr-EDTA recovery (24 h urine + dialysate)	Significant less recovery of Cr-EDTA: CAPD <i>vs</i> controls <i>P</i> < 0.0005	Both small and large intestine
Szeto <i>et al</i> ^[27]	New PD patients <i>vs</i> IgAN patients (mild to moderate CKD) and healthy controls Mean creatinine level IgAN group: 151.2 \pm 116.68 μ mol/L	PD <i>n</i> = 30 IgAN <i>n</i> = 10 Controls <i>n</i> = 6	LPS (plasma)	CAPD patients: Mean 0.57% (0%-1.24%) Healthy controls: Mean 1.99% (0.59-3.48) Significantly higher LPS levels PD <i>vs</i> IgAN <i>P</i> < 0.0001 PD <i>vs</i> controls <i>P</i> < 0.0001 IgAN <i>vs</i> controls: Not provided PD: 0.44 \pm 0.18 EU/mL IgAN: 0.0035 \pm 0.009 EU/mL Controls: 0.013 \pm 0.007 EU/mL	Both small and large intestine
Cobden <i>et al</i> ^[17]	CKD patients <i>vs</i> healthy controls	CKD <i>n</i> = 6 Controls <i>n</i> = 55	Cellobiose and mannitol recovery (urine)	No significant difference recovery cellobiose and mannitol	Small intestine

	CKD group: Serum creatinine levels ranging from 140 to 1050 $\mu\text{mol/L}$			CKD <i>vs</i> controls $P > 0.05$ Cellobiose: CKD: Recovery range 0.09%-0.44% Controls: Not provided Mannitol: CKD: Recovery range 12.8%-52.3% Controls: Not provided	
Magnusson <i>et al</i> ^[28]	Asymptomatic uremic CKD <i>vs</i> healthy volunteers Mean serum creatinine level IgAN group: 503 $\mu\text{mol/L}$, range 274-796 $\mu\text{mol/L}$	CKD $n = 9$ Controls $n = 6$	PEGs (urine) Computer model was used to predict the PEG recovery adjusted for eGFR	Significant lower urinary recovery of PEG's CKD <i>vs</i> controls $P < 0.05$ More heavy PEG's were harvest in urine CKD patients: indicating that intestinal permeability in CKD patients is more increased for larger molecules	Both small and large intestine
Kovacs <i>et al</i> ^[21] and Kovacs <i>et al</i> ^[23]	IgAN patients (both uremic and non-uremic) <i>vs</i> healthy controls	1989: IgAN patients $n = 29$: (uremic $n = 24$ non-uremic $n = 5$) Controls $n = 20$ 1996: IgAN patients $n = 21$ No controls Follow up patients further divided an analyzed in two groups; increased intestinal permeability group <i>vs</i> non-increased intestinal permeability	Cr-EDTA recovery (urine)	Significantly higher Cr-EDTA recovery in IgAN patients <i>vs</i> controls $P < 0.005$, both in 1989 and in follow up after 5 yr	Both small and large intestine
These two studies published results measured in the same patient group. Provided data by the two articles are summarized	Both in 1989 and after a four year follow up in 1994 No mean serum creatinine levels of total IgAN group provided			IgAN (1989): 3.86% \pm 0.29% IgAN (1994): 4.57% \pm 0.63% Controls: 2.72% \pm 0.23% Only in the increased permeability group significant decrease in eGFR (Baseline eGFR 84.4 \pm 6.1 mL/min <i>vs</i> 65.4 \pm 8.6 mL/min after four years, $P < 0.01$)	
Rostoker <i>et al</i> ^[22]	Patients with Primary IgA glomerulonephritis and permanent proteinuria (IgA GN), INS IC-GN: Membranous + membranoproliferative) <i>vs</i> healthy controls and alcohol abusers (positive controls)	IgA GN $n = 30$ INS $n = 25$ IC-GN $n = 20$ Controls $n = 20$ Alcohol abusers $n = 5$	Cr-EDTA recovery (urine)	Significantly higher Cr-EDTA recovery in IgA GN <i>vs</i> controls $P < 0.005$ INS <i>vs</i> controls $P < 0.005$ IC-GN <i>vs</i> controls $P < 0.005$ Alcohol abusers <i>vs</i> controls $P < 0.005$ IgA GN: Median 3.25% (0.7-17.8) INS: Median 3.71% (0.82-10) IC-GN: 3.40% (0.30-16) Alcohol abusers: 4.9% (7-30) Controls: 2% (0.4-3.9)	Both small and large intestine
Layward <i>et al</i> ^[15]	Histologically proven IgAN with proteinuria and microscopic hematuria <i>vs</i> healthy No mean serum creatinine levels provided controls	IgAN patients $n = 18$ Controls $n = 17$	Cellobiose/mannitol ratio (urine)	No significant difference cellobiose/mannitol ratio IgA NP patients <i>vs</i> controls $P = 0.42$ IgA NP: 0.015 \pm 0.008 Controls: 0.022 \pm 0.015	Small intestine
De Maar <i>et al</i> ^[14]	Renal transplant patients assessed before transplantation and in the follow up during active CMV infection and CMV negative controls	Permeability assessed before transplantation $n = 104$ Permeability assessed during active infection $n = 12$ (primary infections: 5, secondary infections: 7) Controls (CMV-): $n = 9$	Lactulose/mannitol ratio (urine)	L/M ratio increased during active CMV infection in 9/12 patients $P < 0.01$ L/M ratio active CMV infection compared to patients without CMV $P < 0.01$	Small intestine Small intestine
Ponda <i>et al</i> ^[16]	CKD stadium III patients <i>vs</i> healthy controls CKD patients: mean eGFR: 51 mL/min per 1.73 ² All patients and controls	CKD $n = 5$ Controls $n = 4$	Endotoxin activity; expressed as fraction of the maximum response to endotoxin (plasma)	No significant difference endotoxin activity CKD <i>vs</i> controls $P > 0.05$ CKD: 0.23 \pm 0.15 Healthy controls: 0.20 \pm 0.13	

had a vitamin D deficiency	Lactulose/mannitol ratio (urine)	L/M ratio increased with D3 therapy $P = 0.02$ (reflecting an increase in permeability) L/M ratio not assessed in control group
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AVF: Arteriovenous fistula; CAPD: Continuous ambulatory peritoneal dialysis; CKD: Chronic kidney disease; CVC: Central venous catheter; ESRD: End stage renal disease; HD: Hemodialysis; IgAN: IgA nephropathy; IgA GN: IgA glomerulonephritis; IC-GN: Immunocomplex glomerulonephritis; INS: Idiopathic nephrotic syndrome; Li: Lithium; LPS: Lipopolysaccharide; PD: Peritoneal dialysis; PEG: Polyethylene glycols; TER: Trans epithelial electrical resistance. ¹Value not provided in article. Calculated as followed: $\text{sqrt}((6-1) \times 1.47^2 + (24-1) \times 2.28^2) / (6-1 + 24-1)$. $S_p = \sqrt{(n1 - 1) \times S1^2 + (N2 - 1) \times S2^2 / (n1 - 1 + n2 - 1)}$.

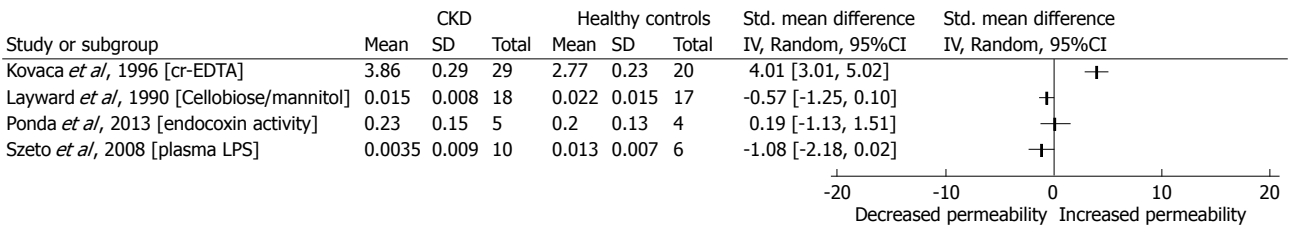
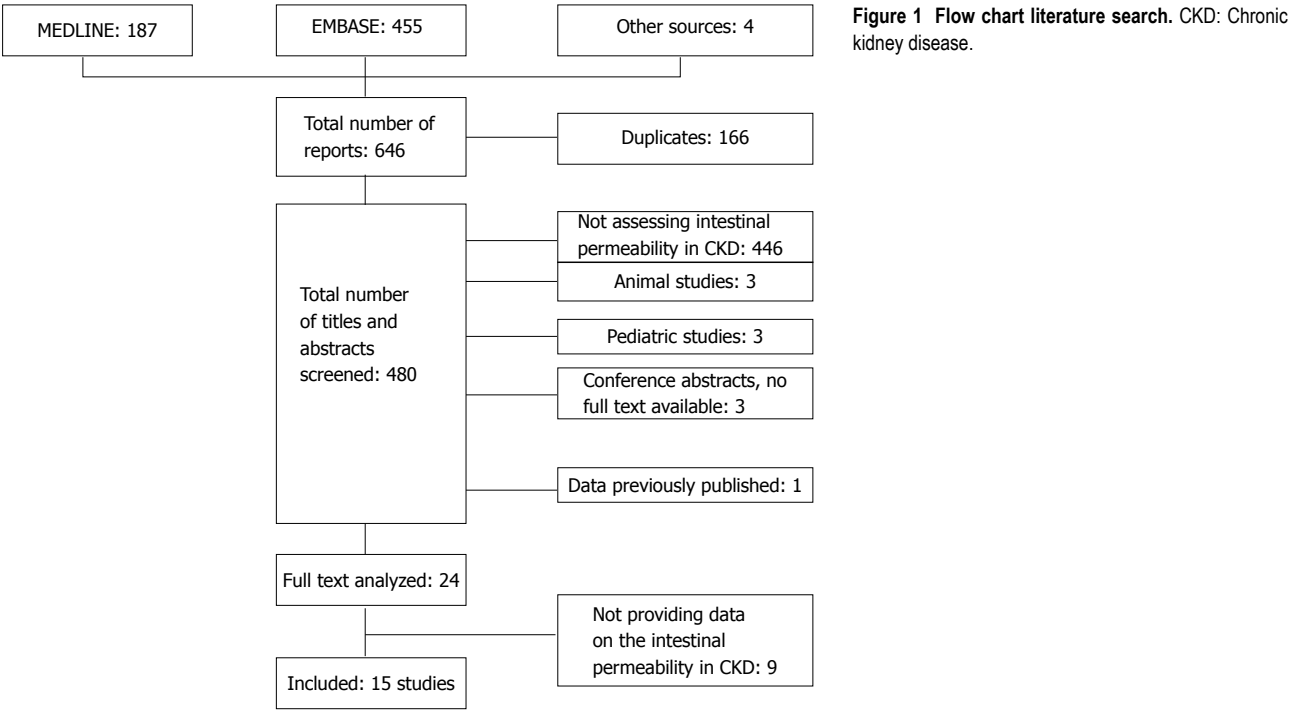


Figure 2 Intestinal permeability mild to moderate chronic kidney disease patients (epidermal growth factor receptor 15-90) vs healthy controls. CKD: Chronic kidney disease.

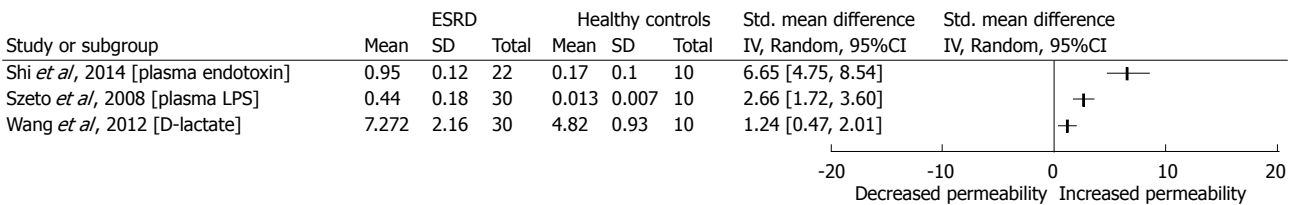


Figure 3 Intestinal permeability end-stage renal disease patients (epidermal growth factor receptor < 15) vs healthy controls. ESRD: End stage renal disease.

moderate CKD patients; results are less convincing, whilst some do point out a significant increased intestinal permeability other studies did not report a statistical difference.

There was one study also comparing the peritoneal dialysis (PD) and HD groups^[25]. They reported increased endotoxin levels in the HD compared to the PD group ($P < 0.008$), reflecting a higher permeability in the HD

group.

DISCUSSION

In this review we focused on the intestinal permeability, how it can be assessed and whether there is an increased intestinal permeability in CKD. The currently used methods for intestinal permeability assessment are primarily used and validated in gastroenterological research; patient with renal failure are often excluded due to the possible bias caused by the reduced eGFR. Discriminating between an altered renal clearance of the used marker and an actual increased permeability is challenging, since for most methods the influence of renal function loss has not been evaluated.

Table 1 summarizes the possible influence of renal function on each test. For most used markers and substances the influence of a decreased renal function remains unclear to a certain extent since there are no data evaluating the renal clearance of the substance.

The tests measuring bacterial products such as endotoxins and bacterial derived DNA are the least likely to be influenced by renal function as they are not actively excreted by the kidney. However these methods are not validated in renal failure. Furthermore, whether these products determined in the circulation actually represent an increased intestinal permeability is open for discussion, as the source of those bacterial products is not precisely known and could for example be the dialysate in dialysis patients. However, the hypothesis that these bacterial products in plasma are derived from the gut and are trans located into the bloodstream due to an increased intestinal permeability is supported by several findings. Shi *et al.*^[18] compared the endotoxin levels of plasma to the levels in the dialysate of HD patients. Endotoxin levels were markedly lower in the dialysate than in the plasma samples, suggesting another bacterial source than the dialysate. Bacterial phyla in the blood samples appeared to be similar to the samples obtained from the gut, which supports the hypothesis that these bacterial compounds are derived from the intestinal tract. Furthermore Bossola *et al.*^[24] reported that only five out of twelve plasma samples from HD patients contained the same bacteria as those in the dialysate, also suggestive for another source of the blood bacteria than the dialysate. They proposed the biofilm on the surface of the central venous catheter (CVC) as a possible source, as the percentages of patients with circulating bacterial DNA fragment tended to be higher in patients with CVCs (4 out of 15) than in patients with an arteriovenous fistula (AVF) (7 out of 44). This difference was however not statistically significant, which can be the result of the small number of patients. However, the species found in the patients with a CVC were *Escherichia coli* (2 patients), *Proteus mirabilis* (1 patient), *Enterococcus faecalis* (1 patient) and *Streptococcus Haemolyticus* (1 patients). These strains indicate rather an intestinal source. Interestingly and in accordance with the results published by Shi

et al.^[18]: In none of the blood samples obtained from healthy controls bacterial DNA was identified.

It is likely that direct demonstration of bacterial DNA in blood through qPCR is more accurate for determining the intestinal permeability compared to endotoxin level measurement, since endotoxins are bacterial surface products while presence of bacterial DNA in blood definitively indicates bacterial presence.

Another marker that is possibly valuable in the CKD population is D-lactate. D-lactate is usually present in human blood at very low concentrations as a product of methylglyoxal metabolism, which is produced in small amounts from fat, protein and carbohydrate metabolism. However, it is also produced by bacteria in the gastrointestinal tract and absorbed in the small intestine and colon. Only 10% of D-lactate is excreted in urine^[29], marking a relatively low influence of renal clearance on plasma levels. In case of bacterial overgrowth a possible increased fermentation of undigested carbohydrates to D-lactate is nevertheless an important factor that might cause bias.

Considering the sugar absorption test, various combinations of oligosaccharides (lactulose, cellobiose) and monosaccharides (mannitol, L-rhamnose) are being used. The percentage of the substance excreted in urine is defined as the urinary recovery and is often expressed as the ratio of the recovery of the administered sugars. Even though renal clearance of these sugars is assumed to be of little or no influence on the ratio since both sugars are equally affected by a reduced eGFR^[17], van Nieuwenhuizen *et al.*^[30] observed different results in their study evaluating the influence of pre- and postabsorptive factors on the lactulose/rhamnose ratio. The urinary excretion of lactulose and rhamnose was measured in 10 healthy males after intravenous administration of different quantities of each sugars. Equal renal clearance of both sugars would assure an unchanged ratio after administering a higher dose of both sugars. The investigators found a significant ($P = 0.021$) increase in lactulose/rhamnose ratio after administration of the high dose compared to the regular dose; a higher quantity of lactulose administration resulted in a lower recovery. These findings suggest that the process of renal clearance is different for the two sugars and thus that renal function might influence test results. Furthermore, in a study in endotoxaemic rats^[31], fluid loading increased the urinary recovery of lactulose, but not of L-rhamnose. This also suggests that renal clearance of both sugars might not be equal. In conclusion, literature results on the recovery of both sugars are conflicting^[30,32]. Differences in administration methods and dosage might be an explanation. The exact renal excretion of the different sugars is not clarified and thus might be affected differently when the eGFR is altered.

Furthermore, a decreased bowel motility has been reported to influence test results^[33]. This test could however be valuable as a follow up method with patients being their own controls.

The studies that used ^{51}Cr -EDTA as a marker for intestinal permeability corrected for renal function by dividing the 24 h ^{51}Cr -EDTA excretion by the plasma creatinine level^[20-23,34]. The radioactivity is nevertheless a major disadvantage that has caused this method to be considered out of date.

For the urinary recovery of different sized polyethylene glycols (PEGs), large inter- and intra-individual variations have been reported, even in healthy controls^[35]. Combined with the influence of renal function on this test we consider it to be less suitable for the CKD patient population than other available methods.

Considering the results provided by the included studies, we divided the studies in categories based on the included patient population before results were compared. In our forest plots both the mild to moderate CKD patients (eGFR 15-90) and the ESRD patients (eGFR < 15) were compared to healthy controls. Seven studies have been published comparing the intestinal permeability specifically in patients with end stage renal disease, with or without dialysis, to healthy controls^[18-20,24,27,36]. One of these studies included both HD and PD patients and also compared these groups.

From the studies comparing ESRD to healthy controls, three were providing sufficient data to calculate the standardized mean difference. Markers that were used in these studies were D-lactate, bacterial DNA, and endotoxins levels. Independent of the method that was used, all studies showed a significantly increased permeability in the ESRD group. These consistent results, despite the variety in the methods used, supports the hypothesis that renal failure is associated with increased intestinal permeability. The significant results published by studies measuring bacterial DNA and endotoxins are unlikely to be influenced by renal function.

The study also comparing the PD and HD groups^[25] reported a significant increased permeability in the HD group compared to the PD group, $P < 0.008$. This was however the only study evaluating the difference between these two groups. All included studies including HD or PD patients reported a significant difference compared to the healthy controls. Further research is required to evaluate difference between the influence of HD vs PD on the intestinal permeability.

Studies assessing the intestinal permeability in mild to moderate CKD, mostly IgA nephropathy patients^[15,23,27,34], yielded conflicting results. Even though some studies^[21,34] reported a significantly increased permeability compared to the healthy controls, other studies could not confirm this finding^[15,16,27]. Not all studies provided data on the exact renal function, but in general the eGFR was mildly decreased. This is an important difference compared to the studies assessing the intestinal permeability in end stage renal disease. Szeto *et al.*^[27] compared new peritoneal dialysis (PD) patients to both patients with mild to moderate CKD due to IgA nephropathy and healthy controls. Average serum creatinine levels of the IgA nephropathy group were $151.3 \pm 116.2 \mu\text{mol/L}$. He found significant higher endotoxin levels when comparing

the PD patients (who suffer from a later stage of CKD) to the IgA group and the healthy controls. There was no significant difference between the IgA group and the healthy control group. This suggests that the intestinal permeability might only increase in later stages of CKD.

This systematic review outlines the lack of a gold standard to determine the intestinal permeability in the CKD patient population. Even though we aim to oppose the most reliable method, the lack of a gold standard is a limitation of this systematic review. In addition to this, unfortunately none of the included studies used more than one method to measure the intestinal permeability in CKD patients in order to be able to actually compare different methods.

In conclusion, assessing the intestinal permeability in CKD patients remains challenging as the influence of decreased renal function on the test results remains unclear. Quantitative PCR for bacterial DNA in blood and D-lactate levels in plasma seem the least likely to be influenced by a decreased eGFR. It should be noted though that also these methods have not been validated in the CKD patient population and results should still be interpreted with caution^[37].

However each included study measuring the intestinal permeability in patients with ESRD pointed out a significant increased permeability. Thus, it seems likely that there is a connection between renal failure and an increased intestinal permeability. How the permeability evolves in time, the possible link with (recurrent) infection(s), cardiovascular complications and prognosis of these patients has not yet been made and requires further exploration.

COMMENTS

Background

In the recent years numerous studies have been published evaluating the intestinal permeability in chronic kidney disease (CKD). Different methods are being used whilst the influence of a decreased renal clearance on these tests is unclear, complicating the interpretation of test results published by these studies. Her aim of this review is: (1) to determine what the best available method to measure the intestinal permeability in CKD; and (2) whether there is an increased intestinal permeability in CKD.

Research frontiers

Noninvasive methods to measure the intestinal permeability have been used for many decades, with the first studies published in the 1950s. Only since the 90s there has however been an increasing interest in the intestinal alterations in renal failure and the possible clinical relevance of this aspect. Even though methods have been improved over the years, still none of the currently available methods has been validated in patients with renal failure. Furthermore it is still unclear whether there actually is an increased intestinal permeability in CKD and what the clinical relevance of this decreased barrier function is. Even though an increased intestinal permeability is proposed as an important prognostic factor, studies evaluating the influence of the intestinal permeability on the long-term prognosis of CKD patients have not yet been published.

Innovations and breakthroughs

Since 2009, three studies have been published using the quantitative amount of bacterial DNA in blood as a marker for intestinal permeability in CKD patients. This method to evaluate the intestinal permeability is unlikely to be influenced by a decreased renal clearance and also points out the exact consequence

of an increased intestinal permeability; bacterial translocation into the bloodstream. This is likely to trigger an inflammatory response and could thus be an important prognostic factor for patients with renal failure.

Applications

This review opposes the most reliable methods to determine the intestinal permeability in CKD and points out that there possibly is a link between an increased intestinal permeability and renal failure. The overview of the advantages and disadvantages of the currently available methods could help fellow researcher to determine what the most reliable method to measure the intestinal permeability in their study population. Future research is necessary specifically considering the role of the intestinal permeability as a prognostic factor in CKD. In this prospect restoration of the intestinal barrier function could also become a possible therapeutic target.

Terminology

Even though the exact pathophysiology is not yet clarified, CKD is accompanied by a chronic inflammatory response, meaning that the immune system appears to be constantly triggered. A chronic inflammatory status is associated with many complications such as cardiovascular disease, which are in turn frequently observed in CKD.

Peer-review

In this systematic review the authors have presented a critical analyse of the currently available methods to determine the intestinal permeability in CKD in order to guide fellow researcher in their choice of methods applicable to measure the intestinal permeability in CKD in future research projects. Furthermore the need for studies evaluating the intestinal permeability with reliable methods is emphasised, especially considering the lack of knowledge on the prognostic consequence of an increased intestinal permeability.

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Latin American Dialysis and Transplant Registry: Experience and contributions to end-stage renal disease epidemiology

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Abstract

In 2015, 634387 million people (9% of the world's population) resided in Latin America (LA), with half of those populating Brazil and Mexico. The LA Dialysis and Transplant Registry was initiated in 1991, with the aim of collecting data on renal replacement therapy (RRT) from the 20 LA-affiliated countries. Since then, the Registry has revealed a trend of increasing prevalence and incidence of end-stage kidney disease on RRT, which is ongoing and is correlated with gross national income, life expectancy at birth, and percentage of population that is older than 65 years. In addition, the rate of kidney transplantation has increased yearly, with > 70% being performed from deceased donors. According to the numbers reported for 2013, the rates of prevalence, incidence and transplantation were (in patients per million population) 669, 149 and 19.4, respectively. Hemodialysis was the treatment of choice (90%), and 43% of the patients undergoing this treatment was located in Brazil; in contrast, peritoneal dialysis prevailed in Costa Rica, El Salvador and Guatemala. To date, the Registry remains the only source of RRT data available to healthcare authorities in many LA countries. It not only serves to promote knowledge regarding epidemiology of end-stage renal disease and the related RRT but also for training of nephrologists and renal researchers, to improve understanding and clinical application of dialysis and transplantation services. In LA, accessibility to RRT is still limited and it remains necessary to develop effective programs that will reduce risk factors, promote early diagnosis and treatment of chronic kidney disease, and strengthen transplantation programs.

Key words: Latin America; Chronic kidney disease; Renal replacement therapy; Kidney transplantation; Prevalence; Incidence; Epidemiologic registries; Risk factors

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Core tip: In Latin America (LA), patients with end-stage renal disease on renal replacement therapy (RRT) are tracked by the LA Dialysis and Transplant Registry. Data from the Registry shows increasing prevalence and incidence, which are correlated with gross national income, life expectancy at birth, and percentage of population over 65 years. The Registry represents the only source of such data in many LA countries. Its contributions to the knowledge of RRT epidemiology in LA as well as to the education and training of nephrologists are highlighted in this article, and the need for its evolution towards population-based Registries is discussed.

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INTRODUCTION

Latin America (LA) is a conglomerate of nations sharing a common Latin ancestry and languages (mainly Spanish or Portuguese). It includes South and Central America, Mexico and the Hispanic Caribbean Islands. Multiethnic and multicultural, its population displays a great genetic diversity, resulting from multiple admixture events that occurred among the original European immigrants (mainly from Spain and Portugal but including other nationalities that arrived in large numbers during the so-called "Italian diaspora" in the late 19th and the early 20th century and also represented by those escaping the World Wars) and Native Americans (who currently represent the majority in Bolivia and very high percentages in Guatemala, Peru and Mexico) as well as the descendants of slaves that migrated from Africa (with very high numbers in Brazil in particular, and fewer in Colombia and Uruguay). The racial admixture is so robust that genetic studies arrived at the conclusion that physical appearance has poor reliability as an indicator of genetic ancestry for the LA peoples in general^[1]. In Uruguay, specifically, it was reported, "the data show that almost every population is dihybrid or trihybrid, and when African influence is not detected, it is probably due more to the method than to an absence of that contribution"^[2]. Thus, each Latin American country is considered to encompass its own unique ethnic characteristics.

The 2015 population estimate for LA is 634387 million (including Puerto Rico, a political territory of the Northern American-situated United States), accounting for roughly 9% of the global population^[3]. Approximately one-half of the LA populations reside in Brazil and Mexico. Brazil, itself, is the biggest and most populous LA country and the 5th largest country in the world, both by geographical

area and by population. LA annual growth is 1% per year, with 7.4% of its inhabitants being older than 65 years^[3-5]. Approximately 8% of LA peoples identify themselves as indigenous, representing more than 522 groups dispersed broadly throughout the continent and speaking around 420 languages (e.g., Quechua, Aymara, Guarani, among others)^[6,7].

LA has experienced significant social and financial progress over the past decades; however, the improvements have happened in an inequitable way, contributing to striking disparities in health and economic conditions among social classes and geographic regions, between countries as well as within countries. As a result, LA is currently characterized by the Gini index (which describes how far away a country's income distribution is from complete equality) as having the highest socioeconomic inequality in the world. As a whole, however, socioeconomic indexes of LA countries have improved throughout the current century.

Gross national income (GNI) per capita (as calculated by the Atlas method) increased from an average of 3300 USD to 9941 USD between 2000 and 2014, but ranging from 830 USD in Haiti to 19210 USD in Puerto Rico^[5]. Most of the LA countries have reached the status of upper middle income (4126 USD to 12735 USD), including Brazil, Colombia, Costa Rica, Cuba, Dominican Republic, Ecuador, Mexico, Panamá, Paraguay and Peru. Only one country - Haiti - qualifies as a low-income country (< 1045 USD). Five countries are included in the group of low middle income (1046 USD to 4125 USD; Bolivia, El Salvador, Guatemala, Honduras and Nicaragua), and five are included in the group of high income (> 12736 USD; Argentina, Chile, Puerto Rico, Uruguay and Venezuela)^[8].

Life expectancy at birth has also shown an increasing trend in LA countries, from 70 years in 2000 to 75.5 years in 2015, approaching the observed indexes of more developed nations^[3]. LA countries have seen increases in life expectancy, with Chile having the highest (80 years) and with Bolivia (67.8 years) and Haiti (62.6 years) having lower proportions of increase compared to the other countries. The overall improvement has been achieved largely through a reduction in child mortality. In contrast, the percentage of people living below the poverty line in LA countries continues to be high (28.1% in 2013), as does the percentage of people leaving under the line for extreme poverty (11.7%)^[3]. Finally, the human development index (HDI; an indicator of the quality of life of a country, defined by the United Nations Development Program and which combines the three basic dimensions of life expectancy at birth, education and income per capita) is 0.748 for LA countries as a whole, but ranges from as low as 0.484 in Haiti to very high in Argentina (0.836) and Chile (0.832)^[9].

Eighty percent of the populations of LA countries reside in urban areas, making LA the most urbanized region in the world^[10]. The rate of urbanization ranges from 53.6% in Honduras to 91.8% in Argentina. Moreover, approximately 24% of LA inhabitants of

Table 1 Cardiovascular and renal risk factors, extracted from national surveys in 7 Latin American countries¹

	Argentina	Brazil	Chile	Mexico	Uruguay	Ecuador	Paraguay
Survey year	2013	2013	2010	2012	2013	2013	2011
Hypertension	34.1	24.1	26.9	31.5	38.7	15.6	32.2
High cholesterol	29.8	20.3	38.5		23.9	24.5	25.5
Overweight	37.1	33.3	39.3	38.8	ND ¹	ND	34.8
Overweight + Ob	57.9	50.8	64.4	71.3	64.7	ND	57.6
Ob	20.8	17.5	25.1	32.4	ND	50	22.8
Diabetes	9.8	6.9	9.4	9.2	7.8	ND	9.7
Current smokers	25.1	11.3	40.6	19.9	28.8	25.9	14.5
Sedentarism	55.1	66.2	88.6		70	ND	74.5

¹In which approximately 68% of the Latin American population resides. ND: Not detailed; Ob: Obesity.

large cities live in slum neighborhoods. More than 60 cities in LA have recorded populations of > 1 million inhabitants, including several cities that are characterized as megalopolis (defined as more than 10000000 people, including neighborhoods), such as San Pablo (Brazil), Mexico City (Mexico), and Buenos Aires City (Argentina)^[3,10,11]. Over the past few decades, the population pyramid has changed its shape into that of a bell, reflecting its evolution towards that of an aging society in general. All of these features of the peoples residing in LA countries present new challenges to each country's healthcare system, which remain under the burden of ongoing challenges otherwise associated with lower socioeconomic regions, such as high rates of infectious diseases.

As in the rest of the world, chronic kidney disease (CKD) is prevalent throughout LA, due to both infectious disease prevalence and immature public health systems. Epidemiological information about the prevalence of early-stage CKD in the general LA population is scarce and of low quality. It is known, however, that CKD constitutes a higher burden in Central America, where a regional epidemic of CKD of unknown origin emerged during the last decade, affecting primarily young male agricultural workers from communities along the Pacific coast and southern México, especially sugarcane workers. In the involved areas, the national mortality rates have reached as high as 5 times the national rates, with El Salvador representing the country with the highest mortality rate from kidney disease worldwide^[12,13].

Information about cardiovascular and renal risk factors is available through national health inquiries in some countries; their results confirm that these risk factors are highly prevalent in LA, particularly in countries with greater percentage of inhabitants over 65-year-old, such as Argentina (11%), Brazil (7%), Chile (10%) and Uruguay (14%) (Table 1).

Renal replacement therapy in LA

Renal replacement therapy (RRT) started as peritoneal dialysis (PD) in Brazil in 1947. Shortly thereafter, the first hemodialysis (HD) was also accomplished in Brazil (in 1949), and the first kidney transplant in Argentina (in 1956)^[14,15]. Initially, HD was considered exclusively

as a therapy to support patients with acute renal failure and who were awaiting transplantation, but it quickly became incorporated as treatment for end-stage renal disease (ESRD). The field of clinical nephrology developed almost simultaneously, with physicians and researchers consolidating into National Nephrology Societies, such as those of Argentina (in 1960), Brazil (in 1960), Chile (in 1964) and Uruguay (in 1967).

The LA Nephrology and Hypertension Society [Sociedad Latinoamericana de Nefrología e Hipertensión (SLANH)] was created in 1970, grouping the various National Nephrology Societies of 20 countries (*i.e.*, Argentina, Bolivia, Brazil, Chile, Colombia, Costa Rica, Cuba, Dominican Republic, Ecuador, El Salvador, Guatemala, Honduras, Mexico, Nicaragua, Panama, Paraguay, Peru, Puerto Rico, Uruguay and Venezuela). In 1991, the SLANH founded the LA Dialysis and Transplant Registry (LADTR) to promote the knowledge and improve the care of ESRD by collects and analyzing data from the 20 member countries. The Registry office was housed in Montevideo (Uruguay) until 2001, when it moved to Buenos Aires (Argentina), where it remained until 2012, when it returned to Montevideo. Currently, the Registry is composed of an Executive Board and delegates from each of the Nephrology Societies that are part of the SLANH.

The methodology used by the LADTR has been reported previously. Briefly, participant countries complete an annual survey to provide data on incident and prevalent cases of patients undergoing RRT by means of all modalities: HD, PD, and living with a functioning graft (LFG), CKD etiology, number and type of kidney transplants, percent of population under RRT coverage, and number of nephrologists, as well as other relevant parameters. Analyses of these variables are performed routinely to determine correlations with GNI and life expectancy at birth as well as other socioeconomic indexes^[16-23].

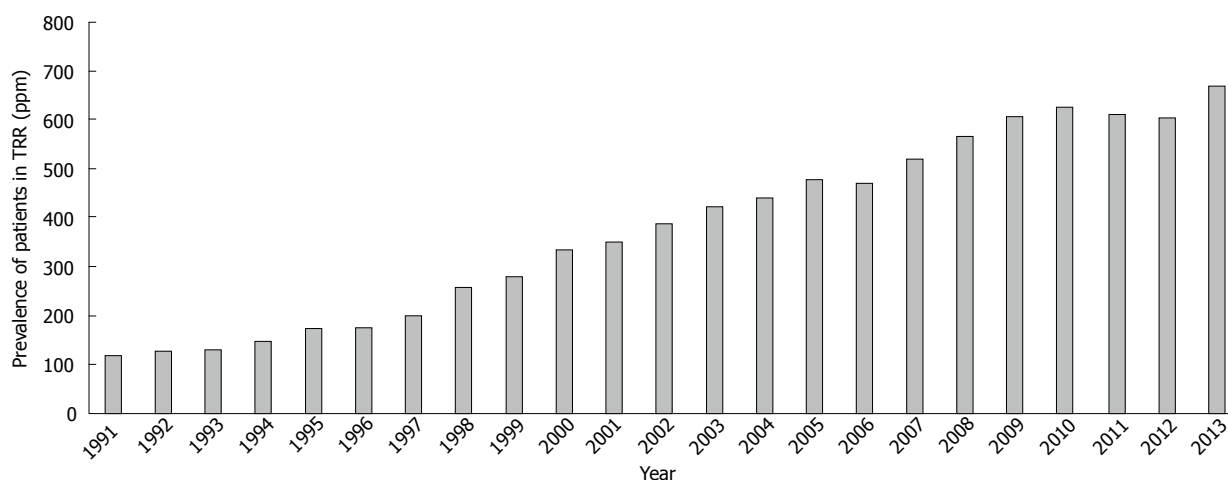
Prevalence and incidence of ESRD under RRT

All 20 SLANH countries have and continue to participate in the annual surveys, providing data for > 90% of the LA populations since the beginning of the century. Table 2 describes the most prominent variables analyzed in

Table 2 Socioeconomic indexes, prevalence and incidence of renal replacement therapy and transplantation rate

Country	Population ¹	GNI	LEB	Prevalence rates, pmp					Incidence rate	Kidney Tx, <i>n</i>	Tx by deceased donors, %	Kidney Tx rate	Nephrologists, <i>n</i>	Nephrologists, pmp
				HD	PD	Total dialysis	LFG	Total RRT						
Argentina	42202935	13690	76	626.6	36.0	662.7	197.2	859.9	160.2	1287	68.4	30.5	1150	27.2
Bolivia	10448913	2220	67	195.2	18.3	213.5	31.6	245.1	94.8	75	24.0	7.2	24	2.3
Brazil	202740000	11640	74	449.6	45.6	495.2	212.6	707.8	180.3	5433	74.7	26.8	3300	16.3
Chile	17819054	14290	80	1019.1	61.2	1080.3	205.1	1285.4	182.4	234	74.8	13.1	132	7.4
Colombia	47661787	7020	74	349.0	143.6	492.6	111.3	603.9	89.7	680	99.7	14.3	95	2.0
Costa Rica	4773730	8850	80	42.3	76.0	118.4	282.6	400.9	ND	105	48.6	22.0	24	5.0
Cuba	11163934	6051	79	259.1	10.1	269.3	78.4	347.6	103.1	174	ND	15.6	524	46.9
Ecuador	16100000	3600	76	481.8	48.0	529.8	20.4	550.2	177.6	127	81.1	7.9	143	8.9
El Salvador	6401240	5360	72	232.5	288.7	521.1	73.6	594.7	390.1	20	0.0	3.1	47	7.3
Guatemala	16173133	3130	72	157.7	221.3	379.0	54.0	433.0	124.8	90	13.3	5.6	54	3.3
Honduras	8500000	2140	73	186.9	14.4	201.3	8.4	209.6	176.7	0	0.0	0.0	18	2.1
Jalisco (Mexico)	7742303	ND	ND	599.4	486.7	1086.1	567.4	1653.5	420.9	447	16.1	57.7	45	5.8
Nicaragua	6146000	1690	74	211.5	24.4	235.9	21.2	257.1	24.4	11	0.0	1.8	28	4.6
Panamá	3975404	9030	77	495.0	90.3	585.3	110.7	696.0	462.1	48	73.1	12.1	25	6.3
Paraguay	6783374	3310	75	165.7	4.0	169.7	19.9	189.6	20.2	26	79.0	3.8	46	6.8
Perú	30297279	5680	72	272.2	43.1	315.3	63.2	378.5	30.0	184	75.0	6.1	301	9.9
Puerto Rico	3615000	18370	79	1362.1	106.2	1468.3	378.4	1846.7	432.9	80	86.3	22.1	97	26.8
Rep Dominicana	12000000	5570	73	178.8	47.3	226.1	52.8	278.9	208.3	84	92.9	7.0	135	11.3
Uruguay	3406545	13670	77	692.2	71.6	763.8	323.5	1087.3	157.3	105	91.4	30.8	173	50.8
Venezuela	30389596	12460	74	505.1	0.0	505.1	60.8	565.9	ND	281	69.8	9.2	502	16.5
Totals	488340227	147771	75	442.0	67.0	509.0	159.0	669.0	149	9491	70.4	19.4	6863	14.0

¹Most population data were provided by the participating countries; when not provided, data from the Latin American and Caribbean Demographic Centre-Population Division of Economic Commission for Latin America and the Caribbean were used instead. LEB: Life expectancy at birth; ND: Not detailed; Tx: Transplant.

**Figure 1** Prevalence of renal replacement therapy in Latin America, 1991-2013.

the last available survey.

From 1991 to 2013, the prevalence of ESRD under RRT increased from 119 patients per million population (pmp) to 660 (HD, 436 pmp; PD, 67 pmp; LFG 157 pmp) (Figures 1 and 2). Only six countries have RRT prevalence above the mean: Argentina, Brazil, Chile, Jalisco (Mexico), Puerto Rico and Uruguay, with the reported rates ranging from 778 pmp to 1847 pmp. The prevalence rates among the population over 65-year-old is particularly high, especially in the six countries accounting for the highest amounts of this population (Argentina, Brazil, Chile, Colombia, Puerto Rico and Paraguay) and reaching as high as 2400 pmp. As

expected, the overall prevalence correlated with the percentage of people over 65-year-old (Figure 3). Moreover, every time it was analyzed, the prevalence correlated significantly with GNI and life expectancy at birth^[16,19-21] (Figure 4).

An increase in RRT patients has occurred for all modalities, but HD in particular has proportionally increased more than PD and transplantation (Figure 2)^[24]. HD continues to be the treatment of choice in the LA region (90%) and 43% of HD patients are located in Brazil. PD prevailed in 2013 only in Costa Rica (64.2%), El Salvador (55.4%) and Guatemala (58.4%). PD was also common in Colombia, although the percentage of

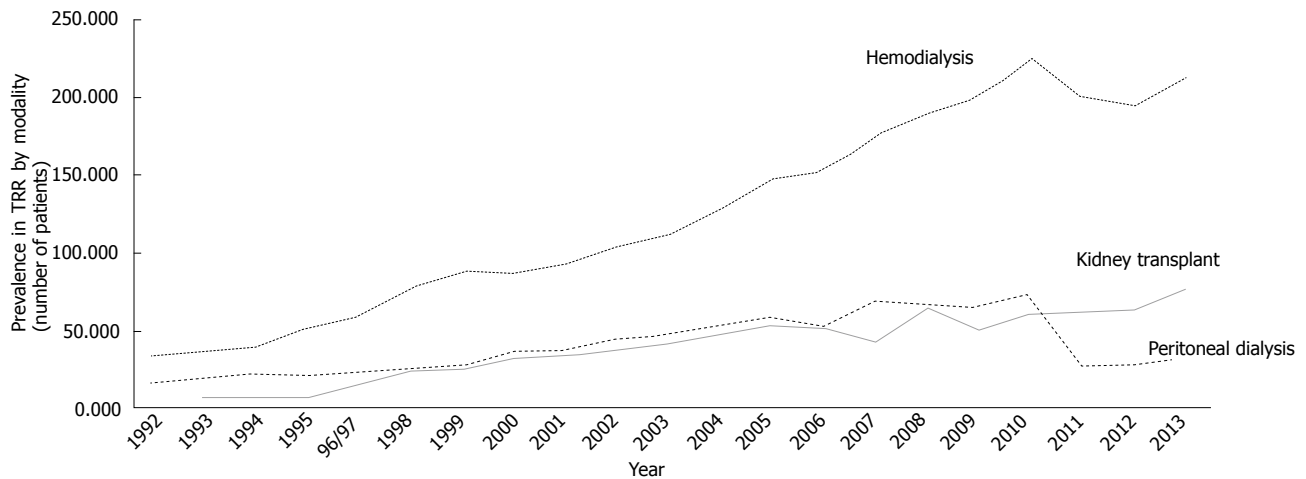


Figure 2 Prevalence of renal replacement therapy in Latin America by modality.

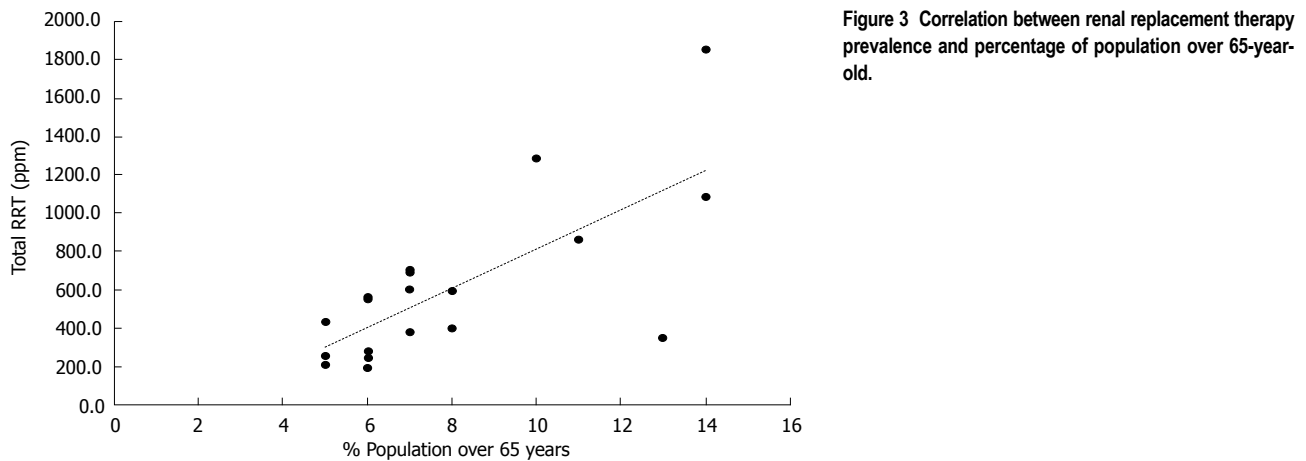


Figure 3 Correlation between renal replacement therapy prevalence and percentage of population over 65-year-old.

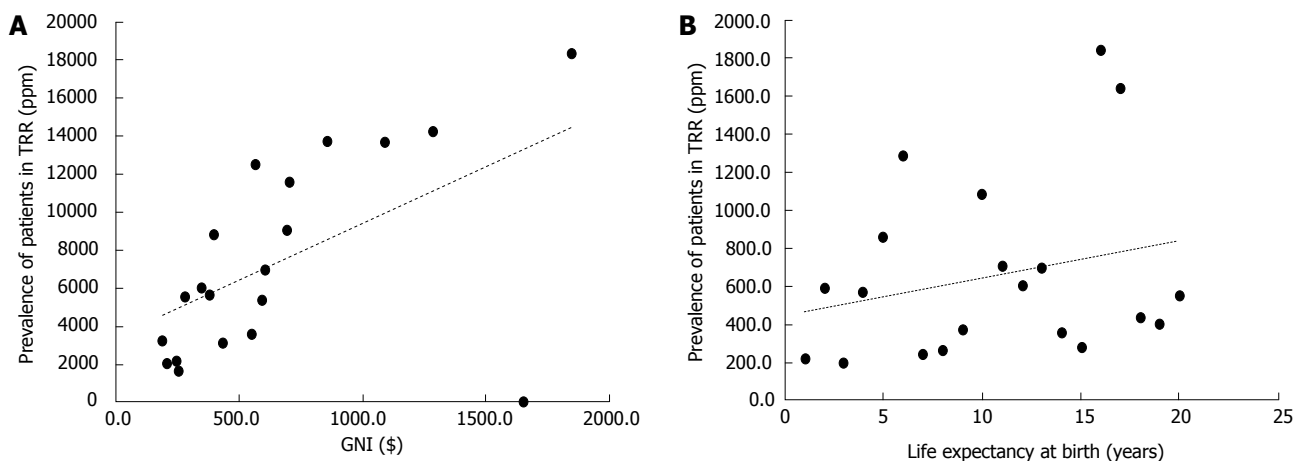


Figure 4 Renal replacement therapy prevalence and correlation with gross national product and life expectancy at birth. A: For gross national product (GNI), the correlation coefficient is 0.43; B: For life expectancy at birth, the correlation coefficient is 0.34.

Colombian PD patients has consistently decreased over the last years (from 54% in 2000 to 29.2% in 2013)^[24].

Data for incidence of RRT were provided to the LADTR by 18/20 countries in 2013, accounting for 92.7% of the population of LA. A wide rate variation was observed, from 462.1 pmp in Panama to 20 in Paraguay.

A tendency towards rate stabilization/little growth was found for most countries, except in the Central America countries of El Salvador, Guatemala, Honduras and Panama, which showed a significant increase in incidence. Diabetes continues to be the leading indication for dialysis. The LADTR received data on RRT incident

Table 3 Total incidence rates and percentages of new diabetic renal replacement therapy patients

Country	Population	Incident patients, <i>n</i>	Incident rate	Diabetics, %
Argentina	42202935	6760	160.2	35.1
Bolivia	10448913	991	94.8	30.0
Brazil	202740000	36548	180.3	40.0
Chile	17819054	3250	182.4	16.7
Colombia	47661787	4274	89.7	33.5
Ecuador	16100000	2860	177.6	30.0
Guatemala	16173133	2018	124.8	30.0
Jalisco (Mexico)	7742303	3259	420.9	58.0
Nicaragua	6146000	150	24.4	41.6
Paraguay	6783374	137	20.2	45.3
Peru	30297279	910	30.0	32.2
Puerto Rico	3615000	1565	432.9	66.9
Uruguay	3406545	536	157.3	27.7

diabetic patients from 13 countries, accounting for 84.2% of the entire LA population. The reported percentage of diabetic patients from each country ranged from 16.7% to 66.9% (mean, 37.5%). The highest diabetes incidence rates were reported by Puerto Rico (66.9%), Jalisco (Mexico) (58%) and Paraguay (45.3%), while the lowest rates were reported by Uruguay (27.7%) and Chile (16.7%) (Table 3). The overall RRT incident diabetics rate was 58 pmp, lower than that reported from the United States Renal Data System in the same year (158.4 pmp), but more than double that from the European ERA/EDTA Registry (24 pmp)^[25,26]. In addition, the LADTR indicated that 19.4% of the RRT population was placed on a kidney transplant waiting list.

When compared with the United States data from 2013, incidence in LA, as a whole, was substantially lower (149 vs 363)^[25], but when looking individual countries, Jalisco (representing Mexico), Puerto Rico, Panama and El Salvador have similar rates (421, 433, 462 and 390, respectively). When compared to the European ERA/EDTA registry (112 pmp)^[26], the rate is higher in most LA countries (Table 2). Probably, there is more than one reason for the striking differences in incidence in LA, such as higher prevalence of diabetes mellitus (in Mexico and Puerto Rico, in particular) (Table 2) or of CKD of unknown origin (termed as Mesoamerican Nephropathy, involving the Pacific coast and southern Mexico)^[12,13], or varying access to RRT among the countries (Nicaragua and Paraguay). Overall, LA prevalence rates (660 pmp) are very far from those reported for the United States (2014 pmp) and are closer to those reported from the European ERA/EDTA (738 pmp)^[24,25].

The data in the LADTR indicates that the overall kidney transplant rate increased from 3.7 pmp in 1987 to 6.9 in 1991 and then to 19.4 in 2013; although, there were remarkable disparities among the various countries in the last year: 57.7 pmp in Jalisco (Mexico), 32 pmp in Uruguay and 1.8 pmp in Nicaragua. The highest number of transplants (*n* = 5433) occurred in Brazil, which had a transplant rate of 26.8 pmp for 2013. A total of 244 double kidney-pancreas transplants

were performed in LA in 2013: Brazil, *n* = 120; Argentina, *n* = 63; Costa Rica, *n* = 51; Colombia, *n* = 5; Uruguay, *n* = 3; Ecuador, *n* = 1; Chile, *n* = 1. The total number of all kidney-related transplants in 2013 was 9491, with 70.4% from cadaveric donors; the highest percentages of the latter were reported by Colombia (99.7%), Dominican Republic (92.9%) and Uruguay (91.4%).

Even though kidney transplantation is feasible, available, and an increasingly used modality for RRT in all LA countries, its growth rate is not as fast as it should be in order to compensate for the increased prevalence of patients on waiting lists for transplantation. This fact further strengthens the need to implement transplant programs and procurement of suitable organs. Moreover, the key issues identified in this study - specifically, the increased incidence of patients in RRT for all modalities in the LA region, and diabetes continuing to be the leading clinical cause for RRT - highlight the crucial nature of prevention programs for CKD to achieve early diagnosis and treatment. Yet, there is a wide gap in the amount of nephrologists among each LA country (from 2 pmp in Colombia to 50.8 pmp in Uruguay) that must be taken into consideration.

Contributions for improvement of nephrology and ESRD knowledge and care in LA

Since its creation, the LADTR has fulfilled its mission, providing valuable information on epidemiology and burden of ESRD under RRT in the region and correlating the data with socioeconomic indexes. The results of the LADTR annual surveys have been published, providing consistent data and trends about ESRD under RRT and national variations inside the region, thereby transforming the registry into a powerful tool for health authorities and highlighting the necessity of guaranteeing full access to RRT while establishing transplantation and procurement programs^[16-24]. In addition, the observation that the primary etiologies of ESRD are preventable (*i.e.*, diabetes and kidney hypertensive disease) has prompted the development of National Clinical Practice Guidelines

for CKD Diagnosis and Treatment and the creation of programs aimed at accomplishing early detection and treatment. Recently, based upon the work of the LADTR, the Pan American Health Organization (PAHO) included in its Strategic Plan a specific target for ESRD treatment – namely, to reach, by the year 2019, a RRT prevalence of, at least, 700 pmp for all ESRD cases in all LA countries^[27]. To achieve this objective, the PAHO estimates at least 20 nephrologists pmp will be needed in each country.

Moreover, the LADTR, through its participation in National, LA, Hispanic American and International Congresses and Meetings and in the International Society of Nephrology Kidney Health in Disadvantaged Populations Committee, has contributed to spreading knowledge of the epidemiology of RRT throughout the nephrology community in the LA region, and to enable productive comparisons with the rest of the world. The LADTR has also stimulated the development of voluntary and obligatory national renal registries. At present, 10 countries have consolidated national registries: Argentina, Brazil, Chile, Colombia, Cuba, Ecuador, Puerto Rico, Paraguay, Uruguay and Venezuela; some of these were initiated in the 1980s, such as the Chilean (1980) and the Uruguayan (1981) ones, so that they are providing a rich database of information today^[28].

The LADTR has simultaneously improved the training of nephrologists in epidemiology, having organized, in conjunction with the European ERA/EDTA Registry, the Introductory Course in Epidemiology in Buenos Aires (2007, Argentina), Mexico City (2009, Mexico) and Cartagena de Indias (2011, Colombia). It is appropriate to recognize, here, the permanent collaboration of the Spanish Society of Nephrology, in particular its fellowship program for Latin Americans, which has allowed young nephrologists to train abroad.

The continuity of the LADTR along the years, whose members participate without any fee, has implied a sustained effort of the entire Latin American nephrology community.

Future of the LADTR

The LADTR recognizes several limitations. In most Latin American countries, reporting to the registry is not mandatory, not all the countries report all the data each year, data are collected from each country on a global basis, and, in the past, data from some province or region have been extrapolated to the whole country. This last feature was the case for Mexico, wherein prevalence and incidence were extrapolated from the data of the Mexican states of Jalisco and Morelos until the year 2010; moreover, since 2011, only the data from Jalisco has been deposited in the LADTR. Furthermore, the LADTR cannot report survival data for any of its participating countries, as it currently collects only aggregated data. Finally, the number of LFG patients in many countries is estimated and not definitive.

In spite of its limitations, the LADTR has provided current knowledge about trends of RRT prevalence and

incidence in a defined geographical zone (LA) and in defined countries (the 20 that comprise the SLANH), has shown that, in part, the increase in incidence is related to the expansion of the burden of kidney diabetic disease, and has revealed the striking differences in prevalence and incidence that are associated with the countries' wealth status and health coverage.

Heterogeneity or even the absence of registries in some Latin American countries is congruent with the inequities in access to RRT in such countries, as well as the limited availability of skilled human resources. The inclusion by the PAHO Strategic Plan of a goal of 700 pmp under RRT by 2019 undoubtedly will contribute to increased health coverage and the implementation of obligatory national registries, all of which, added to the sustained contribution of the nephrology community, will undoubtedly result in improved quality of national-based registries and the LADTR itself, supporting the development of population-based registries.

The LADTR has sustained its continuity over the years and, at present, it is the only source of data about RRT in the region and for many of its member countries. Its future depends on the quality of its data, which in turn depends on the data provided by the respective national registries and their (and its) quality control procedures. To continue its tasks, in the future, the LADTR will likely require funding that is sufficient to strengthen quality-controlled data.

CONCLUSION

Since its creation in 1991, the LADTR has provided valuable information on epidemiology and burden of ESRD under RRT and continues to be, at present, the only source of RRT data for this region. Prevalence and incidence of RRT continue to increase throughout LA. Prevalence correlates with GNI, life expectancy at birth and percentage of the population over 65-year-old. In the LA region, as a whole, it is still necessary to increase full accessibility to RRT and to develop programs that will facilitate better control of risk factors and early diagnosis and treatment of CKD, as well as implementation of effective transplantation programs.

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Vascular calcification: When should we interfere in chronic kidney disease patients and how?

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Abstract

Chronic kidney disease (CKD) patients are endangered with the highest mortality rate compared to other chronic diseases. Cardiovascular events account for up to 60% of the fatalities. Cardiovascular calcifications affect most of the CKD patients. Most of this calcification is related to disturbed renal phosphate handling. Fibroblast growth factor 23 and klotho deficiency were incriminated in the pathogenesis of vascular calcification through different mechanisms including their effects on endothelium and arterial wall smooth muscle cells. In addition, deficient klotho gene expression, a constant feature of CKD, promotes vascular pathology and shares in progression of the CKD. The role of gut in the etio-pathogenesis of systemic inflammation and vascular calcification is a newly discovered mechanism. This review will cover the medical history, prevalence, pathogenesis, clinical relevance, different tools used to diagnose, the ideal timing to prevent or to withhold the progression of vascular calcification and the different medications and medical procedures that can help to prolong the survival of CKD patients.

Key words: Chronic kidney disease; Uremia; Calcification; Sevelamer; Calcific uremic arteriopathy; Fibroblast growth factor 23; Klotho; Phosphate binders; Kidney transplantation

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Core tip: The last 2 decades witnessed the failure of all intervention studies targeting different risk factors of vascular calcification in chronic kidney disease (CKD) patients on regular hemodialysis. The main aim of all these studies was to decrease cardiovascular morbidity and mortality among such patients. These disappointing results criticized the value of such interventions in clinical practice. On the other hand, when similar trials were run on patients at an earlier stage of CKD, most of these

trials showed a significant impact on patient survival and/or cardiovascular morbidity. Such discrepancy indicates the value of timing of interference. We are trying in this review to develop the ideal strategy that would optimize the management of CKD patients to avoid the devastating vascular calcification, highlighting the value of different medicines used in this plan. Meanwhile we are showing the update in guidelines concerned with this issue.

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INTRODUCTION

Vascular calcification (VC) affects either the arterial tree or cardiac valves. Deposits of hydroxyapatite in the arterial wall occur within either tunica intima or tunica media. VC is a strong predictor of increased cardiovascular mortality among chronic kidney disease (CKD) patients. However, the different clinical studies that tried to manipulate the different risk factors of VC in dialysis patients failed to show a significant impact on patient survival. On the other hand, when pre-dialysis patients underwent similar studies, there was a significant decrease of cardiovascular and overall mortality rates, beside a comparable effect on vascular calcification progress rate. These results have probably two explanations. Dialysis patients might have advanced VC rendering their arteries permanently and irreversibly damaged [approaching end stage arterial damage (ESAD^[1])] or they might have additional pathologic problems exceeding in their survival impact the VC. The authors of this review are inclined towards the 1st possibility and will try to outline the best way to tackle this devastating pathology.

HISTORY

In 1855, "metastatic calcification" was described in three patients with renal disease^[2]. Eight years later, Virchow^[3] reported that this calcification is a definite ossification.

The first reported VC in infants was probably that of Durante^[4] describing aortic and pulmonary artery calcification. Calcification of peripheral vessels has been described in a few children with CKD as early as 1942^[5,6].

VC affected humans in ancient history. The most ancient calcification so far reported is 5000 years ago, "identified in the recently discovered ice man"^[7]. On the other hand, the earliest coronary calcification reported is that of an Egyptian mummy who was living 4000 years ago^[8].

VC IN CKD PATIENTS

A high percentage of CKD patients show VC. The prevalence among predialysis CKD G3-5 patients was 79% of cases in one study^[9]. It might approach 100% in patients starting dialysis^[10]. VC significantly contributes to morbidity and mortality of CKD^[11-13]. Up to 3- to 4-fold increase in VC has been reported in the earliest phases of CKD^[14].

CKD patients show VC in almost all arteries whether large, medium or small-sized vessels, including the coronary arteries^[10,15-17]. VC can affect the tunica intima and/or the tunica media of the arterial wall^[18]. Intimal calcification is mainly a feature of atherosclerosis^[19]. CKD patients can have intimal and medial calcification. Medial calcification is reported in CKD of any age^[20,21]. When epigastric arteries of patients with end-stage renal disease (ESRD) were examined at the time of kidney transplantation, vessel calcification was detected regardless of patient age and/or the presence of other risk factors for atherosclerosis^[22]. In one study, hemodialysis (HD) patients have higher calcification scores than either peritoneal dialysis (PD) or CKD G4. More heavily calcified patients were significantly older and mostly male^[23]. In HD patients, coronary calcification progresses steadily^[24]. High serum phosphate concentration was a strong independent risk factor only in non-diabetic patients. Diabetic patients lack similar association^[25].

Fifty percent of CKD patients die out of cardiovascular events^[26]. Cardiovascular mortality is 20- to 30-times of controls matching in age, race and gender^[27]. Patients starting dialysis at age of 25-29 years have a median life expectancy of 18.5 years. This means that their survival is 33 years less than normal personnel^[28]. Arterial calcification is one of the predictors of this increased cardiovascular mortality^[28]. Patients with CKD should, therefore, receive aggressive preventive measures to reduce this cardiovascular disaster^[29].

Coronary artery calcification (CAC) is common among CKD patients whether adolescents, adults or old aged, starting in early stages of CKD and steadily progressing in HD patients^[30-32]. Postmortem study of atheromatous lesions in ESRD patients found more intense calcification of such lesions compared to age- and sex- matched controls^[33].

Calcification of the internal iliac arteries in CKD patients was greater compared with controls^[34].

Large vessel disease is associated with decreased arterial compliance as detected by ultrasound and accounts for the increased mortality^[35,36].

Calcific uremic arteriolopathy (CUA), also called calciphylaxis is an obliterative vasculopathy affecting cutaneous arterioles. It occurs almost exclusively in ESRD patients. Affected arterioles show medial calcification^[37]. Ischemia and necrosis of the skin, subcutaneous fat, visceral organs and skeletal muscles eventually ensues. The skin



Figure 1 Male patient, 36-year-old, on regular hemodialysis for 8 years, presenting with multiple skin ulcers affecting both legs. A: His corrected serum calcium is 10.28 mg/dL and serum phosphorus 8 mg/dL. Serum PTH is 2588 pg/mL. He initially experienced itching papules that eventually ulcerated; B: Another ulcer with necrotic floor in the same patient. PTH: Parathyroid hormone.

manifests by necrotic foci and painful ulcers (Figure 1)^[38].

VC IN KIDNEY TRANSPLANT RECIPIENTS

Death with a functioning graft is one of the major causes of graft loss (accounting for 42% of graft loss) in kidney transplant recipients (KTRs). Cardiovascular events are the first cause of death in this population affecting 36% to 55% of patients. The impact of VC on morbidity and mortality of KTRs is not appreciated enough^[39-41]. Three point five percent to five percent of KTRs experience fatal or non-fatal cardiovascular events annually. This rate is much higher than in the general population. The prevalence of coronary artery calcification (CAC) in KTRs is higher (61%-75%) than that assessed in stage 3 CKD^[42-44] and lower than that found in HD patients^[45]. Moe *et al.*^[37] did not observe CAC progression after a successful kidney transplant. On the other hand, Oschatz *et al.*^[46] observed a significant progression within the first 6 mo, but no significant change between months 6 and 12 after a kidney transplant. All these trials were short term. When longer-term follow-up trials were performed, kidney transplant was found to favorably affects but does not halt CAC progression, with an annual rate of CAC progression ranging between 11% and 12.5%^[47-49]. The risk of progression was higher in Caucasian race, with increased body mass index, higher baseline CAC score, higher diastolic blood pressure and lower glomerular filtration rate 3 mo after transplantation^[50]. Other risk factors included inflammation, hyperparathyroidism and dialysis duration^[47,51,52]. CAC score was significantly lower in KTR who had a pre-emptive transplant in comparison to those who underwent dialysis before transplantation (3.7 vs 102.9, $P < 0.001$)^[52]. According to these studies, it seems that pre-emptive kidney transplant gives ESRD patients their best chance to avoid progressive VC.

PATHOGENESIS OF VC

Many factors summate the pathogenesis of VC in CKD. Such factors are either traditional or CKD related. The factors related to CKD include high serum calcium

and phosphorus, increased dialysis vintage, increased duration of uremia^[53], low serum fetuin-A level^[53], and high serum level of fibroblast growth factor 23 (FGF23)^[10,54-63]. Dialysis vintage, disturbed mineral metabolism and FGF23 are the most relevant factors having impact in the VC of CKD^[37]. There is an association between VC and indices of low bone turnover in dialysis patients^[64].

Is VC an active process?

More than 150 years ago, Virchow^[2] was the first to report that vascular calcium deposits were real ossification. In CUA, vascular smooth muscle cells express osteopontin, bone sialoprotein, and osteonectin^[37,65]. In non-calcified arteries in the same skin biopsy section, osteopontin or other bone proteins were not observed^[65]. It seems that the deposition of these proteins predispose calcification^[37,66].

Role of phosphorus

Vascular smooth muscle cells and osteoblasts originate from the same mesenchymal cell. Core binding factor α -1 (Cbfa1) turns the mesenchymal cell into osteoblast^[37,67]. β -glycerophosphate is a phosphate donor. Vascular smooth muscle cells mineralize in the presence of this phosphate donor and increased Cbfa1 activation^[37,68]. Calcific arterial lesions in patients devoid of CKD showed increased expression of Cbfa1 while normal arteries failed to show similar finding^[37,69]. The findings of Cbfa1 in both CKD vascular lesions and non-CKD arterial disease might denote a common pathogenesis of VC. A significant relationship between increased serum phosphorus and obstructive atherosclerotic coronary artery disease was observed in non-CKD patients^[37,70,71].

Bone morphogenetic protein-2

When bovine vascular smooth muscle cells (BVSMCs) were incubated in uremic serum and healthy control serum, upregulation of Cbfa1 was significantly higher with uremic serum. When β -glycerophosphate was added to increase the inorganic phosphorus within culture media, Cbfa1 significantly increased in normal control serum culture and

the significant difference in Cbfa1 was muffled^[72]. This increase in Cbfa1 was completely inhibited after addition of foscarnet (an inhibitor of sodium/phosphate co-transport) to the normal serum. In case of uremic serum, inhibition was partial, denoting other factors might have an action on Cbfa1 beside hyperphosphatemia^[37]. Bone morphogenetic protein-2 (BMP-2) concentration is doubled in CKD serum. BMP-2 was detected in human calcified arteries^[37,73-75] and human uremic serum can induce *in vitro* calcification that increases as the CKD advances^[37,76].

Fibroblast growth factor 23 - klotho axis

Fibroblast growth factor 23 (FGF23) was isolated 16 years ago^[77]. FGF23 is responsible for autosomal dominant hypophosphataemic rickets (ADHR) in humans^[78] and is the humoral factor secreted by tumors inducing hypophosphatemia and osteomalacia (TIO)^[79]. FGF23 plays an important role in the regulation of serum phosphate level. FGF23 is secreted by osteocytes in bone^[80]. Other sites might share in FGF23 synthesis, including bone marrow, thalamus, lymph nodes and thymus^[81]. The serum levels of FGF23 are derived mainly from bone^[82]. FGF23 exerts its hypophosphatemic effect through inhibition of phosphate reabsorption by proximal tubular epithelial cells. It down-regulates the luminal sodium-phosphate co-transporters. FGF23 also inhibits 1 α hydroxylase^[83]. It was not clear if FGF23 stimulates secretion of parathyroid hormone (PTH)^[82] or PTH stimulates FGF23 secretion. Klotho acts as a co-receptor for FGF23 by markedly increasing the affinity of FGF23 for ubiquitous FGF receptors (FGFR)^[84]. Klotho, is highly expressed in the kidney and the parathyroid glands^[84,85].

Klotho is an anti-senescence protein^[86]. It exists in 2 forms: The transmembrane and the soluble secreted form^[87,88]. Klotho is detected as soluble protein in body fluids including blood, urine^[89-91] and cerebrospinal fluid^[89].

The highest expression of Klotho is in kidney and brain^[86,90,91], but it is also expressed in parathyroid gland^[92,93] and heart^[94] with less abundance.

The similarity of the phenotypes between *Kl*^{-/-} mice^[86] and *Fgf23*^{-/-} mice is striking^[95], which strongly suggests a common signaling pathway shared by these molecules^[96,97]. Now it is well documented that membrane Klotho functions as the coreceptor for FGF23, which amplifies and confers specificity of FGF23 action^[84,85,98,99].

In contrast, soluble Klotho protein functions independently of FGF23^[91] and plays an important role in modulation of ion transporters or channels^[91,100], antioxidation^[101] and anti senescence^[102,103], in addition to simply supporting FGF23 action^[104]. The protective effect of Klotho against soft tissue calcification is mediated by at least 3 mechanisms: Increasing urine phosphate excretion, renal protection and inhibition of phosphate uptake by vascular smooth muscle cells (VSMCs) and their dedifferentiation^[104].

Klotho and FGF23 are likely responsible for calcium and phosphate homeostasis^[105,106]. *In vitro* PTH secretion

and mRNA transcription are inhibited by FGF23^[107]. On the contrary, primary hyperparathyroidism in rodents is associated with increased FGF23 levels that are reduced by parathyroidectomy. PTH stimulates osteocytes to secrete FGF23^[108]. In physiological settings in which there are normal Klotho and FGFR expression, FGF23 decreases PTH production, increases expression of both the parathyroid Ca-sensing receptor and the vitamin D receptor, and decreases cell proliferation^[92].

In Klotho mutant mice, the different pathologic manifestations could be reversed when deficient Klotho is replaced^[109-111]. Exogenous klotho was found to ameliorate kidney injury and renal fibrosis in a rat model of CKD^[112]. It can also ameliorate endothelial cell senescence and muffles the binding of NF κ B to nuclear DNA^[113].

Patients with stages 3b-5 CKD and dialysis patients often develop high serum FGF23^[114]. This elevation can even occur as early as stage 2 CKD, long before any changes in calcium, phosphate, or PTH are apparent^[115]. Elevation in FGF23 stimulates the excretion of phosphorus by surviving nephrons. This would prevent the early onset of hyperphosphatemia in spite of increased bone turnover and the progressive decline in functioning nephrons. Development of CKD is associated with significant decline of Klotho mRNA expression^[116]. This deficiency might explain the increased serum FGF23 levels in CKD as a result of end-organ resistance to the action of FGF23. By the time the patients reach ESRD, FGF-23 concentrations are often 100- to 1000- fold above the normal range^[117], and moreover, circulating FGF-23 in ESRD patients is mostly intact and biologically active^[118]. Three possible explanations could account for such elevation. First, increased secretion into and decreased removal of FGF23 from the circulation. Treatment with corticosteroids could activate osteocytes in pediatric CKD patients, and then significantly stimulate FGF-23 synthesis^[119]. FGF-23 levels and estimated glomerular filtration rate (eGFR) were inversely correlating among individuals with CKD stage G4-5^[120]. Second, the other cause of increased levels of FGF-23 may be related to decreased klotho and end organ resistance to FGF23 action in CKD^[121]. Treatment of CKD patients with vitamin D may be the third cause. In 5/6 nephrectomized rats, intravenous administration of 1,25-(OH)2D, three times a week increased serum FGF-23^[122].

The first report of a positive correlation between FGF23 and VC among HD patients was 6 years ago^[10]. Similar results were reported in cases with CKD stages 2-5D. Patients with higher aortic and coronary calcification scores had elevated FGF23 levels^[62]. Similar results were found in healthy older men irrespective of traditional risk factors^[123]. Pediatric studies confirmed the same results in children with CKD^[124]. The same association was recorded in patients kept on HD for more than one year^[125].

Klotho deficiency in CKD vessels likely potentiates the development of accelerated calcification^[126]. Restoration of Klotho and FGFRs by vitamin D receptor activators

renders human vascular smooth muscle cells FGF23-responsive, and that may be the mechanism of their anti-calcific effects^[126].

Increased FGF23 level is associated with increased risk for mortality among incident HD patients, during their first year of treatment^[127]. This association was also confirmed in prevalent dialysis patients^[128]. Neutralization of FGF23 in CKD rats was found to accelerate VC and increases mortality^[129].

Inflammation

Atherosclerosis and VC accelerate in states of chronic inflammation. The later is one of the hallmarks of uremia. Uremic status was incriminated in the pathogenesis of chronic inflammation, however, the exact pathogenesis was not fully understood. Altered gut microbiome might affect the integrity of the intestinal barrier leading to facilitated blood translocation of bacteria and uremic toxins^[130]. Inflammation also results from multiple co-morbid conditions activating inflammation (like infections and autoimmune systemic diseases)^[131]. Many of the inflammatory markers and mediators are found to promote VC in CKD patients. These factors include interleukin 1 (IL-1), IL-6, C-reactive protein and tumor necrosis factor alpha (TNF α)^[132-137].

The association between FGF-23 and vascular calcification was mitigated when corrected for inflammation markers^[138]. In spite of this important role of inflammation that might underlie the role of Klotho-FGF23 axis, no intervention studies to target inflammation to prevent or stop VC progression in CKD were done.

Inhibitors of vascular calcification

All CKD patients are exposed to the uremic environment, however, not all of them will develop VC, suggesting that protective mechanisms also exist^[139].

Fetuin-A inhibits precipitation of calcium-phosphate^[140]. Fetuin-A synthesis is mainly hepatic. Its serum concentration falls with activation of cell mediated immunity^[141]. Fetuin-A calcium phosphate complex is called calciprotein particles (CPP). In comparison to hydroxyapatite, CPP induce significantly less cytokine secretion when macrophages are exposed to equimolar concentrations of hydroxyapatite and CPP^[142]. Mice deficient in fetuin-A develop extensive renal, myocardial, pulmonary, lingual and cutaneous calcifications^[140]. CKD patients with fetuin-A deficiency develop increased cardiovascular mortality^[140].

Matrix G1a protein (MGP) is a vitamin K dependent protein, synthesized in the bone^[143]. MGP has an inhibitory role in VC^[144,145]. MGP inhibits the formation of calcium crystal^[73]. CKD is associated with decreased uncarboxylated MGP level with subsequent increased rate of VC and atherosclerosis^[146].

Osteoprotegerin (OPG) is another anti-calcific agent. High OPG level is reported in patients with vascular calcification^[147,148]. Increase in OPG level may be a self-defensive mechanism against factors promoting VC^[148].

Vitamin K likely prevents post-menopausal fractures^[149]. Vitamin K deficiency increases the chance of

severe aortic calcification^[150]. Treatment of rodent with vitamin K2 reduced VC^[151]. Treatment of HD patients with vitamin K increases serum MPG and osteocalcin levels^[140]. Dietary menaquinone might be more effective compared to phyloquinone, in prevention of the progression of vascular calcification. Studies linking vitamin K status to calcification outcomes in CKD are needed to determine the therapeutic value in such cases^[152].

Pyrophosphate (PPi) directly blocks hydroxyapatite formation. PPi is synthesized in VSMCs^[153]. PPi deficiency results in excessive arterial calcification^[154]. Plasma PPi is deficient in HD patients, and is negatively correlating with VC^[155,156].

Vitamin D deficient mice develop excessive VC^[157]. Vitamin D deficiency is frequent among CKD patients. Decreased dietary intake, decreased synthesis in the skin and decreased 1 α -hydroxylase activity in the failing kidney are the main causes. Further inhibition of 1 α -hydroxylase ensues when serum FGF23 rises^[158]. In CKD G 3-4, CAC was elevated in both the mild and severe vitamin D deficient cases^[159]. Serum levels of 25(OH)D is negatively associated with VC in CKD G4-5^[160]. Low plasma level of 25-hydroxy - vitamin D is associated with increased mortality in different stages of CKD. Progression to ESRD was accelerated in vitamin D deficient patients^[161-163]. At therapeutic dosages sufficient to correct secondary hyperparathyroidism, VDR activator (VDRA) treatment of mouse model of CKD protected the vasculature from calcifying, but higher doses stimulated aortic calcification^[164]. The latter was probably caused by indirect, endocrine VDRA effects resulting in hyperphosphatemia and hypercalcemia. Organ cultures of human arteries from patients with CKD exhibited significant upregulation of Klotho mRNA levels following 48 h of calcitriol or paricalcitol treatment. This treatment effect was not observed in arteries from healthy individuals. Therapeutic dosages of VDRA were also found to reduce VSMC phenotype transformation in the aorta^[124].

To sum up, it seems clear that VC is triggered by different promoting factors that increase in CKD together with the deficiency of different protective factors. In other words, VC in CKD patients is the result of the interaction of this collection of offenders and inhibitors^[165].

CLINICAL RELEVANCE OF VC

Sudden cardiac death, arrhythmia, congestive heart failure, or stroke are the major causes of death in patients with VC^[166,167]. Most of the data on prognostic value of VC are extrapolated from studies in patients with normal kidney function. CKD patients still need prospective clinical trials evaluating the prognostic impact of aortic, coronary and carotid calcification in different CKD stages^[168]. The European Renal Best Practice (ERBP) work group recommends screening of incident dialysis patients^[169], whereas some national guidelines dictated the screening of any CKD patient^[170]. KDIGO guidelines, issued during 2009, considered that patients with CKD stages 3-5D and with known VC as

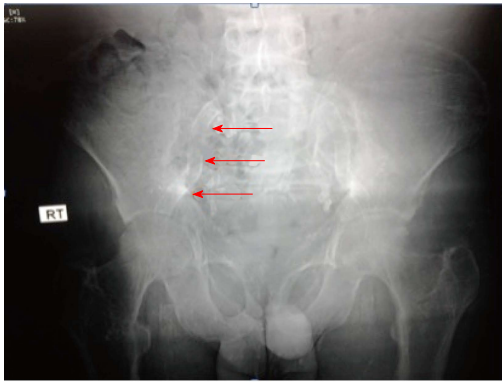


Figure 2 Plain X-ray of the pelvis in hemodialysis patient for 52 mo showing extensive calcification of the right common and external iliac arteries (arrows).

highest vascular risk and that this information should guide the management^[171]. On the other hand, Zoccali *et al.*^[172] denied VC as a risk factor for ongoing vascular disease. Their opinion relies on many studies, one of them is the recent meta-analysis of different clinical trials on the impact of different phosphate binders on mortality in CKD^[173], the ADVANCE trial^[174] and the EVOLVE trial^[175]. In addition, Wanner^[176] criticized any effort offered for diagnosis or treatment of VC as long as all the last mentioned trials failed to change the prognosis in HD patients.

In our opinion, the medical practitioners should do their best effort to prevent this devastating pathology in every CKD patient and not to wait to diagnose its end stage in the dialysis population. This means energetic preventive measures should be offered to every CKD patient all through different stages.

IMAGING OF VC

In 2009, the Kidney Disease Outcomes Quality Initiative (KDOQI) guidelines did not recommend the routine screening of VC as long as there is no clear clinical utility^[177]. However, in some cases, imaging of VC might help to guide the treatment plan^[178]. The gold standard for the quantification of calcification in different vessels is by computed tomography imaging. Plain X-rays can help to identify aortic and peripheral arterial calcifications (Figure 2); Doppler ultrasound is helpful in imaging the carotid, femoral and popliteal arteries and the aorta (Figure 3); echocardiography is valuable tool for visualizing valvular calcification and mammography for breast arterial calcification (BAC). BAC is a useful radiologic sign. It indicates tunica media calcification commonly encountered in CKD^[179].

Quantification of VC is achieved using either the Kauppila score, the Adragao scores, the Agatston score, the volume score or the mass score. The Kauppila scores are used to quantify calcification of abdominal aorta, an indicator of intimal calcification^[180,181]. The Adragao score is used to quantify VC in the iliac, femoral, radial, and digital arteries. Adragao score reflects me-

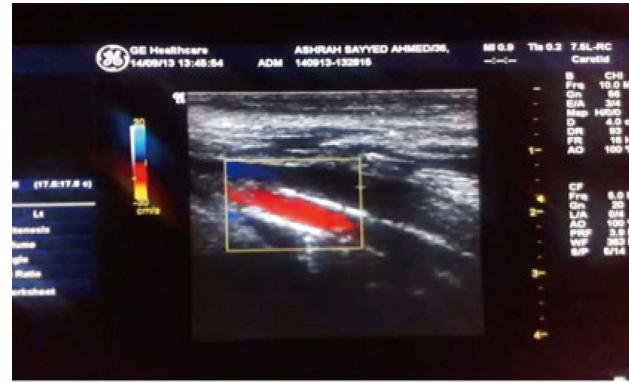


Figure 3 Doppler study of popliteal artery, the vessel wall shows linear calcification.

dial calcification^[182]. The volume and mass scores are quantitative and more reproducible measurements (mm³ or mg, respectively)^[183-185], in addition to being more appropriate for use with modern CT scanners than the Agatston score^[186]. However, the Agatston score (semiquantitative) is the most frequently used and reported method in the medical literature so far.

WHEN TO INTERFERE?

Interference for the traditional risk factors must start very early while the patient is still in stage G1. For nontraditional risk factors, we should start in the very early days of stage G2. The severity of arterial stiffness (as an index of atherosclerosis and VC) was found to increase steadily with more advanced CKD from stages 1 to 5^[187]. The different factors concerned with VC begin very early when renal damage is still trivial and before hyperphosphatemia ensues. Therefore, the earlier the intervention the better is the impact on morbidity and mortality^[188]. The changes in these factors are sequential. FGF23 is the earliest starting during stage G2. Decline of serum calcitriol follows when GFR falls below 60-70 mL/min per 1.73 m². PTH elevation follows in the late phase of stage G3a while changes in serum phosphate occur in stage G3b^[189]. We would like to emphasize that this sequence of events is triggered by the decreased capability of the injured kidney to manipulate phosphate excretion. FGF23 triggers the increase in the fractional excretion of phosphorus by the surviving nephrons. FGF23 inhibits 1- α hydroxylase enzyme, with subsequent decrease in synthesis of 1 α calcidol. Decreased calcitriol synthesis will result in the decline of its serum level, and later in stimulation of parathormone synthesis and secretion. Being the earliest sign of disturbed renal handling of phosphorus, interference should start as soon as FGF23 starts to rise, at a much earlier stage than we do in the time-being^[81,190-196].

HOW TO MANAGE?

VC management aims at improvement of survival and

morbidity in the CKD population^[197]. However, there is a lack of data to guide management strategies in these patients based on CAC scores^[198]. KDOQI guidelines recommend special care of CKD patient if VC is detected in more than one site^[199]. VC is not reversible, so far. Accordingly, the successful management is based on how to prevent or to stabilize existent lesions^[200].

Management of traditional risk factors among dialysis patients still faces concern about its value. Such factors were found correlating with better survival^[201]. Initially, treatment of different traditional risk factors in pre-dialysis CKD patients was based on studies mainly done in cohorts without renal disease, many trials tackling many of such factors in CKD patients have evolved but are still limited^[202].

The strict control of blood sugar carries little benefit, if any, to CKD G5D patients with or without diabetes mellitus^[203]. However, such control has a positive impact on survival of pre-dialysis diabetic CKD patients. Glycemic control might also delay CKD progression and postpones the need for dialysis^[204,205].

Blood pressure control muffles the rate of decline in GFR in pre-dialysis CKD patients^[206]. Hypertensive CKD patients should be treated according to KDIGO guidelines^[207]. The problem is much debatable when discussing hypertension control in dialysis patients^[208]. Home BP carries better prognostic impact when compared to recordings in the dialysis unit. Systolic home BP of 115-145 mmHg is associated with the best prognosis in HD patients^[209]. Renin-Angiotensin-system (RAS) blockers stimulate Klotho gene expression in CKD patients. This novel mechanism might clarify the vascular, cardiac and renal protective benefits of such agents^[210,211]. The RAS mediated renal damage might be through Klotho gene manipulation^[212]. Through their manipulation of Klotho gene, RAS blockers can add a new exciting mechanism for their cardiovascular and renal protective effect.

Aldosterone might induce vascular calcification. We are still waiting for clinical studies to evaluate if there is a protective effect of aldosterone antagonists^[213].

CKD patients frequently develop dyslipidemia. Treatment with statins to lower LDL cholesterol is recommended by KDOQI and KDIGO in all adult patients with diabetic CKD and in hypercholesterolemic non-diabetic CKD patient. Such treatment can reduce different cardiovascular events complicating atherosclerosis. However, this treatment does not impact overall mortality in these patients^[214,215]. Many trials targeting CKD patients were done using different statins or statin-ezetimibe combination. In CKD G3 patients, pravastatin treatment was associated with significant reduction of coronary events^[216]. However, another trial using the same statin failed to show any significant impact on 2nd prevention in patients with early CKD^[217]. When statins are used for primary prevention, instead, they reduced the risk of cardiovascular events in stages 1-3 CKD by 41%^[218]. On the other hand, all trials comparing statins with placebo in HD patients failed to demonstrate any significant impact

on clinical outcome or overall mortality. These trials used atorvastatin, 20 mg daily, in the 4D study, rosuvastatin, 10 mg daily, in the AURORA trial and simvastatin, 20 mg plus ezetimibe 10 mg, in the SHARP study^[219-221].

Lifestyle modifications including regular muscle exercise, salt restriction, decrease of calorie intake, and smoking cessation carry significant cardiovascular benefits in the general population. However, we lack data supporting such interventions at all CKD stages^[202].

The very early elevation of FGF23 during CKD G2 should stimulate the attending physicians to reduce phosphorus intake in CKD patients starting in the early days of stage 2^[222]. Phosphate binders, whether calcium containing or calcium-free, should be avoided in this early stage as long as serum phosphorus level is normal or near normal. The very early use of the phosphate binders might be associated with progression of VC while lowering serum phosphorus and attenuating the progression of secondary hyperparathyroidism^[223].

Calcium-based phosphate binders are still very useful to control hyperphosphatemia, but can lead to hypercalcemia and/or positive calcium balance and cardiovascular calcification^[224].

Sevelamer hydrochloride and carbonate are resin-based binders that appear to have profiles that would prevent or muffle VC^[224]. Treatment of non-diabetic stage 3 CKD patients that have normal serum phosphorus with sevelamer did not lower cardiovascular-related outcomes^[225]. These findings reinforce the trend to avoid phosphate binders in early stages of CKD where the serum phosphorus is still normal. On the other hand, when sevelamer was used in hyperphosphatemic stage 3-4 CKD patients, a significant impact on all-cause mortality and the need of dialysis was observed in comparison to calcium carbonate^[226]. The main drawback of all calcium-containing phosphate binders is the tendency to increase serum calcium level. The higher the dose ingested the greater the extent of VC^[227,228]. Thus their use in cases suffering VC, hypercalcemia, low level of parathormone (PTH) and/or adynamic bone disease has to be restricted^[229]. In the US Sevelamer is mainly used in dialysis patients to decrease progression of coronary artery and aortic calcifications^[230-235]. On the other hand, the European Medicines Agency recommended its use in hyperphosphatemic patients with CKD not yet on dialysis^[236-238]. When incident HD patients were assigned to either calcium-based phosphate binders or sevelamer, and were followed for 44 mo, all-cause mortality was lower in subjects assigned to sevelamer compared to patients assigned to calcium-based binders. However, results were of borderline statistical significance. Another important finding in this study is the significant predictive value of baseline CAC score concerning all-cause mortality^[239]. In the "Treat to Goal Study", coronary and aortic calcification progressed in dialysis patients receiving calcium-containing phosphate binders while those receiving sevelamer did not show progression^[232]. On the other hand, sevelamer failed to improve mortality rate among prevalent HD

patients when compared to calcium-based binders in the multicenter, randomized trial “the DCOR”^[240].

We like to emphasize that while the hyperphosphatemic stage 3-4 CKD patients showed benefits in all-cause mortality^[226], and the incident HD showed borderline significantly lower mortality after sevelamer use^[239], the same agent failed to show a similar benefit in prevalent HD subjects^[240]. We should remember that these different groups are in different stages of evolution as regards VC^[9,10,160] and that the baseline score of coronary calcification is a strong predictor of all-cause mortality^[239]. This confirms that the earlier the approach the better would be the impact on CKD patient survival.

Sevelamer is not just a calcium-free phosphate binder, but also has additional pleiotropic effects such as correcting certain abnormalities of lipid metabolism^[241], significant decrease in inflammatory parameters including IL-6, sCD14 and hs-CRP^[242,243], reduction of serum uric acid concentration^[244], decrease of serum FGF23^[123,245,246], increase of serum level of fetuin-A^[236,247] and Klotho^[246]. Compared to calcium based phosphate binders, sevelamer improves endothelial function in CKD patients^[248]. These results suggest that sevelamer has, beside its hypophosphatemic and calcemic actions, important metabolic, and anti-inflammatory actions that help in decreasing uremic vasculopathy. Sevelamer is more expensive compared to calcium-based phosphate binders^[249]. The significant reduction in all-cause mortality and the significantly fewer hospitalizations in the sevelamer group can offset the higher acquisition cost for sevelamer^[250].

Lanthanum carbonate (LC) is another non-calcium based phosphate binder. It was reported to improve aortic VC progression^[251]. There are no trials studying the effect of LC on either coronary or valve calcification^[252]. LC had no impact on over all mortality in CKD patients^[251,253]. However, the mortality was significantly lower in patients above 65 years in the LC treatment group compared with calcium based phosphate binders. A similar observation was reported in patients receiving sevelamer in the DCOR study^[240,254]. In the only trial looking for the impact of LC on the incidence of cardiovascular events, it failed to show any significant difference compared with calcium-based compounds^[251].

Contrary to sevelamer, lanthanum carbonate does not have a consistent effect on FGF23. LC failed to cause reductions in iFGF23 in patients with CKD stage G3-4^[255,256]. On the other hand, other studies showed that LC was effective in reducing FGF23 levels in CKD G3^[257] and CKD G4 - 5 patients^[258]. None of the trials on Lanthanum reported any effect on inflammation or inflammatory biomarkers. Although LC is cheaper and more compliant (Table 1) compared to either sevelamer hydrochloride or sevelamer carbonate^[259], our target is not just to control phosphorus level. Sevelamer compounds have got more comprehensive trials that showed significant impact on patient mortality during predialysis stages and in incident HD. No similar trials could be encountered for lanthanum. We are still waiting for such studies to assure non-inferiority of Lanthanum in this

field.

The value of nicotinamide (NAM) in phosphate control (as well as its effects on lipid levels) in dialysis patients was explored in some short-term trials^[260-262]. However, such trials did not look for either pharmacokinetics or safety. None of these trials studied the impact on VC, FGF23, Klotho or inflammatory mediators.

Iron compounds represent the new class of phosphate binders. Ferric Citrate, Sucroferriic oxyhydroxide, and Fermagate (iron-magnesium hydroxycarbonate) were tested in some clinical trials^[263]. Most of the clinical studies done so far were using ferric citrate, stressing on phosphate binding and ferrokinetics after short periods of trial. So far, no trials have studied the impact on VC^[264-272]. A single study looked for non-inferiority of Sucroferriic oxyhydroxide (PA21) compared to sevelamer carbonate concerning phosphate binding^[273].

Bixalomer is novel non-calcium, amine-functional polymer that binds phosphate in the gastrointestinal tract and inhibits its absorption. It was approved as hypophosphatemic agent in Japan by June 2012. It proved non-inferiority with much lower adverse effects relative to sevelamer hydrochloride^[274].

Salivary phosphorus binding is another approach to reduce serum phosphate level. Chitosan-loaded chewing gum, chewed during fasting periods, may be a valuable add-on to phosphate binders that can lead to a better control of hyperphosphatemia^[275].

The possible beneficial effect of bisphosphonates on VC has evolved during the 1970s when their administration was found associated with decreased calcification of soft tissue in animal and clinical trials^[276,277]. These observations are probably explained by the paradoxical relation between bone mineral density (BMD) and VC^[276-278]. That effect might also be related to the stimulatory action of bisphosphonates on fetuin-matrix Gla protein-mineral complex^[279] and their possible inhibitory action on IL-6. Transformation of VSMCs to osteoblasts and calcification of intimal atheromatous lesions might be triggered by IL-6^[280]. Bisphosphonates were found to inhibit vascular arterial and cardiac valvular calcifications that develop in rats treated with warfarin^[281]. When different members of bisphosphonates were tried in chronic HD patients their anti-calcific effect was favorable in some studies^[282-284] and failed in other more recent one^[285]. In addition, alendronate failed to withhold the progression of VC in G3-4 CKD patients when compared with placebo for 18 mo^[286]. Bisphosphonates are not safe in patients suffering advanced CKD. They can aggravate hyperparathyroidism. They can also lead to adynamic bone disease, osteomalacia or mixed uremic osteodystrophy^[287]. All the trials of bisphosphonates studied their impact on VC. Only one trial studied the impact of bisphosphonate treatment on cardiovascular outcomes in female CKD patients. This study was retrospective^[288].

In the EVOLVE Trial, cinacalcet was tested in chronic HD patients suffering moderate-to-severe 2ry hyperparathyroidism. Inspite of the favorable effects of

Table 1 Different therapeutic interventions used to prevent or withhold vascular calcification progression

CKD stage	Risk factor	Type off interference	Outcome	Ref.
Traditional Risk factors				
G1-G5	Cigarette smoking	Cessation	No evidence	[202]
G1-G5	Overweight	Decrease calorie intake	No evidence	[202]
G1-G5	Sedentary life	Muscle exercise	No evidence	[202]
G1-G5	Diabetes mellitus	Blood sugar control	Improves survival	[204]
			Delays CKD progression	[205]
G1-G5	Systemic hypertension	Blood pressure control	Delays CKD progression	[206]
G1-G5	Dyslipidemia	Statins	Decreased CV morbidity	[221]
CKD Related Risk factors				
G2-G5	↑	Dietary phosphate restriction	↓	[222]
G3b-G4	Hyperphosphatemia	Sevelamer	↓VC, ↓	[226]
G5		Preemptive kidney Tx	↓VC, ↓	[52,295]
Incident G5D	Hyperphosphatemia	Sevelamer	↓VC, borderline↓	[231]
Prevalent G5D	Hyperphosphatemia	Sevelamer or L.C.	↓VC,	[232,240,251]
Prevalent G5D > 65 yr	Hyperphosphatemia	Sevelamer or L.C.	↓VC, ↓	[240,251]

CKD: Chronic kidney disease.

cinacalcet on serum calcium, it failed to decrease the mortality rate or the major cardiovascular events in such patients^[175].

We recommend small dose of vitamin D or vitamin D analogues to be given daily as prophylaxis against VC in spite of the lack of clinical trials favoring the use of either native or active vitamin D analogues to prevent VC progression. The rarity of vitamin D toxicity in general and the privileged survival benefits offered by VDRAs administered in small doses even in cases suffering hyperparathyroidism and/or increased calcium and phosphorus levels supports this concept. Some studies reported the association of low vitamin D serum level with extensive VC^[289,290]. Vitamin D inhibits renin activity, inflammation, suppresses stimulators of VC and stimulates inhibitors of VC in the uremic milieu^[291].

We are still looking for the possible role of vitamin K supplementation in management of VC^[292]. Treatment of CKD rats with vitamin K1 suppressed the development of VC^[293]. A prospective trial is going on in RDT patients suffering coronary calcification. The effect of vitamin K1 supplementation on the calcification progression in the thoracic aorta and coronary artery will be addressed. All-cause mortality is a secondary end-point. This study may offer an inexpensive agent to treat or prevent VC^[294].

Once the patient proceeds to stage 5, pre-emptive kidney transplantation is the best option to improve patient and graft survival in comparison to patients admitted to dialysis or to patients transplanted after starting dialysis^[295-298]. In patients starting dialysis, the shorter the dialysis vintage the better is the post-transplant survival^[299]. The survival benefit of transplantation compared to dialysis is most probably related to the decreased rate of VC post-transplant compared to the accelerated progress in VC observed in dialysis. To further decrease the rate of calcification progression after transplantation, perioperative vascular imaging and analysis of serum FGF23 might help in appointing patients more likely to have progression of VC. Such patients should continue the anti-calcific measures

applied to CKD G3 patients. This advice is based on the previous observation of the strong association between baseline CAC score and CAC progression^[39,300] and on the recent finding of high serum level FGF23 in KTR even when they have normal graft function^[301]. This disturbance of FGF23 appeared to be related to the endothelial cell injury in KTR^[302]. Elevated levels of FGF23 may predict increased risks of death and allograft loss^[303].

Since the pathogenesis of CUA is not fully elucidated, its treatment is still not uniform^[304]. Cinacalcet appeared to reduce the incidence of CUA in HD recipients who have moderate to severe secondary hyperparathyroidism^[305]. Sodium thiosulphate^[38,304] is used successfully in treatment. Bisphosphonates may be also used^[306,307].

CONCLUSION

The new definition and staging for CKD suggested by the NKF-KDOQI in 2002 aimed at stimulation and increased awareness of the medical community to early diagnose CKD^[308]. Early diagnosis of CKD gives a great chance to delay the progression of such disease, and to have better chance to deal with the different complications. VC has evolved as the most serious complication in CKD patients endangering their life. The only successful treatment for VC is preventive. This treatment should start as early as the early days of stage G1. Control of blood sugar in diabetic pre-dialysis CKD patients is a mandate. Recommended hemoglobin A(1c) level should be around 7%. Hypertensive CKD patients should be treated according to KDIGO guidelines. Statin treatment should be prescribed according to KDIGO guidelines.

Screening for FGF23 would pick up CKD patients requiring phosphorus handling at much earlier stage when they benefit maximally. However, we are still waiting for epidemiologic studies that would determine normal and target levels of FGF23 and the ideal method of assay.

In these early days, moderation of dietary phosphate intake might suffice. If Serum PTH level is high, we

should measure serum 25-hydroxy vitamin D level^[309]. If such level is below 30 ng/mL the patient should be prescribed either vitamin D2 or D3. We are waiting for prospective clinical trials studying the value of recombinant Klotho treatment in normalization of serum FGF23 level and preventing the development or progression of VC. Regular estimation of serum calcium, phosphorus, Ca x p byproduct and PTH should be performed with the frequency recommended by guidelines^[310]. Once serum phosphorus starts to rise above normal, strict restriction of dietary phosphorus and prescription of sevelamer should ensue. Other phosphate binders could be used, however, the lack of clear evidence for their effect on Klotho and on cardiovascular morbidity and mortality would postpone their use in the time being till we have strong evidence for these effects. A small dose of vitamin D analogues should be added to all patients passing to stage 3 and beyond. Vitamin K looks promising in preventing or slowing the progression of VC, however, we are still waiting for the results of the ongoing study looking for its efficacy. Once the patient proceeds to stage 5, pre-emptive kidney transplantation is the best option to improve patient and graft survival in comparison to patients admitted to dialysis or to patients transplanted after starting dialysis. In patients starting dialysis, the shorter the dialysis vintage the better is the post-transplant survival. To further decrease the rate of calcification progression after transplantation, perioperative vascular imaging and analysis of serum FGF23 might help in appointing patients more likely to have progression of VC. Such patients should continue the anti-calcific measures applied to CKD G3 patients.

In patients maintained on dialysis, non-calcium phosphate binders still carry the privilege of decreased progression of vascular calcification in spite of their failure to impact either cardiovascular morbidity or mortality. HD patients above 65 years of age showed survival benefit after use of sevelamer or LC, the latter is preferred in this age group based on patient compliance and cost of treatment.

Finally we have to emphasize that huge effort is still needed to support many of the above suggestions by well-designed prospective controlled studies to evaluate either efficacy, safety of such interventions beside the precise definition of optimum dosage and frequency of every individual therapeutic modality.

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Hypertensive disorders in pregnancy

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Abstract

Renal injury or failure may occur in the context of pregnancy requiring special considerations with regard to fetal and maternal health. The condition of pregnancy itself may be a major factor in such injuries. In addition,

for many young women previously known to be healthy, pregnancy may be the first presentation for routine urine and blood testing which may yield previously subclinical renal disease. As such, pregnancy may add complexity to considerations in the management of renal disease presenting coincidentally requiring knowledge of the physiologic changes and potential renal disorders that may be encountered during pregnancy.

Key words: Pregnancy; Hypertension; Preeclampsia; Hemolysis, elevated liver enzymes, and low platelets

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Core tip: Kidney disease and particularly complications of hypertensive disorders is one of the dire threats to successful pregnancy. This review highlights advances in our understanding of the pathophysiological processes that drive the development of hypertensive disorders' complications during pregnancy, potential use of biomarkers in predicting these complications, and novel therapeutic approaches under consideration for their great promise in achieving successful pregnancy.

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INTRODUCTION

Pregnancy is a unique arena in the practice of nephrology in which special considerations must be made for a host of factors such as hemodynamics, immunology, metabolism, pharmacology, and embryology. For many young women previously known to be healthy, pregnancy may be the first presentation for routine urine and blood testing which may yield previously subclinical renal disease. Furthermore, renal injury or failure may occur in

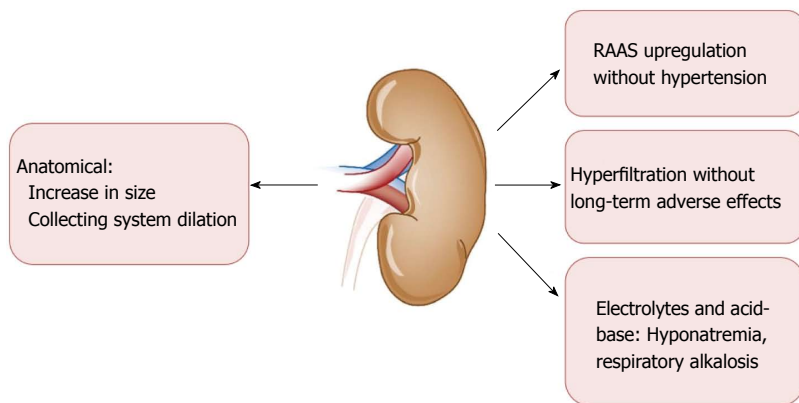


Figure 1 Physiological renal changes during pregnancy.

the context of pregnancy requiring special considerations with regard to fetal and maternal health. The condition of pregnancy itself may be a major factor in such injuries (e.g., as in prerenal injury secondary to hyperemesis gravidarum, or acute cortical necrosis secondary to septic abortion or peripartum hemorrhage) or may simply add complexity to considerations in the management of renal disease presenting coincidentally. The nephrologist may be consulted when patients develop acute kidney injury (AKI) with glomerulonephritic features including refractory hypertension, edema, reduced estimated glomerular filtration rate (eGFR), proteinuria, and occasionally microangiopathy^[1], for which preeclampsia is often on the differential diagnosis. This review aims to explore the anticipated physiologic changes in pregnancy before addressing the hypertensive disorders of gestation, of which pre-eclampsia is the most common; affecting approximately 5% of pregnancies worldwide. Our aim is to present a comprehensive overview of the current knowledge on physiologic changes in pregnancy, with special attention paid to the epidemiology, genetics, pathophysiology, diagnosis, and management of preeclampsia.

NORMAL ADAPTATIONS EXPECTED IN PREGNANCY

Several unique physiologic changes occur in the context of a normal healthy pregnancy (Figure 1). There is an overall up-regulation of the renin-angiotensin-aldosterone system (RAAS) that begins at the time of the luteal phase of the menstrual cycle and is co-incident with rising estrogen and progesterone levels^[2,3]. After fertilization, elaboration of each of these hormones continues in order to support gestation; renin up to eight times, angiotensin up to four times, and aldosterone up to ten to twenty times normal levels^[2,3].

Healthy women do not become hypertensive in this context, however, owing either to an estrogen-mediated decrease in vascular responsiveness to these RAAS components, or possibly owing to the counteracting vasodilatory effect of prostacyclins and the ovarian-secreted gestational hormone relaxin^[4]. This systemic vasodilation tends to decrease systolic blood pressure

by about 10-15 mmHg^[5]. This global vasodilation also dilates the renal vasculature resulting in an early and robust increase in glomerular filtration-initially by about 25% and progressing up to 50% by mid-pregnancy. There is an even larger increase in renal plasma flow, around 60%. The result is a state of hyperfiltration that does not actually engender any pathologic injury as in other states of hyperfiltration such as in diabetic kidney disease^[6]. Pregnancy is associated with reduction in the single nephron filtration fraction compared to the increase encountered in other hyperfiltration conditions. RAAS up-regulation contributes to sodium and fluid retention, which facilitates plasma volume expansion within the dilated vasculature. Intravascular volume expansion results in a mild dilutional anemia and hyponatremia; serum sodium concentrations may be reduced by approximately 4-5 meq/L^[7].

Increased renal volume and length is frequently appreciated on ultrasonography, along with mild non-obstructive hydronephrosis due to uterine compression of the ureters. Pelviectasis is typically more pronounced on the right side, possibly owing to dextrorotation of the uterus and exaggerated by the relative protection of the left ureter provided by the sigmoid colon^[8]. This low-grade obstruction may be symptomatic in approximately 30% of pregnant women and can predispose to urinary tract infections.

HYPERTENSIVE DISORDERS OF PREGNANCY

Preexisting and gestational hypertension

An increasing subset of women enter into pregnancy with pre-existing hypertension in the setting of the usual risk factors for essential hypertension such as obesity, race, and advanced maternal age. An estimated 25% of these patients may develop a superimposed preeclampsia syndrome. Hypertensive disorders of pregnancy increase maternal risk of developing AKI in addition to other etiologies as stratified in Table 1. In this relatively young demographic, essential hypertension is less likely to have been present long enough to manifest any clinically apparent end-organ damage so the development of any proteinuria or other renal dysfunction would potentially

Table 1 Selected renal disorders during pregnancy

Renal disease by trimester		
1 st trimester	Hyperemesis gravidarum	Worsening of preexisting renal disease
Week 1-12	Cortical necrosis due to septic abortion	↓
	Preeclampsia (> after 20 wk)	↓
	AFLP	↓
2 nd trimester	Preeclampsia	↓
Week 13-28	HELLP syndrome	↓
	TTP	↓
	Preeclampsia	↓
3 rd trimester	Preeclampsia	↓
	Polyhydramnios	↓
	Extraureteral obstructive hydronephrosis	↓
Post-partum	Post-partum hemolytic uremic syndrome	↓
	Preeclampsia	↓

AFLP: Acute fatty liver of pregnancy; TTP: Thrombotic thrombocytopenic purpura; HELLP: Hemolysis, elevated liver enzymes, and low platelets.

Table 2 Renal disorders and associated maternal and fetal health risks

Maternal and fetal risk by degree of renal impairment		
Stage	Pregnancy/fetal outcomes	Renal/maternal outcomes
Early CKD I - II sCr < 1.4 mg/dL eGFR < 70 mL/min Normal BP Minimal proteinuria	Higher risk than general population for preeclampsia, SGA, preterm delivery Counseling: May need specialized care Generally good outcomes	Lower risks for accelerated progression
Moderate CKD II -III sCr 1.4-2.4 mg/dL eGFR 40-70 mL/min	With more advanced CKD and higher proteinuria: Higher risks of caesarian section, preterm delivery, SGA, and need for NICU	Increased risk of progression during pregnancy and within 6 wk postpartum Counseling: Pregnancy termination doesn't reliably reduce risks for progression
Severe CKD III-IV sCr > 2.4 mg/dL eGFR < 40 mL/min ESRD	With more advanced CKD and higher proteinuria: Higher risks of caesarian section, preterm delivery, SGA, and need for NICU care Decreased fertility and high fetal mortality except with more intensive hemodialysis Higher risks of preeclampsia, SGA, cervical incompetence, and need for NICU care persist ± increased risk of fetal loss	Increased risk of progression during pregnancy and within 6 wk postpartum Increased need for transfusion, worsening hypertension
Post-transplant	Increased risk of low birth weight and preterm delivery Significantly increased risk of preeclampsia if hypertensive	Blunted renal physiologic adaptations No anticipated decrease in graft survival but may be associated with decreased maternal life span Increased risk of diabetes, urinary tract infection (due to anatomy, insulin resistance, and immunosuppression)

SGA: Small for gestational age; NICU: Neonatal intensive care unit; CKD: Chronic kidney disease; ESRD: End-stage renal disease.

point to the onset of an overlapping preeclampsia syndrome. In women entering pregnancy with pre-existing renal disease, which may potentially be masked by the effects of hyperfiltration on conventional markers of renal function (e.g., serum creatinine) risks to the mother and fetus can be stratified by the severity of renal insufficiency and modes of renal replacement therapy, if applicable (Table 2)^[9].

Clinical features, criteria, and definition of preeclampsia

Preeclampsia is a heterogeneous, multi-system disorder characterized by widespread dysfunction, including glomerular endothelium; it is the most common glomerular disorder in pregnancy. The criteria for diagnosis includes two blood pressure readings at least 4-6 h

apart that are greater than 140/90 occurring after 20 wk' gestation in a woman not known to be previously hypertensive^[10]. The syndrome may also develop in the 4-6 wk postpartum period^[10]. Consequently, preeclampsia is best categorized into early onset/placental (< 34 wk of gestation) vs late onset/maternal (> 34 wk of gestation) reflecting that there are potentially different triggering events in pathogenesis as well as the worse maternal-fetal prognoses of early vs late preeclampsia. Edema and elevated uric acid levels are also frequently among the constellation of findings but are not strictly part of the definition^[10] (Table 3). Either new onset or worsening of pre-existing proteinuria greater than 300 mg in 24 h may be present, but proteinuria itself has been removed from the definition since it is a relatively late marker of

Table 3 Definition of preeclampsia

Updated definition	Supportive clinical signs
2 blood pressure readings ≥ 140/90	Edema ±
Taken ≥ 4-6 h apart	Uric acid level ≥ 7.8 mg/dL
After 20 wk gestation	±
+	Proteinuria (severe ≥ 5 g/g)
Patient not previously known to be hypertensive	Thrombocytopenia Elevated serum aminotransferase levels Acute kidney injury Pulmonary edema Cerebral/visual disturbances (new onset)

glomerular injury. Higher levels of proteinuria above 5 g/g were once considered to be a marker of severity as well but this has fallen out of favor for the same reason. In the absence of proteinuria, preeclampsia is confirmed when *de novo* hypertension after 20 wk of gestation is associated with maternal or fetal end organ damage which may include thrombocytopenia, elevated serum aminotransferase levels, AKI, pulmonary edema, new onset of cerebral or visual disturbances, or uteroplacental dysfunction. AKI or renal failure can occur, however identifying AKI may be fraught with its own challenges given the lack of a consensus definition of AKI in the pregnant population^[10]. Approximately 10%-20% of cases of preeclampsia are severe enough to manifest hemolysis, elevated liver enzymes, and low platelets (HELLP) syndrome, a thrombotic microangiopathic process (TMA) named for its most notable features of red cell lysis and thrombocytopenia^[10]. As such, HELLP is likely a form of atypical HUS that is triggered by pregnancy although transaminitis can occur alone or as part of this syndrome. Adverse cardiovascular and cerebrovascular outcomes may develop if blood pressure is not adequately controlled.

Morphologically, endothelial swelling (Figure 2A) is the cardinal feature on light microscopy which typically resolves approximately eight weeks after delivery, along with the proteinuria and hypertension. However, persistent damage can follow preeclampsia in the form of focal segmental glomerulosclerosis (FSGS) with collapsing features. Loss of glomerular endothelial fenestrae with relative preservation of the podocyte foot processes is expected on electron microscopy along with possible electron-dense deposits in subendothelial and mesangial tissue (Figure 2B). In severe cases or in healing stages one may note increased glomerular cellularity and mesangial interposition. FSGS has been one of the dominant histopathologic lesions in renal biopsies sampled from women with persistent proteinuria following a preeclamptic pregnancy.

Risk factors: Risk of preeclampsia is increased in the setting of maternal endothelial dysfunction. For example,

diabetes mellitus can double or quadruple the risk of developing preeclampsia^[10,11]. There is also an increased risk for preeclampsia in patients with a first degree relative with preeclampsia^[12]. Patients with a history of preeclampsia are known to carry an increased risk of cardiovascular and renal morbidity and there is some evidence that affected women continue to have impaired endothelial-dependent vasorelaxation which may account for this increased risk. However, there are many shared risk factors for preeclampsia and cardiovascular disease such as diabetes, hypertension, obesity, and metabolic syndrome. Another area of growing evidence is the exposure to environmental and innate risk factors that may contribute to increased susceptibility to hypertensive disorders in pregnancy. For example, seasonal variation in preeclampsia and hypertensive disorders of pregnancy have been observed for the better part of a century. Combined findings in a systematic review of 20 preeclampsia studies suggested an increased frequency of episodes during the rainy seasons of tropical climates and the cold seasons of non-tropical climates^[13]. Plausible biological mechanisms explaining this association include higher blood pressures as well as wider daily blood pressure and body temperature variations during these seasons, reduced physical activity and dietary changes, decreased vitamin D levels, and increased infections. Along the same line and not surprisingly, plant-based diets higher in fiber and potassium, cereals, dark bread, and low-fat dairy may be associated with reduced preeclampsia risk^[14]. Similarly, vegetable-laden low protein diets (not more than 0.6-0.7 g/kg per day) seem to confer beneficial effects on clinical variables of renal health during pregnancies in cohorts of women with chronic kidney disease (CKD)^[15].

Genetics and paternity: While much has been published about the maternal and placental pathophysiologic roles in preeclampsia, a growing area of research is contributing to knowledge about the paternal role as well, as summarized in a recent review by Katsi *et al.*^[16]. Among the early implications for a paternal role are the early observations that a family history of preeclampsia places one at increased risk for the syndrome and that multiparity (if prior pregnancies were uncomplicated) decreases one's risk of preeclampsia, unless there is a change in paternity. This latter fact has been understood to be due to maternal mucosal immune tolerance mechanisms as mediated by human leukocyte antigens. Supporting this hypothesis are reports of relatively increased risks of preeclampsia in women using barrier contraceptive methods, couples with shorter durations of sexual relationships prior to conception, and in women undergoing fertility assistive therapies who receive oocytes fertilized with surgically obtained sperm rather than their partner's ejaculated sperm. Other observations suggest that immune tolerance is not likely to be the only key in understanding the paternal role. Interestingly, men who father one preeclamptic pregnancy may be more likely to father a preeclamptic pregnancy with a

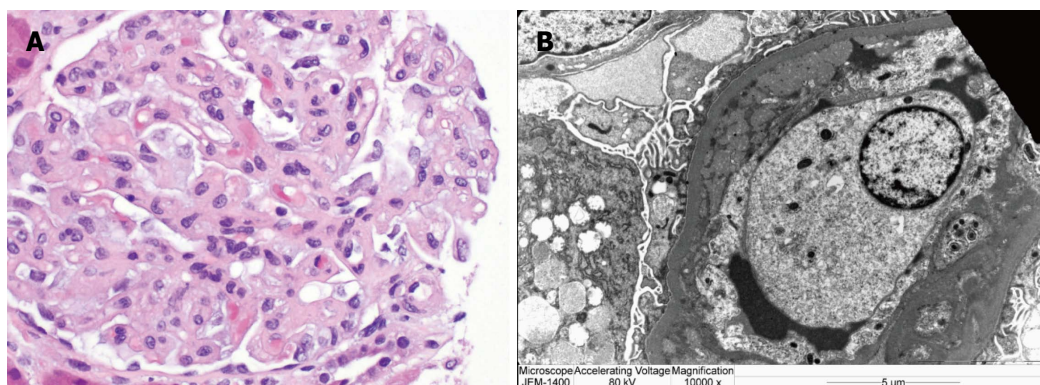


Figure 2 Light (A) and electron (B) microscopy. A: Glomeruli with capillary loops occluded by swollen endothelial cells, e.g., endotheliosis; B: Capillary loop with subendothelial widening by flocculent material and occlusion by swollen endothelial cells. Courtesy of Naima Carter-Monroe.

different woman. Also, in a bimodal pattern, paternal age > 45 or < 25 appears to be another risk factor for preeclamptic pregnancy. Paternal race may factor into risk of preeclampsia, such as when differing from maternal ethnicity; and there appear to be decreased rates of preeclampsia in cases of Asian paternal ethnicity.

Pathophysiology: “Disease of theories”: Numerous mechanisms have been proposed regarding the etiologies of preeclampsia, which has earned it the nickname “the disease of theories”^[12]. Mapping these different mechanisms may help one day to elucidate the different phenotypes and their respective mechanisms. Preeclampsia is a very heterogeneous disease, and it may be better thought of as an umbrella term for multiple different subtypes driven by a variety of different processes to varying extents which have yet to be fully elucidated. The predominant paradigm at present is that there is a fundamental imbalance between proangiogenic drivers and antiangiogenesis. Research in recent years has yielded significant advances in identifying early biomarkers, especially within the proposed pro- and anti-angiogenic pathways. The pathogenesis involves a two stage process wherein in the first stage uterine spiral arteries are incompletely remodeled leading to vasospasm and placental ischemia and in the second stage ischemic placental tissue releases systemic mediators or angiogenesis and inflammation in the the maternal circulation with resulting downstream endothelial injury.

Fetal endovascular cytotrophoblastic cells implant in the uterine endometrium and myometrium during the phase of embryologic development known as placentation, occurring between 8 and 18 wk of gestation. Cytotrophoblasts subsequently invade and remodel uterine spiral arteries to augment blood flow to meet the oxygen and nutrient demands of fetal growth. Remodeling reduces smooth muscle cells in the infiltrated vascular segments, facilitating dilatation and a resulting reduction in uteroplacental pressure and velocity of flow. In the placentas of preeclamptic women-for reasons that are not entirely clear - this trophoblastic

invasion fails to transpire adequately and the result is tortuous, thick-walled, incompletely remodeled vessels prone to spasms among retained vasoactive smooth muscle. The consequence is placental hypoxia, oxidative stress, and subsequent intermittent fetal hypoperfusion. The placental response is expression and secretion of anti-angiogenic and pro-inflammatory factors which may induce maternal hypertension and proteinuria^[18].

Angiogenic imbalance: For more than a decade the preeclampsia syndrome has been understood to be associated with elevated circulating levels the molecule “sFlt-1”; the placental synthesis and release of which is believed to be triggered by the placental ischemic and reperfusion injury alluded to above. Abbreviated for “soluble fms-like tyrosine kinase”, sFlt-1 is a circulating receptor for vascular endothelial growth factor (VEGF), on which it has an antagonistic effect. In the healthy kidney, VEGF is produced by epithelial podocytes to maintain slit diaphragm integrity and to regulate the health of endothelial cells that express receptors to VEGF. Circulating proangiogenic molecules such as VEGF and placental growth factor (PlGF) are scavenged by sFlt-1 (the soluble receptor to VEGF) and as a result perturbs the balance between pro- and antiangiogenesis, skewing this balance towards the latter. Binding sFlt-1 to proangiogenic regulators prevents these vasodilatory effectors from interacting with their receptors on the endothelial cell surface, impairing nitric oxide-mediated vasodilation. The result is widespread endothelial dysfunction with multiorgan implications including notably the renal manifestations of preeclampsia such as hypertension and proteinuric glomerular dysfunction.

Automated assays for the detection and measurement of circulating of sFlt-1 and PlGF levels have been developed but are not yet in widespread use^[19]. In a clinical setting the ratio of these markers may perhaps soon provide indices of overall antiangiogenic activity, offering a potential early prediction tool for the development of preeclampsia well before the clinical onset of disease. Another diagnostic dilemma is the later gestational exacerbations of preexisting hypertension

and proteinuria which can be difficult to interpret and presents a diagnostic challenge in the identification of superimposed preeclampsia in this population. Brahmam *et al.*^[20] recently published their evaluation of the performance of PlGF concentrations as predictors of superimposed preeclampsia (with a clinical endpoint of requiring delivery within 14 d) in patients with CKD and/or chronic hypertension^[20]. In this study, PlGF levels less than the fifth centile performed well in the detection of superimposed preeclampsia in pregnant women with CKD and/or chronic hypertension, with specificity and negative predictive values greater than 80%. sFlt-1, B-type natriuretic peptide, neutrophil gelatinase-associated lipocalin (NGAL), and relaxin were also evaluated but were concluded to have less promising diagnostic discriminatory potential^[20].

Soluble endoglin (sEng) is another known detectable antiangiogenic molecule that behaves as an inhibitory receptor for the cytokine TGF- β 1^[21]. Similar to sFlt-1, it can be found to be at notably increased serum levels as early as 2 to 3 mo prior the clinical onset of preeclampsia. It may offer improved predictive accuracy when used in combination with the ratio of sFlt-1 to PlGF. In normal pregnancies, the sFlt-1 to PlGF ratio is an s-shaped curve with a steep rise in the first 5-10 wk followed by a stagnation and a subsequent third trimester rise that progresses until labor and delivery.

In a nested case control study serum levels of total sFlt-1, free PlGF, and free VEGF in 120 women with preeclampsia were tracked throughout pregnancy and matched to normotensive controls by gestational age^[22]. In the preeclamptic group mean sFlt-1 levels were noted to rise in late gestation; this group also consistently had lower PlGF levels (demonstrating the high sFlt-1 to PlGF ratio associated with an antiangiogenic milieu). Circulating levels of sFlt-1 were noted to increase on average five weeks before the clinical onset of preeclampsia and the degree of rise correlated with disease severity.

Oncology patients treated with drugs targeting VEGF can develop a "preeclampsia-like syndrome" with severe hypertension, proteinuria, and edema^[23]. Anti-VEGF drugs have been an important mainstay in cancer therapy owing to their anti-angiogenic effects, exerted through a variety of pharmacologic mechanisms^[23]. Examples include anti-VEGF monoclonal antibodies (*e.g.*, bevacizumab), decoy receptors (*i.e.*, VEGF-Trap drugs), and multi-target tyrosine kinase inhibitors (MTKIs), which interfere with the VEGF signaling pathway (*e.g.*, sorafenib, sunitinib and brivanib)^[23]. These drugs have been used successfully in renal and gastrointestinal malignancies^[23]. Hypertension is an important side effect, along with GI and skin toxicities. Renal side effects that have been reported include proteinuria and acute renal failure, specifically with bevacizumab and sunitinib^[23]. In some cases, there have been associated renal failure and biopsy-proven TMA along with endotheliosis and effacement of foot processes^[23]. These side effects seem to occur in a dose-dependent manner and have been observed to resolve with treatment cessation^[23].

Podocyte shedding: Another feature noted on kidney biopsy specimens in patients on these anti-VEGF therapies is a down-regulation in the expression of podocyte slit diaphragm proteins such as synaptopodin, nephrin, and podocin^[24]. This helps to strengthen the link between the upstream antiangiogenic environment in preeclampsia and the downstream glomerular injury and proteinuria^[24]. Although proteinuria is no longer considered essential for the diagnosis of preeclampsia, it remains a hallmark of the disorder, differentiating it from other hypertensive disorders of pregnancy as well as from other proteinuric diseases that may be co-incident with pregnancy. Urine sediment is typically "bland" in preeclampsia-without cells or casts-but there may be detectable podocytes and podocyte specific proteins^[25]. There has been a recent diagnostic focus on detecting these sloughed podocytes in the urine of preeclamptic women even before proteinuria develops^[25]. The degree of podocyturia correlates positively with that of proteinuria; and podocyte damage and shedding may affect renal function for years following a pregnancy complicated by preeclampsia^[25,26]. This may someday serve as another methodology for early detection, however currently available lab techniques need more development^[25]. Podocytes can be cultured from urine samples, although not quickly enough to be of clinical utility^[25]. Cytospin techniques for detection may be automated, however there is a loss of sensitivity and specificity owing to contamination with other cellular debris^[25]. Polymerase chain reaction (PCR) and mass spectrometry are anticipated to provide the most sensitive and specific detection methods but are not yet clinically available^[25].

Anti-angiotensin II type 1 receptors: Agonistic antibodies to angiotensin II type 1 receptors (AT1-AA) were first described in 1999^[27]. These are immunoglobulins of the IgG3 subclass which, by agonizing AT1 receptors, lead to enhanced sensitivity to angiotensin II thus influencing increased sodium retention and vasoconstriction. It remains unclear whether these antibodies are the cause or effect; however agonistic antibodies to AT1-AA may be an upstream trigger of increased sFlt-1 expression^[28].

Vasodilatory gases and heme oxygenase pathway:

Other vasodilatory gases (in addition to nitric oxide discussed above) may offer mechanistic insight and therapeutic value^[29]. There exists growing evidence that the enzyme heme oxygenase and its byproduct carbon monoxide may play a protective role in preeclampsia^[29]. Heme oxygenase converts heme to bilirubin and biliverdin; both of which are potent antioxidants^[29]. Carbon monoxide (CO) is released in this process and is thought to be an important mediator in maintaining placental vasodilation and healthy development^[29]. Supporting this are studies demonstrating reduced end-tidal CO levels in women with preeclampsia (perhaps demonstrating decreased heme oxygenase activity); further, women who smoke and live in areas with higher ambient CO appear to have

less epidemiologic risk of preeclampsia^[12,29].

Asymmetric dimethylarginine (ADMA) is an endogenous molecule known to competitively inhibit the activity of nitric oxide synthase. Its metabolism is closely associated with homocysteine (Hcy); which, along with ADMA can be found at elevated concentrations in disease patterns characterized by endothelial injury^[30]. Hcy is an upstream effector of oxidative stress associated with ischemic injury and CKD^[31] and can be found at increased levels in both obesity and vitamin B deficiencies^[30]. López-Alarcón *et al.*^[30] recruited 411 women from two obstetric hospitals in Mexico focused on high risk pregnancies (excluding smokers, diabetics, and women with hypertension) to monitor monthly serum levels of these potential biomarkers. Approximately 20% of the follow-up group went on to develop preeclampsia and tended to have higher Hcy and ADMA concentrations at baseline despite having values within the normal ranges reported for healthy pregnant women. Though there were no detectable differences between groups with varying degrees of preeclampsia severity, serum levels gradually increased throughout pregnancy in the preeclampsia group compared to women who did not develop pregnancy complications (even after adjusting for obesity and nutritional status), allowing authors to postulate that the detection of increases in serum concentrations of these molecules may allow for early prediction of preeclampsia risk^[30].

Placental protein-13: Placental protein 13 (PP-13), first discovered in 1983 by Dr Hans Bohn, is produced by the syncytiotrophoblastic layer in early placental implantation and remodeling, and is thought to be shed in the setting of ischemic placental stress and inflammation^[18]. It has been evaluated in an ever-growing body of literature with regards to its capacity to be used as a clinical marker of placental pathology. Second and third trimester levels of PP-13 have been shown to rise in preeclampsia compared to normal pregnancy in a manner correlated with severity^[18]; however, conflicting reports exist regarding whether detectable levels can be associated in a predictable way with preeclampsia and may vary demographically when accounting for age, ethnicity, and maternal ABO blood type^[32]. According to Seravalli *et al.*^[32], however, first trimester levels of PP-13 are not likely to independently identify increased risk of preeclampsia in a population at low risk for placental dysfunction although in their cohort of 908 women at low risk for preeclampsia, lower levels of first trimester PP-13 were identified in women with higher BMI, perhaps reflective of metabolic syndrome which is thought to be a risk factor for adverse pregnancy outcomes^[33]. Confusing this significance somewhat is the finding that cigarette smoking was associated with a profound decrease in first trimester PP-13 levels; cigarette use has been an environmental exposure consistently associated with a reduced risk of preeclampsia despite other negative

placental effects such as fetal growth restriction^[34,35].

Urine congophilia: Recently reported findings by McCarthy *et al.*^[36] appear to confirm the presence of increased levels of amyloid protein in the urine of women with preeclampsia, CKD, and CKD with superimposed preeclampsia compared to healthy pregnant women and women with chronic and gestational hypertension. "Congophilia" is a term used to describe the retention of Congo red dye in a specimen which indicates the presence of amyloid, an aggregate of inappropriately folded proteins thought to be generated by stressed endoplasmic reticulum in the ischemic placenta. Also noted was a significant positive correlation between the magnitude of congophilia and urine protein to Creatinine ratios. Further research is needed to elucidate how this method may be utilized clinically to distinguish between renal impairment, early and late term preeclampsia, and other pathologic processes that activate the unfolded protein response pathway in endoplasmic reticulum^[36].

CLINICAL MANAGEMENT OF PREECLAMPSIA

Preeclampsia is primarily a placenta-driven disease process; thus delivery is the only definitive treatment. Indeed, levels of key mediators such as sFlt-1 have been noted to fall within 48 h post-partum. The desirability and safety of delivery may depend on clinical considerations such as fetal gestational age, signs of fetal or maternal distress, or severity such as progression to eclampsia as indicated by the presence of seizures^[10].

Pharmacologic

Though it does little to reverse or correct the placental under-perfusion that is thought to be driving preeclampsia, aggressive blood pressure control is another essential mainstay in preeclampsia management^[11]. The primary goals are to prolong gestation in order to allow further fetal growth and development and to prevent maternal cerebro- and cardiovascular catastrophes^[11]. Angiotensin-converting enzyme (ACE) inhibitors and angiotensin receptor blockade (ARB) therapies are very effective but contraindicated during any trimester of pregnancy^[37]. Their use is associated with "fetal renin-angiotensin system blockade syndrome" characterized by impaired tubular development and oligohydramnios, among multiple devastating other effects^[37]. Angiotensin II is essential in the regulation of umbilical-placental blood flow and maintenance of GFR in the low-pressure circulation of the fetus^[37]. Drugs that inhibit renin directly, such as the drug "aliskiren" are expected to have similar effects to ACE inhibitors and ARBs^[37]. There are no case reports of fetal exposure to these drugs, but at present they should be avoided^[37]. Labetalol, hydralazine, methyldopa, and nifedipine are the antihypertensives that have the best safety profile and are the typical go-to agents in

pregnancy^[11,38].

Magnesium: Intravenous magnesium sulfate (MgSO₄) is the treatment of choice for prevention and treatment of recurrent seizures. Infusions are often started 48-72 h prior to delivery induction once preeclampsia is suspected or diagnosed. This allows time for fetal lung maturation after dosing corticosteroids, typically given concomitantly. Dosing may be approached more empirically in patients with normal renal function, however dose serum (at least every 6 h) and clinical monitoring of magnesium levels is advised in renal insufficiency to avoid toxicity. While neuroprotective at therapeutic levels, MgSO₄ levels above 4.8 mg/dL can lead to central and respiratory depression or cardiac arrest. Calcium gluconate is the appropriate antidote^[39]. MgSO₄ also has synergistic blood pressure lowering activity with nifedipine^[40] and may have anti-inflammatory effects as well *via* AT1-AA which has yet to be further elucidated^[17].

Aspirin: Aspirin administration to reduce preeclampsia risk has been an important research question since the 1970s, with more than 50 published trials and several recent meta-analyses^[41]. It has been hypothesized that aspirin facilitates trophoblastic invasion of the uterine spiral arteries^[41]. Some of its benefit may be due to the inhibition of synthesis of platelet thromboxane, a potent vasoconstrictor produced by endothelial cells. However, data to support this strategy has been conflicting^[41]. Since there may be up to 50% risk reduction and there is little harm other than the usual contraindications to aspirin, guidelines currently recommend initiating aspirin in the highest risk patients (such as women with pre-existing diabetes)^[11] early on, ideally in the first trimester^[41,42].

Statin: Statin use has also been under evaluation-plausibility lies with their known anti-inflammatory properties as well as their demonstrated ability in mouse and *in vitro* studies to inhibit cytokine-mediated release of sFlt-1^[43,44]. Statins are also thought to have a positive influence on endothelial health by increasing the bioavailability of nitric oxide, PIGF, and VEGF^[44]. Pravastatin has emerged as the only possible safe agent from this class, due to its inability to cross fetal membranes into the embryonic compartments^[43]. Simvastatin, lovastatin, and atorvastatin are all lipophilic and able to equilibrate between maternal and fetal compartments where these agents may interfere with cholesterol-mediated cell signaling and result in fetal central nervous system, renal, and limb defects^[43].

Metformin: Metformin is known to be safe in pregnancy and is currently used to treat gestational diabetes mellitus^[45]. It is hypothesized to reduce sFlt-1 and sEng secretion by way of its effect on reduction of mitochondrial electron transport chain activity and downstream inhibition of hypoxic inducible factor 1 α ^[45]. Recently, *in vitro* and *ex vivo* experimentation demonstrated reduced

sFlt-1 secretion from metformin-treated endothelial and placental cells in a dose-dependent manner^[45]. Clinical trials assessing the effect of metformin on primary outcomes such as hypertension and preeclampsia have yet to be published^[45].

Calcium: As of 2013, the World Health Organization recommends 1.5-2.0 g of elemental calcium daily in three divided doses from 20 wk' gestation until term in all pregnant women in areas where calcium intake is low, particularly those at higher risk of gestational hypertension^[46]. Evidence to support calcium supplementation to prevent preeclampsia has been conflicting and remains controversial. In 1996, Bucher *et al.*^[47] evaluated 14 randomized controlled trials involving 2459 women and found benefits in blood pressure and preeclampsia incidence reduction supporting the use. The following year, however, the NIH study Calcium for Preeclampsia Prevention (CPEP) concluded from a randomized controlled clinical trial of twice as many healthy nulliparous women that calcium supplementation did not reduce blood pressure, adverse perinatal outcomes, or the incidence or of preeclampsia, nor did it delay onset^[48]. In the decade following, subsequent large-scale meta-analyses have supported the practice, particularly in developing countries where dietary calcium intake may be relatively lacking, as well as in otherwise healthy high-risk populations^[49,50]. Calcium supplementation in this context has not been shown to increase risk of adverse effects such as nephrolithiasis^[48].

Heparin: Heparin has been explored as a possible way to augment the excretion of sFlt-1. A recent systematic review and meta-analysis evaluated six randomized, controlled trials and concluded that the use of low molecular-weight heparin (LMWH) resulted in risk reductions in women who had any previous history of placenta-mediated pregnancy complications^[51]. The mechanism of the potential benefit of LMWH is not yet well understood, but the LMWH molecule is thought to mobilize sFlt-1 into circulation from heparan-bound sites in extracellular matrix^[51]. Heparan is a polysaccharide structurally similar to heparin which is known to sequester and regulate the release of VEGF and other cytokines involved in neovascularization^[51,52].

Extracorporeal

Potential therapeutic solutions may lie within restoration of angiogenic balance, for example *via* the antagonism of sFlt-1 and subsequent blockade of its pathologic effects. One strategy involves infusion of sFlt-1's natural ligands VEGF and PIGF at doses high enough to provide systemic saturation. Attempts have been made in animal trials to induce adenoviral synthesis of VEGF as well as by direct infusion of VEGF in mice^[53,54]. Alternative potential therapeutic strategies may include the administration of anti-sFlt-1 antibodies or small molecules that reduce sFlt-1 production (such as small interfering "siRNA")^[55].

Given the potential adverse effects of novel agents

introduced into maternal circulation and unknowns regarding the ability of such molecules to traverse the placenta, early experiments have instead attempted to remove circulating sFlt-1 with an extracorporeal device. A recent open pilot study was conducted to evaluate the safety and potential efficacy of therapeutic apheresis with a plasma-specific dextran sulfate column to remove circulating sFlt-1 in 11 pregnant women with very preterm preeclampsia^[55]. At physiologic pH, sFlt-1 circulates in blood with a strongly positive charge. The dextran columns used are negatively charged, are approved for safe use in pregnancy, and have already been used in therapeutic apheresis for familial hyperlipidemia. Circulating sFlt-1 can be removed selectively, leaving placental sFlt-1 *in vivo*, which may be essential for placental health maintenance. In the treated group, the average sFlt-1 reduction was 18% and the average proteinuria was decreased by an average of 44%^[55]. Pregnancy continued for eight days in women treated once and 15 d in women treated multiple times. There were no observed adverse effects or infant deaths. Both groups demonstrated similar short-term neonatal outcomes; neonates in the treatment group required fewer days on supplemental oxygen. Without a controlled trial, it remains unknown whether or not some of the therapeutic benefit derives from the removal of other unmeasured factors by the dextran columns, such as LDL and fibrinogen. Studies using ligand-specific apheresis columns (e.g., configured with anti-sFlt-1 Ab or VEGF) would be informative in determining the relative contribution of sFlt-1 depletion vs depletion of other potential mediators^[55].

CONCLUSION

Pregnancy is marked by several key physiologic RAAS driven changes that should not result in hypertensive pathology in normal gestation, yet hypertensive disorders in pregnancy abound. Preeclampsia is the most common among these; it is an exceptionally heterogeneous disease that contributes to at least three million pre-term births each year. Placental dysfunction is the fundamental etiology of preeclampsia and mediates the features of the syndrome *via* the systemic release of angiogenic molecules, typically late in pregnancy and signified by the principal clinical findings of hypertension and proteinuria^[56]. Angiogenic imbalance seems to be at the root of this disorder, resulting in maternofetal endothelial pathology and renal end-organ damage with an almost glomerulonephritic or nephrotic phenotype^[1]. In recent years, knowledge regarding the pathophysiology of preeclampsia has increased markedly. Understanding of this disease process has been significantly advanced by the discovery of the factor sFlt-1 and its placental source, antiangiogenic behavior, and role in diminished glomerular endothelial health and likely holds the key to future advances in prognostication, diagnosis, and treatment of a condition associated with a significant amount of cardiovascular and renal morbidity^[56]. To date,

preventative measures and screening tools are relatively lacking, treatments are directed at the management of overt clinical manifestations, and delivery remains the only definitive cure; thus, a strong need persists for the expansion of detection and treatment options for this disease which has seen few therapeutic advances in recent decades.

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Carbon dioxide: Global warning for nephrologists

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Abstract

The large prevalence of respiratory acid-base disorders

overlapping metabolic acidosis in hemodialysis population should prompt nephrologists to deal with the partial pressure of carbon dioxide ($p\text{CO}_2$) complying with the reduced bicarbonate concentration. What the most suitable formula to compute $p\text{CO}_2$ is reviewed. Then, the neglected issue of CO_2 content in the dialysis fluid is under the spotlight. In fact, a considerable amount of CO_2 comes to patients' bloodstream every hemodialysis treatment and "acidosis by dialysate" may occur if lungs do not properly clear away this burden of CO_2 . Moreover, vascular access recirculation may be easily diagnosed by detecting CO_2 in the arterial line of extracorporeal circuit if CO_2 -enriched blood from the filter reenters arterial needle.

Key words: Acid-base assessment; Bicarbonate; Carbon dioxide; Hemodialysis; Metabolic acidosis; Mixed disorders; Ventilatory response; Expected pressure of carbon dioxide; Vascular access recirculation

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Core tip: Partial pressure of carbon dioxide ($p\text{CO}_2$) should be always taken into account for comprehensive assessment of acid-base imbalances of hemodialysis patients, also because respiratory disorders are very common in this population. To infer a respiratory disorder superimposing to metabolic acidosis, nephrologists should compute the expected $p\text{CO}_2$ complying with the reduced bicarbonate concentration. Moreover, they have to take in account CO_2 load from dialysis solution, because this burden may be harmful if ventilatory compensation does not properly occur. Finally, checking an increase of $p\text{CO}_2$ in arterial line of extracorporeal circuit is an easy and reliable method to discover vascular access recirculation.

Marano M, D'Amato A, Cantone A. Carbon dioxide: Global warning for nephrologists. *World J Nephrol* 2016; 5(5): 429-436 Available from: URL: <http://www.wjgnet.com/2220-6124/full/v5/i5/429.htm> DOI: <http://dx.doi.org/10.5527/wjn.v5.i5.429>

INTRODUCTION

There is widespread awareness about carbon dioxide's (CO₂) effects on global warming of the Earth. A similar recognition would be desirable about the key role of CO₂ in nephrology, but this topic is actually under-recognized. This review aims to issue a global warning about CO₂ in the field of renal replacement therapies.

To date, nephrologists have always focused on serum bicarbonate (HCO₃) concentration and the latter, as marker of metabolic acidosis, has been associated with mortality risk in hemodialysis patients. The finding of a low HCO₃ value has been always regarded as a sign of metabolic acidosis, but respiratory alkalosis also is featured by decreased HCO₃ concentration. Hence, diagnosing metabolic acidosis based on the latter parameter clearly neglects serum HCO₃ modifications that are secondary to respiratory disorders. However, as this "respiratory bias" on serum HCO₃ has been recently highlighted, from now on, a comprehensive assessment of acid-base parameters should be taken into account; it is mandatory, therefore, to include partial pressure of CO₂ (pCO₂) as an important parameter to characterize acid-base imbalances and estimate mortality risk in hemodialysis population. In these patients, respiratory acid-base disorders have been recently found in a large percentage and this should further prompt nephrologists to deal with the pCO₂ complying with the reduced HCO₃ concentration. Mixed disorders occur if measured pCO₂ is not consistent with the expected value.

Next point that will be discussed in this review is the forgotten issue of CO₂ load from dialysis solution. Dialysis solution needs to be acidic to avoid salt precipitations; at the same time, it has to increase patient's blood pH. CO₂ allows to meet both goals, if patients' lungs function is not impaired. In fact the considerable amount of CO₂ in the final diluted dialysis fluid keeps the pH low, preventing salt precipitation. Then, this volatile acid easily and quickly reaches patient's bloodstream and it is cleared by lung ventilation as well. As a result of CO₂ clearance and of HCO₃ addition from dialysis solution, patient's blood pH increases. When this clearance does not happen properly, "acidosis by dialysate" may occur. This syndrome is characterized by early hypercapnia followed by typical, *i.e.*, hypoxic, respiratory failure.

Finally, we will point out that the large amount of CO₂ moving from dialysis solution to the extracorporeal circuit may allow to detect vascular access recirculation if blood returning from the filter reenters arterial needle. Basics of "RecirCO₂lation test" based upon detecting CO₂ in the arterial line of extracorporeal circuit will be outlined.

ACID-BASE STATUS OF HEMODIALYSIS PATIENTS

Bicarbonate and beyond

Since a slightly decreased pre-dialysis HCO₃ concen-

tration has been proven to lead to lower risk of death in hemodialysis patients^[1], many efforts have been made to better characterize such risk. Results from Dialysis Outcomes and Practice Patterns (DOPPS) study^[2] depicted such relationship as a U-shape curve (Figure 1A): Either very low and very high serum HCO₃ concentrations were associated with higher risk of death. The authors of this landmark study concluded that moderate predialysis acidosis seems to be associated with lower relative mortality risk than what observed in patients with normal ranges of midweek predialysis serum HCO₃ concentration or severe acidosis^[2].

In fact the acid-base status of hemodialysis patients was inferred by serum HCO₃, alone; neither pH or pCO₂ were taken into account, because they were unavailable. Furthermore, true serum HCO₃ concentration had not even been measured as the authors dealt with total CO₂ content, however the latter amount is only slightly changed by large fluctuations of partial pressure of CO₂ so that this parameter may be properly used as tantamount to serum HCO₃ concentration. Conversely, it should be noted that serum HCO₃ concentration changes are not exclusively due to metabolic disorders and that this assumption may be misleading because completely neglects the effects of respiratory acid-base disorders on HCO₃ value. These disorders have never been taken into account, but likely exist because DOPPS population was characterized by a burden of comorbidity conditions, including heart and lung diseases known to be associated both with respiratory acidosis and alkalosis.

Another large population study^[3] is based on the same assumption. This study confirmed the high risk of death associated with low HCO₃ concentration, however if it was higher than the reference range risk did not increase (Figure 1B). Again acid-base status was inferred by the HCO₃ value alone, but to answer the question whether it is better for an hemodialysis patient to be acidotic or alkalotic - that authors asked - a complete assessment of acid-base parameters is mandatory. Similar findings (Figure 1C) were later reported by Tentori *et al.*^[4] also in DOPPS cohort, again lacking complete acid-base assessment.

More recently, Yamamoto *et al.*^[5] failed to find any relationship between serum HCO₃ concentration and mortality risk in a Japanese hemodialysis population (Figure 1D), but remarkably they found a strong association between pre-dialysis pH and mortality risk. Moreover, and above all, they provided all acid-base parameters and, in turn, allowed us to have for the first time the picture of acid-base disorders in a large hemodialysis population. As largely expected, the mean pH value was close to the lower limit of normal reference range, mean HCO₃ concentration was 20.5 mEq/L and pCO₂ was slightly under its normal value^[5]. At a first glance, it would seem to be nothing else but mild metabolic acidosis with normal ventilatory response, but looking deeper into their data an unexpected presence of respiratory disorders may

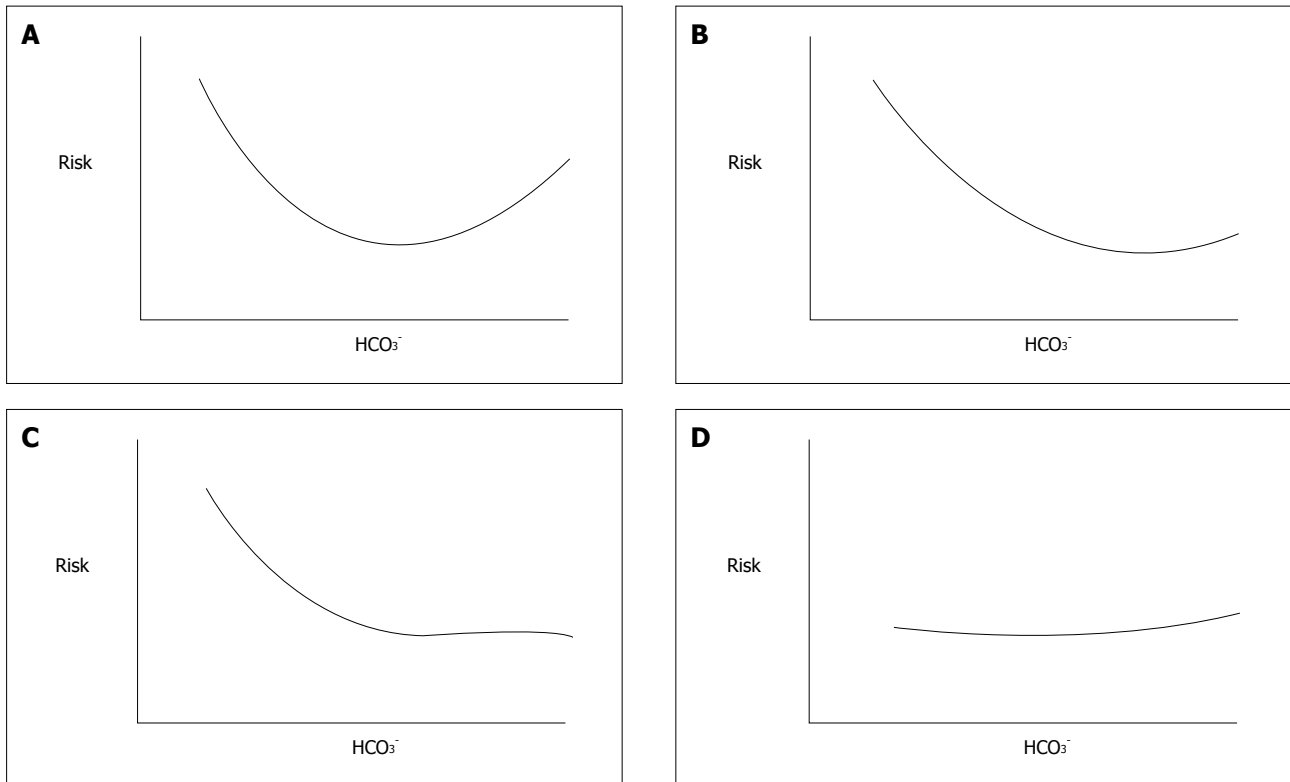


Figure 1 Risk of death and serum bicarbonate concentration in hemodialysis patients. Trend of risk inferred by data from Bommer *et al.*^[2] (A), Wu *et al.*^[3] (B), Tentori *et al.*^[4] (C), Yamamoto *et al.*^[5] (D). HCO₃: Bicarbonate concentration.

be predicted. In fact, patients in the lowest quartile of pH have the lowest mean value of HCO₃ concentration but higher pCO₂ value than patients in the highest pH quartile. This conflicting pattern of pCO₂ with respect to that of HCO₃ can be exclusively due to a superimposing respiratory acidosis in the lowest pH quartile group. Moreover, in the highest pH quartile group (*i.e.*, pH ≥ 7.40) HCO₃ concentration was lower than, not higher than, the normal range and also pCO₂ was decreased as for coexisting respiratory alkalosis. Unfortunately, more detailed data are lacking, hence the existence of respiratory acid-base disorders in hemodialysis patients may be only conjectured. This notwithstanding, it should be acknowledged that Yamamoto *et al.*^[5] moved the spotlight away from serum HCO₃ concentration.

Finally, in a much smaller cohort of patients we have reported the prevalence of all kinds of simple and mixed acid-base disorders^[6]. As expected, metabolic acidosis was the most common acid-base disorder. It was observed that metabolic acidosis as simple disorder was found in 38.7% of measurements and was coupled with respiratory acid-base disturbances in further 23.2%. The latter, as simple or complex disorders, were found in 41% of analyzed blood samples. This finding might be surprising, but the large and growing prevalence in hemodialysis patients of heart^[7] and lung diseases^[8] - known to be possibly associated with respiratory acidosis and alkalosis - accounts for such results. It should be needless to say that to characterize acid-base pattern of hemodialysis patients, as well of all other patients, pCO₂

should always be measured; however, here we want to emphasize that looking at HCO₃ concentration alone is not enough. This is not an academic issue, because a superimposing lung or heart disease can move up or down HCO₃ concentration toward the lower risk zone, but mortality risk of hemodialysis patient likely increases rather than decreases. Even though these results need further confirmation, the era of blood gas measurements in hemodialysis patients begins, and it perhaps occurs with some delay.

In conclusion, CO₂ as respiratory component of acid-base pattern is at least as important as the metabolic component in acid-base assessment also in hemodialysis patients.

Expected pCO₂ in metabolic acidosis

Metabolic acidosis is the commonest acid-base disorder occurring in hemodialysis patients^[9-11]. Often it results in acidemia and consequently in increased ventilation to keep pH close to normal. As a result, pCO₂ decreases, and the magnitude of this reduction is closely dependent on how much serum HCO₃ concentration is decreased. Clearly, concurrent respiratory disorders may affect ventilatory compensation to metabolic acidosis, but this issue never received attention in this population. However mixed acid-base disorders - *i.e.*, respiratory acid-base disturbances superimposing to metabolic disorder - likely occur and, according to recent reports^[6], they are not a rare occurrence. This finding is all but unexpected, as these patients carry a burden of heart and lung comorbid

conditions^[2]. Accordingly, mixed disorders deserve full and prompt recognition, also in hemodialysis patients. To infer and diagnose mixed acid-base disorders clinicians must first evaluate the physiologic respiratory response to metabolic acidosis, namely they must estimate the value of partial pressure of pCO₂ complying with the reduced HCO₃ concentration. Ventilatory response to chronic metabolic acidosis is very predictable indeed; if the measured pCO₂ value is greater or lower than the computed "expected" one, then the presence of a mixed disorder can be inferred. Ventilatory response to chronic metabolic acidosis is independent of the disease causing acid-base derangement^[12], hence rules for the general population and all other patient also apply to hemodialysis population. However, in textbooks^[13-15] and in current literature^[10,16] more than one formula and rule are available, but recommendations on what should be used are lacking. As formulas are different each other, results are often inconsistent; this notwithstanding, selecting the proper formula, *i.e.*, computing the proper value - is mandatory, to avoid wrong diagnosis and inappropriate treatment.

According to the long-lasting and widely used Winters' formula^[17,18] pCO₂ can be predicted as serum HCO₃ × 1.5 + 8. This formula was derived by Albert, Dell and Winters in the 60' in patients with severe acidosis and nowadays is still recommended, even though it lacks at all of any validation in patients with minor reductions of HCO₃ concentration. Intuitively, a slight reduction of HCO₃ is consistent with minor activation of the compensatory mechanisms whereas sizable decrease of serum HCO₃ elicits large increase of ventilation, hence a linear relationship - as Winters' formula is - might be not reliable throughout the acidosis spectrum.

Taking into account that serum HCO₃ in modern hemodialysis patients ranges around 20 mmol/L^[2,3,6,11] which is exactly twice the mean value in Albert's population^[17] - applying Winters' formula in this scenario is at least questionable. Even though it is recommended across-the-board to apply Winters' formula to hemodialysis population, that was associated with a larger error in prediction than other formulas.

A reliable alternative may be the common practical rule that reads "the reduction of pCO₂ with respect to its normal value equals 1.2 multiplied by the reduction of bicarbonate with respect to its normal value"^[11,12,15,16]. This rule reliably predicts pCO₂ in mild-to-moderate acidosis; as a matter of fact, it has always been adopted in hemodialysis population^[11,10]. If 40 mmHg and 24 mmol/L are the normal values of pCO₂ and of HCO₃, respectively, the rule can be read as pCO₂ = 40 - (24 - HCO₃) × 1.2 and equivalently rewritten as pCO₂ = 1.2 × HCO₃ + 11.2. Besides, it requires quite a few computations - and therefore the label practical is not very fitting - also this rule is a linear relationship between pCO₂ and HCO₃, hence it cannot be conveniently applied to all degrees of severity of metabolic acidosis.

In this case the slope of linear equation is reduced to 1.2. The use of different multipliers for acidosis of

different degree fulfills the concept that activation of compensatory mechanisms is gradual and progressive, hence non-linear. In other words ventilatory compensation to chronic metabolic acidosis varies with severity of acidosis and a quadratic or cubic equation, *i.e.*, a curve, better depicts the whole relationship between pCO₂ and HCO₃^[12].

Unfortunately, this is an unfeasible option for physicians. However, as Bushinsky *et al.*^[12] highlighted, by restricting the analysis to HCO₃ values below 10 mmol/L ventilatory response can be predicted with good approximation by the linear equation with a slope equal to 1.5 - just the multiplier of Winters' formula - whereas if HCO₃ values range between 10.1 and 24 mmol/L the linear equation with a slope close to 1.2 - the multiplier of practical rule - allows to properly calculate the expected pCO₂ value. Accordingly, as we already suggested elsewhere^[19,20], a reliable method to correctly predict pCO₂ may be the use of two different linear formulas depending on severity of metabolic acidosis (Figure 2).

Beyond the well-known and widely used above-mentioned formulas, several textbooks provide some tips to easily calculate the expected pCO₂. One of these rules - quite surprisingly - allows a very easy and valid prediction of pCO₂ value in hemodialysis population^[19]. It simply suggests to add "15" to HCO₃ concentration to obtain the expected pCO₂ value, the so called "Bicarbonate plus 15" rule. With this very simple formula only 1 mmHg difference arises compared to practical rule when HCO₃ ranges between 14 and 24 mmol/L, as commonly occurs in almost all hemodialysis patients. In this population the very simple formula was associated with same (low) mean error exhibited by the practical rule (Table 1)^[19] and therefore in this scenario it could be suggested as a valid and reliable alternative formula as it has the undeniable advantage of making CO₂ prediction easier and also attractive to physicians reluctant to approach the acid-base troubles.

CARBON DIOXIDE LOAD FROM DIALYSIS SOLUTION

Dialysis-related acidemia

The acid-base pattern of dialysate and of blood coming back from dialyzer to patient during bicarbonate hemodialysis has been recently recalled and has been labeled "dialysis-related acidemia"^[21].

It has been above mentioned the compensatory response to metabolic acidosis that ultimately leads to hypocapnia - a common feature of hemodialysis patient - here we want to recall that pCO₂ in the final diluted dialysate is two-to-three folds the quantity found in the uremic blood entering the extracorporeal circuit. This large dialysate-blood difference accounts for very high CO₂ dialysance and in turn for the sizeable transfer of CO₂ from dialysate into the blood coming back to patient^[22]. Even though high HCO₃ concentration, blood reaching patient's bloodstream is featured by low pH

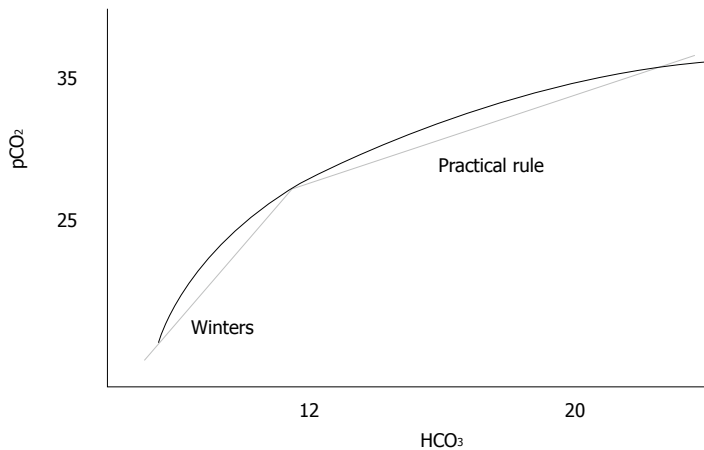


Figure 2 Relationship between pressure of carbon dioxide and bicarbonate concentration in chronic metabolic acidosis. The relationship between $p\text{CO}_2$ and HCO_3^- during metabolic acidosis is graphically depicted as a curve. Two linear approximations (straight lines) equivalent to Winters' formula ($p\text{CO}_2 = 1.5 \times \text{HCO}_3^- + 8$) and to the practical rule ($p\text{CO}_2 = 1.2 \times \text{HCO}_3^- + 11.2$) are also shown. HCO_3^- : Bicarbonate concentration; $p\text{CO}_2$: Pressure of carbon dioxide.

Table 1 Errors in prediction of the expected pressure of carbon dioxide in hemodialysis patients

	Blood samples featuring $\text{HCO}_3^- < 24$ mmol/L	Blood samples claimed for metabolic acidosis
"Winters' formula" $p\text{CO}_2 = 1.5 \text{HCO}_3^- + 8$	4.85	2.06
"Practical rule" $p\text{CO}_2 = 1.2 \text{HCO}_3^- + 11.2$	3.14	1.50
"Very simple formula" $p\text{CO}_2 = \text{HCO}_3^- + 15$	3.09	1.56

Errors (root mean square errors in mmHg) in computing the expected $p\text{CO}_2$ in a dataset of blood gas measurements from chronic hemodialysis patients. Reproduced with permission from Ref. [19]. HCO_3^- : Bicarbonate concentration; $p\text{CO}_2$: Pressure of carbon dioxide.

due to very high $p\text{CO}_2$ ^[22,23]. This pattern looks like respiratory acidosis but it has nothing to do with the lung. Moreover in hypercapnic acidosis partial pressure of oxygen ($p\text{O}_2$) is always decreased, whereas in dialysis-related acidemia does not, because a gain of oxygen across the filtering membrane also occurs. Dialysis-related acidemia vanishes as soon as CO_2 is breathed away by lung (hyper)ventilation, thus HCO_3^- coming from dialyzer counteracts uremic acidosis. The source of CO_2 is dialysate itself, indeed mixing acid concentrate with HCO_3^- -containing solution the acid - commonly acetic acid - reacts with buffer leading to acetate anion and CO_2 . The more the acid in acid concentrate, the more the CO_2 in the final diluted dialysate. As a typical example 3 mmol/L of acetic acid (or a mixture of citric and acetic acid) are in the concentrate and as a result 3 mmol/L of CO_2 are in dialysate. This leads to $p\text{CO}_2$ ranging between 80 and 100 mmHg and in turn to dialysate pH lower than 7.30. This allows calcium and magnesium bicarbonate salts to remain in their soluble form. The presence of CO_2 is actually mandatory and in the same way "an adequate ventilatory capacity is imperative to excrete the excess CO_2 generated during high efficiency bicarbonate hemodialysis"^[23].

Acidosis by dialysate

If patients are unable to increase their ventilatory rate and in turn to breath away CO_2 overload from dialysate, then systemic $p\text{CO}_2$ increases leading to reduction of peripheral vascular resistance^[24,25], harmful hypotension and severe dyspnea poorly relieved by oxygen administration for the time being. Dialysis treatment should be slowed

down or even stopped to avoid more severe effects. As hypercapnia superimposes to metabolic acidosis, a mixed (metabolic plus respiratory) acidosis occurs with abrupt fall of blood pH. Hypoxia is only a later event. A few of such cases are reported in the literature^[26-28], likely due to poor awareness of the syndrome, recently labeled "acidosis by dialysate"^[21].

The burden of CO_2 in renal replacement therapies

The issue of CO_2 load during renal replacement therapy has been for long time neglected and has not in depth investigated. However theoretical considerations and some findings from literature allow to briefly comment on.

Acetate-free hemodiafiltration is an alternative dialysis technique claimed for allowing better hemodynamic stability and paucity of dialysis-related symptoms. It is featured by lack of any buffer in dialysate, indeed any acid is needed. Accordingly, the final diluted dialysate is " CO_2 -free" other than "acetate-free" and this represents an important difference between acetate-free biofiltration and all other dialysis techniques. Even though some amount of CO_2 comes back to patients from sodium bicarbonate infusion, acetate-free biofiltration should be claimed for providing a lighter CO_2 load compared to conventional bicarbonate hemodialysis^[29]. Outstandingly, $p\text{CO}_2$ in blood from dialyzer is very close to physiological amount, meaning that AFB might be suggested as the more advisable technique for patients unable to handle CO_2 overload as those with chronic obstructive lung disease, an increasingly prevalent comorbid condition^[8].

On the other side online hemodiafiltration - regarded

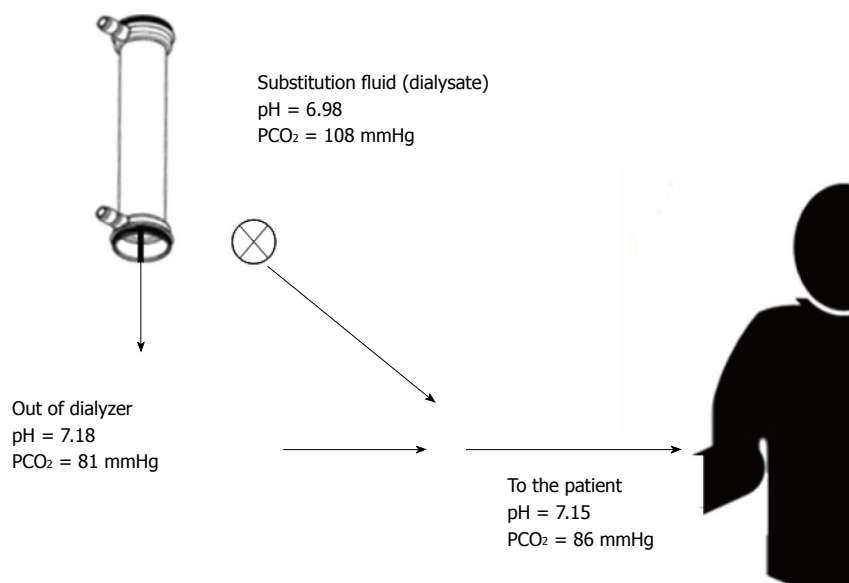


Figure 3 Example of gas analysis in on-line hemodiafiltration. Additional CO₂ load delivered via substitution fluid infusion during online hemodiafiltration. Reproduced with permission from Marano *et al*^[30]. CO₂: Carbon dioxide.

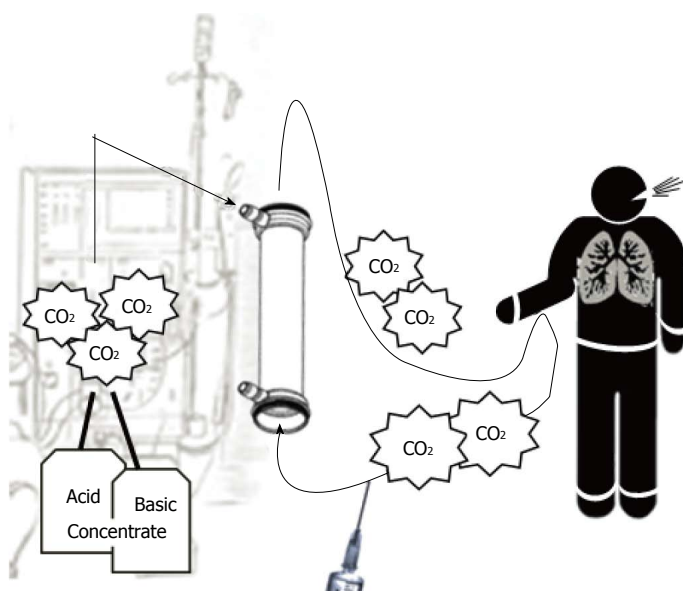


Figure 4 Course of carbon dioxide in presence of vascular access recirculation. pCO₂ from dialyzer re-entering the extracorporeal circuit reveals vascular access recirculation. pCO₂: Pressure of carbon dioxide.

as the new gold standard of renal replacement therapy - implies an heavier CO₂ load than bicarbonate hemodialysis does^[30]. An additional CO₂ load is delivered by infusing dialysate, with its burden of CO₂, directly in patient's bloodstream (Figure 3). As the largest infusion volume possible has been recommended^[31], the issue of CO₂ overload during online hemodiafiltration should be taken in account. Whether different CO₂ loads should be taken in account to withhold or in the opposite to recommend a certain replacement therapy to a certain hemodialysis patient is a question never asked.

RecirCO₂lation test

If CO₂-enriched blood coming from the dialyzer reenters extracorporeal circuit, then vascular access recirculation may be detected by means of gas analysis of blood withdrawn from arterial line^[32] (Figure 4). The typical acid-base picture of blood out the dialyzer - "dialysis-related acidemia" - is actually found in arterial line. As

hypercapnic acidosis is coupled with normal or high pO₂, this acid-base pattern is unique and it is not suggestive of any human illness. Accordingly, vascular access recirculation may be easy and profitably discovered by means of easy blood sampling from arterial line of dialysis circuit. A pCO₂-increase > 4.5 mmHg (with respect to pre-dialysis value: "two samples technique") discovers vascular access recirculation with absolute specificity (100%) and high sensitivity (86.7%). A reliable alternative chance ("one sample technique") consists of a single blood sampling (5 min from dialysis start) to check whether pCO₂ is over or below a certain threshold. For both approaches, receiver operating characteristic analysis showed remarkable areas under curves (Figure 5). As a special feature of this novel test - labeled "RecirCO₂lation test" - the use of CO₂ as indicator offers the undeniable chance of overcoming the issue of cardiopulmonary recirculation, because the excess of CO₂ coming from the dialyzer is time by time cleared away by

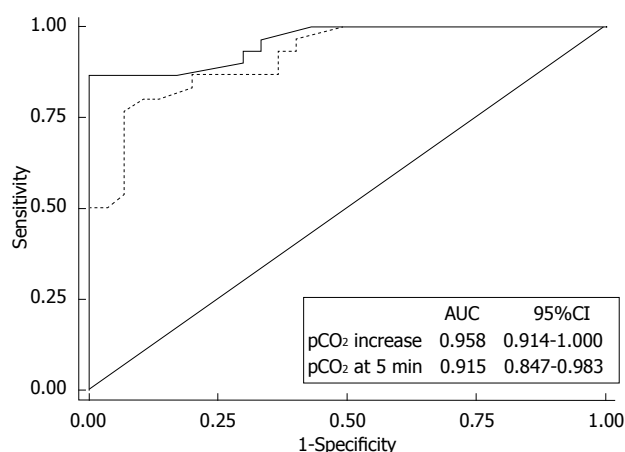


Figure 5 Performance of “RecirCO₂lation” test to detect vascular access recirculation. Receiver operating curves of pCO₂-increase (solid line) and pCO₂ at 5 min (dotted line) for diagnosis of vascular access recirculation using Glucose Infusion Test (> 0.3%) as reference. Reproduced with permission from Marano *et al.*^[32]. AUC: Area under curve; pCO₂: Pressure of carbon dioxide.

lungs and therefore if recirculation does not occur, it can never reaches arterial line.

CONCLUSION

CO₂ as respiratory component of acid-base pattern is at least as important as the metabolic component in acid-base assessment also in hemodialysis patients. To infer and diagnose mixed acid-base disorders, physiologic respiratory response to metabolic acidosis should be considered and the expected pCO₂ value should be computed. To do it, a very simple formula - “bicarbonate plus 15” - is a reliable alternative to the common practical rule, not so practical.

The acid-base pattern of blood coming back from dialyzer to patient during bicarbonate hemodialysis is featured by low pH due to very high pCO₂. Increasing ventilation rate is mandatory to excrete CO₂ overload, otherwise harmful “acidosis by dialysate” may occur. Among renal replacement therapies, acetate-free bio-filtration is featured by a more physiological load of CO₂, whereas online hemodiafiltration implies an additional CO₂ load.

Finally, vascular access recirculation may be detected by means of gas analysis performed on blood withdrawn from arterial line of extracorporeal circuit. This novel method has been labeled “RecirCO₂lation test”.

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Retrospective Cohort Study

Parathyroid ultrasonography and bone metabolic profile of patients on dialysis with hyperparathyroidism

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Abstract

AIM

To evaluate the parathyroid ultrasonography and define parameters that can predict poor response to treatment in patients with secondary hyperparathyroidism due to renal failure.

METHODS

This cohort study evaluated 85 patients with chronic kidney disease stage V with parathyroid hormone levels above 800 pg/mL. All patients underwent ultrasonography of the parathyroids and the following parameters were analyzed: Demographic characteristics (etiology of chronic kidney disease, gender, age, dialysis vintage, vascular access, use of vitamin D), laboratory (calcium, phosphorus, parathyroid hormone, alkaline phosphatase, bone alkaline phosphatase), and the occurrence of bone changes, cardiovascular events and death. The χ^2 test were used to compare proportions or the Fisher exact test for small sample frequencies. Student *t*-test was used to detect differences between the two groups regarding continuous variables.

RESULTS

Fifty-three patients (66.4%) had parathyroid nodules with higher levels of parathyroid hormone, calcium and phosphorus. Sixteen patients underwent parathyroidectomy and had higher levels of phosphorus and calcium \times phosphorus product ($P = 0.03$ and $P = 0.006$, respectively). They also had lower mortality (32% *vs* 68%, $P = 0.01$) and lower incidence of cardiovascular or cerebrovascular events (27% *vs* 73%, $P = 0.02$). Calcium \times phosphorus product above 55 mg²/dL² [RR 1.48 (1.06, 2.08), $P = 0.03$], presence of vascular calcification [1.33 (1.01, 1.76), $P = 0.015$] and previous occurrence of vascular events [RR 2.25 (1.27, 3.98), $P < 0.001$] were risk factors for mortality in this population. There was no association between the occurrence of nodules and mortality.

CONCLUSION

The identification of nodules at ultrasonography strengthens the indication for parathyroidectomy in patients with secondary hyperparathyroidism due to renal failure.

Key words: Secondary hyperparathyroidism; Parathyroid ultrasonography; Calcium; Phosphorus; Parathyroid hormone; Alkaline phosphatase; Chronic kidney disease; Bone alkaline phosphatase

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Core tip: We aimed to evaluate the parathyroid ultrasonography and defined parameters to predict poor response to treatment in 85 patients with chronic kidney

disease stage V and parathyroid hormone (PTH) levels > 800 pg/mL. Fifty-three patients had nodules, higher PTH, calcium and phosphorus. Sixteen underwent parathyroidectomy and had significant higher levels of phosphorus and calcium phosphorus product; lower mortality and lower incidence of cardiovascular or cerebrovascular events. Calcium phosphorus product above 55, vascular calcification and previous vascular events were risk factors for mortality. There was no association between nodules and mortality. We concluded that nodules at ultrasonography strengthens the indication for parathyroidectomy in those patients.

Ribeiro C, Penido MGMG, Guimarães MMM, Tavares MS, Souza BN, Leite AF, de Deus LMC, Machado LJC. Parathyroid ultrasonography and bone metabolic profile of patients on dialysis with hyperparathyroidism. *World J Nephrol* 2016; 5(5): 437-447 Available from: URL: <http://www.wjgnet.com/2220-6124/full/v5/i5/437.htm> DOI: <http://dx.doi.org/10.5527/wjn.v5.i5.437>

INTRODUCTION

Chronic kidney disease-mineral and bone disorder (CKD-MBD) is a growing health care concern associated with secondary hyperparathyroidism (SHPT), mineral abnormalities and increased risk of cardiovascular disease^[1]. Therefore, prevention and control of SHPT is one of the main objectives in the management of chronic kidney disease patients, particularly those on dialysis.

SHPT develops from the early stages of CKD due to disturbances in calcium (Ca), phosphorus (P) and vitamin D metabolism, characterized usually as high turnover bone disease^[2]. Besides stimulating the synthesis and secretion of parathyroid hormone (PTH), hyperplasia of parathyroid cells, initially diffuse and then nodular^[3], can also be observed. Patients with nodular parathyroid hyperplasia exhibit reduced number of Ca-sensing receptor (CaSR) and vitamin D receptor (VDR), and they are often resistant to vitamin D therapy^[3,4]. In early stages of CKD, even without persistent hyperphosphatemia, elevated net body P load is associated with increased production of fibroblast-growth-factor 23 (FGF23) by bone osteoblasts and osteocytes. Serum FGF23 correlates to PTH in predialysis CKD patients and in patients with early CKD when levels of P and Ca are maintained within normal range^[5-7]. However, in advanced stages of CKD, there is an inability of FGF23 to suppress PTH secretion.

A total transient elevation in P levels may be associated with reduction of serum Ca and thereby stimulate the secretion of PTH (tradeoff theory). Theories suggest that the hyperfiltration of the remaining nephrons could raise the P concentration in the proximal tubule cells, inhibiting the 1- α -hydroxylase. Experimental studies have shown a direct effect of P in increased PTH secretion^[5] and have also demonstrated an independent association between phosphatemia and PTH in CKD patients^[7,8].

Reduction of CaSR and VDR expression in parathyroid

cells occurs with the progression of CKD^[9,10]. This process is more prominent in patients with nodular parathyroid hyperplasia than those with diffuse hyperplasia^[9,11-13]. However, it is unclear why diffuse parathyroid hyperplasia develops to nodular hyperplasia. In this kind of hyperplasia (nodular hyperplasia), the parathyroid gland contains nodules of rapidly proliferating parathyroid cells. Each nodule, formed by monoclonal proliferation of parathyroid cells, produces excessive amounts of PTH. Because of the low density of CaSR and VDR, these cells are refractory to medical treatment (activated vitamin D, calcimimetics, control of hyperphosphatemia). During the progression of parathyroid hyperplasia, the ability of PTH synthesis increases progressively while a quantitative reduction of VDR and CaSR occurs, resulting in autonomous function of this gland, which characterizes the tertiary hyperparathyroidism^[9,13,14].

The Kidney Disease Outcomes Quality Initiative proposes strict targets for the control of PTH, Ca and P in CKD patients^[15] considering studies showing that patients with serum PTH levels above 800 pg/mL have severe and refractory hyperparathyroidism^[14]. Considering that parathyroid hyperplasia is resistant to the action of vitamin D, it is important to identify among CKD patients those with high PTH levels and low response to this treatment, since they may be candidates for parathyroidectomy (PTX) in case of hypercalcemia (Ca > 10 mg/dL) and/or hyperphosphatemia (P > 5.5 mg/dL) in association with clinical symptoms, such as bone pain, fractures or spontan tendon rupture, untreatable chronic pruritus, erythropoiesis stimulating agents (ESA) resistant anemia without other causes and calciphylaxis^[16,17].

Ultrasonography (US) is an economically feasible and noninvasive imaging method able to identify nodular hyperplasia^[18,19] and serves as a marker of prognosis and response to treatment of SHPT with vitamin D analogues^[20,21]. Increased parathyroids size, despite several aspects of the US and possible ectopic locations, are generally characterized by a distinct echogenic capsule, independent of the thyroid capsule, surrounding a hypoechoic area due to progressive hypercellularity and the disappearance of adipose tissue^[22,23]. Gland weighing more than 0.5 g (equivalent to 0.5 cm³) or larger than 1.0 cm or greater in diameter correspond to nodular hyperplasia in more than 90% of cases^[24].

Severe SHPT is associated with higher mortality in patients with moderate and advanced CKD^[25] as well as with disorders of bone metabolism (especially hyperphosphatemia and increased Ca × P product), and worse prognosis in CKD. Elevated PTH is responsible for serious long-term consequences, such as renal osteodystrophy, vascular and valvular calcification, changes in myocardial structure and function, immune dysfunction and anemia unresponsive to ESA^[26,27], bone pain, abnormal bone histology and fractures among patients with SHPT^[28,29].

The present study aimed to evaluate the usefulness of US of parathyroids in CKD patients on hemodialysis as a predictor of clinical outcome in severe hyperparathyroidism

and correlate these sonographic findings and data related to CKD-MBD with clinical, epidemiological, laboratory and mortality data and response to treatment. As secondary endpoint, the study aimed to investigate the association between PTX and mortality of patients with CKD and BMD.

MATERIALS AND METHODS

Patients

It was a cohort study of patients on hemodialysis regularly followed at the Nephrology Center of Santa Casa de Belo Horizonte, Brazil, from January 2005 to January 2009. Inclusion criteria were patients with CKD on hemodialysis, age equal to or older than 18 years and at least one measuring serum intact PTH above 800 pg/mL. Patients with severe neuropsychiatric disorders, anatomical and/or functional changes that would interfere with the US examination and patients acutely unstable, such as with uncontrolled infection, hemodynamic and/or metabolic instability, as well as those who refused to participate in the study were excluded.

During the clinical follow-up, patients were submitted to monthly clinical and laboratory examinations according to Resolution - No. 154 of June 15, 2004, the Brazilian National Health Surveillance Agency (ANVISA).

This study was submitted to and approved by the Research Ethics Committee of the Graduation Center of Santa Casa de Belo Horizonte, Minas Gerais, Brazil (011/2005), and was performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

CKD patients received calcitriol according to resolution SES # 267 of the State Health Authority of the State of Minas Gerais, Brazil (Table 1).

In case of no response (reduction of 30%-50% of the initial PTH), patients received vitamin D analogues according to Table 2.

Vitamin D administration was withdrawn under the following conditions: Hypercalcemia (plasma Ca above 10.0 mg/dL), hyperphosphatemia (plasma P above 5.5 mg/dL), Ca × P product above 55 mg/dL or PTH less than 200 pg/mL.

The parathyroid US was performed by an examiner and a medical resident in training with a Siemens Versa Plus Sleep Line and 7.5 Mhz transducer. Both unaware of any patient data. The gland volume was considered increased when presented a dimension of 1.0 cm in any axis, or a volume higher than 0.5 cm³^[3,20,24].

Laboratory data were collected at Time 1, from January 2005 to January 2006, and at Time 2, from October 2008 to January 2009. The following parameters were evaluated: Total Ca, P, alkaline phosphatase (AP), bone specific AP (BAP) at Time 1 only, PTH and Ca × P product. Blood samples were collected immediately before the first dialysis session of the week. PTH was measured by chemoluminescence and bone AP by the method of thermal inactivation.

The clinical and demographic data were: Age, sex,

Table 1 Initial dose of vitamin D analogue (calcitriol) to treat secondary hyperparathyroidism due to chronic kidney disease

PTH level	Vitamin D analogue dose (calcitriol)
PTH 200-499 pg/mL	0.25 mcg qd once a day
PTH 500-999 pg/mL	0.50 mcg qd or 1 mcg qd once a day/3 times a week
PTH > 1000 pg/mL	2.0 mcg qd once a day/3 times a week

Source: Minas Gerais Health Authority (Secretaria do Estado da Saúde de Minas Gerais). Available from: URL: http://www.saude.mg.gov.br/atos_normativos/resolucoes/2003. PTH: Parathyroid hormone.

Table 2 Maintenance dose of vitamin D analogue (calcitriol) to treat secondary hyperparathyroidism due to chronic kidney disease in initially unresponsive patients

PTH level	Vitamin D analogue dose (calcitriol)
PTH 200-299 pg/mL	0.25 mcg qd once a day
PTH 300-399 pg/mL	0.50 mcg qd or 0.75 mcg qd once a day, 3 times a week
PTH 400-999 pg/mL	1.0 mcg qd once a day 3 times a week
PTH > 1000 pg/mL	2.0 mcg oral or IV 3 times a week

Source: Minas Gerais Health Authority (Secretaria do Estado da Saúde de Minas Gerais). Available from: URL: http://www.saude.mg.gov.br/atos_normativos/resolucoes/2003. PTH: Parathyroid hormone; IV: Intravenous.

time on hemodialysis, CKD etiology, type of access for hemodialysis and the presence of bone and/or vascular changes detected by imaging method. Figure 1 summarizes the patients selection and follow-up.

Statistical analysis

The two groups (with and without nodules) were subjected to a descriptive analysis, and nominal/categorical variables as well as frequency distribution were represented in tables. Continuous variables and measures of central tendency and variability were used.

Univariate analysis of categorical variables were performed with the χ^2 test to compare proportions or the Fisher exact test when small sample frequencies were used. Student *t*-test was used to detect differences between the two groups regarding continuous variables. In all analyzes a significance level of 5% level was considered and SPSS v. 12.0 software was used for the analysis.

RESULTS

Patient characteristics

From January 2005 to January 2006 (Time 1), the 85 patients with PTH levels above 800 pg/mL underwent US parathyroid glands, 53 (62.4%) had at least one nodule and 32 (37.6%) showed no abnormality.

Mean age was 44.5 ± 13 years (range 18-76 years), 52% were female. Mean time on hemodialysis in patients without nodules (104.4 ± 54 mo) was similar to the

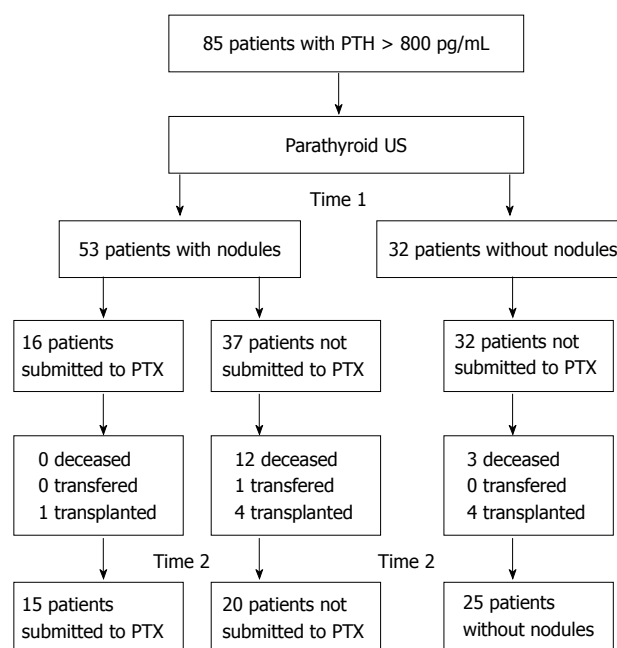


Figure 1 Fluxogram of follow-up of 85 patients with chronic kidney disease stage V submitted to ultrasonography of parathyroids between January 2005 to January 2006 (Time 1) and then until January 2009 (Time 2). PTH: Parathyroid hormone; PTX: Parathyroidectomy; US: Ultrasonography.

group with parathyroid nodules (106 ± 65 mo; $P = 0.91$). There was no difference between groups regarding the type and dialysis access. Most patients were on hemodialysis with arteriovenous fistula as vascular access (89.4%, $P = 0.47$) (Table 3). Glomerular and vascular diseases were the most common causes of CKD in both groups.

Regarding the use of vitamin D-analogues, 6 patients (7%) did not follow the treatment, and four failed to achieve adequate levels of P and two did not achieve adequate levels of both Ca and P, which did not allow the treatment of SHPT. There was no difference between the groups regarding the use of calcitriol ($P = 0.402$). Patients with parathyroid nodules had higher PTH, Ca, P and Ca \times P product. There was no significant difference between the levels of AP and BAP (Table 4).

There was no difference between groups when outcomes were analyzed, considering the 16 patients who underwent PTX in this period (Table 5).

At Time 2 (October 2008 to January 2009), 60 patients remained in the study; 9 were transplanted, 15 deceased and one was transferred to another facility. In this period there was no change regarding PTH, Ca, P, Ca \times P and AP between the groups with and without nodules (Table 6).

In the comparison between Times 1 and 2, there was no difference in any variable in the group without nodule. In the group with nodules, there was a reduction in the levels of P (5.8 vs 5.1, $P = 0.008$ by paired *t* test) and Ca \times P product (51.6 vs 44.6, $P = 0.007$ by paired *t* test).

At Time 1, there was a significant difference in AP and BAP variables, with higher values in the group

Table 3 Clinical and demographical characteristics of the studied population

	Without nodule (<i>n</i> = 32)	With nodule (<i>n</i> = 53)	Total	<i>P</i>	RR (95%CI)
Sex					
Male	16	25	41	0.80 ¹	
Female	16	28	44		1.12 (0.46-2.69)
Total	32	53	85		
Age	43 ± 14	46 ± 13		0.18 ²	1.02 (0.99-1.06)
Time on dialysis	104.4 ± 53.6	106.0 ± 64.7		0.91 ²	1.00 (0.99-1.01)
Access for RRT					
Others	2	7	9	0.47 ¹	2.3 (0.4-11.7)
AVF	30	46	76		1.00

¹Pearson χ^2 test; ²*t*-Student test. AVF: Arteriovenous fistula; RRT: Renal replacement therapy.

Table 4 Comparison of laboratory results of patients without and with nodule

Surgery	PTH	Ca	P	Ca × P	AP	BAP
No nodule (<i>n</i> = 32)	796.2 ± 229.7	8.6 ± 0.7	4.8 ± 1.2	41.5 ± 12.4	246.7 ± 128.7	100.8 ± 69.6
With nodule (<i>n</i> = 53)	1106.4 ± 462.5	9.0 ± 0.6	5.9 ± 1.0	53.1 ± 10.3	283 ± 254.4	106.4 ± 91.9
<i>P</i>	< 0.001	0.006	< 0.001	< 0.001	0.387	0.783

Student's *t*-test. PTH: Parathyroid hormone; BAP: Bone specific alkaline phosphatase.

Table 5 Observed outcomes until end of follow-up in January 2009 (*n* = 85)

	Nodule in US		<i>P</i>	RR (95%CI)
	No (<i>n</i> = 32)	Yes (<i>n</i> = 53)		
Deceased				
No	29 (90.6%)	41 (77.4%)	0.120 ²	1.00
Yes	3 (9.4%)	12 (22.6%)		2.83 (0.73; 10.93)
Transplant				
No	28 (87.5%)	48 (90.6%)	0.723 ²	1.37 (0.54; 5.53)
Yes	4 (12.5%)	5 (9.4%)		1.00
Cardiovascular and/or cerebrovascular event ⁴				
No	25 (78.1%)	43 (81.1%)	0.737 ¹	1.20 (0.41; 3.56)
Yes	7 (21.9%)	10 (18.9%)		1.00
Vascular calcification ⁵				
No	23 (71.9%)	33 (62.3%)	0.633 ¹	1.00
Yes	7 (21.9%)	13 (24.5%)		1.29 (0.40; 4.28)
No register	2 (6.3%)	7 (13.2%)		
Bone disease ⁶				
No	18 (56.3%)	34 (64.2%)	0.202 ¹	1.89 (0.63; 5.56)
Yes	12 (37.5%)	12 (22.6%)		1.00
No register	2 (6.3%)	7 (13.2%)		
Surgery				
No	32 (100%)	37 (69.8%)	< 0.001	³
Yes	0 (0%)	16 (30.2%)		

¹Pearson's χ^2 ; ²Fisher's exact test; ³It was not possible to calculate OR due to null cases; ⁴Patients' records: Acute myocardial infarct, acute coronary syndrome, unstable angina, acute arterial occlusion, congestive heart failure, abnormal findings in heart catheterism, coronary angioplasty and myocardial and coronary angioplasty and myocardial revascularization; ⁵Vascular calcification in imaging (ultrasonography, computed tomography or plain radiographies); ⁶Imaging or notes on them, with evidence of bone disease attributed to SHPT, and bone deformities or fractures attributed to the disease. US: Ultrasonography; SPTH: Secondary hyperparathyroidism.

submitted to PTX (Table 7). At Time 2, they got higher and lower values of P and Ca × P product, as well of AP, compared to non-operated group (Table 8).

Comparing Time 1 with Time 2, in patients with nodular and non-operated, there were elevated levels of Ca (8.7 vs 8.9, *P* = 0.033 by Mann-Whitney test).

Those submitted to PTX, a reduction of Ca, P and Ca × P product was observed (Table 9).

In relation to outcomes, a higher incidence of death and vascular events in patients with nodules not submitted to PTX was observed. Patients who died by the end of the study had higher mean values of Ca, P

Table 6 Comparison of parathyroid hormone, calcium, phosphorus, calcium × phosphorus, alkaline phosphatase between October 2008 and January 2009 for each group (*n* = 60)

Nodules		PTH	Ca	P	Ca × P	AP
Without nodules (<i>n</i> = 25)	Mean	833.2	8.70	4.80	41.4	249.6
	SD	602.2	0.7	1.40	12.50	255.6
With nodules (<i>n</i> = 35)	Mean	1031.2	8.70	5.10	44.60	396.2
	SD	775.1	0.9	1.50	13.60	535.8
	<i>P</i> ¹	0.271	0.974	0.341	0.350	0.210

¹Student's *t*-test. PTH: Parathyroid hormone; Ca: Calcium; P: Phosphorus; AP: Alkaline phosphatase.**Table 7 Comparison between patients submitted or not to parathyroidectomy**

Surgery	PTH	Ca	P	Ca × P	AP	BAP
No (<i>n</i> = 37)	1030.8 ± 391.8	9.0 ± 0.6	6.0 ± 1.0	53.7 ± 10.9	212.6 ± 121.5	82.7 ± 55.3
Yes (<i>n</i> = 16)	1281.1 ± 571.4	9.1 ± 0.6	5.7 ± 1.0	51.6 ± 9.0	445.9 ± 385.1	160.5 ± 132
<i>P</i> ¹	0.125	0.605	0.354	0.497	0.030	0.050

¹Student's *t*-test. PTH: Parathyroid hormone; Ca: Calcium; P: Phosphorus; AP: Alkaline phosphatase; BAP: Bone specific alkaline phosphatase.**Table 8 Comparison of results of parathyroid hormone, calcium, phosphorus, calcium × phosphorus product, alkaline phosphatase levels from October 2008 to January 2009, patients with nodules, with and without parathyroidectomy (*n* = 35)**

Surgery		PTH	Ca	P	Ca × P	AP
No (<i>n</i> = 20)	Mean	992.4	8.90	5.60	49.90	214.9
	SD	639.5	0.80	1.20	11.70	251.7
Yes (<i>n</i> = 15)	Mean	1082.9	8.40	4.50	37.60	638
	SD	948.2	1.10	1.60	13.10	708.3
	<i>P</i> ¹	0.752	0.126	0.030	0.006	0.041

¹Student's *t*-test. PTH: Parathyroid hormone; Ca: Calcium; P: Phosphorus; AP: Alkaline phosphatase.**Table 9 Comparison of laboratory results of patients with nodules submitted to parathyroidectomy (*n* = 15) in 2 different periods: 2005/2006 and 2008/2009**

	2005/2006			2008/2009			<i>P</i>
	Mean	Median	SD	Mean	Median	SD	
PTH	1281.1	1155.7	571.4	1082.9	811.0	948.2	0.173 ¹
Ca	9.1	9.1	0.7	8.4	8.7	1.1	0.017 ¹
P	5.7	5.8	1.0	4.5	4.3	1.6	0.009 ¹
Ca × P	51.8	53.0	9.3	37.6	37.6	13.1	0.004 ¹

¹Mann-Whitney's test. PTH: Parathyroid hormone; Ca: Calcium; P: Phosphorus.

and Ca × P product at Time 1. Analysis of categorical variables, Ca × P product above 55 mg²/dL² was associated with a 48% higher risk of death. Patients who experienced cardiovascular events and vascular calcification in imaging also had a higher risk of death. Those patients who died and had visible nodules in US, had higher mean values of Ca and Ca × P product at Time 1, but no demographic differences was observed in relation to those who did not die. In this group, patients who experienced cardiovascular and/or cerebrovascular events had almost 200% higher chance of death.

There was no difference in demographic and clinical

characteristics between patients with and without nodule on US. At Time 1, patients with nodules had higher levels of Ca, P, Ca × P product and PTH. There was no difference in the occurrence of death, vascular events or bone disease. Patients with nodules submitted to surgery had longer time on dialysis. They also had significantly reduced levels of Ca, P and Ca × P product, without variation of PTH levels. Among patients with nodule, mortality and the occurrence of cardiovascular events was lower in those who underwent surgery. Those who died had higher levels of Ca, P and Ca × P product and highest occurrence of events and vascular

calcification.

DISCUSSION

The present study shows that the identification of parathyroid nodules on US may be an indication criteria for PTX in patients with severe hyperparathyroidism associated with CKD, because this surgery is associated with lower morbidity and mortality in these patients. This finding is highly relevant in view of the simplicity and low cost of US and the great difficulty the treatment of SHPT in CKD. Despite the extensive knowledge about its pathophysiology, treatment of SHPT is complex, involving many factors to achieve therapeutic success. In this sense, achieving optimal levels of Ca, PTH and targets have been a challenge for everyone. The Dialysis Outcomes and Practice Patterns Study, which involves European countries and the United States, shows a large percentage of patients with controlled SHPT^[16].

The presence of parathyroid nodules is associated with poorer bone metabolic profile in patients with severe hyperparathyroidism. In fact, corroborating the observation of other researchers^[30-34], in the first moment of our study (Time 1) the levels of PTH, Ca, P and Ca × P product were higher in patients with parathyroid nodules. Furthermore, we did not find significant differences related to sex, age, time on renal replacement therapy (RRT), underlying disease or vascular access between groups with and without nodules. This in part could be attributed to the high cut-off level of PTH (> 800 pg/mL) in our study. These data would predict an increased difficulty in achieving the therapeutic goal in patients with severe hyperparathyroidism, despite the use of vitamin D-analogues. In fact, experiments have shown that the increase in parathyroid predict correlates with resistance to therapy with vitamin D-analogues (p.o. or parenteral)^[3,20,35].

However, the analysis of bone metabolic profile at Time 2 showed that our patients without nodules also demonstrated that resistance and did not show improvements in mean values of Ca, P and PTH. Non-pharmacological factors may have interfered as poor adherence, supply failures and errors in prescriptions and their interpretation, as well as the initial severity of bone metabolic disorder itself, suggested by the high cut-off level used as inclusion criteria. In fact, despite a better bone metabolic profile at Time 1, this initial severity is suggested by the same proportion of outcomes observed in patients with and without parathyroid nodules.

Nevertheless, the group with nodules showed a reduction of P and Ca × P product probably due to PTX. Patients undergoing PTX had a reduction of Ca, P and Ca × P product. Patients with nodules and non-submitted to surgery had increased Ca levels, while other parameters remained stable. In any group a decrease of PTH was observed.

This observed differences concerning the osteometabolic profiles between the groups with and without parathyroid nodules cannot be attributed to a more

severe osteometabolic disease observed in patients who died, as there was no difference in mortality between these groups. The declining profile among patients with nodules who were not operated, not observed among those without nodules, also not operated, suggests that this beneficial effect of PTX on bone metabolic profile in patients with severe hyperparathyroidism is more prominent and perhaps unique to patients with SHPT with nodules. In this sense, we must consider that PTX is the last therapeutical approach to severe SHPT, meaning there was clinical treatment failure. Patients undergoing PTX have high rates of early mortality, despite lower rates on long-term^[36,37].

The PTX allowed an improvement of bone metabolic profile in our patients. It was not our objective to evaluate success of PTX rates, the surgical techniques used or pathology results. In general, studies show that different surgical techniques have different recurrence rates, persistence and varied complications. However, one study showed similar changes in postoperative patients undergoing total PTX with or without autograft, except for a greater need for Ca replacement^[38]. Comparing patients with nodules, submitted or not to PTX, we observed that only dialysis vintage was different, higher in those submitted to PTX, with no difference concerning age, sex and type of vascular access. In other studies, different factors were associated to PTX: Younger age, female gender, non-diabetic etiology of CKD, longer dialysis vintage, parenteral use of vitamin D and peritoneal dialysis. Remarkable are younger age and the dialysis vintage^[27,36,37,39]. In our study no predominance of a determined cause of CKD was observed, nor differences regarding the use of vitamin D analogues.

Our data showed that the group of patients with nodules was not associated with an increased risk of unfavourable outcome - death, or vascular events and bone changes or differences concerning the chance of transplantation. On the other hand, in the group with nodules not submitted to PTX, a higher mortality and further vascular events was observed. This could be attributed to a lower chance of surgery in patients with a critical state, who eventually died. However, patients with nodules and underwent surgery at Time 1 had poorer bone metabolic profile (higher levels of AP and BAP). The better metabolic control after surgery may have contributed to better survival and lower morbidity. In addition, other studies have shown that, in relation to clinical treatment, PTX is associated with higher early mortality (up to 6 mo), probably to a higher postoperative risk, but lower in the long-term, which could be explained by an improvement in the metabolic and cardiovascular profile with reduced vascular calcification in imaging methods^[40,41]. There was also improvement in symptoms related to bone metabolic metabolism, among those submitted to surgery^[42,43].

Patients who died in Time 1 had higher levels of Ca, P and Ca × P product. They did not differ, however, from those who survived regarding gender, age, dialysis

vintage, type of vascular access, treatment with vitamin D-analogues, presence of parathyroid nodules and PTH levels. Within the group of patients with nodules, those who died presented at Time 1 higher values of $\text{Ca} \times \text{P}$ product, but did not differ from those who survived concerning the other parameters. A higher risk of death was observed in patients with cardiovascular events or vascular calcifications. In the population with nodules, the occurrence of death was higher among those with vascular events during the study. There was also a higher incidence of death and vascular events in patients with nodules and not submitted to PTX. These data suggest that bone metabolic profile can be a predictor of death in patients with severe HPT.

We cannot affirm that the presence of parathyroid nodule can also be a predictor of death in patients with severe hyperparathyroidism. However, as the operated group, with worse metabolic profile had improved this profile and had better outcomes, there is the possibility that the presence of parathyroid nodules can also be predictor of death.

In fact, studies have shown increased risk of death with P levels above 5 and 6.5 mg/dL, $\text{Ca} \times \text{P}$ product $> 72 \text{ mg}^2/\text{dL}^2$ and $\text{PTH} > 600 \text{ pg/mL}$ ^[44,45]. The most common cause of death among patients on RRT is cardiovascular, with cardiac calcification in 60% of them. Joins P mortality, cardiovascular morbidity and mortality and progression of renal disease with or without CKD^[46,47]. Elevated phosphate induces calcification of smooth muscle cells (SMC) *in vitro*. Pit-1, a sodium-dependent phosphate cotransporter is essential for SMC calcification and phenotypic modulation in response to elevated phosphate. P induces the expression of bone markers and extracellular matrix mineralization^[46]. Calciphylaxis is the worst expression of the phenotypic modulation.

There is a strong association between high levels of P, $\text{Ca} \times \text{P}$ product and PTH and cardiovascular morbidity and mortality. The main mechanisms involved in this process are accelerated calcification and atherosclerotic instability, Ca accumulation in the tunica media, hypertension, left ventricular (LV) hypertrophy by direct trophic effects and secondary hypertension and coronary heart disease, anemia, and macro/microangiopathies^[48,49]. PTH has been implicated in abnormalities in CKD-MBD: Immune dysfunction, refractory anemia, lower secretion of insulin^[27]. Even in patients without CKD and SHPT, PTH has been associated with higher mortality^[50,51] and P linked to higher mortality as well, and LV hypertrophy, cardiovascular events and coronary calcification^[52,53].

Although it seems obvious the association of high levels of Ca, P and PTH in several possible combinations and morbidity and mortality, mainly related to cardiovascular events, it is unclear how the PTX can reduce these rates. In our study patients submitted to surgery and with a 49 mo follow-up had lower mortality rates and reduced levels of Ca and P. However, the effect on

PTH was not significative and there was no significant correlation between PTH and mortality. It is plausible to question the correspondance between the success of a PTX as measured by PTH fall, and, therefore, the clinical improvement of a population in which the surgery was not effective. Other unevaluated factors had probably played a role.

The hormone fibroblast growth factor (FGF23) was first described in 2000 and it is mainly produced by osteocytes as a protein that reduces serum P through downregulation of the Na-P-cotransporter type 2 and leads to inhibition of the synthesis of 1-alpha-hydroxylase, with consequent reduction of calcitriol and increased serum PTH levels. The increase of FGF23 occurs in early stages of CKD, even before changes in serum levels of P and PTH and has been an independent factor of mortality in this population. In patients on hemodialysis, FGF23 levels can predict refractory SHPT^[54-56]. Although not evaluated in our study, FGF23 could have explained some issues raised by the results.

Our study has limitations. It was retrospective and based on not standardized data and medical records which may be critical to the results. Moreover, another criticism is due to the fact that none of the patients have used other vitamin D analogs, such as paricalcitol and calcimimetics, whose results have demonstrated better clinical response than with traditional analogs of vitamin D. Two large studies have shown good responses to reduce PTH levels but failed to demonstrate reduction of morbidity and cardiovascular mortality: The EVOLVE and the PRIMO^[54-56]. The first was done with cinacalcet and the second with paricalcitol. Although calcimimetics have reduced the need for surgical PTX, a Cochrane recent review showed no reduction in mortality in the population with CKD stage V with the use of cinacalcet^[57].

In conclusion, patients with $\text{PTH} > 800 \text{ pg/mL}$ and parathyroid nodule presented worse bone metabolic profile than those without nodules. Hypercalcemia, hyperphosphatemia and elevated $\text{Ca} \times \text{P}$ product were associated with higher mortality in this population and PTX was associated with improvements in the control of Ca, P levels and $\text{Ca} \times \text{P}$ product, a lower occurrence of vascular events and longer survival in patients with severe SHPT.

Thus, the presence of nodules on US could be used as a criterion for PTX indication in this group of patients. Furthermore, it is possible that the presence of parathyroid nodule at US may be useful for the prediction of mortality or vascular events in patients with PTH levels higher than 800 pg/mL, although our study did not show this association.

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COMMENTS

Background

Secondary hyperparathyroidism has a high prevalence in the chronic renal dialysis population as cause of cardiovascular morbidity and mortality. The presence of nodular hyperplasia in parathyroids in ultrasound could predict response to drug treatment and guides the surgical treatment.

Research frontiers

To identify prognostic factors of response to pharmacological treatment with non invasive tests, in order to propose new therapies or indicate surgical treatment.

Innovations and breakthroughs

Ultrasonography could be an adjuvant exam in the follow-up of patients with severe hyperparathyroidism, in order to plan the treatment.

Applications

This study confirms the role of secondary hyperparathyroidism in cardiovascular mortality in chronic kidney disease patients in dialysis, associated with bone mineral disorders.

Terminology

Secondary hyperparathyroidism is a hormonal disorder triggered by many metabolic abnormalities that are related to renal function impairment.

Peer-review

The authors support that "The identification of nodules at ultrasonography strengthens the indication for parathyroidectomy in patients with secondary hyperparathyroidism due to renal failure".

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Observational Study

Is overhydration in peritoneal dialysis patients associated with cardiac mortality that might be reversible?

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Abstract

AIM

To study the relationship between overhydration (OH) in peritoneal dialysis (PD) patients and cardiac mortality.

METHODS

OH, as measured by body composition monitor (BCM), is associated with increased mortality in dialysis patients. BCM has been used to guide treatment on the assumption that correcting OH will improve cardiac morbidity and mortality although data demonstrating causality that is reversible is limited. We wished to determine if OH in PD patients predicted cardiac mortality, and if there was a correlation between OH and cardiac troponin-T (cTnT) levels. Finally, we wished to determine if improving OH values would lead to a decrement in cTnT. All prevalent PD patients over the study period of 57 mo who had contemporaneous BCM and cTnT measurements were followed irrespective of transplantation or PD technique failure. We also studied a cohort of patients with who had severe OH ($> +2L$).

The Fresenius Body Composition Monitor was used to obtain hydration parameters. cTnT levels were done as part of routine clinical care. Data was analysed using SPSS version 20.0.

RESULTS

There were 48 deaths in the 336 patients. The patients that died from cardiac or non-cardiac causes were similar with respect to their age, incidence of diabetes mellitus, gender, ethnicity and cause of renal failure. However, the patients with cardiac causes of death had significantly shorter dialysis vintage (10.3 mo *vs* 37.0 mo, $P < 0.0001$) and were significantly more overhydrated by BCM measurement (2.95 L *vs* 1.35 L, $P < 0.05$). The mean (standard error of the means) hydration status of the 336 patients was +1.15 (0.12) L and the median [interquartile range (IQR)] cTnT level was 43.5 (20-90) ng/L. The cTnT results were not normally distributed and were therefore transformed logarithmically. There was a statistically significant correlation between Log (cTnT) with the OH value (Spearman r value 0.425, $P < 0.0001$). We identified a sub-group of patients that were severely overhydrated; median (IQR) hydration at baseline was +2.7 (2.3 to 3.7) L. They were followed up for a minimum of 6 mo. Reduction in OH values in these patients over 6 mo correlated with lowering of cTnT levels (Spearman r value 0.29, $P < 0.02$).

CONCLUSION

Patients that were overhydrated had higher cTnT, and had deaths that were more likely to be cardiac related. Reduction in OH correlated with lowering of cTnT.

Key words: Bioimpedance; Fluid status; Peritoneal dialysis; Mortality; Overhydration; Cardiac troponin

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Core tip: Overhydration measured by bioimpedance spectroscopy is an independent predictor of death in peritoneal dialysis patients. Most studies on this topic provide only a single baseline bioimpedance assessment. We present longitudinal data showing increased cardiac mortality in overhydrated patients, and significant correlation of overhydration with cardiac troponin-T (cTnT) levels. Over 6 mo, these patients had a mean of 7.4 body composition monitor readings and 3.4 cTnT assessments. Patients whose hydration status improved showed a corresponding improvement in cTnT. While observational studies cannot define causality, our results show overhydration is associated with cardiac mortality, and suggest overhydration may be a reversible risk factor.

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INTRODUCTION

Fluid overload measured by bioimpedance spectroscopy (BIS) is an independent predictor of death in peritoneal dialysis (PD) patients^[1] and is highly prevalent^[2]. This was also shown in haemodialysis (HD)^[3]; patients with severe overhydration (OH) had a hazard ratio for all-cause mortality of 2.1, second to only diabetes. However, causality cannot be determined from these retrospective observational studies. Nevertheless, there is circumstantial evidence to support the belief that correcting OH will improve patient outcomes; correcting OH was shown to be associated with improvements in blood pressure, arterial stiffness and left ventricular mass index^[4,5]. But very strict attention to fluid restriction was also shown to increase loss of residual renal function^[6] and there is an association between low hydration status and intra-dialytic hypotension^[7].

While it is often assumed that the observed relationship between OH and mortality is related to cardiovascular damage, an important caveat is that the ratio of extracellular fluid to total body water is also increased in the setting of muscle wasting. Thus, a negative correlation between BIS-OH parameters and malnutrition have been found in several studies^[8,9] and it is possible that the increased mortality associated with OH relate to its association with protein energy wasting (PEW)/malnutrition inflammation atherosclerosis (MIA) Syndrome. Thus, in a study of 72 stable HD patients, although N-Terminal pro-Brain Natriuretic Peptide (NTpro-BNP) correlated with BIS OH, the authors concluded that NTproBNP was also elevated in malnourished patients^[10]. Others have also expressed reservation about the proposed causal relationship between overhydration measure by BIS and mortality^[11].

We wished to explore this subject further by determining if PD patients who died from cardiac causes were more severely OH compared to patients that died from other causes. We also wished to determine if there is a relationship between OH and cardiac troponin-T (cTnT) which remains a highly sensitive biomarker for cardiac injury in dialysis patients^[12,13]. Thus, we studied patients over a 6-mo period to determine if severe OH improved, did it lead to a corresponding decrements of cTnT. We also examined the changes in cTnT over 6 mo against time-average values of biochemical nutrition parameters (serum albumin and haemoglobin) as well the inflammatory marker of C-reactive protein (CRP).

MATERIALS AND METHODS

The study was conducted in accordance with the principles set out by the local ethical committee according to United Kingdom National Health Service audit and clinical service development. We studied a cohort of patients from a single PD unit, consisting of all continuous ambulatory PD (CAPD) and automated PD (APD) patients between 1 January

2008 and 30 March 2012 who had contemporaneous baseline BIS/cTnT readings. All patients with amputations, cardiac pacemakers or defibrillators were excluded as we were unable to perform BIS measurements. Patients were followed up until 15th September 2012 or death. Patients who were transplanted or switched to HD were still followed up. Only patients that recovered renal function or who were transferred to another dialysis unit for geographic relocation reasons were censored at that time point, as their survival follow-up could not be accurately determined. In all cases, baseline characteristics were collated through review of case notes and included primary cause of renal failure, dialysis vintage and presence or absence of diabetes mellitus. To comply with the mandatory United Kingdom Renal Registry submissions, we held weekly multidisciplinary meetings to review and assign causes of death for dialysis patients.

In a subgroup analysis, we identified patients that were severely overhydrated (OH > 2 L). Using the time when their OH was first found to be > 2 L as baseline, we prospectively collected data on their hydration status and their cTnT readings over the subsequent 6 mo.

Bioimpedance analysis

The Fresenius Body Composition Monitor (BCM - Fresenius Medical Care, Bad Homburg, Germany) was used to obtain hydration parameters such as OH and nutritional indices, namely Fat and Lean Tissue Mass (FTM and LTM respectively). This BIS device employed 50 frequencies between 5 and 1000 kHz, and measurements were performed by placing electrodes on one hand and one foot, with PD dialysate *in situ*.

Cardiac troponin assay

Over the duration of the study, cTnT was measured every 3-4 mo as part of routine clinical care. During this study, the cTnT assay (Roche Diagnostics GmbH, Mannheim, Germany) was changed to the high sensitivity assay, though the upper limit of normal was the same (< 14 ng/L, upper 99th percentile). In patients with normal renal function, values > 3 ng/L were suggestive of myocardial injury and values > 14 ng/L were considered diagnostic for myocardial infarction if there were consistent clinical features. Precision of the assays across the range were: 0.5 ng/L (1.9%); 0.0399 (1.6%); 0.133 (1.7%).

Statistical analysis

Categorical variables are expressed as a number and a percentage. Continuous variables are expressed as means and standard error of the means (SEM) or median with quartile ranges depending on whether the results of the parameters were normally distributed (determined by the D'Agostino and Pearson omnibus normality test). If not normally distributed, these parameters were analyzed on a logarithmic scale. Correlation coefficients and multivariate logistical regression analyses were undertaken with SPSS software for Windows version 20.0 (SPSS Inc., Chicago, IL, United States). The regression

model was created based on those clinical variables known to effect survival on PD.

RESULTS

Demographics

There were 336 APD and CAPD patients who had at least 1 contemporaneous BCM/cTnT assessment during the study period. The median age of patients was 57.9 [interquartile range (IQR): 47.9-69.0] years with a median dialysis vintage of 7.6 (IQR: 0.9-31.4) mo (Table 1). 62% were male, and 37% had diabetes mellitus.

There were 74 patients who had an OH reading > 2 L and a subsequent BCM/cTnT assessment between 6-9 mo later. We excluded 8 patients that had documented acute cardiac events (acute rise in cTnT associated with cardiac pain, pulmonary oedema or haemodynamic instability). For the remaining 66 patients, the median (IQR) "baseline" OH value was 2.7 (2.3-3.7) L. Over the follow-up period, the mean number of BCM measurement per patient was 7.4, whilst the mean number of cTnT measurements per patient was 3.4. Demographic details for this cohort are listed in Table 2.

Hydration status correlation with serum troponin

For our cohort of 336 patients, the mean (SEM) hydration status was +1.15 (0.12) L and the median (IQR) cTnT level was 43.5 (20-90) ng/L. The cTnT results were not normally distributed and were therefore transformed logarithmically. There was a statistically significant correlation between Log (cTnT) with the OH value (the Spearman *r* value was 0.425, *P* < 0.0001, Figure 1).

Association of overhydration with cardiac death

Over a median follow-up period of 23.9 mo, 48 patients (14.3%) of the 336 PD patients died. Cardiac causes of death (sudden cardiac death, cardiac failure or myocardial ischaemia) were assigned in 13 (27%) of cases. The patients that died from cardiac or non-cardiac causes were similar with respect to their age, incidence of diabetes mellitus, gender, ethnicity and cause of renal failure (Table 1). However, the patients with cardiac causes of death had significantly shorter dialysis vintage (10.3 mo vs 37.0 mo, *P* < 0.0001) and were significantly more overhydrated by BCM measurement (2.95 L vs 1.35 L, *P* < 0.05). The OH status appeared to predict cardiac death that occurred at a mean of 15.5 mo subsequent to the BCM readings. The mean duration between the BCM reading and non-cardiac death was 16.1 mo.

Correlation between dynamic changes in OH and dynamic changes in cTnT over 6 mo

We identified a sub-group of patients that were severely overhydrated; median (IQR) hydration at baseline was +2.7 (2.3 to 3.7) L. They were followed up for a minimum of 6 mo. For each individual, the rate of change of OH (Δ OH) was calculated using the "least squares" method to estimate the straight line that best fitted that patient's

Table 1 Baseline demographic

	All	Survivors	Non-cardiac death	Cardiac death	P-value (comparing cardiac vs non cardiac death patients)
No.	336	288	35	13	
Age ¹	57.9 (48.1-69.0)	55.4 (46.9-66.6)	68.9 (61.8-77.0)	68.9 (62.9-76.5)	NS
Male	207 (62%)	167 (58%)	27 (77%)	13 (100%)	NS
Diabetes mellitus	123 (37%)	99 (34%)	15 (43%)	9 (69%)	NS
Assessed as suitable for transplantation	159 (47%)	148 (51%)	10 (29%)	1 (8%)	NS
Dialysis vintage (mo) ¹	7.6 (0.9-31.4)	6.5 (0.8-24.0)	37.0 (4.0-57.4)	10.3 (2.9-23.1)	< 0.00001
Body composition measurements: Mean (SEM)					
OH (L)	1.15 (0.12)	1.04 (0.13)	1.35 (0.32)	2.95 (0.78)	< 0.05
Lean tissue index	13.7 (0.5)	13.9 (0.5)	11.9 (0.5)	12.3 (1.6)	< 0.0001
Fat tissue index	13.2 (0.3)	13.3 (0.3)	11.9 (0.6)	13.3 (1.0)	< 0.01
Ethnicity					
Whites	112 (33%)	97 (34%)	14 (40%)	1 (8%)	NS
Blacks	67 (20%)	59 (20%)	5 (14%)	3 (23%)	
Asians	139 (41%)	117 (41%)	15 (43%)	7 (54%)	
Others	18 (5%)				
Cause of renal failure: n (%)					
Unknown	82 (24%)	66 (30%)	8 (23%)	2 (15%)	NS
GN	51 (15%)	26 (12%)	1 (3%)	0 (0%)	
Cancer/trauma	1 (0%)	3 (1%)	0 (0%)	0 (0%)	
Congenital/familial	8 (2%)	5 (2%)	0 (0%)	0 (0%)	
Diabetes	105 (31%)	69 (31%)	14 (40%)	9 (69%)	
Hypertension	28 (8%)	16 (7%)	5 (14%)	2 (15%)	
APKD	12 (4%)	11 (5%)	1 (3%)	0 (0%)	
TIN/chronic pyelonephritis	49 (15%)	29 (13%)	6 (17%)	0 (0%)	
Blood results					
Baseline log(CRP) (mg/L)	0.57 (0.03)	0.52 (0.04)	0.79 (0.12)	0.81 (0.20)	NS
Time av log (CRP) (mg/L)	0.67 (0.04)	0.61 (0.04)	1.09 (0.11)	0.98 (0.17)	NS
Baseline albumin (g/L)	38.9 (0.3)	39.3 (0.3)	36.1 (1.0)	36.8 (1.0)	< 0.05
Time av albumin (g/L)	37.6 (0.2)	38.2 (0.2)	33.8 (1.0)	35.6 (1.1)	< 0.002
Baseline haemoglobin (g/dL)	11.0 (0.1)	10.9 (0.1)	11.8 (0.3)	11.0 (0.5)	NS
Time av haemoglobin (g/dL)	11.2 (0.1)	11.2 (0.1)	11.7 (0.2)	10.7 (0.3)	NS

Values represent mean (SEM) or number (%) unless denoted by¹ (values shown are median, interquartile ranges). OH denotes the “overhydration” reading from the body composition monitor. GN: Glomerulonephritis; APKD: Adult polycystic kidney disease; TIN: Tubular-interstitial nephritis; OH: Overhydration.

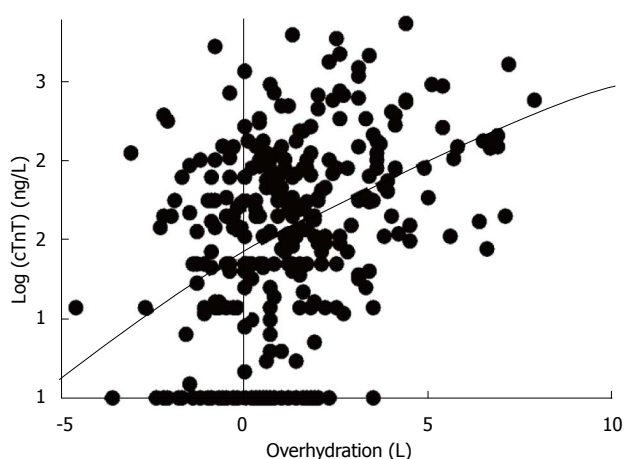


Figure 1 Regression analysis of baseline overhydration vs cardiac troponin T levels. cTnT: Cardiac troponin-T.

data. The median (IQR) change of OH over 6 mo was -0.7 (-0.3 to -1.5) L. The median (IQR) baseline cTnT value was 60 (37-100) ng/L (Table 2). We plotted the Δ OH with Δ cTnT and found a statistically significant correlation

(Spearman r value = 0.29, P < 0.02; Figure 2). The rates of change of FTM and LTM were also calculated using the “least squares” method. Over a 6-mo period, there was a FTM increase of +2.3 kg (IQR: 0.1-3.7, P < 0.0001, Wilcoxon Signed Rank test). By contrast, patients showed a statistically significant loss of LTM during the follow-up, equivalent to -1.5 kg (IQR: -4.5 to -1.7, P < 0.05; Table 2). There were no significant correlation between Δ OH and any of the biochemical nutrition or inflammatory markers (either baseline or time-average values of serum albumin, haemoglobin and CRP).

DISCUSSION

In our current study, patients who died from cardiac causes were more severely overhydrated than patients who died from other diseases although this association does not indicate causality or reversibility. This is in keeping with a recent pre-dialysis study that also showed an association between cardiac (as well as all-cause mortality) for patients with chronic kidney disease stages 4 and 5 with fluid overload determined by BIS^[14]. We

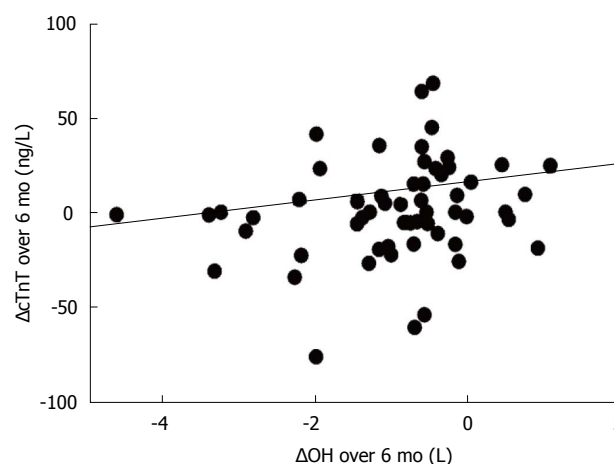
Table 2 Baseline demographic of cohort that had overhydration > 2 L

Sub-group: Patients with severe OH	
Number	66
Age ¹	60.1 (51.1-71.1)
Male (%)	44 (67%)
Diabetes mellitus (%)	27 (41%)
Assessed as suitable for transplantation (%)	26 (39%)
Dialysis vintage (mo) ¹	1.79 (0.5-32.1)
Body composition measurements:	
Baseline OH (L) ¹	2.7 (2.3-3.7)
OH over 6 mo (L/6 mo) ¹	-0.7 (-0.3--1.5)
Baseline FTM (kg) ¹	23.6 (17.3-28.9)
FTM over 6 mo (kg) ¹	2.3 (0.1-3.7) ³
Baseline LTM (kg) ¹	37.2 (29.6-44.6)
LTM over 6 mo (kg) ¹	-1.5 (-4.5-1.7) ²
Number of readings	7.4 (0.4)
Cardiac troponin T measurements (ng/L)	
Baseline cTnT ¹	60 (37-100)
Final cTnT ¹	71 (37-115)
Number of readings	3.4 (0.2)
Ethnicity: n (%)	
Whites	26 (39%)
Blacks	14 (21%)
Asians	23 (35%)
Others	3 (5%)
Cause of renal failure: n (%)	
Unknown	15 (23%)
GN	9 (14%)
Cancer/trauma	2 (3%)
Congenital/familial	2 (3%)
Diabetes	23 (35%)
Hypertension	6 (9%)
APKD	0 (0%)
TIN/chronic pyelonephritis	9 (14%)
Blood results	
Baseline log (CRP) (mg/L)	0.67 (0.08) ⁴
Time average log (CRP) over 6 mo (mg/L)	0.73 (0.08) ⁴
Baseline albumin (g/L)	36.4 (0.6) ⁴
Time average albumin over 6 mo (g/L)	36.0 (0.6) ⁴
Baseline haemoglobin (g/dL)	10.5 (0.2) ⁴
Time average haemoglobin over 6 mo (g/dL)	10.6 (0.2) ⁴

Values represent mean (SEM) or number (%) unless denoted by¹ (values shown are median, interquartile ranges). ² $P < 0.05$ by Wilcoxon Signed Rank Test to determine if Median is $\neq 0$; ³ $P < 0.0001$ by Wilcoxon Signed Rank Test to determine if Median is $\neq 0$; ⁴There were no significant correlation with OH by Pearson r correlation. OH denotes the "overhydration", FTM denotes fat tissue mass and LTM denotes lean tissue mass from the body composition monitor. GN: Glomerulonephritis; APKD: Adult polycystic kidney disease; TIN: Tubular-interstitial nephritis; OH: Overhydration.

also note that a recent report^[15] showed that patients randomized to having their hydration status managed with BCM had lower mortality.

Cardiac cTnT has been repeatedly shown to be predictive of cardiac death in patients on dialysis^[16,17]. It is therefore significant that we found a direct correlation between OH status and Log cTnT ($r = 0.425$, $P < 0.0001$). These results alone do not prove causality and it remains possible that this association is due to PEW/MIA. After all, a large database of haemodialysis patients (MONDO) confirmed that the BIS parameters of FTI and LTI were also associated with mortality^[18]. Unfortunately, for a retrospective study, it was difficult to define the exact

**Figure 2** Regression analysis of the change in overhydration vs cardiac troponin T levels. OH: Overhydration; cTnT: Cardiac troponin T levels.

causes of deaths that were ascribed at the time to be "cardiac/sudden cardiac deaths".

However, uniquely we also found a statistically significant correlation between the improvement in hydration status and decrement in cTnT levels over 6 mo ($r = 0.29$, $P < 0.02$; Figure 2). Patients in this cohort study had a median baseline OH value of +2.7 L and showed a median decrement over 6 mo of -0.7 L. It is unlikely that this magnitude of change is a consequence of correcting malnutrition/PEW. In fact, the BIS data suggested a small but significant loss of LTM over these 6 mo, which may have been due to patients' salt and fluid restriction with consequent diminished dietary protein intake. We also found that there were no correlation between either baseline or time-averaged biochemical markers of nutrition and inflammation suggesting the improvement in cTnT is likely to be a consequence of fluid status and not nutrition. Of course, it must be acknowledged that using albumin, CRP and presence of anaemia to be indicators of nutrition is imprecise. Nevertheless, we found no signal to suggest changes in nutrition status was a cofounder for the change in cTnT.

It is important to note that our cohort exhibited an increase in FTM that was equivalent to +2.3 kg (IQR: 0.1-3.7) over 6 mo. It is possible that the fat gain was exacerbated through increased use of hypertonic glucose dialysate to improve hydration status.

Although our results do not prove that correction of overhydration will lead to reduced cardiac mortality, it supports the finding by Onofriescu *et al.*^[15]. We hope these findings provide added impetus for clinicians to focus on OH particularly as we have previously reported that BCM guided reduction of OH does not cause excessive loss of residual renal function^[19,20]. We note that improved hydration status in this study appeared to come at the expense of increasing FTM but the clinical impact of increasing obesity is unclear. In the normal population, obesity is associated with glucose intolerance and cardiovascular risk but in HD, "reverse epidemiology" has been reported (HD patients with high BMI have a survival

advantage)^[21]. Similarly, studies have shown that PD patients with high FTM have a survival advantage^[18,22].

Limitations of this study include the retrospective nature of the data collection and the fact that we included both incident and prevalent patients. BCM measurements were performed with dialysate *in situ* and this may have reduced the precision of the measurements^[23] (although we were consistent in how we performed the measurements). Most critically, our retrospective study can only demonstrate associations and cannot determine causality. The study would have also benefited if we had a formal assessment of cardiac function. Future studies could include simple assessments such as echocardiography but unfortunately contemporaneous echo studies with bioimpedance measurements were not available for all subjects in our study. Equally, it was difficult in a retrospective study to accurately determine “cause” of fluid overload.

In conclusion, we believe our data provides indirect evidence to suggest that overhydration impacts negatively on the heart. Intriguingly, our data also suggests that correcting overhydration is possible and may lead to improved cardiac prognosis but perhaps at the expense of increasing obesity. Randomized controlled studies on this subject will be difficult, but it will be interesting to await the results of two studies that are designed to explore the impact BIA might have on left ventricular mass^[24] and survival^[25,26].

COMMENTS

Background

Despite the importance of euvoalaemia, there is still much debate on the most clinically useful method of volume assessment. Overhydration (OH) as measured by body composition monitor (BCM) is associated with increased mortality in dialysis patients. BCM has been used to guide treatment on the assumption that correcting OH will improve cardiac morbidity and mortality, although data demonstrating causality that is reversible is limited.

Research frontiers

It has often been assumed that the observed relationship between OH and mortality is related to cardiovascular damage. However, an important caveat is the ratio of extracellular water to total body water is also increased in the setting of muscle wastage. Thus it is possible that the increased mortality associated with OH relate to its association with protein energy wasting (PEW)/malnutrition inflammation atherosclerosis (MIA) Syndrome.

Innovations and breakthroughs

Most research into this area has focused on single time point measurements of OH and cardiac biomarkers. While cardiac troponins-T (cTnT) have been repeatedly shown to be predictive of cardiac death in dialysis patients, the effect of malnutrition on the observed relationship between OH, cardiac biomarkers and outcomes is difficult to establish. More recent trials have shown that targeting a reduction in OH is associated with better survival. However, the temporal relationship of cardiac biomarkers and reduction in OH has not been well described.

Applications

In this study, peritoneal dialysis patients who died of cardiac causes had higher OH, compared to patients that died from other causes. Over a 6-mo period, the authors found that reducing OH in severely overhydrated patients was associated with corresponding decrements in cTnT. There was no significant

correlation between change in OH and any of the biochemical or nutritional markers studied, suggesting that the improvement in cTnT is likely to be a consequence of fluid status and not nutrition. Although the results do not prove that correction of OH will lead to reduced cardiac mortality, the temporal association observed between OH and cTnT supports the role of fluid status in cardiac risk management of dialysis patients.

Terminology

OH is a mathematically derived estimate of excess fluid. The BCM expresses the body weight in terms of lean tissue mass (LTM-mainly muscle), adipose tissue mass (ATM-mainly fat) and OH. Each of these compartments has a specific composition and contains a known quantity of water per mass of tissue. The water of LTM and ATM consist of differing proportion of extracellular and intracellular water in addition to solid components. Excess fluid represents an expansion of only the extracellular water, whereas ICW remains unchanged. The excess fluid may reside within adipose tissue or lean tissue raising the hydration of the respective tissue above the “normal” values (e.g., oedema). Alternatively, excess fluid may simply appear as a distinct compartment without altering the hydration of the major tissues (e.g., ascites, pleural effusion). As the extracellular hydration of LTM and ATM is known, the expected “normal” volume of ECW of these tissues can be calculated. The difference between “normal” ECW and measured ECW is the excess fluid, OH.

Peer-review

OH is gaining popularity as an objective measurement of fluid status due to its relatively low cost and ease of measurement. There have been many studies on this topic, but it is uncommon to find studies on repeated measurements of OH and cardiac biomarkers. This study provides indirect evidence to suggest that OH is associated with worse cardiac outcomes, and importantly, that correcting OH may lead to improved cardiac prognosis.

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Observational Study

Factors associated with regular dental visits among hemodialysis patients

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Abstract

AIM

To investigate awareness and attitudes about preventive dental visits among dialysis patients; to clarify the

barriers to visiting the dentist.

METHODS

Subjects included 141 dentate outpatients receiving hemodialysis treatment at two facilities, one with a dental department and the other without a dental department. We used a structured questionnaire to interview participants about their awareness of oral health management issues for dialysis patients, perceived oral symptoms and attitudes about dental visits. Bivariate analysis using the χ^2 test was conducted to determine associations between study variables and regular dental check-ups. Binominal logistic regression analysis was used to determine factors associated with regular dental check-ups.

RESULTS

There were no significant differences in patient demographics between the two participating facilities, including attitudes about dental visits. Therefore, we included all patients in the following analyses. Few patients (4.3%) had been referred to a dentist by a medical doctor or nurse. Although 80.9% of subjects had a primary dentist, only 34.0% of subjects received regular dental check-ups. The most common reasons cited for not seeking dental care were that visits are burdensome and a lack of perceived need. Patients with gum swelling or bleeding were much more likely to be in the group of those not receiving routine dental check-ups (χ^2 test, $P < 0.01$). Logistic regression analysis demonstrated that receiving dental check-ups was associated with awareness that oral health management is more important for dialysis patients than for others and with having a primary dentist ($P < 0.05$).

CONCLUSION

Dialysis patients should be educated about the importance of preventive dental care. Medical providers are expected to participate in promoting dental visits among dialysis patients.

Key words: Hemodialysis; Questionnaire; Oral health; Dental visit; Health management

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Core tip: We investigated dialysis patients' awareness and attitudes about preventive dental visits, and tried to clarify the barriers to visiting the dentist. Subjects included 141 dentate outpatients receiving hemodialysis treatment. We interviewed participants using a structured questionnaire. The common reasons dialysis patients cited for not seeking dental care were lack of concern and/or lack of awareness of the importance of preventive dental visits. Medical practitioners rarely refer dialysis patients for dental care. Our findings suggest that dialysis patients should be educated about the importance of preventive dental care. Medical providers are expected to participate in promoting dental visits among dialysis patients.

Yoshioka M, Shirayama Y, Imoto I, Hinode D, Yanagisawa S, Takeuchi Y, Bando T, Yokota N. Factors associated with regular dental visits among hemodialysis patients. *World J Nephrol* 2016; 5(5): 455-460 Available from: URL: <http://www.wjgnet.com/2220-6124/full/v5/i5/455.htm> DOI: <http://dx.doi.org/10.5527/wjn.v5.i5.455>

INTRODUCTION

As of December 31, 2013, there were 314180 dialysis patients in Japan, a number that has been increasing yearly^[1]. Most dialysis facilities do not have dental departments^[2]. Prior to this study, we hypothesized that inconvenient accessibility could be a barrier to dental visits among dialysis patients. Dialysis patients have a high risk of dental caries and periodontitis^[3,4]. Recently, several studies have reported a significant association between moderate to severe periodontitis and mortality among hemodialysis patients^[5-7]. Therefore, preventive dental care should be considered very important for this population.

In this study, we tried to determine factors associated with regular dental visits and to determine barriers to preventive dental care among hemodialysis patients, to promote improvement in holistic oral health management.

MATERIALS AND METHODS

Outpatients receiving hemodialysis treatment at two dialysis facilities, one with a dental department (Facility A) and the other without (Facility B), were included in this study. The total number of patients receiving hemodialysis at Facility A was approximately 600; approximately 150 received hemodialysis at Facility B. The inclusion criteria for this study were outpatients receiving hemodialysis treatment three times per week, and who agreed to participate in the study. Because we needed to interview patients at their bedsides, we excluded patients who had difficulty conversing independently. We used a structured questionnaire to interview 141 dentate patients about their awareness of oral health management issues, their perceived oral symptoms and their attitudes about seeking dental care (Figure 1). Written informed consent for participation was not obtained from the participants in this study; we regarded replying to the interview questions as signifying agreement to participate, as we explained in the document that was provided to each patient at the start of the interview.

Statistical analyses were performed with the SPSS 17.00 statistical package (SPSS Japan Inc., Tokyo, Japan). Bivariate analysis using the χ^2 test was conducted to determine associations between study variables and regular dental check-ups. Binominal logistic regression analysis was used to determine factors associated with regular dental check-ups. Statistical significance was accepted at a level of 0.05

Questionnaire

Age sex (male/female)

How long have you been receiving dialysis treatment? Years months

Are you employed? (yes/no)

Is there a dental department at the facility where you receive dialysis treatment? (Yes/No/Not sure)

Have you ever been referred to a dentist by a medical practitioner? (Yes/No)

Do you think that oral health management is more important for patients receiving dialysis treatment than for others? (Yes/No) If "Yes," why do you think so?

How many teeth do you have? [Most (≥ 20) / half (10-19) / few (< 10) / zero]

Do you have dentures? (Yes/no/unused)

Do you have any oral symptoms? (1) toothache/sensitivity; (2) loose tooth; (3) gum swelling/bleeding; (4) food impaction; (5) bad breath; (6) sticky mouth; (7) crooked teeth; (8) malocclusion; (9) clicking of the jaw; (10) missing tooth; (11) dry mouth; (12) rough lips; (13) abnormal taste; (14) frequent stomatitis; (15) odd feeling to dentures; (16) other

Do you have a primary dentist? (Yes/no)

Do you receive a dental check-up once a year or more? (Yes/no)

When did you last visit a dentist? Years, months ago

When do you visit the dentist? (1) I visit regularly, even without a specific problem. (2) I visit irregularly, when I have a specific problem. (3) I occasionally do not visit the dentist, even if problems are present. (4) I never visit the dentist.

If you answered, "I do not receive dental check-ups," why? (1) no perceived need; (2) it is burdensome; (3) lack of time; (4) anxiety about dental treatment; (5) physical barrier (fatigue); (6) psychological barrier (fear/pain/hate); (7) economic burden; (8) lack of accessibility; (9) no attendant; (10) no reliable dentist; (11) other

Please suggest ideas that would make it easier for dialysis patients to receive dental check-ups.

Figure 1 Questionnaire.

and lower.

RESULTS

Distribution of participants

The distribution of respondents is shown in Table 1. The age of the respondents ranged from 29 to 86 years, with a mean age of 63.1 years (SD 11.0). The mean duration of dialysis was 10.3 years (SD 8.7); 42.6% had been receiving dialysis for more than 10 years. The percentage of employed patients was 34.8%.

There were no significant differences in patient demographics between the two participating facilities, including attitudes about dental visits. Therefore, we included all patients in the following analyses.

Awareness of oral health management issues

Only 4.3% of subjects had been referred to a dentist by their medical practitioner. Twenty-three percent of the respondents considered oral health management to be important for dialysis patients; most of these were aware of the association between periodontitis and general health conditions.

Self-reported oral health status

Self-reported oral health conditions are shown in Table 2. Oral health problems reported by dialysis patients included dry mouth (39.0%), bad breath (34.8%) and gum swelling/bleeding (20.6%).

Factors associated with dental visits

Eighty percent of subjects had a primary dentist, but only 34% of participants received regular dental check-ups. However, 66.0% of subjects had visited a dentist in the past year, suggesting that a considerable number of oral problems had arisen. As for the timing of dental visits,

56.0% of subjects answered that they visited a dental office only when symptoms arose; 5.7% answered that they sometimes refused to visit a dentist even if oral symptoms were present. The reasons cited for not seeking a dental check-up are shown in Table 3. The most common reasons given were "it is burdensome" and "no perceived need", followed by "lack of time" and "psychological barrier (fear/pain/hate)". As shown in Table 4, χ^2 testing demonstrated that receiving regular dental check-ups was significantly associated with awareness of oral health management issues related to dialysis and with having a primary dentist ($P < 0.01$). The prevalence of self-reported gum swelling/bleeding was higher among those not receiving dental check-ups than among those receiving dental check-ups ($P < 0.01$). Binominal logistic regression analysis using "receiving dental check-ups" as the outcome variable demonstrated that receiving dental check-ups was significantly associated with awareness of oral health management issues related to dialysis treatment, having many teeth, having dentures and having a primary dentist (Table 5).

DISCUSSION

Because the interviewer in this study was from a third party, not from a dialysis facility or a private dental clinic, and because personal information was completely anonymized, we believe that we were able to elicit patients' opinions and thoughts without bias. Barriers to visiting the dentist included a lack of awareness of the need for care, cost and fear of dental procedures^[8,9]. Especially among patients with special health care needs, dental fear and/or anxiety is considered the most common barrier to accessing oral health care^[10]. Prior to this study, we had hypothesized that time restrictions or general fatigue would be the main reasons that dialysis

Table 1 Demographic profiles of participants

Facility	Facility A With dental department	Facility B Without dental department	Total
Number of subjects	88	53	141
Age	61.9 ± 11.6	65.1 ± 9.7	63.1 ± 11.0
Sex			
Male	59	31	90
Female	29	22	51
Duration of dialysis			
< 1 yr	6	4	10
1-4 yr	23	12	35
5-9 yr	21	15	36
≥ 10 yr	38	22	60
Employment			
Employed	35	15	50
Unemployed	53	38	91
Primary dentist			
Yes	68	46	114
No	20	7	27
Dental check-up			
Yes	31	17	48
No	57	36	93

Table 2 Self-reported oral health status (*n* = 141)

	No.	% of Subjects
Number of teeth		
≥ 20	101	71.6
10-19	25	17.7
1-9	15	10.6
Possession of denture		
Yes	34	24.1
No/unused	107	75.9
Oral symptom		
Toothache/sensitive	26	18.4
Shaking tooth	22	15.6
Gum swelling/bleeding	29	20.6
Food impaction	104	73.8
Bad breath	49	34.8
Sticky mouth	30	21.3
Crooked teeth	21	14.9
Malocclusion	28	19.9
Clicking of jaw joint	14	9.9
Lack of tooth	13	9.2
Dry mouth	55	39.0
Rough lip	22	15.6
Wrong taste	15	10.6
Frequent stomatitis	20	14.2
Odd feeling to denture	2	1.4
Other	8	5.7

patients do not seek dental care. As shown in Table 3, some patients answered "no time to go" as a reason for not seeking dental care. However, we found that lack of concern and/or lack of awareness of the need for preventive dental visits were common reasons in this population. In Japan, most dental care is covered by medical insurance. In fact, dialysis patients are sometimes provided with additional insurance benefits. Therefore, nobody answered "economic burden" as a reason for not seeking dental care.

Table 3 Reasons for not seeking dental care (*n* = 93)

	No.	% of subjects
No perceived need	33	23.4
Burdensome	36	25.5
No time to go	16	11.3
Anxiety for dental treatment	2	1.4
Physical burden (fatigue/tired)	3	2.1
Psychological burden (fear/painful/hate)	13	9.2
Economic burden	0	0.0
Uneasy accessibility	0	0.0
No attendant	2	1.4
No reliable dentist	0	0.0
Others	10	7.1

Recently, the close relationship between periodontal disease and systemic disease has been highlighted^[11,12]. It has been reported that severe periodontitis can affect mortality in hemodialysis patients^[5-7]. Studies involving patients with chronic kidney disease found that efficient initial periodontal therapy lowered serum levels of some inflammatory biomarkers^[13,14].

Our results showed that awareness of the oral health management issues of dialysis patients led to preventive dental visits in this population. Therefore, providing dialysis patients with information about the relationship between periodontitis and systemic conditions might effectively promote preventive oral health care.

Dialysis patients tend to be at high risk for tooth decay and periodontal disease^[15]. Oral surgical procedures require extra precautions in these patients because of associated medications (e.g., anticoagulants) and complications (e.g., hypertension, diabetes). Therefore, dialysis patients must be informed of their greater need for preventive dental care compared with the general population.

Medical history and/or drug use can impact oral health; however, we did not investigate those parameters and therefore cannot draw conclusions on that subject. However, we found that patients with gum swelling or bleeding were much more likely to be in the group of those not receiving routine dental check-ups. This finding suggests that gingival inflammation caused by other illnesses and/or drug use might not lead to routine dental visits.

The percentage of subjects receiving regular dental checkups was 34.0% in this study. According to the National Health and Nutrition Survey of 2012, 47.8% of adults and 55.3% of individuals in their sixties had received a dental check-up in the past year^[16]. A survey in 2010 in Tokushima, the same prefecture in which the present study was carried out, reported those percentages to be 43.6% and 51.0%, respectively^[17]. Therefore, the percentage of dialysis patients who sought dental checkups in this study was lower than that of the general population.

In a previous study, we showed that most hemodialysis outpatients in Japan received dialysis treatment at a facility without a dental department^[2]. The present

Table 4 Distribution of subjects receiving *vs* not receiving dental checkups, according to study variable (χ^2 test)

Variable	Receive dental check-up		Not receive dental check-up		P
	n ¹	% ²	n ¹	% ²	
Sex					
Male	29	60.4	61	65.6	0.545
Female	19	39.6	32	34.4	
Employment					
Employed	15	31.3	34	36.6	0.502
Unemployed	33	68.8	59	63.4	
Referral to dental visit by medical practitioner					
Yes	4	8.3	2	2.2	0.102
No	44	91.7	91	97.8	
Possession of denture					
Yes	17	35.4	17	18.3	0.024
No/unused	31	64.6	66	71.0	
Gum swelling/bleeding					
Yes	4	8.3	25	26.9	0.007
No	44	91.7	68	73.1	
Consciousness of oral health management because of dialysis treatment					
Yes	18	37.5	15	16.1	0.005
No	30	62.5	78	83.9	
Having a primary dentist					
Yes	46	95.8	68	73.1	0.001
No	2	4.2	25	26.9	

¹n: Total number of subjects corresponding to each answer; ²%. The percentage of subjects who answered “receive a dental check-up” or “not receive a dental check-up”.

Table 5 Factors associated with receiving dental check-ups, according to binominal logistic regression analysis¹ (n = 141)

Variable	OR	95%CI	P-value
Consciousness of oral health management because of dialysis treatment	3.241	1.298-8.125	0.012
Number of teeth	2.361	1.060-5.258	0.035
Possession of denture	4.209	1.271-13.933	0.019
Having a primary dentist	6.138	1.279-29.456	0.023
Gum swelling/bleeding	5.831	1.659-20.499	0.006

¹Binominal logistic regression analysis was conducted using each of five variables as the dependent variable.

study included dialysis patients at facilities with and without dental departments. We found no difference between the facilities in the percentage of patients receiving dental check-ups. Few patients at either facility had been referred for a dental visit by their medical practitioner. Education on the importance of regular dental care is necessary for dialysis patients. Moreover, medical providers are expected to participate in promoting dental visits among dialysis patients.

In conclusion, recognition that oral health management is more important for dialysis patients than for the general population might increase regular dental visits in this population. We found that patients who received dental check-ups had fewer symptoms of gum swelling or bleeding, suggesting that periodic dental visits could be effective in preventing an inflammatory response.

Medical providers should participate in promoting dental visits among dialysis patients.

COMMENTS

Background

In Japan, a number of dialysis patients have been increasing yearly. Since dialysis patients have a high risk of dental caries and periodontitis, preventive dental care should be considered very important for this population. In this study, they tried to determine factors associated with dental visits and to determine barriers to preventive dental care among hemodialysis patients.

Research frontiers

Recently, several studies have reported that severe periodontitis can affect mortality in hemodialysis patients. Studies involving patients with chronic kidney disease (CKD) found that efficient initial periodontal therapy lowered serum levels of some inflammatory biomarkers in CKD patients. Therefore, oral health management towards dialysis patients gets attention. The research hotspot is to elucidate the factors associated with dental visits among hemodialysis patients in order to resolve the barriers for dental visits.

Innovations and breakthroughs

Recently, the close relationship between periodontal disease and systemic disease has been highlighted. Many studies describe the oral health conditions of hemodialysis patients. However, there are very few English language literatures sources concerning preventive dental visit among dialysis patients. The present study elucidated the barriers to visiting the dentist, which the authors must manage with first in order to promote a preventive dental care among dialysis patients.

Applications

The data in this study suggested that awareness of oral health management issues should be strengthened among not only dialysis patients but also medical providers. Furthermore, this study suggested that periodic dental visits could be effective in preventing an inflammatory response.

Terminology

"Preventive dental visits" means that patients visit dental clinic periodically without a specific problem. The purpose of preventive dental visit is often oral examination and professional mechanical tooth cleaning to maintain the favorable oral health condition. "Primary dentist" should offer preventive dental care to their patients in Japan, however, many patients only visit their primary dentist when they have a specific problem in their mouth.

Peer-review

Factors associated with regular dental visits among hemodialysis patients is an absorbing manuscript; the research design is well established and fulfills all the requirements for a clinical study. Besides, the conclusion emphasizes the importance of a multidisciplinary approach to hemodialysis patients attain healthy oral conditions.

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Observational Study

Immunofluorescence on paraffin embedded renal biopsies: Experience of a tertiary care center with review of literature

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Abstract

AIM

To describe the technique of immunofluorescence on paraffin embedded tissue sections and discuss the potential pitfalls with an in depth review of literature.

METHODS

Immunofluorescence is integral to diagnostic renal pathology. Immunofluorescence on paraffin embedded renal biopsies (IF-P) after enzyme treatment has been described in literature, however has not found widespread use in renal pathology laboratories. In our laboratory proteinase K digestion of paraffin embedded renal biopsy material was standardized and applied prospectively in cases where immunofluorescence on fresh frozen tissue was non contributory or not possible. Diagnostic utility was assessed and in a cohort of cases comparison of intensity of staining with routine immunofluorescence was performed.

RESULTS

Over the 5-year study period, of the 3141 renal biopsies received IF-P was performed on 246 cases (7.7%) and was interpretable with optimal digestion in 214 cases (6.8%). It was of diagnostic utility in the majority of cases, which predominantly included glomerular disease. Non-diagnostic IF-P was found in membranous nephropathy (2 of 11 cases), membranoproliferative glomerulonephritis (2 of 32 cases), lupus nephritis (1 of 25 cases), post infectious glomerulonephritis (1 of 11 cases) and chronic glomerulonephritis (3 of 8 cases). Comparing cases with both routine IF and IF-P, 35 of 37 showed either equal intensity or a minor difference in intensity of staining

(1+) for the diagnostic immunoglobulin/complement. Technically assessment of immunofluorescence on the paraffin embedded tissue was found to be easier with clearly observed morphology, however a false positive staining pattern was observed in under-digested tissue.

CONCLUSION

As a "salvage" technique, immunofluorescence on paraffin embedded renal biopsies is of great diagnostic utility, however not without pitfalls.

Key words: Immunofluorescence on paraffin section; Renal biopsy; Salvage technique; Enzymatic digestion; Proteinase K

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Core tip: Immunofluorescence on formalin fixed paraffin embedded tissue is a useful "salvage" technique for renal diagnostic pathology, in case of non-availability of representative fresh frozen tissue. This article describes the technique of immunofluorescence on paraffin embedded tissue sections, discusses the potential pitfalls with an in depth review of literature.

Singh G, Singh L, Ghosh R, Nath D, Dinda AK. Immunofluorescence on paraffin embedded renal biopsies: Experience of a tertiary care center with review of literature. *World J Nephrol* 2016; 5(5): 461-470 Available from: URL: <http://www.wjgnet.com/2220-6124/full/v5/i5/461.htm> DOI: <http://dx.doi.org/10.5527/wjn.v5.i5.461>

INTRODUCTION

Immunofluorescence (IF) is an indispensable technique for rendering an accurate diagnosis in renal pathology. Diseases such as IgA nephropathy (IgAN), C1q nephropathy (C1qN) and C3 glomerulopathy (C3G) cannot be diagnosed without IF. Direct immunofluorescence (DIF) on fresh frozen tissue (IF-F) is the most widely used IF technique. Not uncommonly, however IF-F is not satisfactory due to non-representative sampling (medulla) or is not possible due to unavailability of fresh unfixed tissue, such as in referral cases and archived tissue. This leads to incomplete diagnosis and suboptimal patient management. To overcome these hurdles a method of enzymatic digestion of formalin fixed paraffin embedded tissue was standardized and introduced in our laboratory in 2011.

Enzymatic digestion breaks the protein cross linkages formed during formalin fixation^[1] thereby exposing the antigenic immune complexes to staining with FITC (fluorescein isothiocyanate) labeled antibodies. Though this technique has been described in literature using different enzymes with the earliest report in 1976^[2], it is still not in widespread use in laboratories handling renal

biopsies.

We discuss our experience with this technique in day-to-day diagnostic renal pathology, its utility in reaching final diagnoses and compare it with usual IF-F where available. Technical and interpretation issues faced are described in detail, and may be helpful to any laboratory planning to introduce this technique.

MATERIALS AND METHODS

Standardization: In a case of diffuse proliferative lupus nephritis, proteinase K (Sigma Aldrich, United States) enzymatic digestion was standardized (at concentrations according to manufacturer's protocol) with variation in timing of exposure at room temperature. Results were compared for the adequacy of digestion and intensity of staining for FITC-IgG.

Selection of cases

IF-P was performed prospectively in cases where there was inadequate/non representative fresh frozen tissue, in referral blocks where fresh frozen tissue was not available and in cases where the renal pathologists wanted to confirm the findings of routine IF-F. The FITC labeled antibodies to be applied were dictated by light microscopic differential diagnoses in the case and included both full panel (IgA, IgG, IgM, C3, C1q, kappa and lambda) as well as limited panels.

Interpretation of immunofluorescence

In cases where there was optimal digestion and adequate material the IF-P results were evaluated by 2 renal pathologists (LS and GS) and semiquantitatively graded on a 0-3+ scale. In cases where IF-F was available for comparison, these were graded independently in a blinded manner and compared to the grading of IF-P results. All immunofluorescence images were digitally captured and archived.

RESULTS

Enzyme digestion with proteinase K was standardized and the protocol followed is described in Table 1. Standardization was performed at room temperature and slight variations in enzyme exposure depending on ambient temperature (ranging from 15 to 20 min) gave optimal digestion results. This obviated the need for maintaining slides at 37 °C in a water bath.

In the 5-year study period between March 2011 and May 2015, 3171 biopsies (both native and transplant) were received. IF-P was performed on a total of 246 cases (7.7%). The results could not be interpreted in 32 cases (13%) due to technical issues of under digestion (18 cases) and floating of tissue/inadequate tissue (14 cases).

Therefore in 214 cases with adequate tissue, optimal digestion was achieved. Optimal digestion was determined on each individual slide by observing the

Table 1 Protocol for immunofluorescence on paraffin embedded renal biopsies

Cut formalin fixed paraffin embedded tissue at 3-4 μ thickness on poly-L-Lysine coated slides
Deparaffinize and rehydrate tissue sections
Immerse in Tris EDTA pH 9 for 30 min at room temperature
Perform enzymatic digestion with proteinase K 1.25 mg/mL (Sigma Aldrich, United States) at room temperature for 15 min ¹
Stop digestion by immersing in Tris EDTA at 4 °C
Leave in Tris EDTA for 40 min at 4 °C
Rinse in PBS for 10 min
Apply FITC conjugated polyclonal rabbit antibodies directed against IgG (dilution 1:50), IgM (1:60), IgA (1:60), C3 (1:30), C1q (1:30), kappa (1:25), and lambda (1:40) (BIOSSB, Santa Barbara, CA, United States). Incubate for 2 h in a moist chamber in the dark
Rinse with PBS
Mount in glycerine
Examine slides under a dark field immunofluorescence microscope

¹Varied based on room temperature. PBS: Phosphate buffered saline; FITC: Fluorescein isothiocyanate.

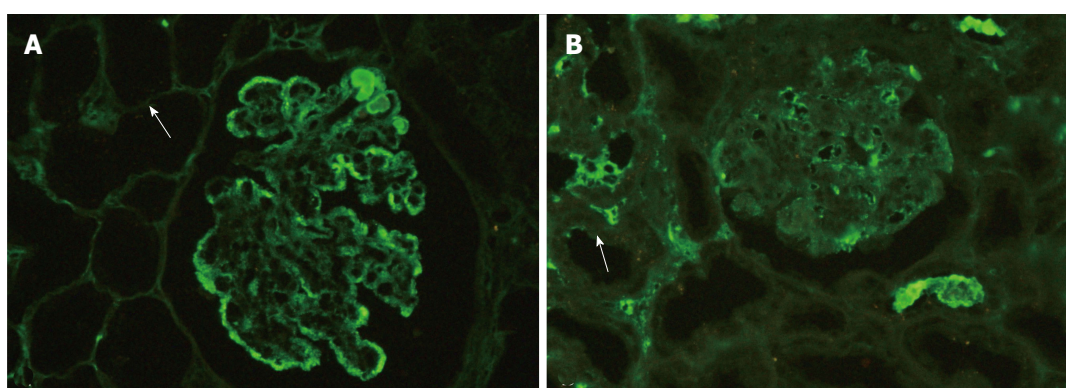


Figure 1 Examples of technically adequate and inadequate digestion. A: Immunofluorescence staining on a paraffin embedded tissue section in a case of diffuse proliferative lupus nephritis after enzymatic digestion with proteinase K. Note the adequate digestion evidenced by disappearance of tubular epithelial cells (arrow) (FITC IgG, $\times 200$); B: Immunofluorescence staining in a case with inadequate digestion with visible tubular epithelial cells (arrow). Note the antibody sticking to the surface of the capillary wall (FITC IgG, $\times 200$). FITC: Fluorescein isothiocyanate.

tubules. From experience, the disappearance of tubular epithelial cell outline with only visible tubular basement membranes correlated with optimal digestion and detection of immune complexes in the tissue (Figure 1A). In under-digested glomeruli, a non-specific staining pattern was observed (Figure 1B) with the antibody appearing to stick to the surface of the capillary walls in a blotchy manner rather than labeling immune complexes/complement with granularity. This staining pattern was recognized as false positive.

The major utility of this technique was in classifying glomerular diseases, with limited utility in tubulointerstitial diseases. Table 2 demonstrates the range of renal pathologies that were diagnosed.

In membranoproliferative glomerulonephritis (MPGN, 32 cases), 30 cases could be adequately diagnosed and sub-classified based on the results of IF-P into immune complex mediated (18 cases) and complement mediated (12 cases) MPGN. In two cases with intramembranous dense transformation of the glomerular basement membrane on electron microscopy (dense deposit disease) no significant C3 deposition was noted on IF-P. One of these cases had a comparative IF-F with the diagnostic C3 dominant pattern and intensity 2+ (0-3+ scale) (Figure 2). One case of IC-MPGN showed 1+ IgG, kappa

and lambda with 2+ C3 deposition, but characteristic MPGN type 1 pattern on ultrastructure examination; no comparative IF-F was available. The rest of the cases of MPGN showed at least 2+ intensity of diagnostic immunoglobulin/complement.

Diffuse proliferative glomerulonephritis (12 cases) were diagnosed as post infectious glomerulonephritis (PIGN) in 10 cases based on classical lumpy bumpy deposits of C3 and IgG. In one case with diffuse proliferative exudative pattern of injury and prior episode of febrile illness, a limited IF-P panel of IgA, IgG and C3 was applied to differentiate a PIGN from a proliferative IgA nephropathy. The intensity of IgG and C3 was only 1+, while IgA was negative. The IF-P results were deemed noncontributory in this case. Tissue for electron microscopy was not available.

Within the lupus nephritides (LN, 25 cases) localization of the deposits as mesangial and/or capillary wall aided in accurate classification of the glomerulonephritis (Table 2, Figure 3). The lack of deposits was also significant, as demonstrated by two cases of systemic lupus erythematosus (SLE) presenting with proteinuria and nonspecific light microscopic findings. Further electron microscopic examination confirmed a lupus podocytopathy. In one case of class II LN significant

Table 2 Renal pathologies diagnosed by immunofluorescence on paraffin embedded biopsies

Diagnosis	Total number of cases	Number of cases with non diagnostic IF-P (%)	Remarks
MPGN	32	2 ¹ (6.2%)	Classification into immune complex mediated MPGN (18 cases) and complement mediated MPGN (12 cases) was possible ¹ In two cases C3 was not demonstrated and electron microscopy showed features of dense deposit disease
Membranous nephropathy	11	2 ¹ (22.2%)	In one case staining intensity of IgG was only 1+, however staining pattern was classical ¹ In 2 cases significant fine granular immunofluorescence was not noted
Lupus nephritis	25	1 ¹ (4%)	Classification into Class II (2 cases), Class III /IV (15 cases) and Class V (5 cases) was possible Two cases of lupus podocytopathy were diagnosed ¹ In one case of lupus nephritis only IgM was demonstrated significantly, though electron dense deposits were noted on electron microscopy ¹ In one case only 1+ IgG and trace C3 deposition noted No tissue for electron microscopy was available
Diffuse proliferative glomerulonephritis - post infectious glomerulonephritis	12	1 ¹ (8.3%)	
Pauciimmunecrescentic glomerulonephritis	7	-	-
IgAN	39	-	In 64 cases (minimal change morphology, mesangial proliferation or FSGS), IgAN was excluded by IF-P In one case of diabetic nephropathy IF-P was used to exclude secondary IgAN
C1q nephropathy	2	-	-
Light chain deposition disease	1	-	Tubular basement membrane and vascular deposits were also noted in addition to the glomerular deposits
Amyloidosis	4	-	2 cases of AL amyloid (demonstrating light chain restriction) and 2 cases of AA amyloid
CGN	8	3 (37.5%)	The immune complexes could not be demonstrated in 3 cases of chronic glomerulonephritis, one of these was a case of biopsy proven MPGN and the other was a case of IgAN. In one case of CGN no immune complexes were seen, however no previous renal biopsy record was available
Cast nephropathy	2	-	One case also demonstrated light chain restriction
Tubulointerstitial nephritis	9	-	Associated immune complex mediated glomerular disease was excluded

¹Non diagnostic cases and details of their immunofluorescence pattern. MPGN: Membranoproliferative glomerulonephritis; CGN: Chronic glomerulonephritis; IgAN: IgA nephropathy; FSGS: Focal segmental glomerulosclerosis.

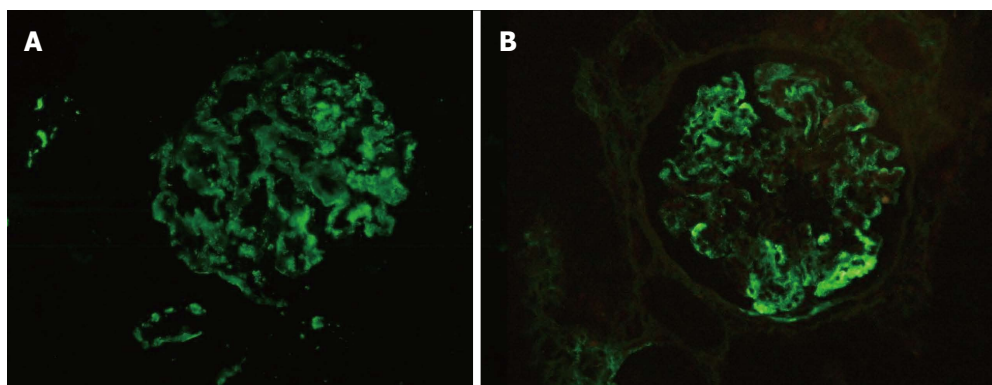


Figure 2 Comparable staining pattern on immunofluorescence on frozen and paraffin embedded tissue. A case of dense deposit disease showing bright C3c deposition 3+ (0-3+ scale) on IF-F (A, FITC C3c, × 200). Note the comparative coarse granular capillary wall staining of C3c (3+) on paraffin embedded tissue section after enzymatic retrieval (B, FITC C3c, × 200). FITC: Fluorescein isothiocyanate; IF-F: Immunofluorescence on fresh frozen tissue.

full house positivity could not be demonstrated. Only IgM showed 2+ mesangial staining and the rest of the immunoglobulins and complements were focal. The EM of this case however revealed numerous predominantly mesangial electron dense deposits along with few subepithelial and subendothelial deposits.

Of 11 cases of membranous nephropathy (MN, 11 cases) diagnostic immunofluorescence with IgG was noted in 8 cases. One case showed weak (1+) staining with characteristic fine granularity and two cases were negative for IgG.

To make the diagnosis of IgA nephropathy (39 cases,

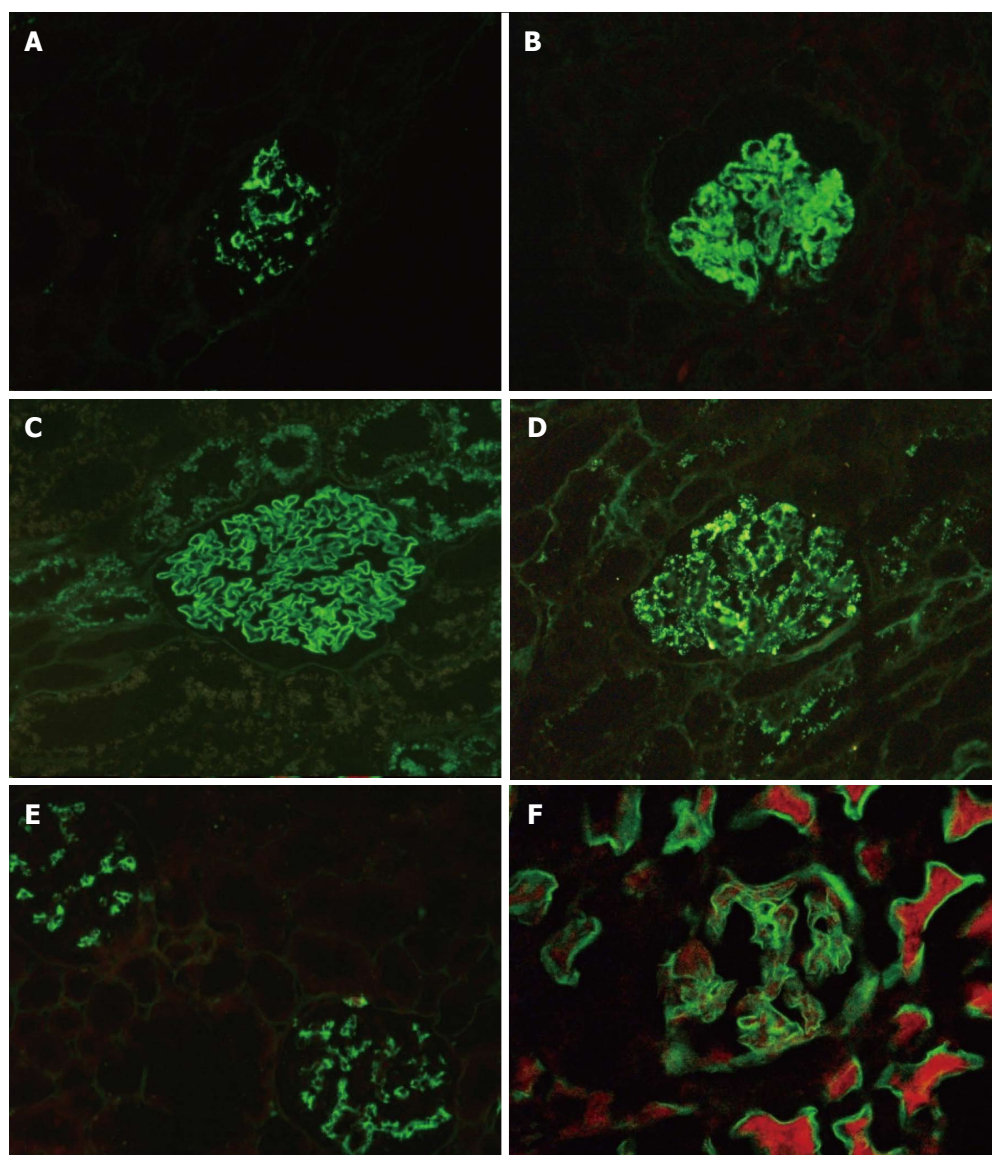


Figure 3 Glomerulonephritis diagnosed on immunofluorescence on paraffin. A: IgA nephropathy: There is predominantly mesangial deposition of IgA (FITC IgA $\times 100$); B: Class IV or diffuse lupus nephritis: Immunofluorescence reveals coarsely granular deposition of immunoglobulins in both mesangium and in the peripheral capillary wall (FITC IgG $\times 200$); C: Membranous nephropathy: Immunofluorescence reveals fine granular capillary wall deposition of IgG (C, FITC IgG $\times 200$); D: Post infectious glomerulonephritis: Garland pattern with elongated peripheral loop deposits is depicted, along with occasional mesangial deposits (D, FITC C3c, $\times 200$); E: C1q nephropathy with mesangial deposition of C1q (FITC C1q $\times 100$); F: Diabetic nephropathy with linear accentuation of glomerular capillary wall and tubular basement membrane (FITC IgG $\times 200$). FITC: Fluorescein isothiocyanate.

16%) or to exclude it in cases with minimal change morphology, mesangial proliferation or focal segmental glomerulosclerosis (64 cases, 26.3%) constituted the bulk of indication for IF-P in our routine practice. In two cases with isolated hematuria, suspected IgA nephropathy and nonspecific light microscopy, IF-P was negative for immunoglobulins which prompted ultrastructural examination of the cases. Classical glomerular basement membrane changes of collagenopathy consistent with Alport syndrome and thin basement membrane disease were identified. IF-P was also performed in patients of diabetic nephropathy with hematuria to exclude secondary IgAN.

In this series there were 8 cases which were diagnosed as chronic glomerulonephritis (CGN), the under-

lying etiology could be established in 5 cases (IgAN = 3, IC-MPGN = 1 and C-MPGN = 1). The immune complexes could not be demonstrated in 3 cases of chronic glomerulonephritis, one of which was a case of biopsy proven MPGN and the other was a case of IgAN. In one case no immune complexes were seen, however no previous renal biopsy record was available.

In one case of post transplant recurrence of nodular glomerulosclerosis of undetermined cause, IF-P resulted in confirming the diagnosis of light chain deposition disease (LCDD) with kappa restriction^[3]. Deposits were identified in the glomerular nodules, tubular basement membranes, arterioles and arteries (Figure 4A and B). Primary amyloidosis was identified in 2 cases demonstrating light chain restriction (Figure 4C and D). Light chains were

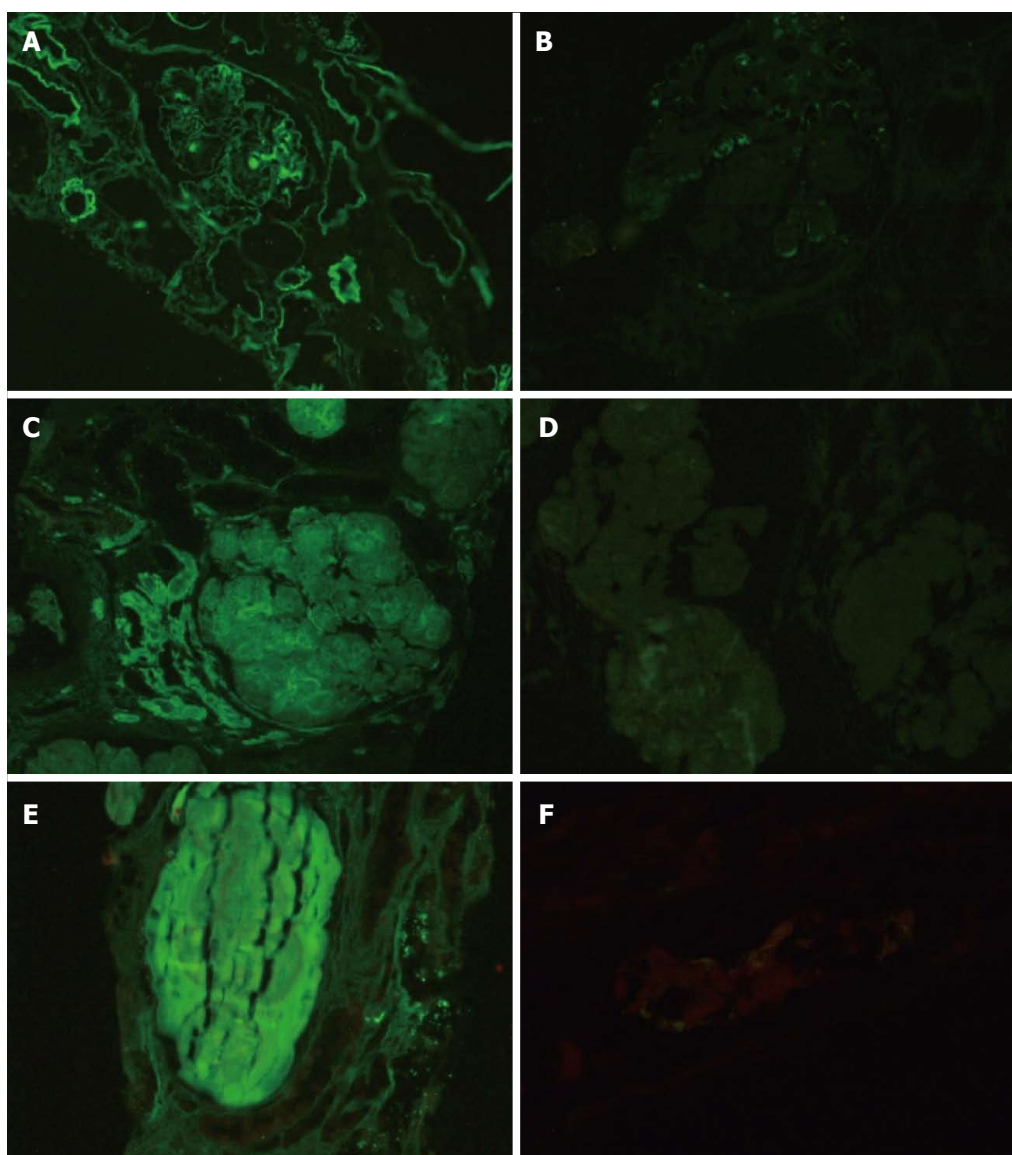


Figure 4 Immunofluorescence on paraffin to demonstrate monoclonal deposits. A case of light chain deposition disease with kappa light chain restriction. There is nodular mesangial, capillary wall and tubular basement membrane deposition of kappa light chain (A, FITC kappa, $\times 100$) while no deposition of lambda is noted (B, FITC lambda, $\times 200$); C: A case of primary amyloidosis with lambda light chain restriction. The lambda deposition is noted in the mesangium (FITC lambda $\times 200$); D: There is no deposition of kappa (D, FITC kappa $\times 200$); E: A case of cast nephropathy with kappa light chain restriction. Note the brightly positive casts for kappa (FITC kappa $\times 200$) with no traces of lambda (F, FITC lambda $\times 200$). FITC: Fluorescein isothiocyanate.

also identified in tubular casts, confirming the diagnosis of cast nephropathy in two cases. One of these cases demonstrated light chain restriction (Figure 4E and F). Other than suspected cast nephropathy, IF-P was performed in cases of primary tubulointerstitial disease with significant proteinuria or hematuria to exclude concomitant glomerular disease.

Comparison between immunofluorescence on frozen and paraffin embedded tissue

Comparative IF-F and IF-P was available in 37 cases. Thirty-five of these cases (93.8%) had either equal intensity or a minor difference in intensity of staining (1+) for the diagnostic immunoglobulin/complement. Significant difference was observed in just 2 cases; a

case of C-MPGN and a case of MN (Table 3).

Technically assessment of immunofluorescence on the paraffin embedded tissue was found to be less challenging than IF-F with clearly observed morphology and ease of comparison with light microscopic findings.

DISCUSSION

IF studies are integral to diagnostic renal pathology and renal pathologists are often left frustrated by a lack of representative tissue in material sent for routine IF. The technique of IF-F is well established however requires a separate representative core of kidney tissue, a cryostat and technical expertise for satisfactory results. Descriptions of enzyme treatment of formalin fixed

Table 3 Comparison of immunofluorescence intensity on fresh frozen and paraffin embedded renal biopsies

Disease	Number of cases with no difference in intensity of diagnostic immunoglobulin/complement (%) IF-F = IF-P	Number of cases with difference in intensity of diagnostic immunoglobulin/complement (%) IF-F > IF-P		Total number of cases
		Difference of 1 +	Difference of 2 +	
IgA nephropathy	7 (78%)	2 (22%)	-	9
C-MPGN	1 (25%)	2 (50%)	1 (25%)	4
IC-MPGN	4 (100%)	-	-	4
Lupus nephritis	3 (50%)	3 (50%)	-	6
C1q nephropathy	2 (100%)	-	-	2
Membranous nephropathy	3 (43%)	3 (43%)	1 (14%)	7
Post infectious glomerulonephritis	1 (100%)	-	-	1

IF-F: Immunofluorescence on fresh frozen tissue; IF-P: Immunofluorescence on paraffin embedded tissue; C-MPGN: Complement mediated membranoproliferative glomerulonephritis; IC-MPGN: Immune complex mediated membranoproliferative glomerulonephritis.

paraffin embedded (FFPE) tissue followed by IF studies (IF-P) can be found in literature from as early as 1976, however the technique has still not found a place in most renal pathology laboratories^[2].

The use of a cross linking fixative like formaldehyde leads to masking of antigens. In addition calcium and other divalent ions form complexes with proteins during fixation and these complexes can block the antigenic determinants^[1]. It is to unmask these determinants that enzyme treatment of FFPE tissue is necessary before applying antibodies. In our laboratory the technique of IF-P was standardized using proteinase K and it was applied prospectively as a "salvage" technique with good results.

Proteinase K is an enzyme that exhibits broad substrate specificity. It is isolated from a fungus, *Engyodontium album* (formerly *Tritirachium album*) and is able to digest keratin hence the name proteinase "K"^[4]. Different proteolytic enzymes including pronase, trypsin and pepsin have been tried in various studies as demonstrated in Table 4^[5-15].

As evident from Table 4 multiple studies comparing IF-P and IF-F have clearly established that IF-P is a feasible and valuable "salvage" technique. Comparable staining intensities have been demonstrated for immunoglobulins, with albeit lower sensitivity for detection of complement.

In an early study by Fogazzi *et al*^[8] paraffin embedded sections were treated with pronase (0.75 g/L for 60 min) and the fluorescence intensity and location was compared with frozen sections in cases of IgAN ($n = 10$), membranous nephropathy ($n = 8$) and proliferative lupus nephritis ($n = 10$). The diagnostic immunoglobulins were detected with equal or increased intensity in 100% cases with a slightly reduced immunoreactivity for C3 in enzyme treated tissue. Structural details were better assessed in terms of location and morphology of deposits. On retrospective digestion of 1 and 2 year old blocks identical staining patterns were obtained in approximately 86% of cases.

Using a similar protocol as Fogazzi *et al*^[8], Nasr *et al*^[10] compared IF-F and IF-P in 71 renal biopsies including a spectrum of renal diseases. In glomerular

diseases diagnostic findings were obtained in 100% of cases of lupus nephritis, acute post-infectious glomerulonephritis, cryoglobulinemic glomerulonephritis, fibrillary glomerulonephritis, primary amyloidosis, 88% of cases of IgAN, 80% cases of LCDD, 60% of cases of MPGN type 1, 50% cases of idiopathic MN and 20% of cases of anti-glomerular basement membrane (anti-GBM) disease. In all disease categories studied IF-P was less sensitive than IF-F for the detection of C3 similar to Fogazzi *et al*^[8]. In addition they found reduced sensitivity for the detection of IgG in cases of MN (50%) and anti-GBM (20%) disease. They also demonstrated utility of the technique in tubulointerstitial diseases such as myeloma cast nephropathy and light chain proximal tubulopathy and found IF-P satisfactory in demonstrating light chain restriction.

More recently Messias *et al*^[15] studied paraffin immunofluorescence in 304 native renal biopsies. The false positive staining on the surface and within capillary lumina attributed to sera adsorption secondary to fixation by the authors was also recognized in our cases and was more pronounced in under-digested tissue. They described a novel utility of the technique in evaluating masked paraprotein and immune complex deposits. The light chain crystals in light chain proximal tubulopathy were only demonstrated after enzyme digestion. Out of 61 cases where IF-P was performed to unmask immunoglobulins, it was helpful in 20 cases which included 9 cases of membranous like glomerulopathy with masked IgG-kappa deposits (MGMIDK) a novel entity first described by Larsen *et al*^[16], 4 cases of MPGN with light chain restriction and 7 cases of MPGN with mixed essential cryoglobulinemia, which would have been misdiagnosed as C3 glomerulopathy. They recommended that all cases of C3 glomerulopathy based on routine immunofluorescence should be subjected to paraffin immunofluorescence to reach the correct diagnosis and avoid unnecessary investigations into complement abnormalities. In addition any case where the routine immunofluorescence findings do not match the ultrastructural findings should undergo paraffin immunofluorescence. However the authors reiterated, and we concur that IF on paraffin embedded

Table 4 Studies using the technique of immunofluorescence on enzyme digested paraffin embedded tissue in literature

Ref.	Year	Enzyme used	Cases (n)	IF panel applied	Significant results
[2]	1976	Trypsin for 120 min	NA	Immunoglobulins and complement	Feasible to demonstrate immunoglobulins but not complement Reduced background immunofluorescence
[5]	1979	Trypsin	52 renal biopsies	IgG, IgA, IgM, C3, Fibrinogen	Accurate detection of immunoglobulins (90%) and complement (75%) in comparison with IF on frozen
[6]	1980	Trypsin	21 (LN, MN, IgAN)	IgG, IgM, IgA	IF on trypsin-digested tissue was as sensitive as IF-F for immunoglobulins but less sensitive for complement
[7]	1980	Pepsin (0.4%) and trypsin	Experimental mice model of anti GBM disease	IgG	Pepsin +/- trypsin digestion better than trypsin alone Enzyme digested tissue showed trivial decrease in sensitivity but good preservation in comparison with IF on frozen
[8]	1989	Pronase (0.75 g/L for 60 min at 37 °C)	IgAN (10), MN (8), Proliferative LN (10)	IgG, IgA, IgM, C3, C1q	Correct diagnosis possible in all cases Better structural details and less fading of IF Lower intensity staining for C3 Retrospectively performed digestion on 1 and 2 yr old blocks, satisfactory in 86% cases
[9]	2005	Microwave treatment (10 min) followed by Protease VII (0.05% for 30/60 min) Trypsin (0.25% for 120 min)	IgAN (7), LN (7), MN (7), MPGN (3)	IgG, IgA, IgM, C3	Microwave treatment followed by protease digestion better than trypsin digestion Diagnostic immunoglobulin found in more than 80% cases
[10]	2006	Pronase (0.75 g/L for 60 min at 37 °C)	MN (8), MPGN (5), LN (5), PIGN (5), IgAN (8), Cryo GN (5), Fibrillary GN (5), Anti GBM (5), Cast nephropathy (5), Amyloid (5), LCDD (5), LCFS (10)	IgG, IgA, IgM, C3, C1q, kappa and lambda	Diagnostic utility in 83% cases Useful in dysproteinemia related renal disease particularly LCFS Less sensitive for staining with C3 in MPGN type I, Cryo GN, PIGN
[11]	2007	Proteinase XXIV	LN (5), antiGBM (5), MN (9)	NA	Less sensitive for IgG in MGN and anti-GBM disease IF-P on proteinase XXIV is more sensitive than IF-P with pronase In LN, better intensity staining for C1q and IgG In anti GBM, 80% sensitivity for detection of IgG In MGN, 55% sensitivity for detection of IgG
[12]	2009	Microwave treatment and/or Proteinase K - (30 or 60 min)	IgAN (24), MN (22), LN (24)	IgG, IgA, IgM, C3	Rate of agreement between immunofluorescence on paraffin sections and immunofluorescence on frozen sections with respect to the presence of IgA was 56.5%, IgM - 44.4%, IgG - 73.9%, and C3 - 51.5% IF-P may be used as a salvage technique when frozen tissue is not available
[13]	2011*	Trypsin (30 min), Pepsin	IgAN (20), MN (25)	IgA, IgG, HBsAg, HbcAg	Trypsin digestion better than pepsin digestion IF-P slightly weaker signal than IF-F
[14]	2012	Heat - Tris/Citrate buffer Pronase RTU (60 min at 37 °C)	LN (15), MN (11), IgMN (10), MPGN (2), IgAN (2)	IgG, IgA, IgM, C3, C1q	Heat based retrieval using Tris buffer showed superior results Pronase digestion shows less sensitivity for detection of immunoglobulins and complement
[15]	2015	Proteinase K for 20 min	304 cases (207 cases as salvage and 97 cases for antigen unmasking)	IgG, IgA, IgM, C3, C4, C1q, fibrinogen, kappa and lambda	Not only a good salvage technique but prevents misdiagnosis due to masked immune complex or light chain deposition

LN: Lupus nephritis; MN: Membranous nephropathy; IgAN: IgA nephropathy; IgMN: IgM nephropathy; MPGN: Membranoproliferative glomerulonephritis; anti GBM: Anti glomerular basement membrane nephritis; PIGN: Post infectious glomerulonephritis; Cryo GN: Cryoglobulinemic glomerulonephritis; LCDD: Light chain deposition disease; LCFS: Light chain fanconi syndrome; RTU: Ready to use; HBsAg: Hepatitis B surface antigen; HbcAg: Hepatitis B core antigen; IF: Immunofluorescence.

tissue cannot supplant routine IF-F in renal biopsy interpretation.

In the present series we found comparable results for staining with IF-F and IF-P. As described in other studies in a few cases expected immunofluorescence results were not obtained by IF-P, including two cases of membranous nephropathy, two cases of dense deposit disease, one case of lupus nephritis, one case

of suspected PIGN and three cases of chronic glomerulonephritis; even in the presence of optimal enzyme digestion. Most of these cases (except PIGN and CGN) had electron microscopic confirmation of presence of electron dense deposits, thus they were truly false negative results. We opine that this variability may be a result of differences in time of exposure of the renal biopsy to formalin, making the unmasking of antigenic

determinants more difficult. This of course becomes a limitation of IF-P in a “salvage” scenario, as a negative result in the presence of optimal digestion would always be questionable; however a positive result will always aid in the diagnosis^[3,17].

Nonetheless in the majority of cases undergoing routine fixation and processing, IF-P was successful in providing immunofluorescence results which added to the final diagnosis. Based on our results, we also now offer this technique for skin biopsies and for amyloid characterization in extra renal sites.

Based on the experience in our laboratory, we conclude that immunofluorescence on formalin fixed paraffin embedded tissue is a useful “salvage” technique in case of non-availability of representative fresh frozen tissue; however it is not without pitfalls. Technically assessment of enzyme digestion on each slide is mandatory for accurate interpretation of staining. Antibody staining of under digested tissue can result in both false positive as well as false negative results. Even with optimal digestion expected immunofluorescence results are sometimes not obtained and there is a yet unexplained reduced sensitivity for complement as demonstrated in multiple studies; all of which may result in a misdiagnosis. The extra slices of the renal biopsy taken for IF-P from the paraffin block apart from the routine stains result in insufficient tissue remaining in the block for any further staining or review. Within these limitations, we have demonstrated a significant diagnostic utility of this technique particularly in glomerular diseases and continue to offer it as a “salvage” option.

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COMMENTS

Background

Immunofluorescence (IF) is an indispensable technique for rendering an accurate diagnosis in renal pathology. Diseases such as IgA nephropathy, C1q nephropathy and C3 glomerulopathy cannot be diagnosed without IF. Direct IF on fresh frozen tissue (IF-F) is the most widely used IF technique.

Research frontiers

IF on paraffin embedded renal biopsies after enzyme treatment has not found widespread use in renal pathology laboratories. This leads to incomplete diagnosis and suboptimal patient management.

Innovations and breakthroughs

To overcome the hurdles above, a method of enzymatic digestion of formalin fixed paraffin embedded tissue was standardized and introduced in the authors' laboratory in 2011.

Applications

The authors discussed their experience with this technique in day-to-day diagnostic renal pathology, its utility in reaching final diagnoses and comparing it with usual IF-F where available. Technical and interpretation issues faced are

described in detail, and may be helpful to any laboratory planning to introduce this technique.

Peer-review

It is an interesting paper that could be very useful for pathologists or nephrologists involved in renal pathology.

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Prospective Study

What is the optimal level of vitamin D in non-dialysis chronic kidney disease population?

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drafted the manuscript, designed the research and supervised the study; Molina P and Molina MD performed statistical analysis; Beltrán S, Vizcaíno B, Escudero V, Kanter J, Ávila AI, Bover J, Fernández E, Nieto J, Cigarrán S, Gruss E, Fernández-Juárez G, Martínez-Castelao A and Navarro-González JF were involved with data collection, and assisted with data analysis; all authors read and approved the final manuscript.

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Abstract

AIM

To evaluate thresholds for serum 25(OH)D concentrations in relation to death, kidney progression and hospitalization in non-dialysis chronic kidney disease (CKD) population.

METHODS

Four hundred and seventy non-dialysis 3-5 stage CKD patients participating in OSERCE-2 study, a prospective, multicenter, cohort study, were prospectively evaluated and categorized into 3 groups according to 25(OH)D levels at enrollment (less than 20 ng/mL, between 20 and 29 ng/mL, and at or above 30 ng/mL), considering 25(OH)D between 20 and 29 ng/mL as reference group. Association between 25(OH)D levels and death (primary outcome), and time to first hospitalization and renal progression (secondary outcomes) over a 3-year follow-up, were assessed by Kaplan-Meier survival curves and Cox-proportional hazard models. To identify 25(OH)D levels at highest risk for outcomes, receiver operating characteristic (ROC) curves were performed.

RESULTS

Over 29 ± 12 mo of follow-up, 46 (10%) patients dead, 156 (33%) showed kidney progression, and 126 (27%) were hospitalized. After multivariate adjustment, 25(OH)D < 20 ng/mL was an independent predictor of all-cause mortality (HR = 2.33; 95%CI: 1.10-4.91; $P = 0.027$) and kidney progression (HR = 2.46; 95%CI: 1.63-3.71; $P < 0.001$), whereas the group with 25(OH)D at or above 30 ng/mL did not have a different hazard for outcomes from the reference group. Hospitalization outcomes were predicted by 25(OH) levels (HR = 0.98; 95%CI: 0.96-1.00; $P = 0.027$) in the unadjusted Cox proportional hazards model, but not after multivariate adjusting. ROC curves identified 25(OH)D levels at highest risk for death, kidney progression, and hospitalization, at 17.4 ng/mL [area under the curve (AUC) = 0.60; 95%CI: 0.685-0.69; $P = 0.027$], 18.6 ng/mL (AUC = 0.65; 95%CI: 0.60-0.71; $P < 0.001$), and 19.0 ng/mL (AUC = 0.56; 95%CI: 0.50-0.62; $P = 0.048$), respectively.

CONCLUSION

25(OH)D < 20 ng/mL was an independent predictor of death and progression in patients with stage 3-5 CKD, with no additional benefits when patients reached the levels at or above 30 ng/mL suggested as optimal by CKD guidelines.

Key words: Vitamin D; Chronic kidney disease; Mortality; Renal progression; Hospitalization

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Core tip: This study examines the prognosis value of 25(OH)D levels on death, chronic kidney disease (CKD) progression, and hospitalization in a cohort of 3-5 stage CKD subjects not on dialysis. The main findings were the predictor value of vitamin D deficiency (< 20 ng/mL), but not insufficiency (< 30 ng/mL), for the 3-year incidence of death and CKD progression, which remained significant after multivariate adjustments. These results could highlight the need for a revision of the current guidelines, which have defined optimal vitamin D status at ≥ 30 ng/mL based on levels required to suppress parathyroid hormone, as opposed to our study, which evaluates thresholds for serum 25(OH)D concentrations in relation to "hard" endpoints.

Molina P, Górriz JL, Molina MD, Beltrán S, Vizcaino B, Escudero V, Kanter J, Ávila AI, Bover J, Fernández E, Nieto J, Cigarrán S, Gruss E, Fernández-Juárez G, Martínez-Castelao A, Navarro-González JF, Romero R, Pallardó LM. What is the optimal level of vitamin D in non-dialysis chronic kidney disease population? *World J Nephrol* 2016; 5(5): 471-481 Available from: URL: <http://www.wjgnet.com/2220-6124/full/v5/i5/471.htm> DOI: <http://dx.doi.org/10.5527/wjn.v5.i5.471>

INTRODUCTION

There is a high prevalence of vitamin D (VD) deficiency in all stages of chronic kidney disease (CKD)^[1-5]. Observational studies in this population have shown that VD levels correlated with cardiovascular disease and markers of renal injury, including albuminuria^[1,6], renal progression^[4,6-8], vascular calcification^[9,10], left ventricular hypertrophy^[9] and mortality^[8,11-13]. Moreover, growing evidence supports a potential role for VD receptor activation in suppressing the renin-angiotensin system, reducing proteinuria and ameliorating kidney dysfunction^[14-16], showing 25-hydroxyvitamin D [25(OH)D] as an attractive, cheap and feasible treatment target^[17]. As a result of these findings, current guidelines have suggested VD supplementation in CKD patients^[18-21], increasing VD supplementation rates among this population^[22].

Nevertheless, these recommendations are opinion based and the optimal VD levels as well as the upper safe limit of VD intakes remains controversial^[23,24]. Based on the inverse relationship between serum concentrations of 25(OH)D and parathyroid hormone (PTH), most current guidelines have defined VD deficiency and insufficiency, as a serum 25(OH)D level of < 20 ng/mL (50 nmol/L) and 20-29 ng/mL (52-72 nmol/L) respectively^[18,19], suggesting a serum concentration of 25(OH)D above 30-40 ng/mL

(75–100 nmol/L) to be desirable, levels at which PTH is suppressed to a minimum in its relation to 25(OH)D^[25,26]. By contrast, the Institute of Medicine advocates VD repletion as a level of 20 ng/mL^[27]. Determining the 25(OH)D target level for optimal health is especially important in CKD population, where overuse of VD leads to hypercalcemia, hypercalciuria and hyperphosphatemia, which could predispose to vascular calcification, nephrolithiasis and reduced glomerular filtration rate^[28–30]. All these data suggest an optimal level of VD exists that is neither too high nor too low^[31].

Aware of the lack of evidence behind guidelines recommendations, and our concerns about VD over-supplementation, encouraged us to investigate the optimal VD status in non-dialysis CKD patients. The aim of our study was to evaluate thresholds for serum 25(OH)D concentrations in relation to hard end-points such as death, kidney progression and hospitalization in this population.

MATERIALS AND METHODS

Study design and patient selection

OSERCE-2 was a 3-year follow-up prospective, observational, study which enrolled 742 adults with 3 to 5-stage CKD not on dialysis subjects attending 39 centres in Spain, to evaluate the effects of vascular calcifications and CKD-mineral bone disorders on mortality, hospitalization and kidney progression^[32]. Inclusion criteria were age ≥ 18 years and CKD Stages 3–5. Exclusion criteria were acute kidney injury, transplantation, hospitalization in the month previous to the enrollment, and severe comorbidity. In this post-hoc analysis of the OSERCE-2 study, patients on current treatment with active VD (calcitriol, α -calcidol or paricalcitol) were also excluded, so 25(OH)D levels reflected the effect of the exposure to VD.

The study was reviewed and approved by the Dr Peset Hospital Research Ethics Committee. All study participants provided informed written consent prior to study enrollment.

Study protocol and baseline data

The study protocol of the OSERCE-2 study has been previously reported^[32]. All patients were assessed at baseline for blood pressure measurement, lateral lumbar, pelvis and hands X-ray, an ankle brachial pressure index (ABPI) determination and laboratory blood sampling. All blood samples were analyzed in a central laboratory, including 25(OH)D, 1,25(OH)₂ vitamin D, creatinine, calcium, phosphorus, intact PTH, albumin, and high-sensitive C-reactive protein. 25(OH)D levels were assessed by radioimmunoassay (Biosource), which were transformed to the usual method of reference (DiaSorin Liaison chemiluminescent radioimmunoassay) for improving the comparability of the results, as previously described^[32]. To study the renal progression, blood samples for determination of serum creatinine levels were obtained

every 12 mo. Estimated glomerular filtration rate (eGFR) was calculated using the modification of diet in renal disease (MDRD) formula^[33].

Outcomes

Deaths episodes (primary outcome), time to first hospital admission and the appearance of a combined renal end-point, defined as a drop $> 30\%$ in eGFR, or beginning of renal replacement therapy (secondary outcomes), were prospectively gathered over a 3-year period^[32].

Statistics analysis

Summary statistics were reported as frequencies or percentages, and as mean \pm SD, for categorical and quantitative variables, respectively. Skewed quantitative variables were expressed as geometric mean (95%CI), after log transformation. Presence or absence of prominent calcification for Adragao (AS) and Kaupila scores (KS) was reported as $AS \geq 3$ and $KS > 6$, respectively.

Patients were classified further into 3 groups by 25(OH)D level: < 20 ng/mL (deficiency), 20–29 ng/mL (insufficiency) and ≥ 30 ng/mL. Comparison of baseline characteristics in these 3 groups was assessed using one-way analysis of variance (ANOVA) for continuous variables, and χ^2 test for trend, for categorical variables. Analysis of variables independently related to 25(OH)D levels was assessed by lineal regression model. To assess the relationship between the odds of VD deficiency and clinical and laboratory baseline characteristics, a stepwise binary logistic regression was performed between 25(OH)D level < 20 or ≥ 20 ng/mL as dependent variables. PTH and 1,25(OH)₂D levels were considered as posterior variables to 25(OH)D levels and then they were not introduced in the models, to avoid an overadjustment bias. Twenty-four hours urine proteinuria was not included either because it was available in only 50% of the patients.

Kaplan-Meier analysis and log-rank tests were used to estimate the effects of VD status on all-cause mortality, appearance of the composite renal end-point, and hospitalizations. We then used univariate and multivariate Cox proportional hazard regression models to determine the association of VD levels with various pre-specified outcomes. Patients with 25(OH)D levels between 20 to 29 ng/mL were considered as reference group. Covariates significantly associated in the univariate analysis were entered (forward selection: Likelihood ratio) into the models. The relatively small number of deaths limited the list of adjustment variables that were included in the regression analyses. To identify VD levels at highest risk for outcomes, we performed a receiver operating characteristic (ROC) curve. The value associated with the highest accuracy was considered as the cut-off point for defining an increased risk of death, appearance of the composite renal endpoint, and hospitalization.

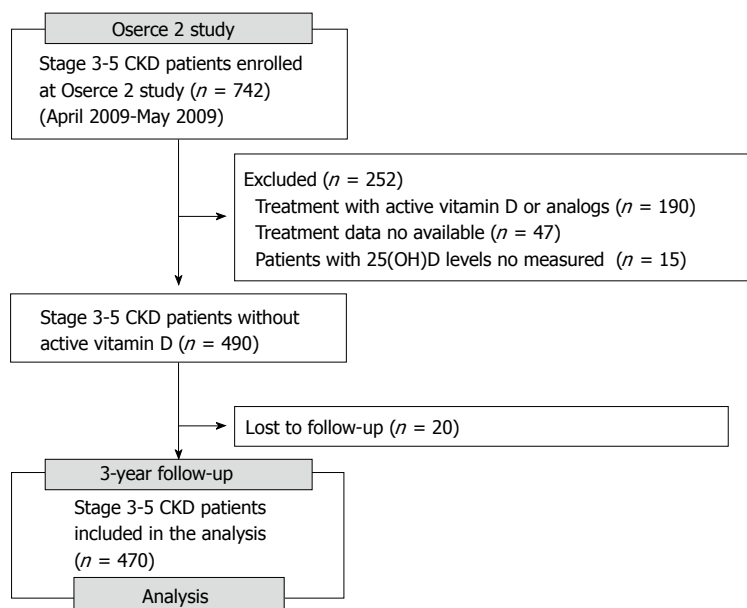


Figure 1 Flow diagram of patient selection for analysis. CKD: Chronic kidney disease; 25(OH)D: 25-hydroxyvitamin D.

The literature indicates that annual mortality in patients with stage 3 to 5 CKD (not on dialysis), is between 3% and 9%. Previous studies have shown a 35% prevalence of VD deficiency in this population^[3]. Compared with the group with VD deficiency, the group with VD insufficiency shows a 57% decrease in mortality^[8]. With 470 patients included, a minimum follow-up of three years, and considering an error of $\alpha = 0.05$, the power estimation of the study is 0.754. The statistical methods of this study were reviewed by MD Molina, from the Department of Mathematics, Universidad de Alicante, Spain, who was included as a co-author. All data analyses were conducted using SPSS, version 15.0 (SPSS Inc., Chicago, IL). A *P*-value < 0.05 was considered statistically significant.

RESULTS

Baseline data

From the 742 subjects enrolled at OSERCE-2 Study, 252 were excluded and 20 were lost to follow-up, leaving 470 patients in the final analysis (Figure 1). Tables 1 and 2 show the patient characteristics and laboratory values, respectively, as a function of vitamin D status. According to 25(OH)D levels, the proportion of patients with deficiency or insufficiency was 53% and 33%, respectively. At baseline, the proportion of patients with 5-stage CKD, diabetes mellitus, diabetic nephropathy and chronic heart failure was higher in the group with less 25(OH)D levels. ABPI, eGFR, PTH, 1,25(OH)₂ vitamin D and albumin levels were increased in groups with better VD status, which showed lower degree of proteinuria. The group with 25(OH)D less than 20 ng/mL was prescribed more frequently treatment with diuretics and erythropoietin-stimulating agents, with a lower proportion of patients under native VD treatment.

Relationship between 25(OH)D levels and baseline characteristics

Linear correlation analysis showed significant correlation between 25(OH)D levels and eGFR ($R = 0.10$; $P = 0.027$), body mass index ($R = -0.10$; $P = 0.046$), serum levels of albumin ($R = 0.10$; $P = 0.027$), calcium ($R = 0.12$; $P = 0.013$), 1,25(OH)₂ vitamin D ($R = 0.20$; $P < 0.001$), PTH ($R = -0.26$; $P < 0.001$), and hemoglobin ($R = 0.13$; $P = 0.005$), proteinuria (log transformed, $R = -0.19$; $P = 0.004$) and ABPI ($R = 0.15$; $P = 0.002$). Multivariate binary logistic regression analysis showed as independent predictors of 25(OH) < 20 ng/mL the albumin levels (OR = 0.61; 95%CI: 0.40-0.92; $P = 0.018$), the ABPI (OR = 0.28; 95%CI: 0.11-0.73; $P = 0.010$), and treatment with native VD (OR = 0.35; 95%CI: 0.17-0.73; $P = 0.005$), and diuretics (OR = 2.03; 95%CI: 1.35-3.06; $P = 0.001$).

Mortality

Forty-six (10%) patients died after a mean follow-up of 29 ± 12 mo. Cardiovascular disease ($n = 16$, 35%) and infections ($n = 8$, 17%) were the most common causes of death. Tumors and others accounted for 11% ($n = 5$) and 13% ($n = 6$) of deaths, respectively. In 11 cases (24%) the cause of death was not identified. The Kaplan-Meier survival analysis (Figure 2A) suggested that patients with 25(OH)D less than 20 ng/mL had significantly higher mortality than the other two groups (log rank test, $P = 0.031$). Univariate Cox regression found a more than twice higher risk of death in the group with the 25(OH)D level less than 20 ng/mL compared with the reference group (HR = 2.47; 95%CI: 1.18-5.18; $P = 0.017$), whereas the group with 25(OH)D at or above 30 ng/mL was not significantly different from that with the 25(OH)D between 20 to 29 ng/mL (HR = 0.78; 95%CI: 0.26-2.32; $P = 0.650$). Multivariate analysis

Table 1 Baseline patient characteristics (*n* = 470), as a function of vitamin D status

	All	25(OH)D < 20 ng/mL	25(OH)D 20-29 ng/mL	25(OH)D ≥ 30 ng/mL	<i>P</i>
<i>n</i>	470	252 (53%)	154 (33%)	64 (14%)	
Age (yr)	66.1 ± 12.9	65.8 ± 13.1	65.9 ± 11.9	68.1 ± 12.1	0.421
Male sex (%)	309 (66%)	162 (64%)	101 (66%)	46 (72%)	0.303
High blood pressure (%)	444 (95%)	242 (96%)	144 (94%)	58 (91%)	0.072
Dyslipidemia (%)	311 (66%)	168 (68%)	101 (66%)	42 (66%)	0.646
Diabetes mellitus (%)	183 (39%)	114 (45%)	53 (34%)	16 (25%)	0.001
Ischemic heart disease (%)	104 (22%)	60 (24%)	33 (22%)	11 (17%)	0.224
Chronic heart failure (%)	43 (9%)	33 (13%)	7 (5%)	3 (5%)	0.005
Stroke (%)	52 (11%)	30 (12%)	15 (10%)	7 (11%)	0.668
Peripheral arterial disease (%)	93 (20%)	59 (24%)	22 (14%)	12 (19%)	0.117
Stage of CKD (%)					
3 (eGFR = 30-59 mL/min per 1.73 m ²)	221 (47%)	103 (41%)	84 (54%)	34 (53%)	0.002
4 (eGFR = 15-29 mL/min per 1.73 m ²)	205 (44%)	105 (46%)	64 (42%)	26 (41%)	
5 (eGFR < 15 mL/min per 1.73 m ²)	44 (9%)	34 (13%)	6 (4%)	4 (6%)	
Etiology of CKD (%)					
Hypertension	108 (23%)	54 (21%)	40 (26%)	14 (22%)	0.039
Diabetes mellitus	108 (23%)	72 (29%)	29 (19%)	7 (11%)	
Tubulointerstitial disease	65 (14%)	24 (10%)	25 (16%)	16 (25%)	
Glomerulonephritis	47 (10%)	26 (10%)	15 (10%)	6 (10%)	
Unknown/others	142 (30%)	75 (30%)	44 (29%)	20 (32%)	
Smoking (%) ¹					
Never	231 (53%)	124 (52%)	82 (58%)	25 (44%)	0.494
Ex-smoker	144 (33%)	81 (34%)	44 (31%)	19 (33%)	
Active	64 (14%)	35 (14%)	16 (11%)	13 (23%)	
Blood pressure (kPa)					
Systolic	19.0 ± 2.9	19.3 ± 2.9	18.6 ± 2.8	19.0 ± 3.1	0.085
Diastolic	10.2 ± 1.5	10.2 ± 1.6	10.1 ± 1.4	10.3 ± 1.7	0.617
Pulse pressure (kPa)	8.8 ± 2.5	9.1 ± 2.5	8.5 ± 2.5	8.7 ± 2.5	0.098
Body mass index (kg/m ²)	28.6 ± 5.1	28.8 ± 5.5	28.6 ± 4.6	27.7 ± 4.4	0.294
Underweight (≤ 18.5)	6 (1%)	4 (2%)	1 (1%)	1 (2%)	0.353
Normal (18.6-24.9)	96 (20%)	50 (20%)	30 (19%)	16 (25%)	
Overweight (25.0-29.9)	210 (45%)	111 (44%)	68 (44%)	31 (48%)	
Obesity (> 29.9)	158 (34%)	87 (34%)	55 (36%)	16 (25%)	
Waist (cm)					
Males	102.2 ± 12.0	102.1 ± 13.0	102.2 ± 10.6	102.4 ± 11.5	0.989
Females	97.8 ± 13.5	98.3 ± 14.7	97.7 ± 12.2	95.7 ± 11.4	0.760
ABPI	1.01 ± 0.21	0.98 ± 0.20	1.04 ± 0.21	1.05 ± 0.22	0.013
Abnormal ABPI ²	194 (41%)	100 (41%)	66 (44%)	28 (44%)	0.539
Abnormal Kauppila score ³	107 (29%)	52 (27%)	35 (29%)	20 (36%)	0.183
Abnormal Adragao score ⁴	121 (32%)	66 (33%)	38 (30%)	17 (29%)	0.474
Vitamin D supplementation (%)	43 (9%)	16 (6%)	17 (11%)	10 (16%)	0.012
Use of phosphate binders (%)	72 (15%)	47 (19%)	16 (11%)	9 (14%)	0.105
Use of ACEI/ARB (%)	365 (78%)	196 (79%)	121 (82%)	48 (76%)	0.947
Use of diuretic (%)	287 (61%)	173 (70%)	88 (58%)	26 (42%)	< 0.001
Use of ESA (%)	124 (26%)	77 (31%)	31 (20%)	16 (25%)	0.015

¹Data available in 439 patients; ²< 0.9 or > 1.3; ³> 6 data available in 370 patients; ⁴≥ 3 data available in 383 patients. If not indicated otherwise, results are presented as mean ± SD, or number (percent). ABPI: Ankle-brachial pressure index; ACEI: Angiotensin-converting-enzyme inhibitor; ARB: Angiotensin II receptor blocker; eGFR: Estimated glomerular filtration rate; ESA: Erythropoietin-Stimulating agents; 25(OH)D: 25-hydroxyvitamin D.

showed the predictive value of 25(OH)D levels as a continuous variable for preventing death when adjusted for multiple covariates in different models (Table 3). Adjusted for age, comorbidity, diabetes mellitus, eGFR and phosphorous and albumin levels, the HR for all-cause mortality for 25(OH)D < 20 vs 20-29 was 2.33 (95%CI: 1.10-4.91; *P* = 0.027; Figure 3). The 25(OH)D ≥ 30 group did not have a significantly different mortality hazard from the reference group (HR = 1.19; 95%CI: 0.37-3.81; *P* = 0.775).

Progression of CKD and renal replacement therapy initiation

During the follow-up, 81 (17%) patients started renal

replacement therapy and 156 (33%) patients showed the composite renal end-point. Kaplan-Meier analysis (Figure 2B) showed that the 25(OH)D < 20 group had significantly more risk than the other two groups (log rank test, *P* < 0.001). Univariate Cox regression found again higher risk of the renal end-point with 25(OH)D level less than 20 ng/mL compared with 20 to 29 ng/mL (HR = 2.78; 95%CI: 1.84-4.16; *P* < 0.001), whereas the group with 25(OH)D above 30 ng/mL did not show different risk from reference group (HR = 1.13; 95%CI: 0.59-2.13; *P* = 0.717). Multivariate analysis showed the predictive value of VD levels as a continuous variable for preventing appearance of renal end point when adjusted for multiple covariates (Table 4). Adjusted for

Table 2 Baseline laboratory values, as a function of vitamin D status

	All (n = 470)	25(OH)D < 20 ng/mL (n = 252)	25(OH)D 20-29 ng/mL (n = 154)	25(OH)D ≥ 30 ng/mL (n = 64)	P
25-hydroxivitamin D (nmol/L)	52 ± 21	36 ± 9	61 ± 7	90 ± 16	< 0.001
1,25(OH) ₂ vitamin D (pmol/L)	103 ± 28	97 ± 27	111 ± 28	107 ± 23	< 0.001
Ca _{alb} (mmol/L)	2.40 ± 0.20	2.40 ± 0.15	2.42 ± 0.23	2.45 ± 0.23	0.163
P (mmol/L)	1.10 ± 0.26	1.10 ± 0.26	1.10 ± 0.26	1.07 ± 0.26	0.517
iPTH (ng/L) ¹	91 (85-97)	106 (96-116)	81 (73-91)	64 (55-74)	< 0.001
Creatinine (μmol/L)	221 ± 97	239 ± 106	212 ± 88	212 ± 88	0.017
eGFR (MDRD, mL/min per 1.73 m ²)	29.4 ± 11.5	28.1 ± 11.9	30.8 ± 10.8	30.5 ± 11.1	0.049
Urine protein excretion (g/24 h) ^{1,2}	0.592 (0.502-0.697)	0.699 (0.573-0.853)	0.448 (0.321-0.626)	0.448 (0.271-0.742)	0.034
hsCRP (nmol/L) ¹	36.2 (29.5-39.1)	37.1 (33.3-41.0)	36.2 (31.4-41.0)	32.4 (26.7-39.1)	0.506
Albumin (g/L)	40 ± 5	39 ± 5	41 ± 5	40 ± 5	0.011
Total proteins (g/L)	77 ± 12	77 ± 11	77 ± 13	76 ± 14	0.877
Total cholesterol (mmol/L)	4.7 ± 1.1	4.7 ± 1.1	4.7 ± 1.0	4.8 ± 1.1	0.603
HDL cholesterol (mmol/L)	1.3 ± 0.4	1.3 ± 0.3	1.3 ± 0.4	1.3 ± 0.4	0.973
LDL cholesterol (mmol/L)	2.7 ± 0.9	2.7 ± 0.9	2.7 ± 0.9	2.9 ± 0.8	0.344
Hemoglobin (g/L)	130 ± 16	129 ± 16	132 ± 16	132 ± 18	0.058
Ferritin (pmol/L) ¹	225 (207-245)	227 (202-252)	216 (187-252)	247 (191-319)	0.635
Transferrin (μmol/L)	3.0 ± 1.2	2.9 ± 1.2	3.0 ± 1.2	3.1 ± 1.3	0.289
Glucose (mmol/L)	6.3 ± 2.2	6.3 ± 2.3	6.4 ± 2.4	5.9 ± 1.5	0.241

¹24 h urine proteinuria obtained in 237 (50%) patients; ²Skewed values are presented as geometric mean with 95%CI. Ca_{alb}: Calcium adjusted for albumin levels; eGFR: Estimated glomerular filtration rate; HsCRP: High-sensitive C reactive protein; iPTH: Intact parathyroid hormone; MDRD: Modification of diet in renal disease; P: Phosphorous; 25(OH)D: 25-hydroxivitamin D. If not indicated otherwise, results are presented as mean ± SD.

Table 3 Adjusted Cox proportional hazards models of patient survival (events = 46)

Model	Covariates controlled for	Adjusted HR (95%CI)	P
0 (Unadjusted)	25-hydroxivitamin D levels (mg/dL)	0.95 (0.91-0.99)	0.009
1	25-hydroxivitamin D levels (mg/dL) + age	0.95 (0.91-0.99)	0.009
2	Model 1 + diabetes mellitus, ischemic heart disease, chronic heart failure	0.96 (0.92-0.99)	0.028
3	Model 1 + peripheral arterial disease, abnormal ABPI ¹ , phosphorous (mg/dL)	0.95 (0.92-0.99)	0.023
4	Model 1 + DBP (mm Hg), 1,25(OH) ₂ vitamin D (pg/mL), estimated GFR (mL/min per 1.73 m ²)	0.96 (0.92-0.99)	0.020
5	Model 1 + vascular calcification [Kauppila score (log), Adragao score (log)], CKD stage 5	0.95 (0.91-1.00)	0.050
6	Model 1 + obesity, hemoglobin (g/L), albumin (g/dL)	0.95 (0.92-0.99)	0.019

¹< 0.9 or >1.3. ABPI: Ankle-brachial pressure index; DBP: Diastolic blood pressure; GFR: Glomerular filtration rate.

Table 4 Multivariate Cox regression analysis in relation to renal end point (events = 156)

	HR (95%CI)	P value
25-hydroxivitamin D (ng/mL)	0.97 (0.95-0.99)	0.004
Age (yr)	0.99 (0.97-1.00)	0.044
Male sex	2.20 (1.47-3.30)	< 0.001
Estimated GFR (mL/min per 1.73 m ²)	0.93 (0.91-0.95)	< 0.001
ABPI (mmHg)	0.23 (0.10-0.53)	0.001
Hemoglobin (g/L)	0.84 (0.78-0.94)	0.001

ABPI: Ankle-brachial pressure index; GFR: Glomerular filtration rate.

age, gender, diabetes mellitus, eGFR, and phosphorous levels, the HR for the composite renal end-point for the 25(OH)D < 20 group compared to the reference group was 2.46 (95%CI: 1.63-3.71; *P* < 0.001; Figure 4). The 25(OH)D ≥ 30 group did not have a significantly different hazard for kidney progression from the reference group (HR = 1.20; 95%CI: 0.62-2.32; *P* = 0.581).

Hospitalization

During the follow-up, 126 (27%) patients were admitted for hospitalization, cardiovascular (49%) and infections (20%) being the most common causes. Kaplan-Meier analysis (Figure 2C) indicated that crude hospitalization event-free period was different between the VD groups (log rank test, *P* = 0.039). Univariate Cox regression found a shorter hospitalization event-free period in patients with 25(OH)D level less than 20 ng/mL compared with 20-29 ng/mL (HR = 1.58; 95%CI: 1.05-2.36; *P* = 0.027), with no difference between the 25(OH)D ≥ 30 and the reference groups (*P* = 0.861). Hospitalization outcomes were predicted by 25(OH) levels (HR = 0.98; 95%CI: 0.96-1.00; *P* = 0.027) in the unadjusted Cox proportional hazards model, but not after adjusting for age, eGFR, diabetes and comorbidity.

Cutoff points to define VD sufficiency based on hard endpoints

ROC curves identified VD levels at highest risk for death,

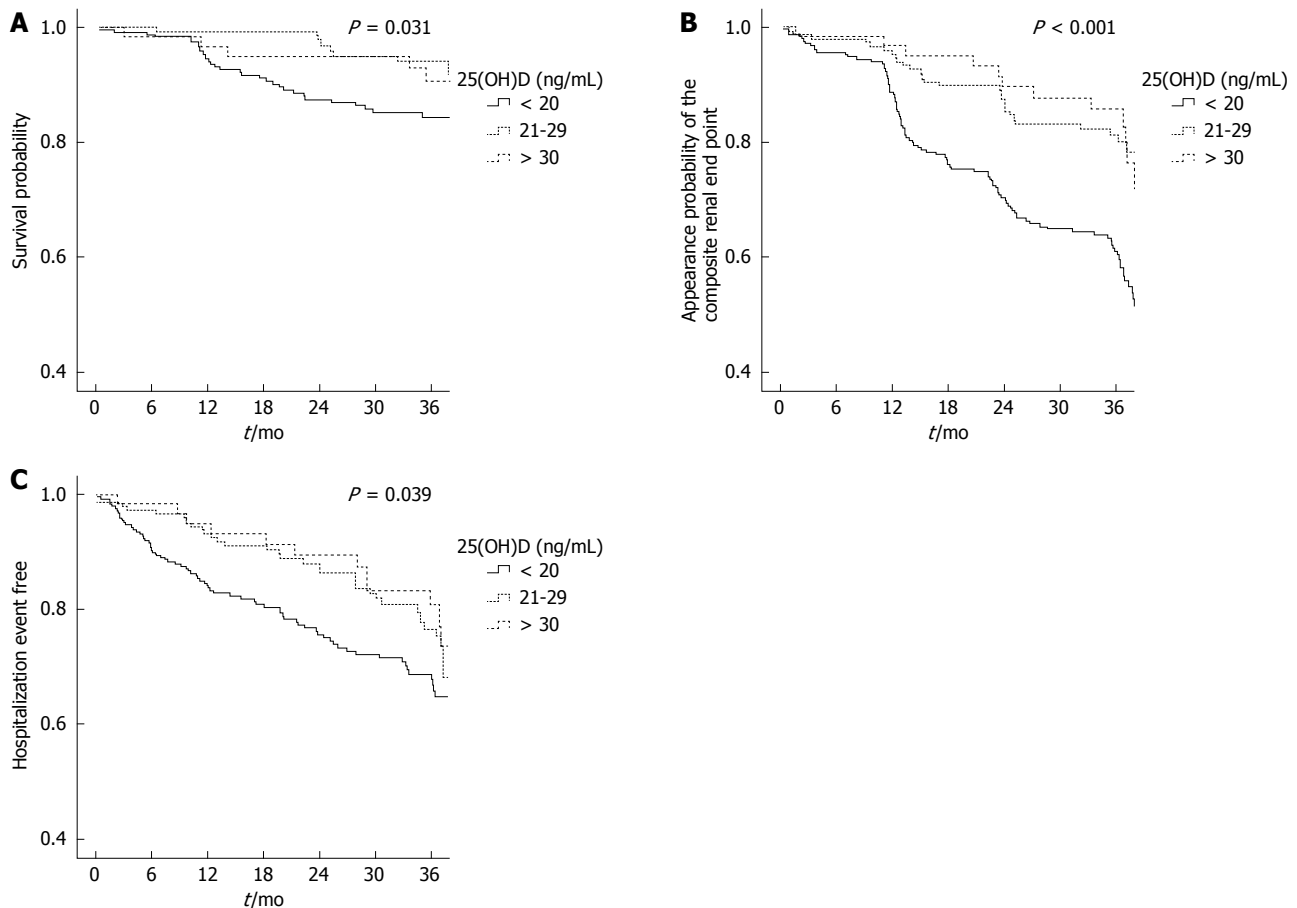


Figure 2 Kaplan-Meier survival (A), and appearance of the composite renal endpoint (B) and the hospitalization (C) curves as a function of 25-hydroxyvitamin D levels (< 20 ng/mL, 20-29 ng/mL and ≥ 30 ng/mL).

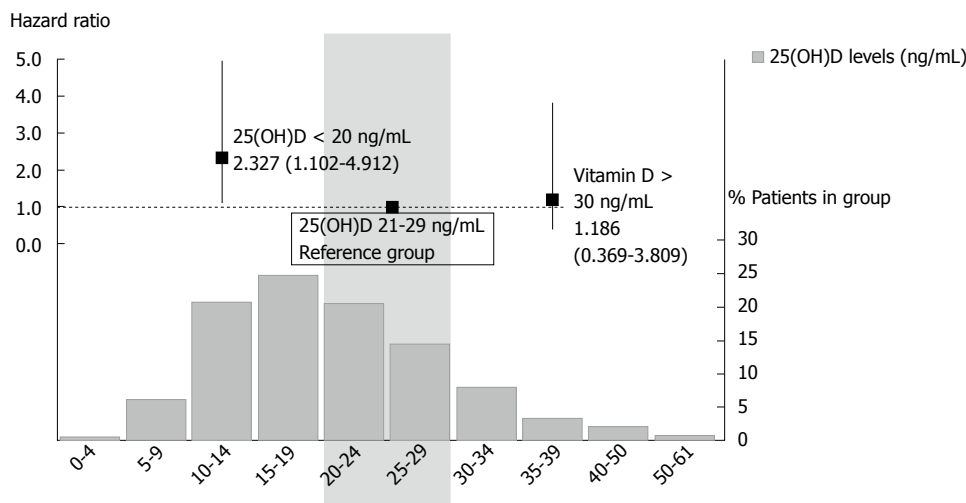


Figure 3 Proportion of patients with different 25-hydroxyvitamin D levels and hazard ratio (95%CI) for mortality after adjustment by age, comorbidity, diabetes mellitus, estimated glomerular filtration rate and albumin levels. 25(OH)D: 25-hydroxyvitamin D.

the composite renal endpoint, and hospitalization, at 17.4 ng/mL [area under the curve (AUC) = 0.60; 95%CI: 0.52-0.69; $P = 0.027$], 18.6 (AUC = 0.65; 95%CI: 0.60-0.71; $P < 0.001$), and 19.0 (AUC = 0.56; 95%CI: 0.50-0.62; $P = 0.048$), respectively.

DISCUSSION

One of the main limitations for the development of evidence-based clinical recommendations for VD supplementation lies in the discrepancies in the criteria

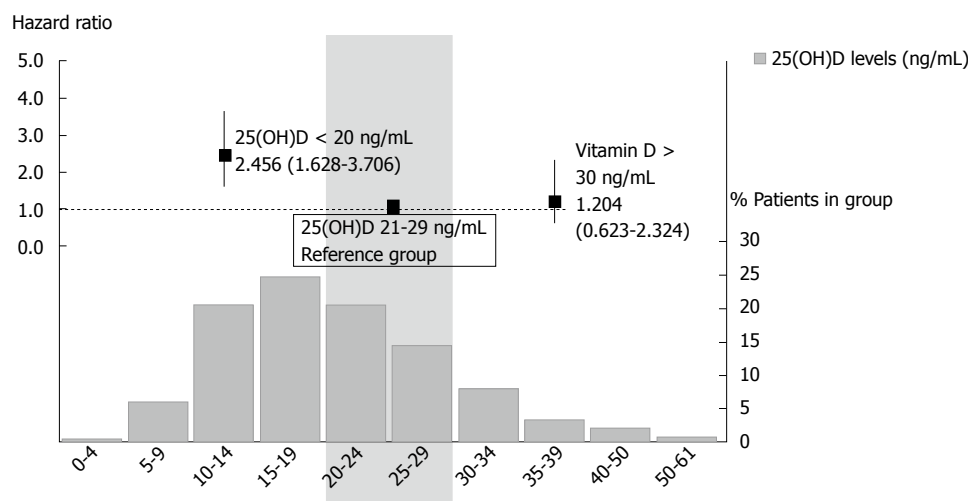


Figure 4 Proportion of patients with different 25-hydroxyvitamin D levels and hazard ratio (95%CI) for composite renal end-point after adjustment by age, sex, diabetes mellitus, estimated glomerular filtration rate and albumin levels. 25(OH)D: 25-hydroxyvitamin D.

for defining VD deficiency and insufficiency, which can explain conflicting results from meta-analysis addressing vitamin D levels and outcomes^[24,34]. These criteria vary among authors and societies, including 25(OH)D levels below which osteomalacia [10 ng/mL (25 nmol/L)] or secondary hyperparathyroidism [20-30 ng/mL (50 to 75 nmol/L)] may appear^[18-20,35,36]. Being aware of their potential clinical significance, the present study examines the prognosis value of 25(OH)D levels in a cohort of 3-5 stage CKD subjects not on dialysis, trying to identify cut-off points for serum 25(OH)D levels to define VD sufficiency. These cut-offs were not based on biological abnormalities as classically noted^[25,26], but on VD levels at highest risk for death, CKD progression and all-cause hospitalization.

Although randomized clinical trials are the best way for generating a high evidence for treatment decisions, trials are rare and suboptimal in nephrology^[37]. Therefore, observational studies have an important role, particularly when the intervention, in this case vitamin D supplementation, is inexpensive and potentially effective. Although there are previous prospective observational studies which examined the prognosis value of 25(OH)D levels in CKD subjects not on dialysis^[8,12], this is the first one, to our knowledge, in which 25(OH)D levels unequivocally reflect exposure to VD, given that patients on treatment with active VD were excluded, as well as including the biggest cohort of non-dialysis CKD subjects with data regarding emerging cardiovascular risk factors as vascular calcification scores and ABPI. In the main analysis of the OSERCE-2 study, low VD levels were associated to worse survival and CKD progression only in the univariate analysis^[32]. However, 26% of patients of the study received activated VD, which may confer a protective effect and therefore may decrease any negative effect of VD levels observed, as it has been stated on dialysis population^[38]. In this context, we conducted this post-hoc analysis of the OSERCE-2 dataset in patients without active VD

treatment. In this selected cohort, the main findings were the independent predictor value of VD deficiency, but not insufficiency, for the 3-year incidence of death and CKD progression, which remained significant after multivariate adjustments, as previously published^[8,12]. In a prospective study involving 94 CKD patients, those with 25(OH)D levels less than 16.7 ng/mL had a higher mortality rate^[12]. 25(OH)D was confirmed as an independent inverse predictor of death in a 6-year follow-up study which included 168 CKD subjects^[8]. In that study patients with ≥ 15 ng/mL of 25(OH)D showed a reduction in mortality by 33% to 60% in the different models, compared to patients with 25(OH)D < 15 ng/mL. Less CKD progression to end-stage renal disease was also reported in the groups of patients with better VD status. All these data are in agreement with our results, which show how low 25(OH)D levels predicted mortality and CKD progression independently of such traditional and non-traditional risk factors, as vascular calcification or inflammation. In this context, it is noteworthy that the lack of association between 25(OH)D levels and vascular calcification observed in our study, is in agreement with some^[12], but not all^[9,10], previously published data. These findings indicate that 25(OH)D may impact on CKD outcomes by additional mechanisms including the suppression of the renin-angiotensin system, albuminuria reduction or amelioration of left ventricular hypertrophy^[6,9,16,31,39]. Of note, we have detected ABPI as an independent predictor of VD deficiency, which could contribute to vascular stiffness and high cardiovascular risk for this population.

More interestingly, our study, as the first prospective which analyzed the upper level associated to better improvement in survival and CKD progression on CKD patients, did not demonstrate additional benefits on these hard outcomes when patients reached the optimal target levels for VD suggested by current guidelines (≥ 30 ng/mL). It is noteworthy that all three cut-off points

for serum 25(OH)D levels at highest risk for death, CKD progression and all-cause hospitalization were between 17 ng/mL and 19, which reinforces the threshold value for abnormally reduced 25(OH)D in 20 ng/mL. These findings confirm the data reported in the biggest retrospective observational study analyzing VD and mortality in CKD patients. Navaneethan *et al*^[40] studied 12763 patients with 3-4 stage CKD, showing 25(OH)D level \leq 15 ng/mL to be associated independently with a 33% increased risk of all-cause mortality, whereas the group with 25(OH)D levels of 15-29 ng/mL did not show a significantly increased risk of mortality compared with patients with 25(OH)D levels \geq 30 ng/mL.

Taking all these data together, we agree with the Institute of Medicine recommendation to consider sufficient 25(OH)D levels of at least 20 ng/mL, given that serum 25(OH)D concentrations above 30 ng/mL are not consistently associated with increased benefit^[27,40]. In addition, most clinical trials have only confirmed the neutral effect of VD supplementation on hard outcomes^[41], whereas some controlled studies have shown positive results in spite of the mean VD concentration not reaching the optimal recommended levels of \geq 30 ng/mL^[16]. Moreover, VD might not be safe in all settings, and supplementing could cause harm in people with CKD, who have a high prevalence of vascular calcification, and a decreasing ability for renal excretion of calcium and phosphorous^[32,42]. Excessive VD supplementation may be particularly harmful in those high risk individuals with serum 25(OH)D levels above 20 ng/mL which are classified as insufficient according to current guidelines, and who then are treated with high-dose supplements of VD containing many times the levels of intake recommended for adults (600-800 UI/d)^[18,27,43]. Although some experts suggest that it is safe to carry higher vitamin D levels (40-70 ng/mL), this recommendation is based on acute and not long-term observations^[44].

Lastly, our study confirmed the high prevalence of low VD status on CKD patients^[1-5]. There are many factors which could contribute to the deficiency that are not related to GFR, including limited exposure to the sun, reduced dietary intake and urinary loss of 25(OH)D and VD-binding protein in proteinuric nephropathies^[24,44,45]. The present study, as others^[8,12,38], has shown significant correlation between 25(OH)D levels and body mass index and albumin, which emphasizes the relationship between nutritional status, VD levels and survival in chronic illness as CKD. Of note, the independent relationship observed, even after adjustment for chronic heart failure, between VD deficiency and diuretic use. VD deficiency is highly prevalent in heart failure patients, being a significant predictor of reduced survival. In addition, loop diuretics treatment may worsen osteoporosis on general population, but no data are available in CKD patients^[46,47].

Strengths and limitations

The strong points of the study include the relatively

high number of patients included and the 3-year follow-up, which strengthens the study's power. To minimize the inter-method and seasonal variability in VD and PTH measurements, blood samples were analyzed by a central laboratory, and patients' recruitment was done in a short period of time (April-May)^[32]. In contrast, there are several limitations to be commented. As a longitudinal study, it is still insufficient to determine whether the association between low 25(OH)D levels and worse CKD outcomes is causal and reversible, which should be tested in future randomized clinical trials. The results may not be valid to non-Caucasian populations living at other latitudes, or to patients on active VD treatment. The multivariate analysis of cardiovascular deaths was limited due to its low incidence. Lastly, it would be interesting to study other relevant bone-related clinical outcomes, such as bone-density changes or fracture risk.

In conclusion, in accordance with previously published data, the present study confirms: (1) a high prevalence of 25(OH)D deficiency and insufficiency in non-dialysis CKD patients; and (2) an independent association between serum 25(OH)D levels and worse clinical outcomes, such as death and CKD progression. The results of this study add to the knowledge of optimal VD status in non-dialysis CKD patients, identifying the threshold value for abnormally reduced 25(OH)D in 20 ng/mL, which is in agreement with the Institute of Medicine recommendations. Whereas high doses of VD supplementation on this population can lead to a calcium and phosphate overload, promoting vascular calcification and CKD progression, our results suggest that, with the limitations inherent to the observational studies, 25(OH)D levels between 20 to 30 ng/mL could be sufficient for CKD patients. Randomized clinical trials are warranted to know the most favorable 25(OH)D level for CKD patients.

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COMMENTS

Background

Although knowledge of the skeletal and non-skeletal effects of nutritional vitamin D (VD) has expanded, no consensus currently exists within the medical community regarding the criteria for defining thresholds for VD supplementation in chronic kidney disease (CKD) patients.

Research frontiers

Based on levels of 25(OH)D required to suppress parathyroid hormone

(PTH), clinical guidelines most commonly recommend a serum concentration of 25(OH)D above 30–40 ng/mL (75–100 nmol/L), levels at which PTH is suppressed to a minimum in its relation to 25(OH)D. However, there is a lack of evidence regarding this target recommendation, and overuse of VD supplementation on this population can lead to a calcium and phosphate overload, promoting vascular calcification and CKD progression.

Innovations and breakthroughs

Being aware of both the therapeutic and iatrogenic power of VD supplementation, the present study examines the prognosis value of 25(OH)D levels in a cohort of 3–5 stage CKD subjects not on dialysis, trying to identify cut-off points for serum 25(OH)D levels to define VD sufficiency. These cut-offs were not based on biochemical abnormalities as classically noted, but on VD levels at highest risk for death, CKD progression and all-cause hospitalization. The results of this study add to the knowledge of optimal VD status in non-dialysis CKD patients, identifying the threshold value for abnormally reduced 25(OH)D in 20 ng/mL.

Applications

The data in this study suggested that the optimal VD level might be lower than is currently recommended, advocating that 25(OH)D levels at or above 20 ng/mL could be sufficient for CKD patients. The authors recommend caution when nutritional VD is prescribed.

Terminology

25(OH)D, also known as calcifediol, is a prehormone that is produced in the liver by hydroxylation of vitamin D3 (cholecalciferol). Serum 25(OH)D levels are considered the best indicator of VD status.

Peer-review

The paper with the title: "What is the optimal level of vitamin D in non-dialysis CKD population?" is an interesting well written article and the authors claim that their study as the first prospective which analyzed the upper level of VD associated to better improvement in survival and CKD progression on CKD patients, did not demonstrate additional benefits on these hard outcomes when patients reached the optimal target levels for VD suggested by current guidelines (≥ 30 ng/mL). So with this study, despite the limitations, the authors provide a new option in this so controversial field of VD treatment in CKD patients.

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Association of blood transfusion with acute kidney injury after transcatheter aortic valve replacement: A meta-analysis

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Abstract

AIM

To assess red blood cell (RBC) transfusion effects on acute kidney injury (AKI) after transcatheter aortic valve replacement (TAVR).

METHODS

A literature search was performed using MEDLINE, EMBASE, Cochrane Database of Systematic Reviews, and clinicaltrials.gov from the inception of the databases through December 2015. Studies that reported relative risk, odds ratio or hazard ratio comparing the risks of AKI following TAVR in patients who received periprocedural RBC transfusion were included. Pooled risk ratio (RR) and 95%CI were calculated using a random-effect, generic inverse variance method.

RESULTS

Sixteen cohort studies with 4690 patients were included in the analyses to assess the risk of AKI after TAVR in patients who received a periprocedural RBC transfusion. The pooled RR of AKI after TAVR in patients who received a periprocedural RBC transfusion was 1.95 (95%CI: 1.56-2.43) when compared with the patients who did not receive a RBC transfusion. The meta-analysis was

then limited to only studies with adjusted analysis for confounders assessing the risk of AKI after TAVR; the pooled RR of AKI in patients who received periprocedural RBC transfusion was 1.85 (95%CI: 1.29-2.67).

CONCLUSION

Our meta-analysis demonstrates an association between periprocedural RBC transfusion and a higher risk of AKI after TAVR. Future studies are required to assess the risks of severe AKI after TAVR requiring renal replacement therapy and mortality in the patients who received periprocedural RBC transfusion.

Key words: Acute kidney injury; Transcatheter aortic valve replacement; Meta-analysis; Mortality; Blood transfusion

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Core tip: We performed this meta-analysis to assess the impact of periprocedural red blood cell (RBC) transfusion on the risk of acute kidney injury (AKI) after transcatheter aortic valve replacement (TAVR). We verified a significant association between peri-procedural RBC transfusion and AKI after a TAVR with an overall 1.95-fold increased risk of AKI compared to those who did not receive transfusion. This study highlights the importance of vigilance when considering transfusions and should impact the clinical management of the high-risk group of patients undergoing TAVR.

Thongprayoon C, Cheungpasitporn W, Gillaspie EA, Greason KL, Kashani KB. Association of blood transfusion with acute kidney injury after transcatheter aortic valve replacement: A meta-analysis. *World J Nephrol* 2016; 5(5): 482-488 Available from: URL: <http://www.wjgnet.com/2220-6124/full/v5/i5/482.htm> DOI: <http://dx.doi.org/10.5527/wjn.v5.i5.482>

INTRODUCTION

Patients with severe symptomatic aortic stenosis have destitute prognosis with medical treatment alone^[1]. Transcatheter aortic valve replacement (TAVR), also known as transcatheter aortic valve implantation, is an exciting new approach to the treatment of high-risk or inoperable patients with severe aortic stenosis^[2-6]. Despite advances in TAVR procedures, acute kidney injury (AKI) is one of the most frequent complications of TAVR, ranging in the literature from 15% to 57%^[3,7-9]. Notably, the subset of patients, who develop AKI after TAVR, also have a high mortality rate of 9%-44% at 30 d and 32%-56% at 1 year^[7,8].

Perioperative anemia has been shown to be independently associated with AKI after cardiac surgery^[10,11]. Anemia can result in decreased renal oxygen delivery, increased oxidative stress and impaired hemostasis^[10]. Thus, perioperative red blood cell (RBC) transfusion is used to improve oxygen delivery. However, stored RBC

transfusion can also promote a pro-inflammatory state, impair tissue oxygen delivery, and induce tissue oxidative stress^[12,13]. The association of AKI with RBC transfusion after TAVR is conflicting. While a few studies have demonstrated a higher incidence of AKI among patients who received periprocedural RBC transfusion^[14-23], the others have shown no such association^[24-29]. Thus, we conducted this meta-analysis to assess the impact of periprocedural RBC transfusion on the risk of AKI after TAVR.

MATERIALS AND METHODS

Search strategy

Two investigators (Thongprayoon C and Cheungpasitporn W) independently searched published studies and conference abstracts indexed in MEDLINE, EMBASE, Cochrane Database of Systematic Reviews and clinicaltrials.gov from the inception of the databases through December 2015. The search strategy used is described in the supplementary material. A manual search for additional relevant studies using the references from these retrieved articles was also performed. We followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines for a systematic review and meta-analysis^[30].

Inclusion criteria

We included studies that: (1) enrolled adult (≥ 18 years old) patients; (2) provided information about periprocedural RBC transfusion and comparator patients who did not receive RBC transfusion; (3) included AKI after TAVR as an outcome; (4) were randomized clinical trials or observational studies (case-control, cross-sectional or cohort studies) published as original studies or conference abstracts; and (5) provided data to calculate odds ratios (ORs), relative risks, hazard ratios (HRs) or standardized incidence ratios with 95% CIs. No language limits were applied.

Study eligibility was independently determined by the two investigators noted previously. Differing decisions were resolved by mutual consensus. The quality of each study was evaluated using the Newcastle-Ottawa quality assessment scale^[31].

Data extraction

A standardized data collection form was used to extract the following information: Last name of the first author, article title, study design, year of study, country of origin, year of publication, sample size, AKI definition, blood transfusion, confounder adjustment, and the adjusted effect estimate with 95%CI.

Statistical analysis

Review Manager software (Version 5.3, Copenhagen, Denmark) from the Cochrane Collaboration was used for data analysis. Point estimates and standard errors were extracted from individual studies and were combined by the generic inverse variance method of DerSimonian

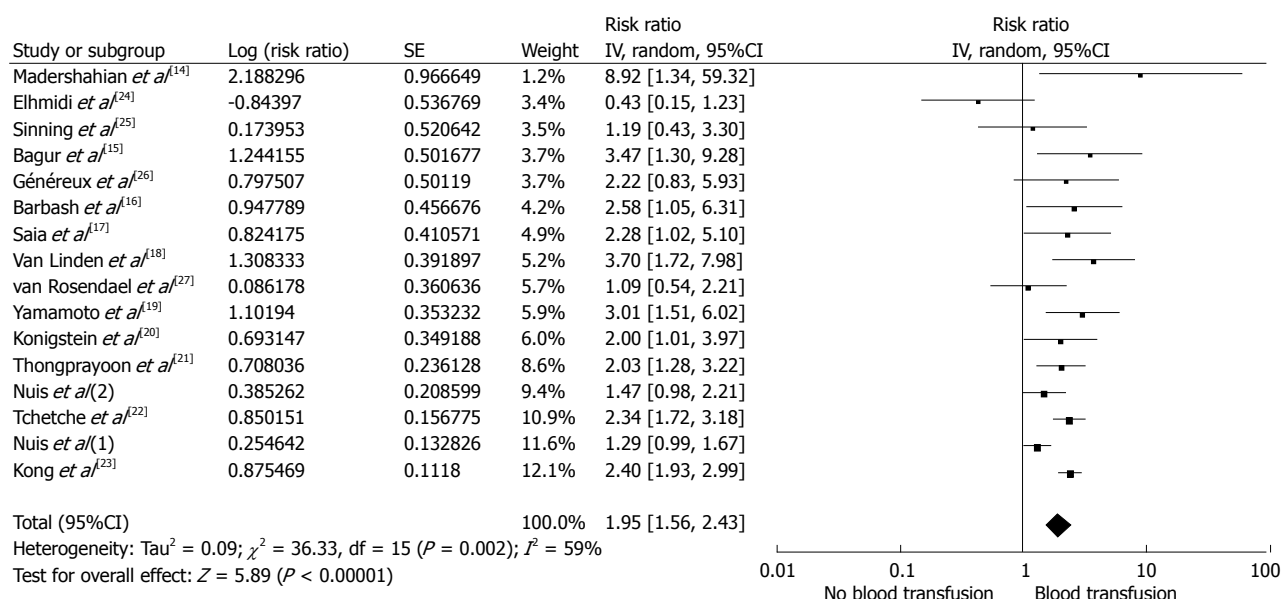


Figure 1 Forest plot of comparing the risk of acute kidney injury after transcatheter aortic valve replacement in patients who received red blood cell transfusion and those who did not. Square data markers represent risk ratios (RRs); horizontal lines, the 95% CIs with marker size reflecting the statistical weight of the study using random-effects meta-analysis. A diamond data marker represents the overall RR and 95%CI for the outcome of interest.

and Laird^[32]. Given the high likelihood of between-study variances, a random-effect model was used. Statistical heterogeneity was assessed using Cochran's Q test. This statistic was complemented with the I^2 statistic, which quantifies the proportion of the total variation across studies that is due to heterogeneity rather than chance. An I^2 of 0%-25% represents insignificant heterogeneity, 26%-50% low heterogeneity, 51%-75% moderate heterogeneity and > 75% high heterogeneity^[33]. The presence of publication bias was assessed by funnel plots of the logarithm of ORs vs their standard errors^[34].

RESULTS

Our search strategy yielded 1327 articles. Of these, 1169 articles were excluded based on their relevance and the eligibility criteria, following the review of their title and abstract. The remaining 158 articles underwent full-length review and an additional 142 were excluded for failing to meet the criteria: 114 articles did not report the outcome of interest; and 28 articles were not observational studies or randomized clinical trials. Sixteen cohort studies were included in the meta-analysis to assess the risk of AKI after TAVR in patients who received periprocedural RBC transfusion (Table 1). Of the 16 cohort studies, eight studies performed adjusted analysis for known risk factors for AKI^[14,15,19,20,23,24,28,29]. Supplementary Item 2 outlines our search methodology and selection process.

Study quality

All observational studies were considered moderate to high quality, with a median Newcastle-Ottawa quality assessment scale of 6.5 (range: 6-8) as shown in Table 1.

AKI definition

All included studies identified the AKI occurrence, based on the change in serum creatinine (SCr) or GFR after TAVR. One of the included studies also used urine output criteria for the AKI diagnosis^[25]. Twelve^[16,17,19-23,25-29] of the 16 included studies used standard AKI definitions (modified Risk, Injury, Failure, Loss of kidney function^[35]; Acute Kidney Injury Network^[36]; or Kidney Disease Improving Global Outcomes criteria^[37]), as shown in Table 1.

AKI risk

The pooled risk ratio (RR) of AKI following TAVR in patients who received a RBC transfusion was 1.95 (95%CI: 1.56-2.43; $I^2 = 59\%$). Figure 1 shows the forest plot of the included studies. When meta-analysis was limited to the studies using standard AKI definitions, the pooled RRs were 1.89 (95%CI: 1.55-2.31; $I^2 = 50\%$). To minimize the effects of confounders, we performed a sensitivity analysis and excluded studies that did not include an adjusted analysis for known risk factors for AKI. The pooled RR of AKI after TAVR remained significant in patients who received periprocedural RBC transfusions (RR = 1.85; 95%CI: 1.29-2.67; $I^2 = 75\%$), shown in Figure 2.

Nuis *et al*^[28] assessed the dose response relationship of a RBC transfusion and AKI, and demonstrated an increased risk of AKI with a higher number of RBC transfusions with ORs of 1.47 (95%CI: 0.98-2.22), 3.05 (95%CI: 1.24-7.53), 4.81 (95%CI: 1.45-15.95) for 1-2 units, 3-4 units, and ≥ 5 units of RBC transfusion, respectively. Reporting of severe AKI requiring renal replacement therapy (RRT) was limited. Van Linden *et al*^[18] reported a higher risk of AKI requiring RRT with an OR of 8.8 (95%CI: 1.7-45.6; Table 1).

Table 1 Main characteristics of the studies included in this meta-analysis

Ref.	Country ¹	Year	n	Transfusion definition	AKI definition (changes in baseline)	RR for AKI	Confounder adjustment	S, C, O ²
Sinning <i>et al</i> ^[25]	Germany	2010	77	RBC in 2 d post-procedure	Increase in SCr of ≥ 0.3 mg/dL or $\geq 50\%$ or U output < 0.5 mL/kg per hour for > 6 h in 48 h post procedure	1.19 (0.43-3.31)	None	3, 0, 3
Bagur <i>et al</i> ^[15]	Canada	2010	213	Peri-procedural blood	Decrease in eGFR of $> 25\%$ at 48 h post procedure or hemodialysis needed during index hospitalization	3.47 (1.30-9.29)	HTN, COPD	3, 1, 3
Van Linden <i>et al</i> ^[18]	Germany	2011	261	Blood > 4 u in 7 d post-operative	Decrease in eGFR of $> 25\%$ or increase in SCr of 50% in 7 d post procedure	AKI 3.7 (1.7-7.9) RRT 8.8 (1.7-45.6)	None	3, 0, 3
Nuis <i>et al</i> ^[29]	Netherlands	2011	118	Peri-procedural RBC	Increase in SCr of ≥ 0.3 mg/dL or $\geq 50\%$ in 72 h post procedure	1.29 (1.01-1.70)	Previous MI, leukocyte count, logistic EuroScore	3, 1, 3
Elhmidi <i>et al</i> ^[24]	Germany	2011	234	Post-operative blood	Decrease in eGFR of $> 25\%$ or increase in SCr of 50% in 7 d post procedure	0.43 (0.15-1.23)	Baseline creatinine, STS score, DM	3, 2, 3
Madershahian <i>et al</i> ^[14]	Germany	2012	50	RBC	Increase in SCr of ≥ 0.5 mg/dL or $\geq 25\%$ from baseline within 48 h post procedure	8.92 (1.34-59.26)	COPD and contrast amount	3, 1, 3
Kong <i>et al</i> ^[23]	Australia	2012	52	Peri-procedural RBC	SCr criteria of RIFLE classification in 48 h post procedure	2.4 (2.0-3.1)	TA, history of HTN	3, 1, 3
Tchetche <i>et al</i> ^[22]	France, Netherlands, Italy	2012	743	RBC	Increase in SCr of ≥ 0.3 mg/dL or $\geq 50\%$ in 72 h post procedure	2.34 (1.72-3.18)	None	3, 0, 3
Barbash <i>et al</i> ^[16]	United States	2012	165	Post procedure blood	Increase in SCr of ≥ 0.3 mg/dL or $\geq 50\%$ in 72 h post procedure	2.58 (1.05-6.29)	None	3, 0, 3
Nuis <i>et al</i> ^[28]	Netherlands, Canada, Germany, Belgium, Columbia	2012	995	RBC in 24 h post procedure	Increase in SCr of ≥ 0.3 mg/dL or $\geq 50\%$ in 72 h post procedure	1-2 u, 1.47 (0.98-2.22); 3-4 u, 3.05 (1.24-7.53); ≥ 5 u, 4.81 (1.45-15.95)	PVD, CHF, maximal leukocyte count, logistic EuroScore	3, 2, 3
Saia <i>et al</i> ^[17]	Italy	2013	102	Peri-procedural RBC	Increase in SCr of ≥ 0.3 mg/dL or $\geq 50\%$ in 72 h post procedure	2.28 (1.02-5.10)	None	3, 0, 3
Konigstein <i>et al</i> ^[20]	Israel	2013	251	Blood	Increase in SCr of ≥ 0.3 mg/dL or $\geq 50\%$ in 72 h post procedure	2.00 (1.01-3.97)	Gender, HTN, DM, dyslipidemia, PVD, CHF, stroke, COPD, PHTN, VC, CKD, valve type and size	3, 2, 3
Yamamoto <i>et al</i> ^[19]	France	2013	415	RBC	Increase in SCr of ≥ 0.3 mg/dL or $\geq 50\%$ in 72 h post procedure	3.01 (1.54-6.15)	Contrast amount and LVEF	3, 1, 3
Généreux <i>et al</i> ^[26]	United States	2013	218	Blood	VARC-modified RIFLE stage 2 or 3 until discharge	2.22 (0.83-5.92)	None	3, 0, 3
Thongprayoon <i>et al</i> ^[21]	United States	2015	386	Intra-operative RBC	Increase in SCr of ≥ 0.3 mg/dL in 48 h or $\geq 50\%$ in 7 d post procedure	2.03 (1.28-3.23)	None	3, 0, 3
van Rosendaal <i>et al</i> ^[27]	Netherlands	2015	210	Peri-procedural RBC	Increase in SCr of ≥ 0.3 mg/dL or $\geq 50\%$ in 7 d post procedure	1.09 (0.54-2.22)	None	3, 0, 3

¹Countries are listed by their three letter country code; ²Quality Assessment Newcastle-Ottawa scale: S: Selection; C: Comparability; O: Outcome. AKI: Acute kidney injury; CHF: Congestive heart failure; CKD: Chronic kidney disease; COPD: Chronic obstructive pulmonary disease; DM: Diabetes mellitus; eGFR: Estimated glomerular filtration rate; HTN: Hypertension; LVEF: Left ventricular ejection fraction; MI: Myocardial infarction; PHTN: Pulmonary hypertension; PVD: Peripheral vascular disease; RIFLE: Risk, injury, failure, loss of kidney function, and end-stage kidney disease; SCr: Serum creatinine; STS: Society of thoracic surgeons; TA: Transapical approach; VARC: Valve academic research consortium; VC: Vascular complication; RBC: Red blood cell.

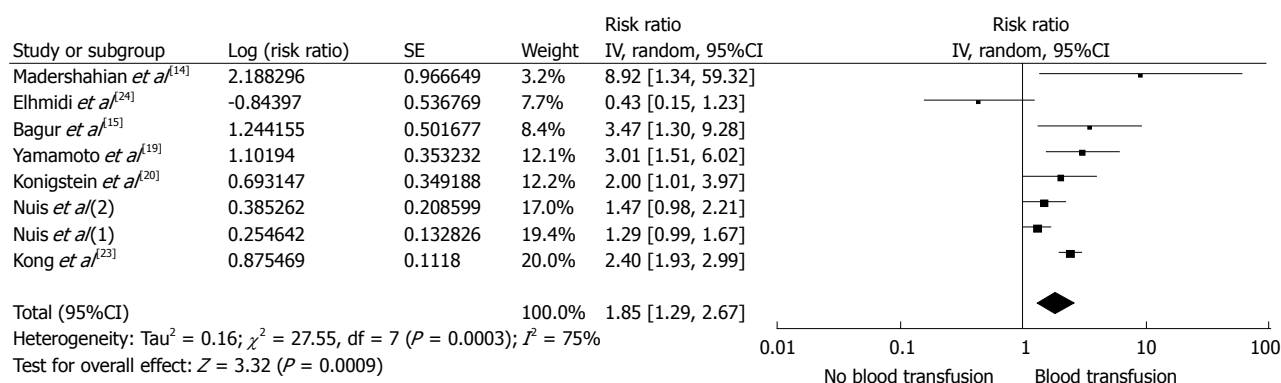


Figure 2 Forest plot of the adjusted analysis comparing the risk of acute kidney injury after transcatheter aortic valve replacement in patients who received red blood cell transfusion and those who did not. The square data markers represent risk ratios (RRs); horizontal lines, the 95% CIs with marker size reflecting the statistical weight of the study using random-effects meta-analysis. A diamond data marker represents the overall RR and 95% CI for the outcome of interest.

Evaluation for publication bias

Funnel plots to evaluate publication bias for the risk of AKI after TAVR in patients who received a RBC transfusion are summarized in Supplementary Figures 1 and 2. The graphs demonstrate no obvious asymmetry and, thus, suggest an insignificant publication bias.

DISCUSSION

In this meta-analysis, we verified a significant association between peri-procedural RBC transfusion and AKI after a TAVR with an overall 1.95-fold increased risk of AKI compared to those who did not receive transfusion. This association remained significant when adjusting for potential confounders.

The mechanism for the higher incidence of AKI after TAVR in patients with a periprocedural RBC transfusion is not well-elucidated. Analysis has shown that preserved RBCs used in transfusions undergo progressive structural and functional changes during storage, such as reduced deformability and increased tendency to aggregate. These changes result in the deterioration of RBC function and viability, and the resultant accumulation of free iron and pro-inflammatory agents^[38] leads to AKI^[8,39-41]. Studies have also shown an association between RBC transfusions and increased leukocyte count in patients who developed AKI after TAVR^[28,29].

Nuis *et al*^[28] reported that the number of RBC transfusions was an independent predictor of AKI following TAVR. In their study, a higher number of RBC transfusions were found to be associated with a higher AKI incidence. Interestingly, the investigators did not find significant associations between AKI and the clinical indications for transfusion (*i.e.*, baseline anemia, bleeding complications, or blood loss).

The risks of transfusion-associated AKI is not limited to TAVR; patients undergoing coronary artery bypass grafting or surgical aortic valve replacements who require transfusions also have a higher frequency of AKI^[41-43].

Although the included studies in our meta-an-

alysis were all of moderate to high quality, there are some limitations of this study that bear mentioning. First, there were statistical heterogeneities among the enrolled studies. The potential sources of these heterogeneities include the variations in the diagnostic methodology of AKI after TAVR and the differences in confounder adjustment methods. Second, the data on severe AKI requiring RRT after TAVR is lacking. Further studies are certainly warranted to further delineate the impact of transfusions after TAVR with specific regard to the severity of AKI. Third, the data on valve size and approaches for TAVR procedure were limited. These factors might have affected the risk of AKI following TAVR. Lastly, this is a meta-analysis of observational studies with the inherent limitation that a causal relationship cannot be inferred.

The threshold for transfusions is constantly changing. The deleterious effects of transfusions are well documented, and many institutions have worked hard to create protocols to diminish unnecessary transfusions. Our meta-analysis demonstrates an association between periprocedural RBC transfusion and a higher risk of AKI following TAVR. In many cases, patients undergoing TAVR have considerable debility and comorbid conditions. This study highlights the importance of vigilance when considering transfusions and should impact the clinical management of the high-risk group of patients undergoing TAVR.

COMMENTS

Background

Transcatheter aortic valve replacement (TAVR) is an exciting new approach to the treatment of high-risk or inoperable patients with severe aortic stenosis. Despite advances in TAVR procedures, acute kidney injury (AKI) is one of the most frequent complications of TAVR, associated with significant morbidity and mortality following the procedures.

Research frontiers

The association of AKI with red blood cell (RBC) transfusion after TAVR is conflicting in the findings of numerous literature. It is thus necessary to assess the impact of periprocedural RBC transfusion on the risk of AKI after TAVR.

Innovations and breakthroughs

In this study, the authors verified a significant association between peri-procedural RBC transfusion and AKI after a TAVR with an overall 1.95-fold increased risk of AKI compared to those who did not receive transfusion.

Applications

The data in this study highlights the importance of vigilance when considering transfusions and should impact the clinical management of the high-risk group of patients undergoing TAVR.

Terminology

PRISMA: Preferred reporting items for systematic reviews and meta-analyses, etc.

Peer-review

This is a reasonable first meta-analysis of association of blood transfusion with AKI after transcatheter aortic valve replacement. The results have potential clinical applications.

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Bacteremia in hemodialysis patients

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leading cause of death in hemodialysis patients. The risk of bacteremia in hemodialysis patients is 26-fold higher than in the general population, and 1/2-3/4 of the causative organisms of bacteremia in hemodialysis patients are Gram-positive bacteria. The ratio of resistant bacteria in hemodialysis patients compared to the general population is unclear. Several reports have indicated that hemodialysis patients have a higher risk of methicillin-resistant *Staphylococcus aureus* infection. The most common site of infection causing bacteremia is internal prostheses; the use of a hemodialysis catheter is the most important risk factor for bacteremia. Although antibiotic lock of hemodialysis catheters and topical antibiotic ointment can reduce catheter-related blood stream infection (CRBSI), their use should be limited to necessary cases because of the emergence of resistant organisms. Systemic antibiotic administration and catheter removal is recommended for treating CRBSI, although a study indicated the advantages of antibiotic lock and guidewire exchange of catheters over systemic antibiotic therapy. An infection control bundle recommended by the Center for Disease Control and Prevention succeeded in reducing bacteremia in hemodialysis patients with either a catheter or arteriovenous fistula. Appropriate infection control can reduce bacteremia in hemodialysis patients.

Key words: Bacteremia; Hemodialysis; Blood stream infection; Epidemiology; Infection control

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Core tip: Infection is common in hemodialysis patients, who are at high risk for bacteremia. The use of a hemodialysis catheter is the most important risk factor for bacteremia. Improvement of standard infection control measures, including the reduction of catheter use, appropriate catheter care, patient and staff education, and hand hygiene could reduce bacteremia in hemodialysis patients.

Abstract

Infection is a common complication and is the second

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INTRODUCTION

Infection is the second leading cause of death in hemodialysis patients in many countries and is the leading cause of death in the first year of hemodialysis in Japan^[1-3]. Furthermore, infection is a major cause of hospitalization in hemodialysis patients. In the United States, infection was observed in approximately 30% of all hospitalizations of hemodialysis patients^[4,5]. These data indicate that infection is a serious threat to these patients. Hemodialysis patients have higher rates of bacteremia, whereas peritoneal dialysis patients have higher rates of peritonitis^[4,6]. In a study in the United States, the rates (per 100 person-years) of specific infection-related hospitalizations of hemodialysis patients were 17.6 for septicemia, 15.3 for pulmonary infections, 3.7 for gastrointestinal infections, 12.3 for genitourinary infections, and 10.2 for soft-tissue infections^[4]. In a cohort study in Denmark, the incidence of blood stream infection was 13.7 per 100 person-years in hemodialysis patients and 0.53 per 100 person-years in a population control^[7]. These data indicate higher risks of bacteremia and a significant need to reduce bacteremia in hemodialysis patients. In this review, we describe the features of bacteremia, including its prevalence, microbiological features, and risk factors in hemodialysis patients. And we describe details of catheter related bacteremia, a characteristic bacteremia in hemodialysis patients. Furthermore we discuss how to reduce the risk of bacteremia in hemodialysis patients.

INCIDENCE OF BACTEREMIA IN HEMODIALYSIS PATIENTS

The incidence of bacteremia in hemodialysis patients is very high compared with its incidence in the general population. A population-based cohort study in Denmark showed that the incidence of bacteremia was 13.7 per 100 person years in hemodialysis patients, whereas that in the general population was 0.53 per 100 person years^[7]. The incidence of *Staphylococcus aureus* (*S. aureus*) bacteremia in hemodialysis patients was 46.9-fold that of the general population in Denmark^[8]. In studies in Canada, the relative risks of *Pseudomonas aeruginosa* and anaerobe infection were 123.3 and 72.7, respectively, in hemodialysis patients^[9,10].

Almost all studies on bacteremia in Japan were case reports^[11-13]. Sepsis was the second leading cause of death in infectious diseases in a study in Japan^[14]. However, microbiological studies have not been conducted. On the other hand, the greater use of arteriovenous fistula and low catheter use are unique characteristics in Japan^[15]. Eighty nine point seven percent of vascular accesses of Japanese hemodialysis patients was native arteriovenous fistula, 7.1% was arteriovenous grafts, 1.8% was super-

ficialization of artery, and only 0.5% was long-term catheters in 2008^[16]. It might be a reason why few studies have conducted on catheter related bacteremia in Japan. Further studies are required to clarify about the bacteremia in Japan.

In the Dialysis Outcomes and Practice Patterns Study (DOPPS), adjusted relative risks of mortality were 2.84 for Europe and 3.78 for the United States compared with Japan, although it was speculated because not all dialysis facilities in Japan enrolled in the study^[17]. The percentage of infection in cause of death was about 18% in Japan, which is higher than North America and comparable with Europe and Australia/New Zealand in the DOPPS^[18]. The other causes of death may contribute to the difference of mortality.

CAUSATIVE ORGANISMS

Half to 3/4 of the causative organisms of bacteremia in hemodialysis patients are Gram-positive bacteria (Table 1)^[19-21]. The remaining less than 1/4 are Gram-negative. Among the causative organisms, *S. aureus*, including methicillin-resistant *S. aureus* (MRSA), is the most common causative organism. Other staphylococci, including *S. epidermidis* and coagulase negative staphylococcus (CNS), are also common Gram-positive organisms. *Escherichia coli* (*E. coli*), *Enterobacter* species and *Klebsiella* species are the common Gram-negative organisms isolated from blood samples. The pattern of causative organisms was similar in vascular access-associated and catheter-related bacteremia^[20,22]. In hemodialysis patients, rate of *S. aureus* was relatively high and rate of *E. coli* was relatively low compared with the general population^[23,24]. It is unclear whether the ratio of resistant bacteria in hemodialysis patients is higher than that in the general population. In a single-center report from Brazil in 2010-2013, 38.5% of *S. aureus* was MRSA^[25], whereas the methicillin resistance percentage was 31.0% in surveillance data from Brazil in 2005-2008^[26]. However, a national surveillance report for England indicated that the relative risk of MRSA bacteremia was approximately 100-fold higher for dialysis patients than for the general population and was 8-fold higher for patients using a catheter than for those with an arteriovenous fistula^[27]. In addition, another surveillance report from the United States also indicated that dialysis patients had a 100-fold higher risk of MRSA infection than the general population^[28].

CAUSES OF BACTEREMIA

In seven years of hospitalization and death records of hemodialysis patients in the United States Renal Data System, the secondary diagnosis codes among all episodes of septicemia were infection/inflammation caused by internal prostheses (18%), other complications of internal prosthetic device (8%), decubitus ulcer (6%), urinary tract infections (5%), pneumonia (5%), gangrene (3%), endocarditis (2%), and cellulitis and abscess of the foot (1%). These data indicated that septicemia

Table 1 Causative organisms of bacteremia in hemodialysis patients

	Hemodialysis patients	Hemodialysis vascular access-associated	Hemodialysis catheter-related bacteremia	General population				
Ref.	Danese <i>et al</i> ^[19] , 2006	Loo <i>et al</i> ^[20] , 2015	D'Amato-Palumbo <i>et al</i> ^[21] , 2013	Aslam <i>et al</i> ^[22] , 2014	Biedenbach <i>et al</i> ^[23] , 2004		Alfandari <i>et al</i> ^[24] , 2016	
Region	United States	Singapore	United States	Meta-analysis	North America	Latin America	Europe	France
n	15618	144	112	1386	42857	11743	26613	519
Gram positive		73.6%	73.2%					39.7%
<i>Staphylococcus aureus</i> (MRSA)	38.4%	47.2%	50.9%	25.9%	26.0%	21.6%	19.5%	15.4%
(MRSA/SA)		13.9%	23.2%					2.9%
		29.4%	45.6%					18.8%
Other <i>staphylococcus</i>	15.4%	20.1%	10.7%	23.4%	11.5%	13.3%	14.6%	8.3%
<i>Streptococcus</i>	11.9%		2.7%		9.5%	6.8%	6.5%	12.5%
<i>Enterococcus</i>			8.9%		10.2%	3.3%	7.2%	3.5%
Gram negative		26.4%	23.2%	22.0%				55.3%
<i>Escherichia coli</i>	6.5%		4.5%		17.7%	18.2%	22.4%	34.5%
<i>Pseudomonas spp.</i>	3.6%	9.0%	9.8%		4.3%	6.5%	6.1%	1.5%
<i>Enterobacter spp.</i>		4.9%			3.7%	5.5%	4.2%	3.7%
<i>Klebsiella spp.</i>			5.4%		7.6%	10.1%	7.3%	7.1%
<i>Proteus mirabilis</i>								2.3%
<i>Candida spp.</i>		1.2%	3.6%					6.2%

MRSA: Methicillin-resistant *Staphylococcus aureus*.

secondary to vascular access infection was the most common cause of septicemia^[29]. In the analysis of the causes of hospitalization for infection, the leading causes of hospitalization for infection were dialysis access or central venous catheter-related infections (30%), bloodstream infections or sepsis (24%), and pulmonary infections (22%)^[6]. These data also indicated that blood access infection is the most common cause of infection in hemodialysis patients.

RISK FACTORS

The most important risk factor for bacteremia in hemodialysis patients is the use of central venous catheters. Hemodialysis catheter uses were at higher risk of bacteremia compared with arteriovenous fistula or graft uses. A single center study in the United States indicated that the rate of positive blood cultures in patients with central venous catheters was 1.86/1000 d and 0.08/1000 d in patients with an arteriovenous fistula and 0.31/1000 d in patients with an arteriovenous graft^[30]. Rate of infection related hospitalization was higher in the patients with catheters or arteriovenous grafts compared with arteriovenous fistula, the rate ratios were 1.59 and 1.37, respectively^[5]. Analysis of the United States Renal Data System showed that hemodialysis patients with a temporary catheter had a 50% higher risk of septicemia than patients with a native fistula. Patients with a GORTEX or bovine graft had a 33% higher risk of septicemia than patients with a native fistula during throughout seven years of follow-up^[29]. In a retrospective study of a hospital in Brazil, a multiple regression analysis showed that the use of a central venous catheter was associated with an 11.2-fold increased risk of bloodstream infections compared with arteriovenous fistula for vascular access^[25]. In addition, a second leading risk factor was previous hospitalizations, which had an odds ratio of 6.63 in a multiple logistic regression analysis^[25]. In patient charac-

teristics, the adjusted risk ratios of age > 65 years, diabetes mellitus, and serum albumin < 3.5 were 1.61, 1.26 and 1.66, respectively^[29]. The patients who reused dialyzers had a 28% higher risk of septicemia than patients who did not reuse membranes^[29].

CATHETER-RELATED BLOOD STREAM INFECTIONS

Long-term catheters are essential for hemodialysis patients whose blood access site is limited. Catheters are a major risk factor for bacteremia as described above, and they cannot be easily changed. Therefore, especially careful handling is needed to prevent catheter-related blood stream infections (CRBSI).

Diagnosis of CRBSI

In the guidelines for the diagnosis and management of intravascular catheter-related infection by the Infectious Diseases Society of America, a definitive diagnosis of CRBSI requires: (1) a set of peripheral blood cultures; (2) blood cultures from a peripheral vein and from a culture of the catheter tip; or (3) cultures from an arterial and venous catheter hub that meet differential time-to-positivity criteria^[31]. A report from Canada indicated that blood cultures drawn from a hemodialysis circuit were the most sensitive, specific, and accurate for diagnosing CRBSIs when all culture data and clinical information were factored into the assessment^[32]. For the hemodialysis circuit, the values for sensitivity, specificity, and accuracy were 93.5%, 100% and 95%, respectively, whereas peripheral veins had a sensitivity of 93.9%, a specificity of 92.5%, and an accuracy of 93%.

Antibiotic lock for hemodialysis catheter

There are many reports and a meta-analysis of antibiotic lock for hemodialysis catheters^[33-39]. The antibiotics

Table 2 Antibiotic concentrations applied in locks^[31]

	Dosage (mg/mL)	Heparin or saline, IU/mL
Vancomycin	2.5	2500 or 5000
Vancomycin	2.0	10
Vancomycin	5.0	0 or 5000
Ceftazidime	0.5	100
Cefazolin	5.0	2500 or 5000
Ciprofloxacin	0.2	5000
Gentamicin	1.0	2500
Ampicillin	10.0	10 or 5000
70% ethanol		0

include gentamycin, minocycline, cefotaxime, cefazolin, and vancomycin (Table 2). Moreover, antiseptics including taurolidine and trisodium citrate have also been tested. Antibiotic lock therapy significantly reduced CRBSI in all studies. In a subgroup analysis of each antibiotic, the reductions in bacteremia rates remained significant for locks containing gentamicin, minocycline, cefotaxime, and vancomycin and gentamicin, but not for those containing taurolidine, or cefazolin and gentamicin, or citrate^[33,39]. Although they are associated with a significant reduction of CRBSI, the guidelines of the Centers for Disease Control and Prevention (CDC) do not recommend the routine use of antibiotic lock, and limit this treatment to patients with long-term catheters who have a history of multiple CRBSIs despite optimal maximal adherence to aseptic techniques because of the potential for side effects, toxicity, allergic reactions, or the emergence of resistance to the antimicrobial agent^[40].

Topical antibiotics

The prophylactic effects of the application of topical antibiotics, including mupirocin and polysporin triple antibiotic ointments, to the exit sites of the catheter were also investigated. These topical antibiotics significantly reduced CRBSI in hemodialysis patients. A randomized trial indicated that the topical use of polysporin triple antibiotic ointment to catheter exit sites reduced the relative risk of bacteremia by 60%, as well as the relative risk of mortality by 78%^[41]. Mupirocin ointment is also effective to reduce CRBSI. In a randomized controlled trial, application of mupirocin ointment to the catheter exit sites reduced CRBSI by 85%^[42]. However, the rapid emergence of resistant *S. aureus* and CNS has been reported^[43-45]. Based on this evidence, the CDC guidelines recommend the antibiotic ointment for only hemodialysis patients.

Antibiotics/antiseptics coated catheters

Antibiotics or antiseptics impregnated or coated catheters can reduce the catheter related bacteremia^[46,47]. The duration of catheter use in these studies were within a month, and the efficacy in long term use of these catheter has not been established^[48].

Treatment of catheter related bacteremia

Generally, the treatment of bacteremia is the administra-

Table 3 Antibiotic dosing for patients who are undergoing hemodialysis^[31]

Empirical dosing pending culture results
Vancomycin plus empirical gram-negative rod coverage based on local antibiogram data
Or
Vancomycin plus gentamicin
(Cefazolin may be used in place of vancomycin in units with a low prevalence of methicillin-resistant staphylococci)
Vancomycin: 20 mg/kg loading dose infused during the last hour of the dialysis session, and then 500 mg during the last 30 min of each subsequent dialysis session
Gentamicin (or tobramycin): 1 mg/kg, not to exceed 100 mg after each dialysis session
Ceftazidime: 1 g <i>iv</i> after each dialysis session
Cefazolin: 20 mg/kg <i>iv</i> after each dialysis session
For Candida infection
An echinocandin (caspofungin 70 mg <i>iv</i> loading dose followed by 50 mg <i>iv</i> daily; intravenous micafungin 100 mg <i>iv</i> daily; or anidulafungin 200 mg <i>iv</i> loading dose, followed by 100 mg <i>iv</i> daily); fluconazole (200 mg orally daily); or amphotericin-B

iv: Intravenous.

tion of systemic antibiotics and the care of the infection site; for CRBSI, this necessitates catheter removal. Initial treatment is empiric systemic antibiotics and antibiotic lock (Figure 1, Tables 2 and 3)^[31]. Recommended empirical antibiotics are vancomycin plus empirical gram-negative rod coverage based on local antibiogram data. Catheter removal is required in cases that bacteremia or clinical symptom persists, or in cases that causative organisms could colonize in the surface of catheters (Figure 1). For hemodialysis patients, catheters sometimes cannot be removed due to limited blood access sites. Antibiotic lock therapy and guidewire exchange of the catheter are attempted in those cases. A meta-analysis compared systemic antibiotics, antibiotic lock therapy and guidewire exchange of the catheter to treat patients with tunneled hemodialysis catheter-related bacteremia^[22]. The cure proportions were significantly higher with antibiotic lock therapy than with systemic antibiotics (OR = 2.08; 95%CI: 1.25-3.45; $P < 0.01$) and were higher with guidewire exchange than with systemic antibiotics (OR = 2.88; 95%CI: 1.82-4.55; $P < 0.001$). In particular, for those with *S. aureus* infections, guidewire exchange achieved a significantly higher cure proportion than both systemic antibiotics and antibiotic lock therapy (OR = 3.33, 95%CI: 1.17-9.46, $P = 0.02$; OR = 4.72, 95%CI: 1.79-12.46, $P = 0.002$). However, guidelines recommend systemic antibiotics and catheter removal, especially for infection with *S. aureus*, *P. aeruginosa*, or *Candida* species^[31,48]. Guidewire exchange is considered to be an alternative only if another insertion site is not available. This study described above was a meta-analysis of observational studies. Randomized controlled studies are required to obtain further insight.

Improvement of infection control measures

In 2009, the CDC established a collaborative project to prevent bloodstream infection in hemodialysis

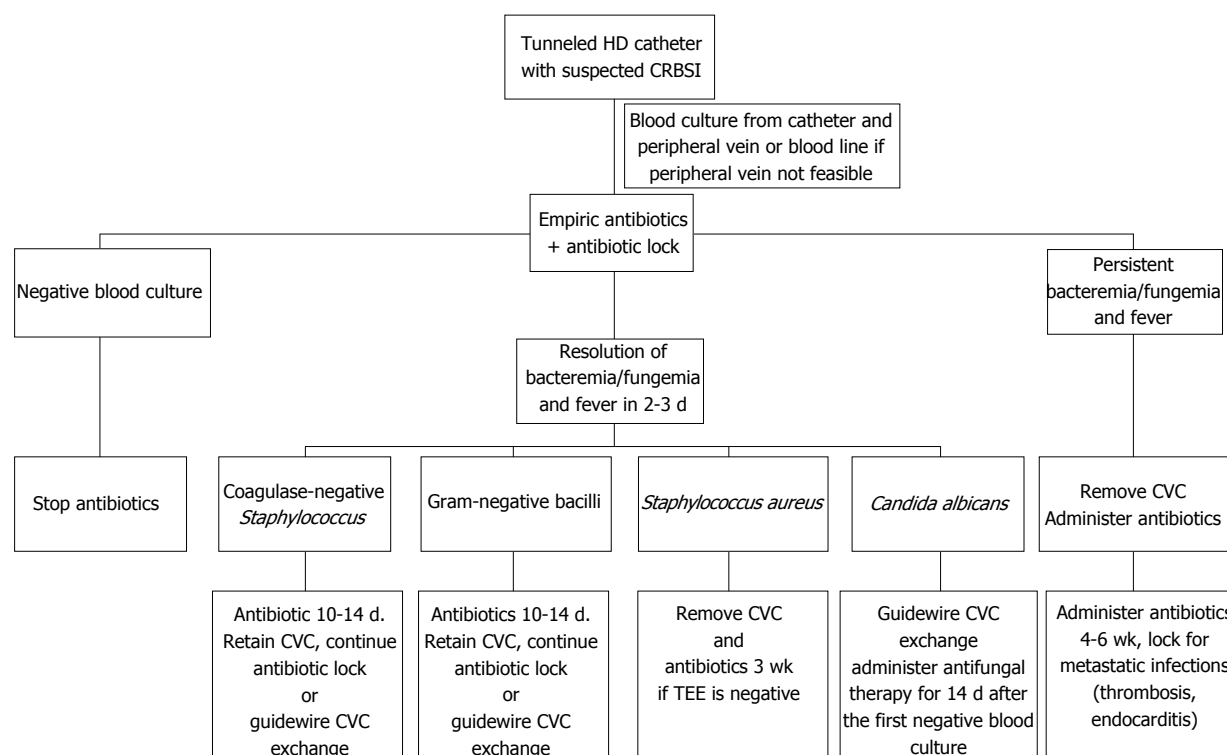


Figure 1 Catheter-related blood stream infection among patients who are undergoing hemodialysis with tunneled catheters^[31]. CVC: Central venous catheter; TEE: Transesophageal echocardiograph; HD: Hemodialysis; CRBSI: Catheter-related blood stream infection.

Table 4 Core interventions of dialysis blood stream infections prevention in collaboration with the Centers for Disease Control and Prevention

Surveillance and feedback using NHSN

Conduct monthly surveillance for BSIs and other dialysis events using NHSN-Dialysis Surveillance. Calculate facility rates and compare to rates in other NHSN facilities. Actively share results with front-line clinical staff. See Data Reports on this website (available from: URL: <http://www.cdc.gov/dialysis/reports-news/data-reports.html>)

Hand hygiene observations

Perform observations of hand hygiene opportunities monthly and share results with clinical staff. See observation protocols for hand hygiene and glove use on this website (available from: URL: <http://www.cdc.gov/dialysis/PDFs/collaborative/Hemodialysis-Hand-Hygiene-Observations.pdf>)

Catheter/vascular access care observations

Perform observations of vascular access care and catheter accessing quarterly. Assess staff adherence to aseptic technique when connecting and disconnecting catheters and during dressing changes. Share results with clinical staff

Staff education and competency

Train staff on infection control topics, including access care and aseptic technique. Perform competency evaluation for skills such as catheter care and accessing every 6-12 mo and upon hire. See staff education on this website (available from: URL: <http://www.cdc.gov/dialysis/clinician/index.html>)

Patient education/engagement

Provide standardized education to all patients on infection prevention topics including vascular access care, hand hygiene, risks related to catheter use, recognizing signs of infection, and instructions for access management when away from the dialysis unit. See patient education on this website (available from: URL: <http://www.cdc.gov/dialysis/clinician/index.html>)

Catheter reduction

Incorporate efforts (e.g., through patient education, vascular access coordinator) to reduce catheters by identifying and addressing barriers to permanent vascular access placement and catheter removal

Chlorhexidine for skin antisepsis

Use an alcohol-based chlorhexidine (> 0.5%) solution as the first line skin antiseptic agent for central line insertion and during dressing changes. Povidone-iodine (preferably with alcohol) or 70% alcohol are alternatives for patients with chlorhexidine intolerance

Catheter hub disinfection

Scrub catheter hubs with an appropriate antiseptic after cap is removed and before accessing. Perform every time catheter is accessed or disconnected. If closed needleless connector device is used, disinfect connector device per manufacturer's instructions

Antimicrobial ointment

Apply antibiotic ointment or povidone-iodine ointment to catheter exit sites during dressing change. See information on selecting an antimicrobial ointment for hemodialysis catheter exit sites (selecting an antimicrobial ointment). Use of chlorhexidine-impregnated sponge dressing might be an alternative

NHSN: National Healthcare Safety Network; BSIs: Blood stream infections.

patients^[49]. Core interventions included surveillance and feedback using the National Healthcare Safety Network, audits of hand hygiene, observation of vascular access care, and other infection control measures (Table 4). In the analysis of 17 outpatient hemodialysis facilities that participated in the project, in the pre-intervention period, the pooled mean blood stream infections (BSI) and access-related BSI rates were 1.09 and 0.73 events per 100 patient-months, respectively. After the intervention, these rates decreased to 0.89 and 0.42 events per 100 patient-months, respectively^[49]. Furthermore, in a report using positive deviance to improve the infection control measures in addition to the collaborative interventions of the CDC, the incidence of all access-related BSIs reduced from 2.04 per 100 patient-months pre-intervention to 0.75 after employing the collaborative interventions and to 0.24 after augmenting the collaborative interventions with positive deviance^[50]. Positive deviance is a behavioral change process based on the observation of those with uncommon, beneficial practices who consequently experience better outcomes than their neighbors who share similar risks^[51]. These data indicated that the improvement of infection control practices could reduce bacteremia in hemodialysis patients.

CONCLUSION

The prevalence of blood stream infection in hemodialysis patients is much higher than in the general population. Furthermore, bacteremia is sometimes life-threatening. Improvement of basic infection control measures, including appropriate hand hygiene, catheter care, and education for medical staff and patients, could reduce the occurrence of bacteremia, although this is difficult because the blood streams of these patients are frequently exposed to extracorporeal devices.

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Retrospective Cohort Study

Outcomes of renal transplant recipients with BK virus infection and BK virus surveillance in the Auckland region from 2006 to 2012

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Abstract

AIM

To evaluate incidence, risk factors and treatment outcome of BK polyomavirus nephropathy (BKVN) in a cohort of renal transplant recipients in the Auckland region without a formal BK polyomavirus (BKV) surveillance programme.

METHODS

A cohort of 226 patients who received their renal trans-

plants from 2006 to 2012 was retrospectively reviewed.

RESULTS

Seventy-six recipients (33.6%) had a BK viral load (BKVL) test and 9 patients (3.9%) developed BKVN. Cold ischaemia time (HR = 1.18, 95%CI: 1.04-1.35) was found to be a risk factor for BKVN. Four recipients with BKVN had complete resolution of their BKV infection; 1 recipient had BKVL less than 625 copies/mL; 3 recipients had BKVL more than 1000 copies/mL and 1 had graft failure from BKVN. BKVN has a negative impact on graft function [median estimated glomerular filtration rate (eGFR) 22.5 (IQR 18.5-53.0) mL/min per 1.73 m², $P = 0.015$], but no statistically significant difference ($P = 0.374$) in renal allograft function was found among negative BK viraemia group [median eGFR 60.0 (IQR 48.5-74.2) mL/min per 1.73 m²], positive BK viraemia without BKVN group [median eGFR 55.0 (IQR 47.0-76.0) mL/min per 1.73 m²] and unknown BKV status group [median eGFR 54.0 (IQR 43.8-71.0) mL/min per 1.73 m²]. The incidence and treatment outcomes of BKVN were similar to some centres with BKV surveillance programmes.

CONCLUSION

Recipients with BKVN have poorer graft function. Although active surveillance for BKV has been shown to be effective in reducing incidence of BKVN, it should be tailored specifically to that transplant centre based on its epidemiology and outcomes of BKVN, particularly in centres with limited resources.

Key words: BK virus; BK polyomavirus nephropathy; Kidney transplantation; Screening

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Core tip: A retrospective analysis of 226 patients from Auckland, New Zealand found BK polyomavirus (BKV) as an uncommon cause of graft loss. Renal units without a formal BKV surveillance programme showed a similar incidence and outcomes for BK polyomavirus nephropathy (BKVN) to centres with an active screening programme. When designing a cost effective screening programme for BKV infection, it should be centre specific in relation to the units immunosuppression and monitoring protocol, epidemiology and outcomes of BKVN.

Hsiao CY, Pilmore HL, Zhou L, de Zoysa JR. Outcomes of renal transplant recipients with BK virus infection and BK virus surveillance in the Auckland region from 2006 to 2012. *World J Nephrol* 2016; 5(6): 497-506 Available from: URL: <http://www.wjgnet.com/2220-6124/full/v5/i6/497.htm> DOI: <http://dx.doi.org/10.5527/wjn.v5.i6.497>

INTRODUCTION

Since the first discovery of BK polyomavirus (BKV) isolated from the urine of a renal allograft recipient with

ureteric obstruction in 1971^[1], BKV infection has emerged as an important cause of renal allograft dysfunction. In the modern era, with the use of more potent immunosuppressive agents, BKV nephropathy (BKVN) has resulted in a significant rate of graft loss^[2].

Seroprevalence of BKV in immunocompetent adults ranges from 65% to 90% and BKV remains latent predominantly in the urinary tract^[3]. Immunosuppression, inflammation and insufficient anti-viral immune responses play an integral role in the reactivation and replication of BKV and progression to BKVN in renal transplant recipients^[4]. Dendritic cell deficiency, seronegativity for BKV and impaired BKV-specific T-cell response are found to be associated with BK viraemia^[5-8]. Other possible risk factors for BK viraemia include a long cold ischaemia time (CIT)^[9], acute rejection^[10,11], placement of a ureteric stent^[12,13], human leukocyte antigen (HLA) mismatch^[11,14], lymphocyte-depleting agents^[15-18], tacrolimus^[19,20] and steroids^[16,19,20]. Given the complexity in the pathogenesis of BKVN, the intensity of immunosuppression may not be solely responsible for the development of BKVN and it may not be appropriate to generalise these predisposing risk factors in all renal transplant recipients. However, as the immunosuppressive burden appears a significant risk factor for the development of BK polyomavirus, it is possible that the immunosuppression regimen used accounts at least in part for the substantial variation in the prevalence of BK viraemia (30%-62%), BK viraemia (11.5%-20%) and BKVN (1%-10%) among transplant centres^[10,12,21,22].

No effective pharmacological treatment has consistently emerged for the treatment of BKV infection apart from a reduction of immunosuppressants^[23,24]. Many investigators have recommended screening BK viraemia and pre-emptive reduction in immunosuppressants as a strategy to preserve graft function and reduce the risk of BKVN occurring^[25-28]. However, a screening programme may not be suitable in a transplant centre with a low rate of BKVN^[29].

We conducted a retrospective review of renal transplant recipients from our region, where no lymphocyte depleting induction treatment is used and the predominant calcineurin inhibitor utilised is Ciclosporin, to determine the incidence of BKVN and/or BK viraemia and to evaluate the characteristics that are potentially associated with the development of BKVN and related treatment outcome.

MATERIALS AND METHODS

Patients

We searched through the Auckland Renal Transplant Group's database to identify patients who received renal transplants from January 2006 to December 2012. Patients resident outside the Auckland region were not included in this retrospective review. We excluded those who died or had primary graft failure within 1 mo of receiving a renal allograft. Patients' clinical notes with

clinical and demographic data were obtained from the clinical electronic portal system, Concerto® and 3M® Health Information Systems, with data censored for 31 December 2013.

Testing for serum BKV viral load is performed at a single laboratory, LabPlus™, in the Auckland region. The laboratory does not routinely perform BKV viral load in urine samples, due to the high prevalence of positive BK viraemia in renal transplant recipients. Recipients are considered to have BK viraemia for any level of viral load. Testing for serum creatinine is performed at both hospital and community laboratories in the Auckland region. Estimated glomerular filtration rate (eGFR) was calculated using the CKD-EPI equation^[30]. The recipients' renal biopsies performed in the Auckland region were processed and interpreted at a single laboratory: LabPlus™.

BKV surveillance

There is no formal screening programme among the three renal units in the Auckland region. One unit routinely screened for BKV from the year 2012. One of these hospitals trialled screening for BKV in 2009, but halted this strategy due to cost, and BK viral load (BKVL) test is performed only if there is a biopsy-proven BKVN. The third unit checks for BKV if there is a clinical indication or a biopsy-proven BKVN.

Immunosuppression protocol

Basiliximab was routinely used for induction from August 2010. All male recipients were given Ciclosporin as their maintenance calcineurin inhibitor, but female recipients were given a choice of either Tacrolimus or Ciclosporin. The tacrolimus trough concentration in each recipient was kept between 10 to 15 µg/L for the first 2 mo after transplant, and then the target concentration was kept between 5 to 10 µg/L. The Ciclosporin (2 h post dose) target concentration was 1700 µg/L for the first month after transplant and 1500 µg/L for the second month. The target concentration was then gradually reduced to equal to or less than 800 µg/L after 12 mo of transplantation. Every recipient received Mycophenolate mofetil (MMF) 1 g twice daily if on Ciclosporin or 750 mg twice daily if on Tacrolimus. Concentration monitoring for MMF is not routinely performed. All recipients received Methylprednisolone 1 g at induction followed by prednisone as maintenance. The prednisone dose was 30 mg daily for all recipients and it was tapered down to 7.5 mg daily or lower at months 4 post-transplant.

Acute rejection treatment protocol

Recipients who have acute rejection Banff grade I receive Methylprednisolone 500 mg daily for 3 d. For Banff grade II, III and steroid-resistant rejections, patients receive lymphocyte-depleting agents. Biopsy-proven rejection in the presence of therapeutic Ciclosporin concentrations necessitates a conversion to Tacrolimus unless contraindicated. The target Tacrolimus concentration is maintained between 7 to 10 µg/L.

Renal transplant biopsy protocol

A protocol biopsy at 3 mo after transplantation is performed in all renal transplant recipients. Patients with a > 20% decline in graft function without a clear cause and all reversible non-renal causes excluded undergo renal biopsy.

Statistical analysis

SPSS version 22 was used to perform statistical analysis. Results are expressed as numbers (percentages), median (interquartile range Q1-Q3) and mean (± SD) unless otherwise stated. The 95% CIs are based on exact confidence limits. Data were compared by χ^2 test, Fisher's exact test, non-parametric Mann-Whitney *U* test or Kruskal-Wallis test as appropriate. Cox regression was used to identify possible risk factors for BKVN. Those who died, developed graft failure unrelated to BKVN and were transferred to outside of the Auckland region were censored in this model.

RESULTS

Description of the study population

Four hundred and twenty-eight patients underwent renal transplantation between January 2006 and December 2012. After excluding 194 patients from outside of the Auckland region and 8 patients who died or developed primary graft failure at one month after transplant, 226 were included in the study (Figure 1).

Seventy-six recipients were tested for BKV (33.6%) at any point in time over the study period. Twenty-eight patients of 76 tested patients had BK viraemia (36.8%). Twenty of these 28 patients were managed with a reduction of their immunosuppressants; by reducing or stopping MMF and maintaining Tacrolimus trough concentration and Ciclosporin trough concentration between 4-6 µg/L and 100-150 µg/L, respectively, depending on BKVLs' responses. Seven patients received Leflunomide concurrently; one BKVN recipient was given additional Ciprofloxacin as the BKVL failed to decline despite reducing immunosuppressants, and another BKVN recipient also received Ciprofloxacin and subsequently intravenous Immunoglobulin due to persistent high level of BK viraemia, worsening graft dysfunction and a repeat graft biopsy showed features of acute rejection in addition to BKVN. The remaining 8 BK viraemic recipients did not have a reduction of immunosuppressants due to low viral loads (all less than 1250 copies/mL).

Of these 28 patients, 16 patients had transplant biopsies for renal allograft dysfunction. Nine patients had biopsy-proven BKV nephropathy equivalent to an incidence of 11.8% (95%CI: 5.6%-21.3%) of the cohort tested for BKV viral load (9/76) or 4.0% (95%CI: 1.8%-7.4%) of the entire cohort (9/226). Eight of these nine patients were tested for BKVLs after their transplant biopsies diagnosed BKVN. The other patient with BKVN had the transplant biopsy and serum BKVL requested concurrently as part of investigation of graft dysfunction. The remaining seven transplant recipients with transplant

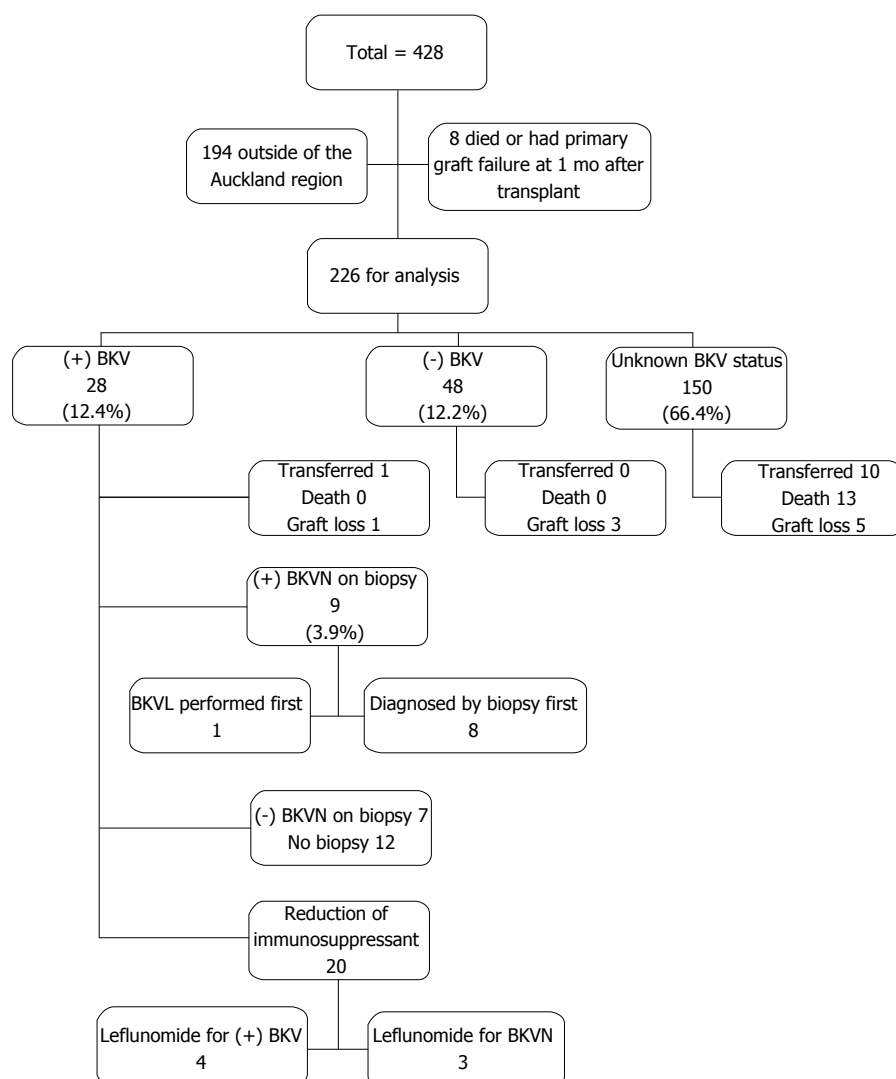


Figure 1 Description of the study population from January 2006 to December 2012 with follow-up until December 2013. BKV: BK polyomavirus; BKVN: BK polyomavirus nephropathy.

biopsies for graft dysfunction did not have BKVN. The other 12 recipients did not have transplant biopsies as their graft functions were stable, and 7 of these 12 recipients had their immunosuppression reduced.

Three (33.3%) of the recipients with BKVN did not have acute rejection prior to the diagnosis of BKVN. The other 6 recipients (66.7%) had at least one episode of biopsy proven acute rejection requiring a pulse methylprednisolone course prior to the development of BKVN. Ten of the 19 recipients (53.7%) with BK viraemia did not have acute rejection prior to the diagnosis of BK viraemia and the others (47.3%) had at least one acute rejection prior.

Associations with BKV nephropathy

When comparing features between recipients with BKVN and without BKVN, we found that BKVN was more common in Māori, Pacific Islanders and Asians than in Europeans (European 1.5% vs Asian 9.3% vs Māori and Pacific Islanders 7.4%, $P = 0.038$). The renal allografts of the recipients that developed BKVN were all from deceased

donors ($P = 0.005$). The BKVN group had a longer CIT (median 19 h vs 7 h, $P = 0.001$). The group was also more likely to have at least 1 episode of acute rejection at any time point, though it was not statistically significant ($P = 0.069$). There was no significant difference observed in age, gender, co-morbidity, dialysis vintage, HLA mismatch, Tacrolimus, Basiliximab induction, or use of lymphocyte-depleting agents for acute rejection between the positive (+) BKVN and negative (-) BKVN groups (Table 1).

Comparison of recipients with BK viraemia only and recipients with BKVN

There was no statistical difference in ethnicity, acute rejection at any time point and other demographics when comparing the BKVN group and the (+) BKV group (recipients with only BK viraemia and no biopsy-proven BKVN). The (+) BKV group had fewer deceased donors (47.4%, $P = 0.01$) and shorter CIT (median 5.4 h, $P < 0.0001$) than patients who developed BKV nephropathy. BK viraemic recipients (47.4%) and recipients with BKVN

Table 1 Demographics and clinical characteristics of renal transplant recipients with/without BK polyomavirus nephropathy and comparison between those with BK polyomavirus nephropathy and BK viraemia *n* (%)

	(-) BKVN (<i>n</i> = 217)	(+) BKVN (<i>n</i> = 9)	<i>P</i> value	(+) BKV (<i>n</i> = 19)	<i>P</i> value
Age (yr)	48 (35.3-56.8)	55 (34.9-60.5)	NS	50 (27.1-60)	NS
Gender (female)	92 (42.3)	3 (33.3)	NS	6 (31.6)	NS
Ethnicity					
European	131 (98.5)	2 (1.5)	0.038	11 (57.9)	0.191
Asian	32 (90.7)	3 (9.3)		4 (21.1)	
Māori and Pacific Islander	54 (92.6)	4 (7.4)		4 (21.1)	
Cause of ESKF					
Glomerulonephritis	91 (41.9)	5 (55.6)	NS	8 (42.1)	NS
Diabetes mellitus	26 (12.0)	2 (22.2)		2 (10.5)	
Others	55 (46.1)	2 (22.2)		9 (47.4)	
Co-morbidity					
Diabetes mellitus	26 (11.9)	2 (22.2)	NS	2 (10.5)	NS
Hypertension	130 (59.9)	5 (55.5)	NS	12 (63.2)	NS
Cardiac disease	31 (14.2)	2 (22.2)	NS	3 (15.8)	NS
Dialysis vintage (yr)	2 (0.2-5)	5 (1-6)	NS	2.5 (0-5)	NS
Donor source (deceased)	118 (54.3)	9 (100)	0.005	9 (47.4)	0.01
HLA mismatch	3 (2-4)	4 (2.5-4)	NS	3.5 (2-5)	NS
Basiliximab induction	95 (43.7)	3 (33.3)	NS	13 (68.4)	NS
Tacrolimus	92 (42.3)	5 (55.6)	NS	10 (52.6)	NS
Cold ischaemia time (h)	7 (4-15)	19 (14.2-20.4)	0.001	5.4 (4-12.5)	< 0.0001
AR at any time point					
0 episode	144 (66.4)	3 (33.3)	0.069	9 (47.4)	NS
≥ 1 episode(s)	73 (33.6%)	6 (66.7)		10 (52.6)	
AR before known BKV/BKVN status					
10 → 0 episode	-	3 (33.3)		10 (52.6)	NS
11 → ≥ 1 episode(s)	-	6 (66.7)		9 (47.6)	
Thymoglobulin for acute rejection	33 (15.2)	0 (0)	NS	5 (26.3)	NS

Values are expressed as numbers (percentages) and medians (interquartile range Q1-Q3). *P* value are calculated using non-parametric test and Fisher's exact test, and actual values are shown if *P* < 0.1. BKVN: BK polyomavirus nephropathy; (+) BKV: Recipients with BK viraemia without biopsy-proven BKV nephropathy; NS: Not significant; AR: Acute rejection; ESKF: End-stage kidney failure; HLA: Human leukocyte antigen.

(66.7%) were more likely to have acute rejection prior to the diagnosis of BKVN and BK viraemia as opposed to recipients without BK viraemia (27.1%, *P* = 0.048).

Risk factors for BKVN

In a univariable Cox regression analysis, Asian recipients had a greater risk of developing BKVN compared with European recipients (unadjusted HR = 6.36, 95%CI: 1.96-38.16, *P* = 0.043), but it was not seen in Māori and Pacific islands recipients (unadjusted HR = 4.75, 95%CI: 0.87-25.94, *P* = 0.072) (Table 2). Because CIT is dependent on donor source, donor source was not used in the analysis. Recipients with longer CIT had a higher risk of developing BKVN (unadjusted HR = 1.18, 95%CI: 1.06-1.32, *P* = 0.003). While acute rejection appeared to be associated with BKVN, this did not reach statistical significance (unadjusted HR = 3.72, 95%CI: 0.93-14.91, *P* = 0.063).

We included only the variables that had *P* value < 0.1 in a multivariable model. Ethnicity was not found to be significant, and Longer CIT was the only risk factor for BKVN (HR = 1.18, 95%CI: 1.05-1.39, *P* = 0.009).

Effect of BKVN and BK viraemia on renal allograft function

Renal allograft function in the BKVN group was signifi-

cantly lower comparing with those in the other BKV status groups (*P* = 0.015), and the median graft function of the recipients who were never checked for BK viraemia was similar to those with and without BK viraemia using the non-parametric Kruskal-Wallis test (*P* = 0.374) (Figure 2). After controlling for those factors (age at transplant, comorbidity, donor source, HLA mismatch, acute rejection, Basiliximab induction, calcineurin inhibitor and eGFR at 1 mo after transplant) that could potentially affect graft function, the mean eGFR of the recipients with BKVN (taken just before the censored date or before recipients were transferred, but those who developed graft failure were not included) was still 17.0 mL/min per 1.73 m² (95%CI: -32.5 to -1.5 mL/min per 1.73 m², *P* = 0.032) lower than that of those without BKVN. However, no significant impact on graft function was found in recipients with only BK viraemia (-4.1 mL/min per 1.73 m², *P* = 0.464) and unknown BKV status (-1.1 mL/min per 1.73 m², *P* = 0.754) comparing with the negative BK viraemic recipients.

One of the 9 recipients (11.1%) with BKVN had graft failure compared to 8 (3.7%) of those without, but there was no statistical difference found using a log-rank test in the Kaplan-Meier survival analysis (*P*_{log-rank} = 0.283) (Figure 3). Similarly, no difference was found between those with known and unknown BKV status (0.92 for positive BKV and BKVN, 0.90 for negative BKV, and 0.92

Table 2 Univariable and multivariable Cox regression to assess potential risk factors for BK polyomavirus nephropathy

	Crude HR (+) BKVN	95%CI	P value	Adjusted HR (+) BKVN	95%CI	P value
Gender (female)	0.66	0.16-2.66	NS			
Ethnicity (reference: European)						
Asian	6.36	1.06-38.16	0.043	3.73 ¹	0.61-22.92	0.154
Māori/Pacific Islander	4.75	0.87-25.94	0.072	2.63 ¹	0.45-15.25	0.279
Co-morbidity						
Diabetes mellitus	2.06	0.42-9.94	NS			
Basiliximab induction	0.75	0.18-3.03	NS			
Tacrolimus	1.64	0.44-6.11	NS			
Thymoglobulin for rejection	0.03	0.00-88.65	NS			
Cold ischaemia time	1.18	1.06-1.32	0.003	1.18 ¹	1.04-1.35	0.009
Acute rejection (≥ 1 episode) ²	3.72	0.93-14.91	0.063	4.05 ¹	0.99-16.53	0.051
HLA mismatch	1.15	0.75-1.77	NS			

¹Variables included in the multivariable Cox regression model; ²Acute rejection at any time point. HR: Hazard ratio; CI: Confidence interval; (+) BKVN: BK polyomavirus nephropathy; HLA: Human leukocyte antigen; NS: No significance.

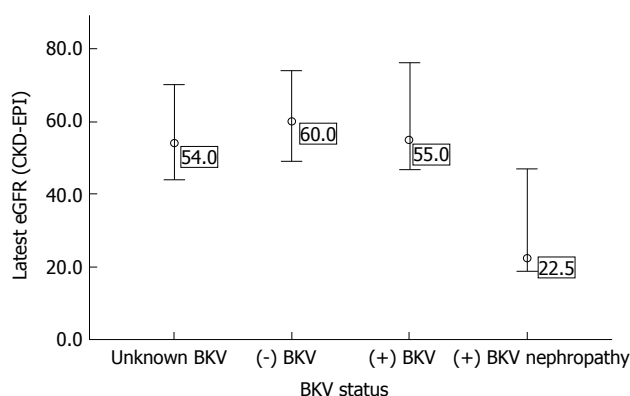


Figure 2 Comparison of unadjusted graft function (median estimated glomerular filtration rate mL/min per 1.73 m²) taken before the censored date among BK polyomavirus status groups, excluding those who developed graft failure. BKV: BK polyomavirus; eGFR: Estimated glomerular filtration rate.

for unknown BKV status, $P_{\log\text{-rank}} = 0.568$) (Figure 4).

Outcome of management of BKVN and BK viraemia

For those diagnosed with BKVN, BKVLs were undetectable in 4 recipients and the BKVL was less than 625 copies/mL in one recipient at the time of censoring. The other 3 patients still had persistent BK viraemia but viral loads were declining at the end of study period (Table 3). One patient developed graft failure due to BKVN, but it is important to note that this recipient's graft function was poor prior to the diagnosis of BKVN (eGFR 23-27 mL/min per 1.73 m²) with no clear explanation despite 4 graft biopsies that did not show any significant abnormality. BKVLs of the recipients with BK viraemia were either undetectable or declining during the study period. None of these 19 patients, including six of them with BKVLs more than 10⁴ copies/mL, progressed to BKVN over the study period.

Of the patients who had a reduction in immunosuppression none of the 11 patients with BK viraemia had acute rejection, while two of the 9 recipients with BKVN developed an acute T-cell mediated rejection grade 1B at 30 mo and 2 mo after their immunosuppressive therapy

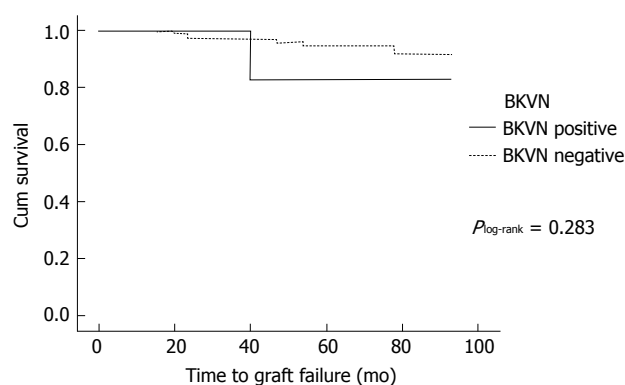


Figure 3 Kaplan-Meier plot showing graft failure rates comparing recipients with/without BK polyomavirus nephropathy censored for death without graft failure and recipients transferred. BKVN: BK polyomavirus nephropathy.

was reduced. The acute rejections were successfully treated with intravenous pulse methylprednisolone, and their calcineurin inhibitors were already switched to Tacrolimus from previous acute rejection prior to the diagnosis of BKVN.

DISCUSSION

This retrospective study has demonstrated that only one patient had graft failure due to BKVN in our cohort of patients. We identified 28 patients with BKV infection with a rate of 36.8% in the cohort that was screened. However, this has likely overestimated the incidence as 150 patients (66.4%) were not tested for BK viraemia. When considering the entire cohort the rate of BKV was 12.4% (28 of 226). The incidence of biopsy proven BKVN was 11.8% (9/76). It is important to note that it is unlikely that any patient with clinically significant BKV did not undergo testing due to the regional protocol to perform renal biopsy. However, it is possible that early BKVN could be missed by renal biopsy due to its focal nature^[25]. Using the overall cohort we noted an incidence of 4.0% (9/226) which is comparable to that of other transplant centres with an active BKV screening programmes (0.8% to 6.4%)^[11,26-28,31-33].

Table 3 Clinical outcome of the renal transplant recipients with BK polyomavirus nephropathy after a reduction of immunosuppressive therapy

Recipients with BKVN	BKVL at diagnosis	eGFR at 1 mo	eGFR at CD	BKVL outcome ¹	Time to outcome ¹	Acute rejection (post reduction of IS)
1	1100000	48.7	73	Undetectable	28 mo	No
2	3250	17.8	22	Undetectable	44 mo	Yes (at 30 mo)
3	364700	36.9	23	Undetectable	31 mo	Yes (at 2 mo)
4	265650	21.5	59	Undetectable	17 mo	No
5	47225	23	18	< 625	7 mo	No
6	179450 ²	57	35	3725	59 mo	No
7	5973650 ^{2,3}	33.4	20	41175	12 mo	No
8	29560875 ^{2,3,4}	50.2	16	30425	8 mo	No
9	56125	27.9	Graft failure	Graft failure	21 mo	No

¹Follow-up censored at December 2013; ²Use of Leflunomide; ³Use of Ciprofloxacin; ⁴Use of intravenous Immunoglobulin. BKVL: BK viral load (copies/mL); BKVN: BKV Nephropathy; IS: Immunosuppressant; CD: Censored date; eGFR: Estimated glomerular filtration rate (mL/min per 1.73 m²).

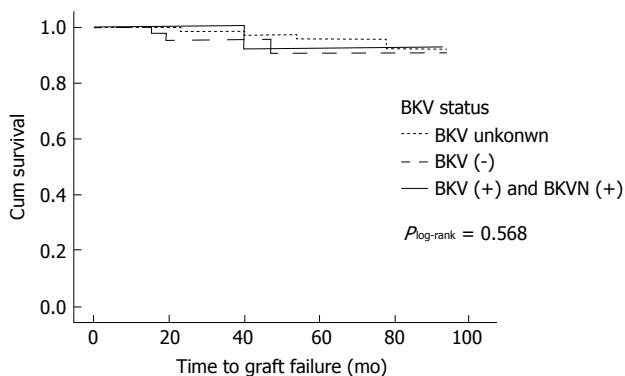


Figure 4 Kaplan-Meier plot showing graft failure rates comparing renal transplant recipients with different BK polyomavirus status censored for death without graft failure and recipients transferred. BKV: BK polyomavirus; BKVN: BK polyomavirus nephropathy.

A long CIT was identified as a potential risk factor for BKVN in our study, and is known to cause severe ischaemia-reperfusion injury resulting in intragraft inflammation, which in turn is found to stimulate BK polyomavirus DNA replication^[34,35]. Acute rejection results in tubulointerstitial inflammation and typically leads to also an increased burden of immunosuppression. However, interestingly in our cohort, acute rejection was not found to be a risk factor for progressing to BKVN. Although others have demonstrated an association between Tacrolimus use and BKVN, Tacrolimus was not found to be associated with BKVN in our cohort. Despite lymphocyte-depleting agents were used for severe acute rejection episodes in this cohort, our analysis did not demonstrate more recipients with BK viraemia and BKVN. Other studies have also failed to show any correlation between lymphocyte-depleting agents given at induction and BKV infection^[26,28].

Because of the difficulty in predicting BKVN in transplant recipients, a latent period from BK viraemia to the development of BKVN, and possible sampling errors in diagnosing early BKVN due to its focal nature^[25], screening of the blood or urine for BKV infection and pre-emptive reduction of immunosuppressants when BKV is detected have been shown to reduce the risk of the development of BKVN (0.8%-1.3%)^[27,28,32,33]. Interestingly, some

centres report that despite having a BKV screening programme in the literature, their incidence of BKVN is greater than that seen in our cohort (4.2%-6.4%)^[11,26,31]. This includes one centre that screened for decoys cells in urine fortnightly for the first 3 mo, then at 6 and 12 mo and yearly after transplant^[31]. It appears that frequent monthly BKV monitoring is essential in early detection of BK viraemia and intervention resulting in the lower incidences of BKVN described in the abovementioned studies, with one centre that performed a cheaper urine cytology screening fortnightly from 0 to 3 mo after transplant, monthly from 3 to 6 mo and then every 2 mo from 6 to 12 mo^[33].

Because all patients with a > 20% decline in renal function undergo routine biopsy and all patients undergo a protocol biopsy at 3 mo after transplantation in our study cohort, we postulate that a large proportion (66.4%) of our cohort with unknown BKV status had no episodes of graft dysfunction due to BKVN. Bohl and his co-investigators looked at other studies and commented that BKVLs exceeding 10⁴ copies/mL only have a positive predictive value of 50%-85% for diagnosing BKVN^[36]. BKVLs that are less than 10⁴ copies/mL do not require intervention^[28,37]. This is reflected not only on the recipients who never had any BKVL tests, but also on the eight patients with low BKVLs who did not progress to BKVN even without a reduction of immunosuppressants and further BKV monitoring.

BKVN impacted on graft function; one patient lost their allograft and the other eight patients had a mean eGFR more than over 18 mL/min worse than patients without BKVN. This finding of poorer graft function from BKVN is also found in a prospective study that adopted a rigorous monthly screening programme - the mean eGFR of recipients with BKVN (39.0 ± 14.3 mL/min per 1.73 m²) was lower than that of those without BKVN (52.3 ± 19.9 mL/min per 1.73 m²), though there was no graft failure due to BKVN^[28].

Frequent monthly BKV monitoring and pre-emptive reduction of immunosuppression have been shown to be beneficial in reducing the risk of BKVN occurring. This strategy is effective in improving overall graft outcomes in renal transplant recipients as there is no definitive medical therapy for BKVN with graft failure still occurring.

However, this approach may be cost prohibitive for those resource restricted centres especially where a low incidence of BKVN is identified^[29]. Screening for decoy cells in the urine first may be a cheaper option^[33], but there is a high prevalence rate of BK viraemia even in immunocompetent adults and not all centres have resources to perform this test. Though cost saving can be achieved by reductions of immunosuppression as described in the literature^[38,39], they may not necessarily cover the cost of screening for BKV if immunosuppressants are inexpensive. To reduce the financial burden by increasing the monitoring interval to every 3 mo or longer, it may not reduce the incidence of BKVN as seen in the other studies. Nevertheless, either screening approach may not necessarily preserve graft function for those with BKVN. It is also interesting to see that the group with unknown BKV status in this cohort had a similar median eGFR comparing to those without BK viraemia, thus questioning the necessity of screening in this context. Our study is likely under-powered and increasing differences among these groups would likely have been observed in a large sample size. Targeting only those recipients who have significant predisposing risk factors could potentially reduce screening cost, but there is no consistency in what these risk factors are in the literature, thereby failing to identify BKVN cases early. Another cost saving option would be to identify recipients with positive BK viraemia early by performing an intensive monthly BKV screening only in the first 3 mo of renal transplant when the degree of immunosuppression is the greatest followed by a 3 mo screening until 12 mo after transplant. Therefore, when designing a BKV surveillance programme, it should be centre specific by taking the epidemiology of BKV infection, immunosuppression and monitoring protocols and related costs in a transplant centre into consideration to make it viable and cost effective.

There are several limitations in this retrospective study. Due to the lack of a comprehensive screening program we may have underestimated the incidence of BKVN. With our current approach of undertaking renal biopsy in all patients with a significant decline in renal function, it is unlikely that there are many patients with clinically significant BKVN that are not recognised. Interestingly, three patients with BKVLs of more than 160000 copies/mL did not have transplant biopsies due to stable graft function. Because of the selected cohort, the sizes of the comparative groups were small and there were only 9 recipients with BKVN which allow only a small number of variables to be included in the multivariable Cox regression model. As a result, the study is likely under-powered. In addition, there might be other risk factors for BKVN that were not identified, and tacrolimus and ciclosporin levels were not included in this study. Due to a large number of the recipients not tested for BKV, we cannot make effective comparisons, evaluate risk factors for BK viraemia or perform a cost analysis in this cohort.

A comprehensive BK virus surveillance program and

reduction of immunosuppressive therapy is the recognised management strategy to reduce the risk of BKVN occurring, because BKVN significantly impairs graft function. This study highlights that in our cohort the incidence of BKVN and graft failure due to BKVN without a formal screening programme is low and comparable to some transplant centres that have a BKV surveillance programme. Long CIT is associated with BKVN. The risk factor for BKVN identified is not consistent with other studies suggesting intricacy in the pathogenesis of BKVN and different protocols adopted by various transplant centres. Though the outcomes of this study remain speculative particularly the incidence of BKVN due to the study's limitations and it requires further validation in larger trials, it provides a similar perspective in BK virus screening to Kiberd's and Smith's studies^[29,38]. Transplant centres should evaluate its immunosuppression and monitoring protocols, the epidemiology of BKV infection and related costs before designing a BKV surveillance programme to make it centre specific and cost effective.

COMMENTS

Background

Screening for BK viraemia is an important strategy in reducing the risk of BK polyomavirus nephropathy (BKVN) and requires monthly monitoring in the first year of transplant in order for a BK virus surveillance to be effective. Applying this surveillance strategy in any transplant centres may not necessarily produce the best possible outcomes due to resource constraints and a low incidence of BK virus infection. This retrospective study was performed to compare outcomes of BK virus infection in the Auckland region without a formal BK virus screening program with those in centres with a BK virus surveillance program reported in literature.

Research frontiers

There are many risk factors, such as immunosuppressive burden, human leukocyte antigen mismatch, implicated in BK virus infection and the development of BKVN in renal transplant recipients. Therefore, immunosuppression and monitoring protocols play a role in BK virus infection. When designing a surveillance program for BK virus, the authors feel that immunosuppression and monitoring protocols, the epidemiology of BK virus infection and related costs should also be evaluated.

Innovations and breakthroughs

Outcomes of BKV infection, particularly BKVN, in this studied cohort are similar to some of transplant centres with a formal BK screening program that has less frequent testing for BKV.

Applications

Screening for BKV infection is important. Some transplant centres with limited resources may not afford frequent BKV testing or have capacity to perform BKV test. The study results suggest that a BKV screening program should be centre specific to be cost effective and achieve best possible outcomes.

Peer-review

This retrospective study looked at the incidence of BK viremia, BK nephropathy and graft outcomes among kidney transplant recipients in Auckland region. Study is well conducted and written clearly.

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Observational Study

Prevalence of risk factors for cardiovascular and kidney disease in Brazilian healthy preschool children

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Abstract

AIM

To investigate the prevalence of nutritional parameters of risk for cardiovascular disease (CVD) and kidney diseases in healthy preschool children.

METHODS

This is an observational cross-sectional study with 60 healthy children, of both genders, aged two to six years old and 56 mothers, in Belo Horizonte, Minas Gerais, Brazil. Preschool children and their families with regular activities at public schools were invited to participate in the study. The following characteristics were assessed: Socio-demographic conditions, clinical health, anthropometric, biochemical, lifestyle and data on food

consumption. The 56 healthy children were divided into two groups, overweight (C1) and non-overweight (C2), as well as their mothers, respectively, in overweight (M1) and non-overweight (M2). Nutritional status was defined according to results obtained through the Anthro® Software for nutritional analysis.

RESULTS

Thirty-five children were male, with mean age of 4.44 ± 1.0 years old. Eighty-nine percent of them were eutrophic, 86.7% were sedentary and they had five meals a day. Body mass index (BMI) for age and total cholesterol (TC) was higher on C1 ($P = 0.0001$) and high density lipoprotein cholesterol (HDL-c) was higher on C2. Mothers were 32.5 ± 7.1 years old, mostly married and employed. Eighty-six percent of them were sedentary and 62.5% were overweight with $BMI = 26.38 \pm 5.07 \text{ kg/m}^2$. Eighteen percent of the overweight mothers had isolated total hypercholesterolemia (TC levels elevated) and 12.5% had low HDL-c levels. The present study showed an association between overweight and obesity during the preschool years and the correspondent mothers' nutritional status of overweight and obesity ($OR = 4.96$; 95%CI: 0.558-44.17). There was a positive correlation between the food risk associated with CVD by children and mothers when their consumption was 4 times/wk ($P = 0.049$; $r = 0.516$) or daily ($P = 0.000008$; $r = 0.892$).

CONCLUSION

Analyzed children showed high rates of physical inactivity, high serum cholesterol levels and high consumption of food associated with risk for CVD and renal disease. Changes in habits should be encouraged early in kindergarten.

Key words: Cardiovascular disease; Kidney disease; Preschool children; Food habits; Lifestyle

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Core tip: This is an observational cross-sectional study with 60 healthy preschool children and 56 mothers. Children were divided in overweight and non-overweight groups, as well as their mothers. There were 35 male children, mean age 4.44 ± 1.0 years old, 89% of all children were eutrophic, 87% sedentary. Body mass index/age and total cholesterol were higher in the overweight group. Mother's age was 32.5 ± 7.1 years old, mostly married and employed, 86% of them were sedentary, 63% overweight. There was an association between overweight and obesity during preschool years and the correspondent mothers' nutritional status of overweight and obesity. There was a positive correlation between food risk consumption associated with cardiovascular disease by children and mothers.

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INTRODUCTION

Child growth is not limited to increased weight and height, but is characterized by a complex process involving body size and number of cells influenced by genetic, environmental and psychological factors^[1,2].

The first process is the formation, when tissues are organized and cellular and tissue functions are defined^[2]. Subsequently, acquisition and improvement of more refined functional abilities arise, depending on the interaction with external stimuli. The progressive development of motor coordination increases physical activity and proportionally energy needs^[3]. Most children are picky about the food and the consumption of inadequate or unbalanced amount of food can interfere with their development^[4]. The formation of eating habits takes place gradually during infancy. There are genetic predispositions to like or dislike certain foods and differences in sensitivity to some tastes and flavors inherited from parents. These genetic influences will be increasingly shaped by the experiences along the person's life and will be combined with cultural, social, affective or emotional and behavioral values.

Brazil is experiencing a period of epidemiological and nutritional transition with increasing prevalence of chronic non-communicable diseases (CND) in all age groups. According to the World Health Organization (WHO), the CND are the leading causes of death and disability worldwide. Obesity, high blood pressure (BP) and hypertension, type 2 diabetes mellitus and dyslipidemias feature a metabolic profile that creates favorable conditions for the development of cardiovascular disease (CVD) and progressive renal disease (RD)^[5-7]. This metabolic profile or risk factors for development of CVD and RD was not important in children in the past. However, profound changes in lifestyle have favored the emergence of these risk factors in pediatric patients.

Studies that focus on risk factors for the development of CVD and RD in children, especially in preschool age, are scarce. Such information is important, since they allow estimating the possible factors associated with worsening health and determine the nutritional inadequacies in individuals early on. Such information allows a better planning of targeted interventions, which may provide better fitness levels of clinical and metabolic control since childhood. In this scope, this study aimed to investigate the relationship between nutritional parameters and risk factors for CVD and RD in healthy preschool children, and whether a combination of these factors and the biological inheritance between mother and child may occur.

MATERIALS AND METHODS

Ethical considerations

The study was approved by the institutional review board

of the Federal University of Minas Gerais, Brazil (ETIC 0030.0.410.203-09) and by the Secretaria Municipal de Saúde de Belo Horizonte (0030.0.410.410-09 A). It was performed in accordance with the ethical standards laid down in 1964 by Declaration of Helsinki. The participants and/or their guardians signed informed consent forms.

Study design and population

This is a cross-sectional, observational, epidemiological school-based survey. Children and their mothers were selected from a total of 80, but 20 participants were excluded due to non-accordance to participation. The sample consisted of 60 healthy preschool children (75% of the original population), from 2 to 6 years old, both gender; and 56 mothers (some had more children in the study) recruited from March to September 2010 in kindergartens and public schools in the Northeast region of the city of Belo Horizonte, capital of the state of Minas Gerais, Brazil, which had 2258096 inhabitants^[8].

Exclusion criteria were with any of the following conditions: Those under 2 years old or over 6 years old, those with chronic degenerative diseases, acute infectious and febrile diseases, with co-morbidities or use of drugs that could interfere with the biochemical analysis of blood and urine. The recruited children and their mothers attended the Primary Care Unit in a pre-scheduled date for obtaining measurements and blood and urine collection.

Data collection

We used a semi-structured questionnaire previously tested and adapted for the studied population. This questionnaire contained social, demographic, economic, clinical data and family history. Anthropometric measurements (weight and height) were performed according to the techniques recommended by Jelliffe (1968)^[9].

The body mass index for age (BMI/A) was calculated for the purpose of evaluating the adequacy of weight for height and age. This index was obtained by dividing the current weight (kg) by height squared (m^2) and through the calculation performed by the WHO Anthro v3.0.1 program for children up to 5 years old, and WHO Anthro Plus v1.0.2, for children older than 5 years old. For the analysis of BMI/A in children we used the cutoffs of WHO (2006) for children between 0 and 5 years old and WHO in 2007 for children between 5 and 10 years old^[10]. Overweight or obesity were characterized as z-score for BMI/A higher than +2 and called C1, with the purpose of comparison to children with z-score for BMI/A below this limit, called C2.

BMI of mothers was also calculated to assess nutritional status. This index was obtained by dividing the current weight (kg) by height squared (m^2)^[11]. For the analysis of BMI of the mothers, the nutritional diagnostic criteria were used as recommended for adults by the WHO (1995 and 1997)^[12]. Mothers were divided into 2 groups, mothers with overweight or obesity (M1) and those with other nutritional classification (M2). Children of mothers with overweight and obesity (O1) were com-

pared with children whose mothers with other nutritional state (O2).

BP was measured according to the recommendations of the VI Brazilian Guidelines on Arterial Hypertension (2010). The cut-off points of systolic blood pressure (SBP) and diastolic blood pressure (DBP) of the children were analyzed according to the percentiles of height for age obtained by the statistical program Epi Info Windows version 3.5.1. It was considered as "BP above the normal values" when the SBP or DBP were above the 90th percentile of the reference population, as recommended by the fourth report on the diagnosis, evaluation and treatment of high blood pressure in children and adolescents. For mothers, the cut-offs of SBP and DBP were also analyzed according to the values described by the VI Brazilian Guidelines on Arterial Hypertension (2010), where BP high values are referred as equal to or above 140 mmHg \times 90 mmHg. For blood sampling, mothers and children were instructed to fast for 12 h and to have at least 6 h sleep the night before. Mothers should not have consumed alcohol in the 48 h prior to the examination, they had not made use of diuretics at least 7 d in advance and should report the use of medications that may interfere with test results^[13].

We evaluated the total cholesterol (TC) and its fractions such as high density lipoprotein cholesterol (HDL-c), low density lipoprotein cholesterol (LDL-c) and very low density lipoprotein cholesterol (VLDL-c), triglycerides (TG), glucose (GL), albumin (ALB), uric acid, hemoglobin (HG) and creatinine (CR) to calculate creatinine clearance by the formula of Schwartz^[14] for children and the formula of Cockcroft Gault for mothers^[15]. Analysis of TC and TG levels was made by an enzymatic method. The colorimetric method without precipitation was used for HDL-c and obtained by calculation of the LDL-c fraction and VLDL-c from the Friedwald formula. The cut-off points for children followed the recommendation of the American Academy of Pediatrics^[16]. For mothers, the adopted cut-off points were the IV Brazilian Directive on Dyslipidemia and Prevention of Atherosclerosis (2007)^[17].

The classification of GL levels was performed according to the American Diabetes Association (2006)^[18] and the method of analysis was the enzymatic. The cut-off point used for the classification of ALB values was 3.5 to 5.0 g/dL. The analysis method used was the colorimetric. For checking the AU was used the colorimetric method, being considered as altered, values above 7.0^[19].

The CR serum level was used as a risk marker for the development of RD and the method of analysis was kinetic. For children, we used the criteria proposed by The National Kidney Foundation's Kidney Disease Outcomes Quality Initiative^[20] and for mothers the reference values was from 0.7 to 1.5 mg/dL^[21].

The concentration of HG was obtained by the colorimetric method and the considered reference values were 11.5 to 13.5 g/dL for children and 12.2 to 18.1 g/dL for mothers.

A random urine sample was collected from children and their mothers after 12 h of fasting for albuminuria

detection and normal values were considered below 30 mcg/g creatinine, according to Cari Guidelines (2004)^[22]. The analysis of ALB was made by the immunoturbidimetry and creatinine by colorimetry.

Eating habits

The food intake of children and their mothers was assessed by a food frequency questionnaire (FFQ) validated and adapted to the studied population. The list of foods in this questionnaire was built considering the foods most commonly consumed by children in Belo Horizonte, based on data retrieving the use of food recall 24 h in 10 children and their mothers.

After setting all parameters, the developed instrument presented 63 food items for group I, classified as risk foods or promoters of CVD, and 39 food items for group II, classified as protectors of CVD food. The foods were divided into 7 time units according to frequency of consumption: Not consuming, 1 time/mo, 2-3 times/mo, 1-2 times/wk, 3-4 times/wk, 5-6 times/wk or consume daily.

The FFQ was answered individually, and the mothers were asked to report the frequency of consumption of foods listed and to add the foods consumed regularly, but not included in the questionnaire. For each item of the questionnaire it was also informed the average frequency of food consumption for the last 6 mo and the respective time unit (if daily, weekly, monthly or not consumed). To minimize the interviewer's information bias, training sessions were conducted to standardize the procedure and school meetings were scheduled with the mothers to demonstrate how to fill the FFQ correctly. Each item of the FFQ was entered in a spreadsheet (Excel, 2007), each time unit converted into scores and obtained the sum of consumption of each food, the average and the frequency of consumption of risk food and protection for CV diseases. To qualify the usual food consumption, a minimal consumption once a week was considered, usual consumption when frequency was ≥ 4 times/wk and daily consumption, with the usual consumption of food ≥ 4 times/wk chosen as ideal considered by approaching the median of week days.

Statistical analysis

The statistical analysis and review of the study was performed by a biomedical statistician. A descriptive analysis of the data and the values were expressed as mean (and SD) or median, according to normal/non-normal distribution; first and third quartiles, otherwise. GraphPad Prism® v.5.0 statistical software was used and GraphPad Software (San Diego, California, United States), adopting a significance level of 5% ($P < 0.05$) for all analyzes. For comparison and correlation purposes, we used the student *t* test and Pearson's coefficient. For the variables without normal distribution the Mann-Whitney test and the Spearman coefficient was used to measure correlation. The association test, when using contingency tables, was the Fisher's exact test.

To estimate the risk odds ratio and 95%CI were chosen (OR)^[23,24].

RESULTS

Social and demographic characteristics

Children: There were 35 (58.3%) male children, with a mean age of 4.44 ± 1.0 years old. The average birth weight was 3.1 kg and height was 49.8 cm. The duration of breastfeeding was 8.3 mo, excluding exclusivity. The BMI/A z-score was 0.30 reflecting adequacy of current weight and height for age. Elevated levels of SBP were found in 18.3% of children and 35% of them had high levels. Most children were eutrophic, despite sedentary.

In the sample, 86.7% had 5 meals a day and between the 3 main meals (breakfast, lunch and dinner) 28.3% skipped dinner. HDL-c values were reduced in 15% of the children (Table 1). When comparing children with overweight (C1) and without (C2) no difference was found except in BMI/A z-score ($P < 0.0001$).

The comparison between C1 and C2 is shown in Table 2 and a difference was observed only for HG, being higher in C1 group ($P = 0.038$). The values of TC, LDL-c, VLDL-c and TG were higher in C1, although not statistically significant. The value of HDL-c was higher in the C2 group, also not statistically significant.

Mothers: The average age of mothers was 32.5 years old and most were married or cohabitating. The educational level ranged from 0 to 15 years of formal study and approximately 2/3 of them had a minimum of 8 years of study. Most of them worked and per capita income ranged from BRL \$85.80 to BRL \$2000.00. Only 23.2% had monthly income above one minimum wage per person (BRL\$510.00) at the time of the survey (Table 1).

We found a high rate of overweight (BMI > 25 kg/m²) mothers and most were sedentary. Consumption was no more than 4 meals/d and among the top 3 (breakfast, lunch and dinner) 53.6% skipped dinner.

The mean SBP was 111 mmHg and for DBP was 75 mmHg, values considered suitable for adults. Just over half of the mothers had a family history of CV event. Laboratory evaluations of M1 and M2 groups are presented in Table 2 and there was no difference. Only 2 mothers had high values of ALB in random urine sample (3.33%), a result that may have been found by chance.

Mother's influence on their children

The children of mothers with overweight/obesity (O1) were compared to those of mothers without overweight/obesity O2 (Table 3). The values for LDL-c and TG were lower in the O2 group, although not statistically significant. The O1 group had higher ALB values but all results within the range considered normal. The likelihood of a child being overweight when this child was the mother's child with the same nutritional status was 85.7%, positive predictive value (PPV) of 0.8571 (95%CI:

Table 1 Clinical, demographic and cardiovascular and renal risk factors in healthy preschool children and their mothers

	Children (<i>n</i> = 60)	Mothers (<i>n</i> = 56)
Clinical and demographic characteristic		
Male	58.33%	
Age (yr)	4.44 ± 1.0 ¹	32.5 ± 7.1 ¹
Gestational age at birth (wk)	38.72 ± 1.83 ¹	
Birth weight (kg)	3.12 ± 0.60 ¹	
Length at birth (cm)	49.83 ± 3.61 ¹	
Breastfeeding duration (mo)	8.25 (4-21.50)	
BMI/A z-score (children) and BMI (mothers)	0.30 (-0.55-1.33)	26.38 ± 5.07
Prehypertension systolic <i>n</i> (%)	11 (18.34)	1 (1.79)
Prehypertension diastolic <i>n</i> (%)	21 (35)	8 (14.29)
Overweight and obesity <i>n</i> (%)	7 (11.67)	35 (62.5)
Physical activity <i>n</i> (%)	8 (13.33)	8 (14.29)
Familial history of cardiovascular event <i>n</i> (%)		32 (57.14)
Marital status <i>n</i> (%)		
Married or cohabiting		41 (73.21)
Divorced, single or widowed		15 (26.79)
Education (> 8 yr of schooling)		38 (67.86)
Occupation (working outside the home)		42 (75)
Income per capita (> 1 minimum wage)		13 (23.21)
Cardiovascular and renal risk factors <i>n</i> (%)		
Total cholesterol > 200 mg/dL	2 (3.33)	10 (17.86)
LDL-c > 130 mg/dL	3 (5)	8 (14.29)
HDL-c < 35 mg/dL	9 (15)	7 (12.5)
Triglycerides > 150 mg/dL	2 (3.33)	6 (10.71)
Uric acid > 7 mg/dL	0 (0)	0 (0)
Albuminuria > 30 mcg/mg of creatinine	2 (3.33)	2 (3.57)

¹Mean ± SD. BMI/A: Body mass index for age; CV: Cardiovascular; Minimum wage: BRZ \$51000; *n*: Number of observations; LDL-c: Low density lipoprotein; HDL-c: High density lipoprotein.

0.4213-0.9964). The proportion of children without overweight whose caregiver also showed that nutritional classification was 96%, with a specificity of 0.96 (95%CI: 0.7965-0.9990); negative predictive value (NPV) of 0.4528 (95%CI: 0.3156-0.5955) and 0.1714 sensitivity (95%CI: 0.06562-0.3365). The analysis of the mother's weight relative to the weight of the child, particularly with regard to the presence of excess weight in the mother, is a predictor of overweight indicator in children. Although the test is very sensitive, it appears to be very specific for predicting normal weight in children.

Analysis of the frequency of food intake of children and mothers

The analysis of consumption of each food by risk group or protection of children and their mothers showed a positive correlation, especially for foods that pose a risk for the development of CVD (Table 3).

The main risk for CVD food most consumed by children and mothers were: Whole milk, coffee, spaghetti, French bread, margarine, sweets like candies, lollipops and chewing gum; broth, artificial juices and soft drinks. As protectors against CVD a higher frequency of consumption of beans, banana, carrots, orange and tomato was described.

Figure 1 demonstrated the positive correlation between the daily consumption of food associated with risk and protective ones, respectively, for CVD between mothers and preschool children.

DISCUSSION

Previous research has shown that the first nutritional experience of a person may influence susceptibility to certain chronic diseases in school age children and adulthood, especially obesity. Even children with an appropriate BMI may have asymptomatic clinical and metabolic changes that contribute to the development of CND^[25]. Physical inactivity in childhood tends to perpetuate in adulthood and physical activity in pediatric patients significantly declined in the last two decades, favoring the onset of risk factors for CVD and RD^[25,26]. The increasing prevalence of overweight reflects the positive energy balance caused by excessive energy intake and/or decrease in physical activity in children and adolescents in the last 3 decades of life^[27].

The world scenario of NCD is a new challenge for public health^[28,29]. The possibility of preschool children to become obese adults, sedentary and with metabolic changes that will culminate in CVD or RD is worrisome to health managers. There are few studies about the prevalence of risk factors for these conditions in preschool children, making it difficult to properly approach and elaborate actions that could change the course and the deleterious effects of these diseases. Most likely, kindergartens and schools would be the most supportive environments to promote healthy lifestyles. This study aimed to assess the prevalence of risk factors for CVD and RD in preschool children from public schools in Belo

Table 2 Comparing the laboratory parameters of preschool children with overweight/obesity (C1) and without overweight/obesity (C2) and mothers with overweight/obesity (M1) and without overweight/obesity (M2)

	Children (n = 60)			Mothers (n = 56)		
	C1	C2	P	M1	M2	P
Uric acid (mg/dL)	2.8 (2.0-3.2)	3 (2.5-3.0)	NS	4.8 (3.1-4.9)	3.7 (3.0-4.3)	NS
Total cholesterol (mg/dL)	166 (119-167)	155 (136-169)	NS	169 (145-189)	163 (133.5-190.8)	NS
HDL-c (mg/dL)	40 (37-45)	49 (42.5-57.5)	NS	52 (45-58)	49.5 (43-57.5)	NS
LDL-c (mg/dL)	92 (57-116)	87 (76-103)	NS	103 (81-123)	94 (73.5-114)	NS
VLDL-c (mg/dL)	14 (9-18)	13 (9.5-18)	NS	14 (11-19)	16 (10-24)	NS
Triglycerides (mg/dL)	72 (45-91)	65 (47.5-92)	NS	69 (53-97)	83 (50-120.5)	NS
Albuminuria ¹	5.0 (2-10.37)	5.0 (3.17-10.56)	NS	3 (1.9-3.9)	4.01 (2.99-6.0)	NS
Estimated creatinine clearance ² (mL/min per 1.73 m ²)	143.7 (123.2-151.9)	129.8 (118-144.4)	NS	-	-	-
CrCl ³	-	-	-	111.4 (105.1-131.2)	109.9 (93.5-147.3)	NS
Glucose (mg/dL)	75 (70-85)	82 (77-85)	NS	84 (79-93)	84 (79-89)	NS
Albumin (g/dL)	4.3 (4.2-4.5)	4.4 (4.3-4.7)	NS	4.3 (3.9-4.4)	4.4 (4.3-4.5)	NS
Hemoglobin (mg/dL)	13.1 (12.8-14.25)	12.7 (12.15-13.1)	0.03	13.6 (13-14.7)	13.4 (12.7-13.9)	NS

¹mcg/mg creatinine; ²Estimated creatinine clearance, Schwartz's formula mL/min per 1.73 m²; ³Estimated creatinine clearance through Cockcroft-Gault's formula in mL/min per 1.73 m². Median, 1st and 3rd quartiles for all variables; Mann-Whitney U test. LDL-c: Low density lipoprotein; HDL-c: High density lipoprotein; VLDL-c: Very low density lipoproteins; NS: Not significant; CrCl: Creatinine clearance.

Table 3 Correlation between the consumption of risk and protection foods for cardiovascular disease between mothers and preschool children

Variables		P	r
Foods associated with risk	Frequency consumption		
	1/wk	0.079	0.516
	4 times/wk	0.049	0.516
	Daily	0.000008	0.892
Foods associated with protection	Frequency consumption		
	1/wk	0.218	0.429
	4 times/wk	0.946	-0.031
	Daily	0.009	0.796

P < 0.05: Spearman's correlation test.

Horizonte, Brazil, and the influence of lifestyle habits of their mothers on them.

Clinical and demographic characteristics of the children point to some health concerns on them. Elevated systolic and diastolic BP levels were observed in 18.3% and 35% of the children, respectively. These pressure levels were higher than a previous studies conducted in Southern Brazil published only locally^[30], which suggested that the best age to intervene in children is before 6 years of age when high BMI/A z-score are diagnosed.

It was also observed that overweight and obesity were independent predictors for arterial hypertension^[31,32]. In the present study the BMI/A z-score was within the normal range for most children and cannot be considered responsible for the higher BP levels observed. Probably, there was influence of other behavioral factors such as lifestyle (sedentary lifestyle) and food (high intake of fat and salt). In agreement with other studies, no difference in DBP between genders was found. The elevated SBP levels (18.3%) and high DBP levels (35%) observed in the study may be related to food intake considered as high risk for CVD (sodium), dinner substitution snacks in 28.3% of the cases (11.7% overweight), and prevalence of physical inactivity (86.7%). Rodríguez-Moran *et al.*^[33]

identified hyperglycemia (0.3%), hypertension (3.4%), metabolic syndrome (10.1%) and hyperinsulinemia (13.4%) in 358 schoolchildren. Hyperglycemia and hyperinsulinemia in children with maternal history of hypertension were also observed, suggesting familial inheritance for the increased risk to develop this comorbidity^[33].

Studies have shown that the reduction in the number of nephrons is associated with development of primary hypertension. According to the "Brenner-Barker Hypothesis", the chronic degenerative diseases of the adult may result from environmental conditions experienced during fetal life. Birth weight was associated with nephronic mass reduction and thus this hypothesis synthesizes the interaction gene-environment-disease in the causation of hypertension^[34,35]. Low birth weight was associated with a reduced number of glomeruli and with increased volume of them^[36].

Hughson *et al.*^[37] found that birth weight is a determining factor in the number of nephrons and consequently postnatal renal size. These findings support the hypothesis that low birth weight is a risk factor for hypertension and chronic kidney disease (CKD)^[37]. In the study of patients with hypertension, Keller *et al.*^[38] found fewer glomeruli per kidney when compared to normotensive patients and the hypertensive patients had a higher glomerular volume than the control group. It is suggested that intrauterine growth would have regulatory influence on the formation of nephrons and renal function in humans, which extends beyond the neonatal period^[39]. In our study, weight and gestational age at birth were suitable for all children and there were no differences when we compared obese children and children with other nutritional status.

Prospective epidemiological studies have shown that active lifestyle and aerobic fitness are independently associated with reduced incidence of NCDs and overall mortality and CVD. This practice is also an important protective factor against obesity, type 2 diabetes, some cancers and some mental disorders^[27]. However, this

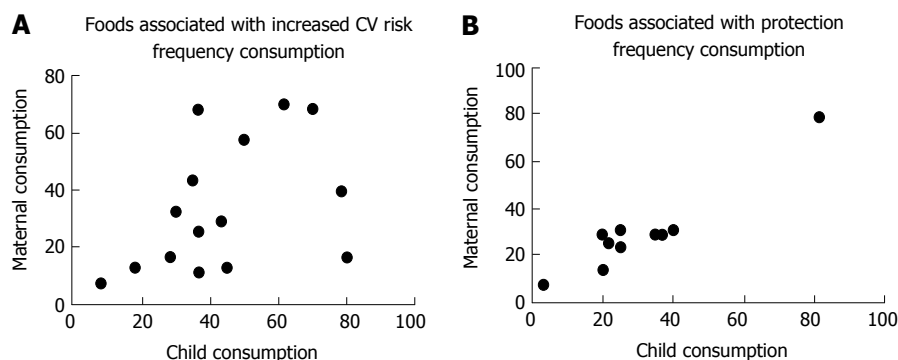


Figure 1 Correlation of the daily consumption of food risk (A) and daily intake of protective foods (B) for cardiovascular disease between mothers and preschool children. CV: Cardiovascular.

is not the reality of children in our study. Only 13.3% of the sample performed some kind of physical activity outside the school. The prevalence of childhood obesity has grown 10% to 40% in the last 10 years in most European countries and occurs in the first years of life, between 5 and 6 years old and adolescence^[40]. In the United States it affects between 20% to 27% of children and adolescents^[41]. In Brazil, obesity is more prevalent in developed regions, where the industrial modernization process is advanced, in children during the first years of life and is associated with early weaning practices and dissemination of incorrect dietary instructions that encourage overfeeding^[42]. This study with supposedly healthy children found 11.7% of overweight. This percentage is high, but it is close to that shown above by Abrantes *et al.*^[43] in children in northeast and southeast regions of Brazil.

The comparison between children classified as overweight and those with normal weight showed no differences in gestational age and birth weight, duration of breastfeeding and current age. However, the BMI/A z-score showed a significant difference ($P < 0.0001$), suggesting that other factors could be involved in the determination of this nutritional status, such as diet, lifestyle and genetic inheritance^[44]. Studies correlating genetic factors and obesity demonstrated interference of these aspects in up to 25% of obese children, suggesting that the accumulation of excessive body fat, in most cases, would be triggered by social and environmental aspects^[6].

According to Barja *et al.*^[45], the prevalence of obesity in families of obese adolescents is related to family history of obesity and possibly by the combined effect of genetic factors and lifestyle habits. The mothers, in our study, were overweight (62.5%), sedentary (85.7%) and with high BMI. Twelve percent of children were overweight, suggesting influence of life and eating habits of mothers on their children. Guo *et al.*^[46], studying obese children and adolescents showed that 33% of boys and 50% girls remained obese in adulthood.

Breastfeeding has been considered protective against obesity, however, there is controversy^[41]. O'Callaghan *et al.*^[47] did not observe association between duration

of breastfeeding and the prevalence of obesity at 5 years of age in 4062 children in Australia. In this study, overweight and obesity children had shorter breastfeeding when compared to those with normal weight, although not statistically significant.

Dyslipidemia may start in childhood and perpetuate. Some children have a metabolic profile characterized by decreased HDL-c, increased LDL-c and TG, reduced activity of the enzyme lipoprotein lipase and increased insulin resistance, favorable to the development of CVD and progressive RD^[48]. A previous study with Brazilian children and adolescents (2 to 12 years old, 12 to 19 years old) found a correlation between dyslipidemia and obesity and overweight^[49].

The Bogalusa Heart Study correlated the finding of atherosclerosis in autopsies of children with risk factors detected before death (elevated serum levels of TC, LDL-c and low HDL-c) and concluded that these alterations were related to atherosclerotic lesions present from the earlier stages in childhood^[50]. In this study 3.3% and 5% of children had serum levels of TC and TG and LDL-c, respectively, above the desirable values and 15% with HDL-c below. It is known that low HDL-c accelerates progression of atherogenesis^[47].

There is evidence that peripheral endothelial cells have modulating effects on vascular reactivity. This condition may disrupt homeostasis, can lead to endothelial dysfunction and contribute to atherosclerosis and CVD^[51]. According to de Oliveira *et al.*^[42], studies have demonstrated the presence of at least one risk factor (hypertension, hyperlipidemia or hyperinsulinemia) for CVD in 60% of children and adolescents overweight, and 20% had two or more risk factors.

Ribeiro *et al.*^[32] evaluated CV risk in children and adolescents aged 6 to 18 and found that 32.9% and 25.1% had TC and LDL-c above normal values, respectively, and 17% had HDL-c below the recommended values. The children in the study were separated into 2 groups according to overweight and higher values for TC, LDL-c and TG and lower values for HDL-c were observed in those classified as overweight compared with eutrophic ones, although not statistically significant. This suggests a positive association between excess weight and

metabolic changes.

Obesity, dyslipidemia and hypertension are increasingly prevalent in the pediatric population and are considered risk factors for KD^[20,52]. A cross-sectional study with 274 healthy school children was conducted to identify risk factors for developing CKD and found 8.1% of low birth weight; 23.6% of obesity in grandparents, 6.3% in parents and in 13.8% of the mothers; and 7.1% of them were hypertensive. There was also a positive correlation between SBP/DBP and BMI as well as with waist circumference^[53].

Excess body weight was associated with the presence of proteinuria and obese individuals had a higher risk for developing glomerulopathy^[54]. Obesity in a parent leads to increased risk of obesity in children and can be almost 2 times higher for individuals with obese father and mother^[55]. In this study, there was a tendency of mothers with overweight towards having children also overweight and obesity in the future (OR = 4.96, 95%CI: 0.56-44.17). The PPV of the test was 85.7% and the NPV was 45.3% (probability of a child to be eutrophic when she or he is mother's daughter without overweight). For the specificity of the same test, the proportion of children without overweight whose caregiver was also classified with the same nutritional status was 96%. The presence of excess weight in the mother was a very sensitive indicator (17.1%) in predicting overweight in children but very specific in predicting normal weight in this population.

Children have their food style heavily influenced by family, friends and media^[4]. Most children in the study had at least 5 meals/d and 51.8% of mothers 4 meals/d. The fact of not having dinner was identified in 28.3% of children and 53.6% of mothers with replacement of that meal for food associated with CVD and RD risk. This finding reinforces the mother's influence on the food quality of their children and the damage on the nutritional status of the individual. Another important finding of this study was the correlation between the consumption of certain food by children and their mothers and the protection for CVD. It was found a close relationship between the profiles of food consumed by mothers and their children's. The risk of developing CVD was higher when specific food consumption (those associated with increased CV risk) was 4 times/wk ($P = 0.049$, $r = 0.516$) or daily ($P = 0.000008$, $r = 0.892$). It was necessary a daily intake of protective foods for adequate protection for CVD ($P = 0.009$, $r = 0.796$).

There are limitations in the present study. The small number of participants, the difficulty of collection of laboratory samples and to perform physical examination at kindergarten/school are some of these limitations. The small number of participants could be justified because we have difficulty to recruit them due to age and because the parents' refusal to accept the study. Data regarding this age group are scarce on cardiovascular risks factors and KD risk factors. In the same way, it is very difficult to collect blood and urine samples from preschool children

as well as it is difficult to perform physical examination without to cause some discomfort to the child.

The results of BP measurements in children is frequently influenced by environmental aspects, such as technical acceptance by the child and difficulty and lack of time of mothers to repeat the measurement, preventing reliable correlations between variables and inferences. Thus, we have to be careful with the interpretation of these results.

Nevertheless, the study is valueable due to assessment of CVD and KD risk in a population of preschool children. Data regarding this age group are few. Day care and pre-school are friendly environments to promote proper lifestyle habits. Programs that provide healthy foods and preparations, the creation of support groups for women who are breastfeeding and the viability of public safe spaces for regular physical activity are essential for prevention of these diseases.

Most children were sedentary, although eutrophic. The group of overweight children had high TC and BMI/A z-score. Most mothers were also sedentary and overweight. The possibility of a child being overweight was 4.96 times higher in cases of being born from obese mothers. There was a positive correlation to the risk of food consumption and protectors for CVD among children and mothers. Considering the importance of maintaining adequate metabolic clinical control and prevention of the increased risk of CVD and RD development, it is necessary the proper guidance of parents, as well as the need for adequacy of life and eating habits of these. For the implementation of such a strategy it is necessary for the nutritional and educational intervention programs to be introduced into the routine of children, their families and educators.

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COMMENTS

Background

The first nutritional experience of a person may influence susceptibility to certain chronic diseases in school age children and adulthood, especially obesity. Physical inactivity in childhood tends to perpetuate in adulthood and physical activity in pediatric patients significantly declined in the last two decades, favoring the onset of risk factors for cardiovascular diseases (CVDs) and renal diseases. The prevalence of risk factors for these conditions in preschool children are not well known making it difficult to properly approach and elaborate actions that could change the course and the deleterious effects of these diseases. Such information allows a better planning of targeted interventions, which may provide better fitness levels of clinical and metabolic control since childhood.

Research frontiers

This study aimed to investigate the prevalence of nutritional parameters of risk for CVD and renal diseases in healthy preschool children as well as their

relationship with food profiles consumed by mothers and their children.

Innovations and breakthroughs

It is important to know the metabolic profile or risk factors for development of CVDs and renal diseases in order to maintain adequate metabolic clinical control and prevention of these diseases. A proper guidance of parents is necessary, as well as the need for adequacy of life and eating habits.

Applications

This study confirms that the nutritional parameters of risk for cardiovascular and renal diseases are already present in preschool children. It also confirms that there is a relationship between the profiles of food consumed by mothers and their children.

Terminology

Traditional risk factors for cardiovascular and renal diseases are high blood pressure, dyslipidemia, overweight/obesity, diabetes mellitus and some habits related to lifestyle (diet high in calories, saturated fats, increased cholesterol, salt consumption and sedentary lifestyle). The study was done based on these risk factors.

Peer-review

This is a well written observational cross-sectional study with 60 healthy preschool children and 56 mothers. The results showed an association between overweight/obesity during preschool years and the correspondent mothers. The design and results are novel.

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Observational Study

Impact of renal transplantation on cardiac morphological and functional characteristics in children and adults

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Abstract

AIM

To compare the effects of renal transplantation on cardiac functions in children and adults.

METHODS

One hundred and ten patients attending the nephrology outpatient clinic were enrolled in this study and were divided into six groups. The first two groups consisted each of 30 renal transplant patients who had a successful renal transplantation more than six months, but less than one year. Group I were less than 18 years and group II were more than 18 years. The third and fourth groups, each were 20 chronic renal failure patients on regular hemodialysis. Again, group III were less than 18 years and group IV were more than 18 years. Group V and VI (The control Groups) consisted each of 5 subjects below and above 18 years of age, respectively with normal kidney functions. All patients were subjected to history and examination. The kidney functions and the hemoglobin were analyzed. After

obtaining informed consent, echocardiography was done to all patients.

RESULTS

There was a statistically significant improvement ($P < 0.0001$) in all cardiac parameters. A regression in left ventricular end diastolic volume (LVED) both in children (4.7 ± 0.8 to 4.2 ± 0.5) and in adults (5.9 ± 0.7 to 4.9 ± 0.6) were found. There was a regression in left ventricular end systolic volume (LVES) both in children (3.1 ± 0.6 to 2.4 ± 0.4) and in adults (4.1 ± 0.9 to 3.1 ± 0.5). Fractional shortening improves both in children (32.6 ± 5.3 to 41.7 ± 7.6) and in adults (29.0 ± 6.6 to 36.5 ± 4.1). The improvement in ejection fraction (EF) was higher in children (59.7 ± 7.0 to 71.9 ± 6.1) than in adults (52.0 ± 12.5 to 64.8 ± 5.9). However, this degree of improvement (in children: 12.2 ± 5.1) did not show statistical difference (P -value 0.8), when compared to adults (12.7 ± 9.8).

CONCLUSION

After renal transplantation cardiac functions and morphology (EF/LVED/LVES) do improve markedly and rapidly in both children and adults.

Key words: Echocardiography chronic renal disease; Renal transplantation; Cardiac problems

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Core tip: Cardiac functions do improve in chronic kidney disease patients after renal transplantation. This improvement is evident even in the early post-transplant period. In our study, we concluded that this improvement is even more marked in children. Renal transplantation in children with end-stage renal disease should, therefore, be encouraged.

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INTRODUCTION

Chronic kidney disease (CKD) affects almost 13% of the United States citizens and is definitely related to increased risk of cardiovascular (CV) diseases. After renal replacement therapy (RRT) became available, it became evident that the cause of death of patients with advanced CKD was more likely related to CV compromise^[1].

In hemodialysis (HD) or peritoneal dialysis patients, Coronary artery disease (CAD) prevalence is estimated at 40% with a 9% annual CV mortality^[2]. Renal transplant recipients (RTRs) have a lower CAD prevalence (15%)

with an annual CV mortality of 0.54%^[3]. Left ventricular hypertrophy (LVH) is an independent risk factor for cardiovascular disease (CVD) related to death in patients on RRT^[4]. In a prospective echocardiographic study, patients with renal insufficiency and clearances of 25 to 50 mL/min and less than 25 mL/min, the prevalence of LVH was 31% and 45% respectively^[5]. Echocardiographic studies of patients on dialysis have revealed severe systolic dysfunction [left ventricular (LV) ejection fraction (EF) of $\leq 25\%$] in 15% of patients; with 74% of patients having LVH and 32% of patients demonstrating LV dilatation^[6]. Although increased awareness of CVD has resulted in a reduction in related deaths over time, yet still they remain the leading mortality cause of in RTRs^[7]. The American Society for Transplantation guidelines has recommended pretransplant risk stratification and noninvasive stress testing for candidates at high cardiac risk^[8].

Although cardiovascular problems in adults were thoroughly investigated, yet much less work has been done in children and adolescents with end-stage renal disease (ESRD) whether before or after renal transplantation. Cardiac changes are believed to be less prevalent in children compared to adults with chronic renal failure (CRF). However, no recent studies discussing their frequency are available. Long-term prognosis of cardiac morphological changes in children with CRF and after renal transplantation is largely unknown. When the pediatric mortality in RRT patients in Europe between 1987 and 1990 was investigated, a CV cause of death was identified in 51% of dialysis and in 37% of transplanted subjects^[9]. Cardiac disease is known to be the second most common cause of mortality in children after infection and is the leading cause of death in young adults who have undergone renal transplantation^[10]. Although CV mortality is relatively high in pediatric RTRs, it does not always follow that the same risk factors as in adults^[11]. According to Johnstone *et al.*^[12] the most common echocardiographic abnormality in ESRD before renal transplantation is LVH (47.9%). This prevalence is higher in comparison to other studies, e.g., in the only large study performed by European Dialysis Transplant Association in children, an incidence of 22% of post-transplant LVH only was found. Mitsnefes *et al.*^[13] found that the prevalence of LVH was 56% among children and adolescents after renal transplantation. Johnstone and his colleagues indicated that LVH was found to be more frequent and severe in children after transplantation; when compared to those on HD or with advanced renal failure^[12]. Alvares *et al.*^[14] found that pretransplant HD resulted in an increased left ventricular mass index (LVMI) in children, especially if dialysis lasted for more than two years. They concluded that although this cardiac hypertrophy was reversible after renal transplantation, children may benefit from an earlier transplantation. Mitsnefes *et al.*^[13] also found that hypertension was a predictor of increased LVMI after transplantation in both children and adolescents and that control of blood

Table 1 Demographic and laboratory data of various groups (mean values)

	Age in years	Male/female	BMI (kg/m ²)	Hb pre Tx	Creatinine ne post Tx (mg/dL)	Hb post Tx (g/dL)	Duration of dialysis in years
Group 1	9.8 ± 3.7	18/12	25.8 ± 4.4	6.9 ± 1.0	0.9 ± 0.3	13.9 ± 0.9	0.6 ± 0.2
Group 2	26.8 ± 5.6	25/5	24.7 ± 3.1	6.9 ± 1.2	1.2 ± 0.2	13.3 ± 1.9	0.9 ± 0.4
Group 3	10.7 ± 2.4	12/8	28.2 ± 9.0	8.7 ± 1.5			3.3 ± 1.2
Group 4	43.9 ± 14.1	8/12	22.7 ± 5.2	9.7 ± 1.9			8.5 ± 4.7
Group 5	12.0 ± 1.5	5 males	18.0 ± 1.5	14.2 ± 0.8			
Group 6	30.2 ± 3.7	5 males	25.7 ± 3.8	13.2 ± 1.3			

BMI: Body mass index; Hb: Hemoglobin; Tx: Transplantation.

Table 2 Echocardiographic data in various groups (mean values)

	LVED pre Tx	LVES pre Tx	FS pre Tx	EF pre Tx	LVED post Tx	LVES post Tx	FS post Tx	EF post Tx
Group 1	4.7 ± 0.8	3.1 mm ± 0.6 mm	32.6% ± 5.3%	59.7% ± 7.0%	4.2 mm ± 0.5 mm	2.4 mm ± 0.4 mm	41.7% ± 7.6%	71.9 ± 6.1
Group 2	5.9 ± 0.7	4.1 ± 0.9	29.0 ± 6.6	52.0 ± 12.5	4.9 ± 0.6	3.1 ± 0.5	36.5 ± 4.1	64.8 ± 5.9
Group 3	4.7 ± 0.8	3.2 ± 0.6	30.9 ± 5.0	56.5 ± 6.3				
Group 4	5.6 ± 0.8	4.0 ± 1.0	29.2 ± 4.3	50.2 ± 7.4				
Group 5	4.2 ± 0.4	LVES 3.0 ± 0.5	41.9 ± 2.3	71.6 ± 4.0				
Group 6	5.0 ± 0.3	3.1 ± 0.6	38.0 ± 1.9	64.8 ± 3.4				

LVED: Left ventricular end diastolic; LVES: Left ventricular end systolic; FS: Fractional shortening; EF: Ejection fraction; Tx: Transplantation.

pressure might help in preventing LVH progression in RTRs.

MATERIALS AND METHODS

To evaluate the impact of renal transplantation on the cardiac morphology and functional characteristics and to study its clinical correlation with renal graft function in children and adults.

Our work was carried out in the Nephrology department at Cairo University. One hundred and ten patients attending the nephrology outpatient clinic were enrolled in this study and were divided into six groups. The first two groups consisted each of 30 renal transplant patients who had a successful renal transplantation in a period more than six months, but less than one year. Group 1 was less than 18 years, and group 2 was more than 18 years. The third and fourth groups, each were 20 CRF patients on regular hemodialysis (RHD). Group 3 were less than 18 years, and group 4 was more than 18 years. Group 5 and 6 consisted each of 5 subjects below and above 18 years of age, respectively with normal kidney functions.

All patients underwent full medical history taking and clinical examination. In all cases, we recorded age, body mass index (BMI) and a complete blood count. Patients in group 1 and 2 had their hemoglobin (Hb) measured before and six months after transplantation. Trans-thoracic echocardiography (TTE) was done to all our patients. Again, patients in group 1 and 2 had their TTE done before and six months after transplantation (Table 1).

We excluded patients older than sixty years, patients with ischemic heart diseases, diabetics, liver cirrhosis, chronic obstructive pulmonary disease and those with

a high serum creatinine (> 1.5 mg/dL) after renal transplantation.

SPSS version 9.0 was used to analyze the data. Data was summarized as mean, SD. *t*-test for dependent and independent variables were used for analysis of two quantitative data. One Way ANOVA test was done for analysis of more than two variables, followed by post HOC test for detection of significance. Pearson correlation was also done. Value was considered significant if < 0.05.

RESULTS

The improvement in all cardiac parameters in the renal transplant groups was statistically significant. Left ventricular end diastolic volume (LVED) decreased in children (4.7 ± 0.8 to 4.2 ± 0.5) and in adults (5.9 ± 0.7 to 4.9 ± 0.6) after renal transplantation. There was a regression in left ventricular end systolic volume (LVES) both in children (3.1 ± 0.6 to 2.4 ± 0.4) and in adults (4.1 ± 0.9 to 3.1 ± 0.5). There was an improvement in fractional shortening (FS) both in children (32.6 ± 5.3 to 41.7 ± 7.6) and adults (29.0 ± 6.6 to 36.5 ± 4.1) (Figure 1). The improvement in EF was higher in children (59.7 ± 7.0 to 71.9 ± 6.1) than in adults (52.0 ± 12.5 to 64.8 ± 5.9). The degree of improvement in pediatrics (12.2 ± 5.1), when compared to adults (12.7 ± 9.8) did not show statistical difference (Table 2).

When the data in post-transplant children were compared to those on RHD and normal children, there were statistically significant differences, regarding Hb levels (*P*-value 0.0001), LVED (*P*-value 0.02), LVES (*P*-value 0.0001), FS (*P*-value 0.0001) and EF (*P*-value 0.0001) (Table 3).

When comparing adults' data post renal trans-

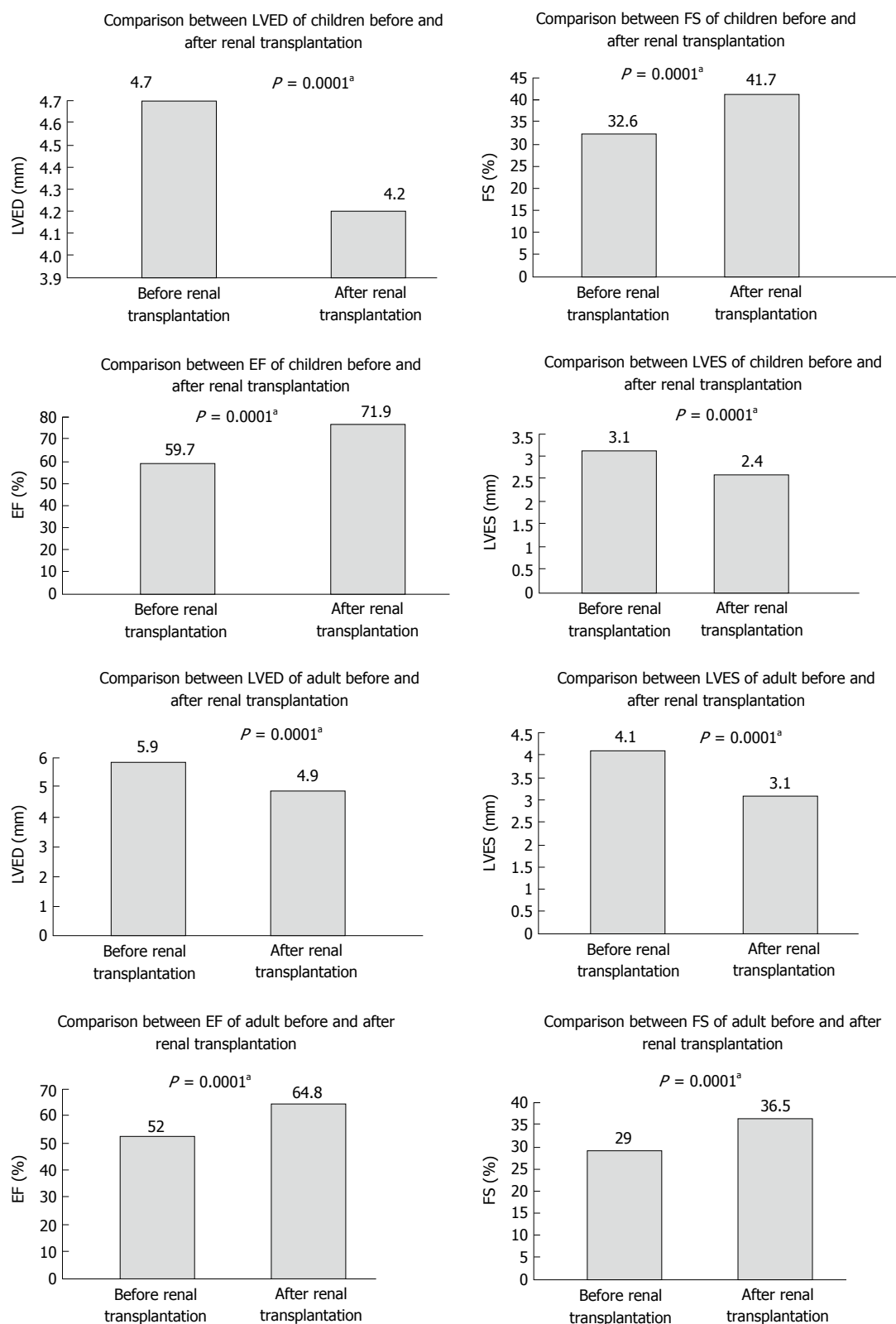


Figure 1 Function graft after renal transplant remains the most beneficial renal replacement therapy for children with end-stage renal disease. ^aP-value is considered significant if < 0.05. LVED: Left ventricular end diastolic volume; EF: Ejection fraction; LVES: Left ventricular end systolic volume; FS: Fractional shortening.

plantation to data of adults on RHD and normal adults, there was a statistically significant difference regarding Hb level, LVED, LVES, FS and EF% (P-value 0.0001 in all cases) (Table 4). When correlating the degree of improvement in EF after transplantation to other

parameters in both children and adults, there was no statistically significant correlation as regards Hb levels (P-value 0.4 in children and 0.1 in adults), age (P-value 0.5 vs 0.1), duration of dialysis (P-value 0.3 vs 0.4), BMI (P-value 0.8 vs 0.7). Comparison of the EF between

Table 3 Comparison of children data before and after renal transplantation

Variables	Before renal transplantation (mean \pm SD)	After renal transplantation (mean \pm SD)	P-value (P-value is significant if < 0.05)
Hemoglobin <i>n</i> (g/dL)	6.9 \pm 1.0	13.9 \pm 0.9	0.0001
LVED (mm)	4.7 \pm 0.8	4.2 \pm 0.5	0.0001
LVES (mm)	3.1 \pm 0.6	2.4 \pm 0.4	0.0001
FS (%)	32.6 \pm 5.3	41.7 \pm 7.6	0.0001
EF (%)	59.7 \pm 7.0	71.9 \pm 6.1	0.0001

LVED: Left ventricular end diastolic; LVES: Left ventricular end systolic; FS: Fractional shortening; EF: Ejection fraction.

Table 4 Comparison of adults' data before and after renal transplantation

Variables	Before renal transplantation (mean \pm SD)	After renal transplantation (mean \pm SD)	P-value (P-value is significant if < 0.05)
Hemoglobin (g/dL)	6.9 \pm 1.2	13.3 \pm 1.9	0.0001
LVED (mm)	5.9 \pm 0.7	4.9 \pm 0.6	0.0001
LVES (mm)	4.1 \pm 0.9	3.1 \pm 0.5	0.0001
FS (%)	29.0 \pm 6.6	36.5 \pm 4.1	0.0001
EF (%)	52.0 \pm 12.5	64.8 \pm 5.9	0.0001

LVED: Left ventricular end diastolic; LVES: Left ventricular end systolic; FS: Fractional shortening; EF: Ejection fraction.

children and adults showed there was a statistically significant difference, whether before *P*-value 0.005 or after renal transplantation *P*-value 0.0001).

DISCUSSION

Compared with the general population, (RTRs) are at a higher risk for morbidity and mortality, largely as a result of (CVD)^[15]. Marked improvements in all cardiac functions are evident after successful renal transplantation. The changes are apparent in the early post-transplant period and continue over time, depending on BP control and renal functions^[16]. The aim of our work is to evaluate the impact of renal transplantation on the cardiac morphological and functional characteristics in children and adults who had renal transplantation and to compare the degree of improvement in children to adults. The study showed that a statistically significant improvement in LVED, LVES and FS and EF occurred 6 mo after renal transplantation in the pediatric population. This goes in agreement with the work previously published by El-Husseini *et al.*^[17] where they reported a 56% increase in FS after transplantation; larger than that expected with correction of anemia. Few studies used EF to reflect improvement in cardiac characteristics after renal transplantation, although many used LVH and LVMI.

In our study, the adult group of renal transplant recipients also showed a statistically significant improvement in LVED, LVES, FS and EF, six months after renal transplantation. Montanaro used LVM and LVMI left compared to the pre-transplantation period to assess cardiac status amelioration. He observed that the prevalence of LVH significantly decreased (78% vs 44%, *P* < 0.03). Systolic 24-h BP was the only predictor of

LVM and LVMI at two years after transplantation. He concluded that successful renal transplantation produced a regression in LVH and that this beneficial effect depended on a decrease in systolic pressure levels.

In our work, we attributed the marked improvement in cardiac morphological and functional characteristics after renal transplantation. In to correction of anemia, control of blood pressure, normalization kidney function and reduction of volume overload.

When comparing pediatric RTRs to those on RHD, there was a statistically significant difference in EF between the two groups (group 1 and 3); with improvement in EF after renal transplantation. These parameters reflect improvement in the degree of left ventricular dilatation and systolic dysfunction in RTR than those on HD. This was in agreement with Chinali *et al.*^[18] Children with CRF have a reduced EF; this was found to be in agreement with work published by Colan *et al.*^[19] who found a markedly reduced EF in children on HD; though many other former studies showed that predialysis or dialysis-dependent CRF children may have a normal or further more a supernormal EF at rest^[20].

Schrier^[21] reported that in ESRD patients the most common CV abnormalities are systolic dysfunction (30%-60%), diastolic dysfunction (17%), LVH (up to 93%) and LV dilatation (27%). Iqbal *et al.*^[22] 2006 stated that marked beneficial alterations in cardiac function and morphology become apparent as early as three months post renal transplantation.

Correction of anemia and proper control of BP contribute to the reduction in cardiac ventricular diameters. Long term maintenance of these changes are definitely more in patients with functioning renal graft. Systolic or diastolic hypertension is risk factors for patients and

graft survival after renal transplantation. The effect of hypertension on kidney grafts has been attributed to amplification of vascular injury^[23]. Salvatierra *et al*^[24] reported that in CRF, cardiomyopathy presents by systolic dysfunction, concentric LVH or LVD. Renal transplantation leads to normalization of left ventricular contractility, regression of LVH and improvement of cavity volume.

A statistically significant increase in Hb level was noted after transplantation in both children and adults; however, when correlating the degree of improvement in Hb with the degree of improvement in EF after transplantation, no statistically significant correlation was found. This was considered to be contrary to Iqbal *et al*^[25] in 2008, who showed that the reduction in BP with correction of anemia and a decreased in creatinine level influenced the improvements in LV parameters. Foley *et al*^[6] reported that after renal transplantation, a 17% increase in LVMI (similar to the degree of regression of hypertrophy found on partial correction of anemia by erythropoietin) occurred. LVH regression may be compromised by hypertension, as a clear association was seen between the fall in blood pressure and the fall in LVMI.

Correction of anemia in CRF patients and RTRs is an important issue as shown by Walker *et al*^[26] 2006 who reported that patients with anemia and CRF had elevated risks for CVD. Risks for hospitalization with myocardial infarction was found to be 2-5 times higher in anemic (Hb < 12 g/dL) patients^[27]. The risks for hospitalization for congestive heart failure reduced from a doubling risk at Hb < 10% to a 61% decrease at Hb 15 g/dL after increasing Hb^[28].

In our study, the comparison of the EF between children and adults showed there was a statistically significant difference, whether before or after renal transplantation. Although the improvement in EF was higher in children than in adults, yet the degree of improvement in children, when compared to the degree of improvement in adults did not show a statistically significant difference. As stated by Salvatierra *et al*^[29] function graft after renal transplant remains the most beneficial RRT for children with ESRD (Figure 1).

Transplanting children is very challenging. Graft and patient survival were often reported to be less promising in young patients compared with older children and adults, yet the results have recently improved significantly^[30].

In conclusion, marked improvement in cardiac morphological and functional characteristics occurs after renal transplantation. These findings were found to be more significant in the pediatric population. Renal transplantation is therefore expected to reduce mortality and cardiovascular deaths than dialysis in CRF patients, although, in both groups, survival remains worse than in the general population. Renal transplantation is the treatment of choice for CRF, especially in children and adolescence. A larger number of patients need to be studied with a longer follow-up period up to five years after transplantation with frequent cardiological assessment

and proper correlation with anemia correction, BP control, lipid profile correction and detection and management of new-onset diabetes.

COMMENTS

Background

Cardiovascular diseases are the principal causes of morbidity and mortality among adults and children with chronic kidney disease (CKD). After renal transplantation, there is a substantial improvement in cardiac morphology and functions. The early and rapid improvement in cardiac status is even more marked in children.

Research frontiers

In Egypt, the number of children with CKD having a renal transplant is increasing. The cardiac problems in children with CKD are among the most common reasons for not choosing renal transplantation for those children. There is a very few English literature documenting the marked improvement in the ejection fraction of Egyptian children especially in comparison to adults. The study hotspot is to emphasize not only the rapid (as early as six months post-transplant) but also the marked improvement in the ejection fraction (EF) in children when compared to adults after have a renal transplant.

Innovations and breakthroughs

In recent years the list of contraindications and relative contraindications to renal transplant has been changing. The impaired cardiac function was among the relative contraindications to renal transplantation. The present study shows that there is a marked improvement in EF after renal transplantation both in adults and in children with CKD. Although the improvement is marked and significant in both adults and children yet, the authors' study also shows that the improvement in children is even more.

Applications

The data in their work suggested that the EF of patients with ESRD improves after renal transplantation, this improvement is marked in children.

Terminology

EF is the amount of blood pumped from the heart by heartbeat. It is low in systolic congestive heart failure. It is a similar but different mathematically when compared to stroke volume, which (together with heart rate) measures cardiac output. EF is often determined by echocardiography, in which the volumes of the heart's chambers are measured during the cardiac cycle. EF can then be calculated by dividing the volume ejected by the heart (stroke volume) by the volume of the filled heart (end-diastolic volume). It can also be measured by computed tomography scan, magnetic resonance imaging, ventriculography, gated SPECT and radionuclide angiography scanning.

Peer-review

Available papers concerning the comparison between the improvements in cardiac function after renal transplantation in children and adults are rare.

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Observational Study

Factors associating with oxygenation of lower-limb muscle tissue in hemodialysis patients

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Abstract

AIM

To evaluate the lower-limb muscle oxygenation in hemodialysis (HD) patients and identify the factors associating with muscle oxygenation.

METHODS

Sixty-seven HD patients (53 men and 14 women; mean age, 67.1 ± 1.2 years; mean HD duration, 5.6 ± 0.9 years) were recruited. In addition, 15 healthy individuals (nine men and six women; mean age, 38.2 ± 4.6 years) were recruited as the control group. Lower-limb muscle regional saturation of oxygen (rSO₂) was monitored on the lateral side of the gastrocnemius muscle before HD using an INVOS 5100C (Covidien Japan, Tokyo, Japan), which utilizes near-infrared spectroscopy. Here, we evaluated the association between lower-limb muscle rSO₂ and clinical parameters.

RESULTS

The rSO₂ values were significantly lower in patients undergoing HD than in healthy individuals (50.0%

$\pm 1.7\%$ vs $76.8\% \pm 2.5\%$, $P < 0.001$). Lower-limb muscle rSO_2 showed significant positive correlations with diastolic blood pressure, blood urea nitrogen concentration, serum creatinine concentration, serum potassium concentration, serum inorganic phosphate concentration, and serum albumin concentration as well as negative correlation with HD duration. We conducted a multiple linear regression analysis using parameters that were significantly correlated with the lower-limb muscle rSO_2 in a simple linear regression analysis. Multiple regression analysis demonstrated that lower-limb muscle rSO_2 was independently associated with serum inorganic phosphate (standardized coefficient: 0.27) and serum albumin concentrations (standardized coefficient: 0.24). In addition, there were no differences in lower-limb muscle rSO_2 between diabetic and non-diabetic HD patients. This study has several limitations. Firstly, its sample size was relatively small. Secondly, we could not evaluate the association between lower-limb muscle rSO_2 and calculated nutritional markers, including normalized protein catabolic rate and body mass index, anthropometric measurements representing nutritional status, and the severity of protein-energy wasting. Finally, we did not routinely examine the arterial vascular status of HD patients without symptoms of peripheral artery disease. As such, it is possible that some HD patients with subclinical peripheral artery disease may have been included in this study.

CONCLUSION

In HD patients, the oxygenation of lower-limb muscle tissue was associated with serum inorganic phosphate and albumin concentrations, both of which represent nutritional status.

Key words: Regional saturation of oxygen; Lower-limb muscle; Sarcopenia; Protein-energy wasting; Nutritional status; Inorganic phosphate; Albumin; Hemodialysis

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Core tip: Sarcopenia, defined by reduced muscle mass and peripheral arterial disease, is common in patients undergoing hemodialysis (HD). Therefore, muscle status, including muscle oxygenation, would deteriorate; however, no muscle status evaluation method has been established and remains under debate. Here we investigated the tissue oxygenation of lower-limb muscles using near-infrared spectroscopy in HD patients. Values of regional saturation of oxygen in the lower-limb muscles were significantly lower in HD patients than in healthy controls and independently associated with serum inorganic phosphate and albumin concentrations, both of which represent nutritional status.

Miyazawa H, Ookawara S, Ito K, Yanai K, Ishii H, Kitano T, Shindo M, Ueda Y, Kaku Y, Hirai K, Hoshino T, Tabei K, Morishita Y. Factors associating with oxygenation of lower-limb muscle tissue in hemodialysis patients. *World J Nephrol*

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INTRODUCTION

According to the increase in numbers of older and diabetic patients undergoing hemodialysis (HD), protein-energy wasting, which includes abnormal serum biochemistry, reduced body mass, reduced muscle mass, and decreased dietary intake, has been a concern in the clinical setting^[1]. In particular, sarcopenia refers to a state of reduced muscle mass^[2] and is reportedly an independent predictor of mortality in HD patients^[3]. However, a standard muscle status evaluation method has not been established and remains under debate. Furthermore, peripheral artery disease is common in HD patients^[4]; therefore, lower-limb muscle oxygenation would deteriorate as a result of a decreased oxygen supply induced by impaired microcirculation.

Near-infrared spectroscopy (NIRS) is recently reported as a way to evaluate the regional oxygen saturation (rSO_2), a tissue oxygenation marker^[5,6], especially including cerebral oxygenation in various conditions. Then, rSO_2 value indicates changes in tissue oxygen metabolisms between oxygen supply from arterial blood flow and tissue oxygen consumption^[7-10]. Furthermore, an adequate measurement of tissue oxygenation of muscle mass using NIRS has been previously reported^[11] and this method was used to evaluate muscle status^[12-14]. However, few reports have examined lower-limb muscle oxygenation using NIRS much less the correlation between these values and clinical parameters in HD patients. Therefore, this study aimed to: (1) monitor lower-limb muscle rSO_2 before HD; and (2) clarify the association influencing the value of lower-limb muscle oxygenation in HD patients.

MATERIALS AND METHODS

Participants recruitments

This was performed as a single-center observational study and included HD patients who met the following criteria: (1) end-stage renal disease and receiving intermittent HD; (2) agreement for the purpose of this study; and (3) presence of arteriovenous fistula as a vascular access for HD. The exclusion criteria were: (1) coexisting disease including chronic obstructive pulmonary disease, apparent neurological disorder, and chronic hypotension (defined as systolic blood pressure < 100 mmHg); and (2) symptomatic ischemia and receiving lower leg amputation. Sixty seven HD patients were recruited (53 men and 14 women; mean age, 67.1 ± 1.2 years; mean HD duration, 5.9 ± 0.9 years). The causes of chronic renal failure were diabetes mellitus (DM; 38 patients), chronic glomerulonephritis (14 patients), nephrosclerosis (four patients), polycystic kidney disease

Table 1 Patients' characteristics and the correlation between lower-limb muscle regional saturation of oxygen and clinical parameters in a simple linear regression analysis

	Mean ± SE	P values	r
Total number of patients (male/female)	67 (53/14)		
Age (yr)	67.1 ± 1.2	0.09	-0.21
Disease			
Diabetes mellitus	38		
Chronic glomerulonephritis	14		
Nephrosclerosis	4		
Polycystic kidney disease	3		
Others	8		
HD duration (yr)	5.6 ± 0.9	0.02	-0.28
Systolic blood pressure (mmHg)	144 ± 3	0.97	-0.01
Diastolic blood pressure (mmHg)	75 ± 2	0.04	0.26
pH	7.39 ± 0.02	0.41	-0.10
pCO ₂ (mmHg)	36.9 ± 0.5	0.14	0.18
pO ₂ (mmHg)	85.6 ± 2.0	0.80	-0.03
HCO ₃ ⁻ (mEq/L)	21.9 ± 0.3	0.70	0.05
SpO ₂ (%)	95.4 ± 0.3	0.88	-0.02
Hb (g/dL)	9.9 ± 0.1	0.30	0.13
Arterial O ₂ content (mL/dL)	12.9 ± 0.2	0.27	0.13
BUN (mg/dL)	51.4 ± 2.2	0.03	0.27
Cr (mg/dL)	8.4 ± 0.3	0.02	0.28
Na (mEq/L)	137.0 ± 0.4	0.19	0.16
K (mEq/L)	4.2 ± 0.1	0.02	0.30
Ca (mg/dL)	8.7 ± 0.1	0.91	-0.01
P (mg/dL)	4.5 ± 0.2	< 0.01	0.31
Total protein (g/dL)	6.1 ± 0.1	0.72	0.04
Serum albumin (g/dL)	3.2 ± 0.1	0.02	0.29
Serum osmolality (mOsm/kg·H ₂ O)	301 ± 1	0.09	0.21
Plasma glucose (mg/dL)	156 ± 8	0.52	-0.08
Kt/V urea	1.18 ± 0.04	0.65	-0.06
Urea reduction ratio (%)	61.3 ± 1.6	0.94	0.01

SpO₂: Oxygen saturation; Hb: Hemoglobin concentration; BUN: Blood urea nitrogen concentration; Cr: Serum creatinine concentration; Na: Serum sodium concentration; K: Serum potassium concentration; Ca: Serum calcium concentration; P: Serum inorganic phosphate concentration; HD: Hemodialysis; SE: Standard error.

(three patients), and other (eight patients). Each patient received maintenance HD two or three times a week, and each session was 3 or 4 h in duration. The patients' general characteristics are summarized in Table 1. Furthermore, the proportions of patients using phosphate binder and vitamin D metabolites were 71.6% ($n = 48$) and 37.3% ($n = 25$), respectively. The HD dialysate comprised 140 mEq/L Na⁺, 2.0 mEq/L K⁺, 114.5 mEq/L Cl⁻, 2.5 mEq/L Ca²⁺, 1.0 mEq/L Mg²⁺, 25 mEq/L HCO₃⁻, and 150 mg/dL glucose. The dialyzer included polysulfone membrane (three patients; 1.0 m², five patients; 1.3 m², four patients; 1.5 m², six patients; 1.6 m², thirteen patients; 1.8 m², nine patients; 2.0 m², sixteen patients; 2.1 m², four patients; 2.3 m²) and cellulose triacetate membrane (two patients; 1.3 m², five patients; 1.5 m²). The dialysate flow rate in all patients was consistently 500 mL/min and the blood flow rate was 183 ± 1.7 mL/min in this study. The dialysate purification process followed the recommendations in the Japanese Society for Dialysis Therapy guidelines^[15]. The dialysate bacteria and endotoxin concentration in this study were less than 0.1 CFU/mL and 0.001 EU/mL respectively. All

participants provided informed consent to participate in this study. This study was approved by the Institutional Review Board of Saitama Medical Center, Jichi Medical University, Japan (No. RIN13-39), and conforms to the provisions of the Declaration of Helsinki (as revised in Tokyo in 2004). In addition, 15 healthy volunteers (nine men and six women; mean age, 38.2 ± 4.6 years) were recruited as the control group.

Monitoring of lower-limb muscle oxygenation and clinical laboratory measurements

Lower-limb muscle rSO₂ was monitored at right side lower-limb on the lateral side of the gastrocnemius muscle with an INVOS 5100C saturation monitor (Covidien Japan, Tokyo, Japan), which is based on the NIRS technology. This monitor utilizes a light-emitting diode that generates near-infrared light at two wavelengths (735 and 810 nm) as well as two photodiodes constructed by silicon, which function as light detectors; these data are interpreted as a single numerical value that expresses the rSO₂ value^[5,6]. All data taken by this monitor were stored in sequence with immediate and automatic manner. Interobserver variance for this monitor, which means a reproducibility of rSO₂ monitoring, is considered acceptable based on the study reported previously^[11]. Thus, rSO₂ values would be reliable in the estimation of the lower-limb muscle oxygen metabolism.

Before HD, included patients in this study lied supine for 10 min to avoid the influence of change in posture. An rSO₂ monitoring probe was attached to the patients' gastrocnemius muscle to take the measurement during resting in the supine. Then, rSO₂ value was monitored for 5 min, and we evaluated the mean rSO₂ for 5 min as a lower-limb muscle oxygenation marker.

Blood samples were drawn from arteriovenous fistula before HD under ambient air. Samples drawn from the radial artery or those from an arterial line at the arteriovenous fistula were previously reported to present similar values during evaluation of the clinical parameters of oxygen status, including pH, oxygen pressure (pO₂), and oxygen saturation (SpO₂)^[16]. Thus, we took all blood samples including blood gas analysis from the arterial site of arteriovenous fistula in each patient prior to HD.

We calculated arterial O₂ content (CaO₂) and serum osmolality (sOsm) using following equations:

$$\text{CaO}_2 \text{ (mL/dL)} = 1.34 \times \text{Hb} \times \text{SpO}_2/100 + (0.0031 \times \text{pO}_2)^{[17]}$$

$$\text{sOsm (mOsm/kg·H}_2\text{O)} = (2 \times \text{Na}) + \text{PG}/18 + \text{BUN}/2.8^{[18]}$$

Where Hb indicates the hemoglobin concentration (g/dL), SpO₂ indicates the oxygen saturation (%), and pO₂ indicates the oxygen pressure (mmHg). Na indicates the serum sodium concentration (mEq/L), PG indicates the plasma glucose level (mg/dL), and BUN indicates the blood urea nitrogen concentration (mg/dL). Furthermore, for evaluating the efficacy of HD, Kt/V by using Daugirdas II formula^[19] and urea reduction ratio^[20] were calculated

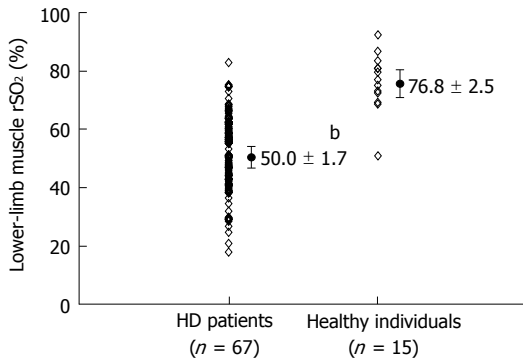


Figure 1 Lower-limb muscle regional saturation of oxygen in hemodialysis patients vs healthy individuals. ^a $P < 0.001$ vs healthy individuals. HD: Hemodialysis; rSO₂: Regional saturation of oxygen.

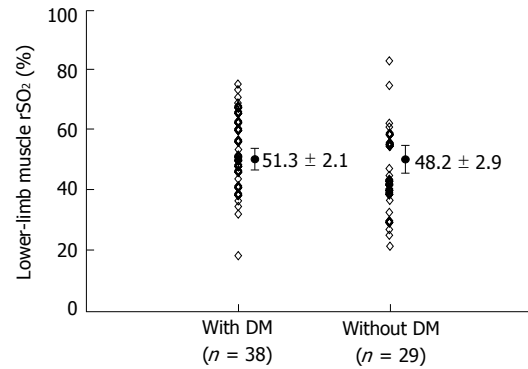


Figure 2 Comparison of lower-limb muscle regional saturation of oxygen in hemodialysis patients with vs without diabetes mellitus. rSO₂: Regional saturation of oxygen; DM: Diabetes mellitus.

Table 2 Multiple linear regression analysis of independent factors of lower-limb muscle regional saturation of oxygen in hemodialysis patients

Parameters	Coefficient (95%CI)	Standardized coefficient	P values
P	3.02 (0.38-5.65)	0.27	0.025
Serum albumin	6.26 (0.15-12.37)	0.24	0.045

P: Serum inorganic phosphate concentration.

in each patient. The rSO₂ in healthy individuals was monitored for 5 min during resting in the supine position according to the same manner of rSO₂ measurement in HD patients.

Statistical analysis

Data are expressed as mean \pm standard error. The Shapiro-Wilk test was used to confirm that all data were normally distributed. Student's *t*-test was used to compare non-paired values between the 2 values. Correlations between the 2 values were evaluated by Pearson's correlation coefficient and linear regression analysis. Parameters that were significantly correlated with lower-limb muscle rSO₂ in a simple linear regression analysis were included in a multiple linear regression analysis in order to identify factors associating with lower-limb muscle rSO₂ in patients undergoing HD. A difference with $P < 0.05$ was considered statistically significant.

RESULTS

Lower-limb muscle rSO₂ values in patients undergoing HD were significantly lower than those of healthy individuals (HD patients: $50.0\% \pm 1.7\%$; healthy individuals: $76.8\% \pm 2.5\%$; $P < 0.001$; Figure 1).

Table 1 shows the patients' characteristics as well as correlations between lower-limb muscle rSO₂ and clinical parameters. According to a simple linear regression analysis, lower-limb muscle rSO₂ showed significant positive correlations with diastolic blood pressure, BUN, serum creatinine, serum potassium, serum inorganic phosphate, and serum albumin as well as negative

correlation with HD duration. We conducted a multiple linear regression analysis using parameters that were significantly correlated with lower-limb muscle rSO₂ in a simple linear regression analysis (Table 2). As a result, lower-limb muscle SO₂ was independently associated with serum inorganic phosphate (standardized coefficient: 0.27) and serum albumin (standardized coefficient: 0.24). In addition, we examined the relationship between presence of DM and lower-limb muscle rSO₂ values, and found no differences in these values between patients with and those without DM ($P = 0.38$; Figure 2).

DISCUSSION

In the present study, lower-limb muscle rSO₂ values in HD patients were significantly lower than those of healthy individuals. Furthermore, multiple linear regression analysis indicated that serum inorganic phosphate and albumin concentrations, both of which represent nutritional status, were independently associated with the lower-limb muscle rSO₂.

Regarding the association between tissue oxygenation and HD therapy, cerebral rSO₂ values in HD patients were significantly lower than those of healthy individuals^[21,22]. These values were maintained during HD and not influenced by blood volume reduction^[22], whereas intradialytic hypotension might be associated with a decrease in cerebral rSO₂^[23]. Therefore, these findings indicate the importance of monitoring systemic tissue oxygenation, particularly cerebral oxygenation as a part of systemic oxygenation, in HD patients.

According to the increasing number of elderly patients on maintenance HD, malnutrition and loss of muscle mass are considered common in the clinical setting of dialysis therapy. The age-related loss of muscle mass and function was termed sarcopenia^[2] and the European working group on sarcopenia in older people defined sarcopenia as "a syndrome characterized by progressive and generalized loss of skeletal muscle mass and strength with a risk of adverse outcome such as physical disability, poor quality of life, and death"^[24]. Therefore, muscle mass condition has been a concern in patients undergoing HD, and here we focused on muscle oxygenation as a method to

define muscle condition. The measurement of muscle rSO₂ using NIRS at the lower extremities was reportedly applicable as a tool for evaluating tissue oxygenation because of the good reproducibility and inter-subject variability of its measurements^[11]. Clinically, in patients with acute compartment syndrome of the leg, the tissue oxygenation decrease of injured leg was reported to reflect the decrease of perfusion pressure at the injured compared to uninjured leg^[25]. Furthermore, in patients with community-acquired pneumonia, values of forearm muscle rSO₂ at intensive care unit admission and those at 24 h after admission were independently associated with mortality^[14]. However, to date, tissue oxygenation at lower extremities in patients undergoing HD has rarely been reported.

The reduction of the lower-limb muscle rSO₂ values in HD patients included in this study was similar to the result of cerebral rSO₂ comparison between HD patients and healthy controls^[21,22]. Although this result is inconclusive because the ages and sexes were not completely matched between the groups, De Blasi *et al.*^[12] reported that the tissue oxygen saturation values at the gastrocnemius muscle are lower in patients undergoing HD with and without DM compared to healthy controls, even though the differences are not significant (tissue oxygen saturation: In HD patients with and without DM, 49.2% ± 16.6% and 51.5% ± 21.4%; in healthy controls, 62.3% ± 7.3%). HD patients with symptomatic lower-limb ischemia who were diagnosed with peripheral artery disease by angiography and/or magnetic resonance imaging were excluded from the study. The prevalence of subclinical peripheral artery disease in HD patients is approximately 20%-25%^[4]. We did not use methods such as the ankle-brachial pressure index to investigate the presence of peripheral artery disease in asymptomatic patients. Therefore, while we have excluded patients with symptomatic peripheral artery disease from the present study, we may have inadvertently included some with subclinical disease. In these patients, muscle rSO₂ might be reduced *via* the decrease in oxygen supply caused by the microcirculatory impairment due to subclinical peripheral artery disease. Thus, it would be necessary to perform arterial vascular examinations in future studies so that such patients can be excluded.

In the fields of chronic kidney disease and dialysis therapy, the concept of protein-energy wasting has recently been proposed^[1]. Furthermore, protein-energy wasting itself was one of the strongest predictors of mortality in patients with chronic kidney disease^[26]. Therefore, to prevent the progression of protein-energy wasting in patients undergoing HD, the initiation of oral nutritional supplementation, including 1.2 g/kg per day of dietary protein intake, is recommended^[27]. On the other hand, there was a significant positive correlation between dietary protein intake and phosphorus intake as well as between phosphorus intake and pre-dialysis serum inorganic phosphate concentration^[28]. Based on these results, serum inorganic phosphate concentration might

be positively associated with dietary protein intake. In this study, lower-limb muscle rSO₂ values were positively and significantly associated with serum inorganic phosphate concentration. The increase in its concentration would reflect the nutritional status improvement *via* increased dietary protein intake; therefore, lower-limb muscle oxygenation might be associated with the change in serum inorganic phosphate concentration, which could be influenced by the nutritional status. However, although serum inorganic phosphate concentration is one of several nutritional markers, hyperphosphatemia may contribute to worsening vascular calcification and a greater risk of cardiovascular morbidity^[29,30]; therefore, further studies will be necessary to confirm the association between lower-limb muscle rSO₂ and other nutritional markers such as normalized protein catabolic rate^[31], which represents the dietary protein intake in anuric HD patients.

Regarding the association between serum albumin concentration and lower-limb muscle rSO₂, serum albumin was reportedly a prognostic marker of survival in patients undergoing HD similar to nutritional status^[32,33]. Serum albumin reduction is included in the criteria in the diagnosis of protein-energy wasting^[1], leading to prognostic worsening in patients undergoing HD. Furthermore, serum albumin itself, which associates with the formation of plasma colloid osmotic pressure, influences the preservation of microcirculation *via* the fluid movement from the interstitial space into vessels at small systemic blood vessels. Therefore, serum albumin itself might be associated with muscle oxygenation through the preservation of muscle microcirculation, in addition to the nutritional status and prognosis in HD patients. Furthermore, in this study, lower-limb muscle rSO₂ values were not significantly different between patients with or those without DM, although cerebral rSO₂ values revealed significant decrease in patients undergoing HD with than those without DM^[21]. Differences in tissue oxygenation associated with DM itself between the brain and the muscle is very interesting; however, its precise mechanism remains uncertain.

This study has several limitations. Firstly, its sample size was relatively small. Secondly, we could not evaluate the association between lower-limb muscle rSO₂ and calculated nutritional markers, including normalized protein catabolic rate and body mass index, anthropometric measurements representing nutritional status, and the severity of protein-energy wasting. Finally, we did not routinely examine the arterial vascular status of HD patients without symptoms of peripheral artery disease. As such, it is possible that some HD patients with subclinical peripheral artery disease may have been included in this study. Thus, additional studies are necessary to fully clarify the correlation between lower-limb muscle rSO₂ and various clinical parameters.

In conclusion, lower-limb muscle rSO₂ values in patients undergoing HD were significantly lower than those in healthy individuals. Furthermore, the oxygenation of lower-limb muscle tissue was associated with serum inorganic phosphate and albumin concentrations, both of

which represent nutritional status.

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COMMENTS

Background

The incidence of protein-energy wasting in patients undergoing maintenance hemodialysis (HD) is high. In particular, sarcopenia characterized by skeletal muscle atrophy and decrease in muscle strength and function correlates with mortality in HD patients. In addition, peripheral arterial disease, including critical limb ischemia, is a major risk factor for amputation and death in HD patients. Therefore, a method for evaluating muscle is required, especially in the lower-limb.

Research frontiers

Recently, near-infrared spectroscopy (NIRS) has been used as a way to measure the regional saturation of oxygen (rSO₂), which is a marker of tissue oxygenation. NIRS is a non-invasive method for measuring tissue oxygenation continuously and mainly used for monitoring brain tissue oxygen saturation during cardiac surgery. In this study, the authors used NIRS to measure the lower-limb muscle rSO₂ in HD patients.

Innovations and breakthroughs

Lower-limb muscle rSO₂ values were significantly lower in HD patients than in healthy individuals, and independently associated with serum inorganic phosphate and albumin concentrations.

Applications

Serum inorganic phosphate and albumin concentrations are markers of nutritional status. Therefore, the authors can conclude that lower-limb muscle oxygenation may be associated with nutritional status in HD patients.

Terminology

NIRS technology uses a light-emitting diode which transmits near-infrared light at 2 wavelengths, as well as 2 silicon photodiodes which act as light detectors. Each result is read as a single numerical value representing the rSO₂.

Peer-review

The authors performed a very interesting and well conducted study aimed at evaluating the factors influencing regional muscular oxygenation in patients underwent hemodialysis. The adopted statistical approach is convincing and the conclusions are supported by the findings reported.

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Observational Study

Six end-stage renal disease patients benefited from first non-simultaneous single center 6-way kidney exchange transplantation in India

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Abstract

AIM

To avoid desensitization protocols and ABO incompatible kidney transplantation (KT) due to high costs and increased risk of infections from intense immunosuppression.

METHODS

We present institutional ethical review board - approved study of single center 6-way kidney exchange transplantation. The participants comprised ABO incompatibility ($n = 1$); positive cross-match and/or presence of donor

specific antibody ($n = 5$). The average time required from registration in kidney paired donation (KPD) registry to find suitable donors was 45 d and time required to perform transplants after legal permission was 2 mo.

RESULTS

Graft and patient survival were 100%, and 100%, respectively. One patient had biopsy-proven acute borderline T cell rejection (Banff update 2013, type 3). Mean serum creatinine was 0.8 mg/dL at 9 mo follow-up. The waiting time in KPD was short as compared to deceased donor KT.

CONCLUSION

We report first non-simultaneous, single center, 6-way kidney exchange transplantation from India. Our experience will encourage other centers in India to undertake this practice.

Key words: Kidney transplantation/methods; Kidney paired donation; Living donors/supply and distribution; Donor selection/methods

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Core tip: We report first non-simultaneous single center 6-way kidney exchange transplantation from India which has the potential to expand the living donor pool and increases kidney transplant opportunity for immunologically sensitized patients. Simultaneous transplant surgery is an accepted standard practice in kidney paired donation and should be encouraged. Non-simultaneous paired exchange transplants should be cautiously performed in carefully selected donors/recipient pairs with "due diligence" and legal permission from institutional ethical review board and written informed consent from the donors/recipients. Counseling to understand the risks and benefits of this procedure is mandatory.

Kute VB, Patel HV, Varyani UT, Shah PR, Modi PR, Shah VR, Rizvi SJ, Pal BC, Shah PS, Wakhare PS, Ghodla VA, Shinde SG, Trivedi VB, Patel MH, Trivedi HL. Six end-stage renal disease patients benefited from first non-simultaneous single center 6-way kidney exchange transplantation in India. *World J Nephrol* 2016; 5(6): 531-537 Available from: URL: <http://www.wjgnet.com/2220-6124/full/v5/i6/531.htm> DOI: <http://dx.doi.org/10.5527/wjn.v5.i6.531>

INTRODUCTION

As per Indian chronic kidney disease (CKD) registry only 2% of end stage renal disease (ESRD) patients underwent kidney transplantation (KT). Due to economic constraints 61% patients did not receive any form of renal replacement therapy; 35% patients were on maintenance hemodialysis while only 5% on continuous ambulatory peritoneal dialysis. Economic constraints

resulting in non-adherence to dialysis therapy leads to higher morbidity and mortality. In absence of national healthcare insurance scheme in India and in other South Asian countries, 75% of CKD patients were referred late in stage 5 CKD. Of these, 90% die within a month of diagnosis and majority (66%) of patients are unable to continue dialysis therapy beyond the first 3 mo due to financial reasons^[1]. Every year 175000 new patients develop ESRD in India. Kidney transplantation rate is 3.25 per million populations (pmp). India does not have a robust deceased donor kidney transplant program (0.08 pmp); 90% of KT was from living donors.

Our center in Ahmedabad, India has pioneered kidney paired donation (KPD) transplantation over the last 10 years^[2-6]. Two way single center KPD has the inherent limitation to increase transplant rate^[2-4]. Domino paired donation (DPD) would increase the number of kidney exchange transplants by about 20%, depending on the size of the donor pool^[7]. Herein, we report six ESRD patients who underwent the non-simultaneous single center 6-way kidney exchange transplantation. We believe this is the first of its kind in India.

MATERIALS AND METHODS

Kidney paired donation allocation

The KPD allocation procedure was facilitated by a nephrologist supervised by ethical review board ensuring equitable allocation. In manual KPD allocation, we took into account the following: Bonus points were given to previous history of positive cross-match more than twice, history of failure to desensitization protocol, history of previous KT, dialysis time, HLA match, pediatric patients, O group patients, waiting time, and failure of vascular access for dialysis. All patients were matched with donors of similar age group. The difference between kidney exchange donors should be ≤ 10 years. All the patients exchanged kidney of similar quality (anatomical, functional and immunological similarity). There should be single renal artery and vein on side of donor nephrectomy, measured glomerular filtration rate ≥ 90 mL/min per 1.73 m² using Tc99m DTPA renal scan should be considered as an acceptable level of kidney function for kidney donation.

Immunological compatibility

We demonstrated absence of the donor specific antibody (DSA) in the each recipient to mismatched antigens of intended donor using data of blood groups, 14 points HLA antigens of donors and recipients and recipients' HLA antibody specificities (by one Lambda single antigen bead assay). Thereafter, actual testing of complement-dependent cytotoxicity (CDC)-lymphocyte cross-match (LCM), flow cross match (FCM), were performed for every patient with the intended donor in transplant workup. It was mandated that all three test (LCM, FCM and DSA) should be negative prior to assigning the final KPD donor.

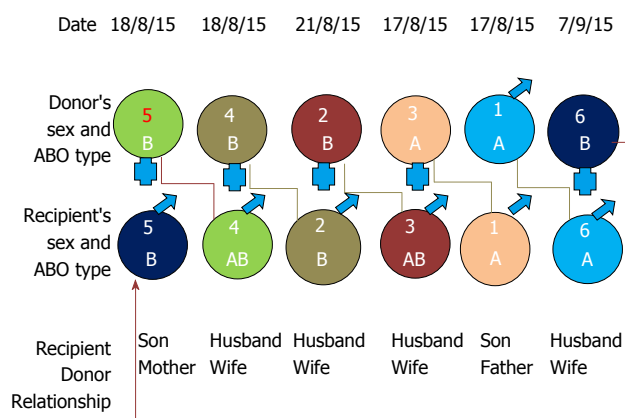


Figure 1 Variables of the 6-way kidney exchange transplantation.

Immunosuppression

Induction immunosuppression consisted of methyl prednisolone 500 mg for 3 d and rabbit thymoglobulin (1-2 mg/kg) and maintenance immunosuppression consisted of tacrolimus (target level 8-10 ng/mL in first 3 mo and 4-7 ng/mL thereafter), prednisolone 20 mg/d and tapered to 10 mg at 3 mo and thereafter) and mycophenolate sodium 1080-1440 mg/d.

Ethics and cost of KT

The clinical study has been reviewed by the ethics committee according to international standards of Good Clinical Practice as well as according to local laws and regulations (transplant human organ act, India). We also abide by the Declaration of Helsinki and Declaration of Istanbul principles. The average monthly family income of the 6 ESRD patients was approximately 100 USD. The cost of kidney transplant in our transplant center is approximately 5000 USD. Two patients received 100% economic support and four patients received 50% economic support from the Government funds for the transplantation. Transplants were done irrespective of the recipient's financial status. All the kidney donors were family members (spouse or parents) and there did not receive any compensation for kidney donation.

RESULTS

Patient demographics

Figure 1 shows variables of the 6-way kidney exchange transplantation. Table 1 shows the demographics of 6 ESRD patients. All were male recipient, 5 donors were females and one donor was male. This gender imbalance in recipient and donors is common in our transplant scenario and not limited to KPD. Reasons for enrollment of donor recipient pair (DRP) in this program included ABO incompatibility ($n = 1$); positive cross-match and/or DSA ($n = 5$). The average time required from registration in our single center KPD registry to find KPD donor was 45 d and time required to perform actual KT after legal permission was 2 mo. Table 2 shows our desensitization protocol and their outcome. Initially, 3 patients who were sensitized were not willing to participate in KPD so we

attempted desensitization protocol. Unfortunately, they did not respond to desensitization protocol and were willing to participate in KPD. Two patients joined KPD without undergoing desensitization therapy as due to the high cost. We initially planned simultaneous KT surgery of 6 pairs which was postponed twice; due to respiratory infection in patient no 1, 3 and on another occasion due to respiratory infection in patient no 2, 6. Later with written informed consent of the 6 pairs, we performed first non-simultaneous KT on 4 different days as shown in Figure 1.

Donor demographics

Table 3 showed HLA data of 6 DRP. Table 4 showed the demographic data of 6 donors. All donors underwent uneventful left laparoscopic nephrectomy.

Outcome data

Table 5 showed the surgical details and outcome. Mean anastomosis time was 27 min, 3 patients underwent robotic KT and 3 underwent open KT. Graft and patient survival was 100% and 100%, respectively, with mean serum creatinine of 0.8 mg/dL at 9 mo follow-up. The allograft biopsy-proven acute borderline T cell rejection (Banff update 2013, type 3) was noted in a single patient who received lower dose of thymoglobulin due to pre-transplant pulmonary infection. We routinely did not perform protocol allograft biopsy. However, rejection episode responded well to methyl prednisolone (500 mg \times 3 doses) with stable graft function at 9 mo follow-up. Two of the 3 patients who underwent pre-transplant desensitization developed more infections [tuberculosis ($n = 2$), urinary tract infection ($n = 2$)] and new onset diabetes after transplantation vs patients who did not undergo pre-transplant desensitization.

DISCUSSION

As of August 2015, all the KPD transplants (103 two-way and 9 three-way exchanges in 233 patients) in our center have been performed simultaneously to eliminate the risk of renege by the intended donor of the recipient who had already received a kidney from a matched donor. We have performed 27 patients in 3 way simultaneous KPD transplants as DRP were not willing for the non-simultaneous KT and this will also avoid the risk of donor renege. However, from our previous experience of 10 KPD transplants (5 pairs of 2-way exchanges) in our single center in single day (on world kidney day 2013); our surgical team proposed that simultaneous KT of more than five-way exchange in KPD transplants is very difficult to perform logistically in our single center^[6]. In addition, patients developed infections leading to break in simultaneous KT twice.

Single center non-simultaneous KPD or multicenter simultaneous in Indian scenario

It is difficult to do simultaneous living donor kidney transplantation (LDKT) beyond the 2-way exchange

Table 1 Demographic of patients

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6
Age (yr)	17	38	45	40	25	45
Gender	Male	Male	Male	Male	Male	Male
Original disease	CGN	HT	HT	ADPKD	HT	DM
ABO group	A	B	AB	AB	B	A
Dialysis	12 mo	8 mo	7 mo	18 mo	7 mo	3 mo
Weight (kg)	40	44	59	68	43	55
Original donor relation	Father	Wife	Wife	Wife	Mother	Wife
Reason for joining KPD						
Sensitized	Yes	Yes	Yes	Yes	Yes	No
ABO incompatible						Yes
Time from KPD registration to find donor	2 wk	1 d	2 wk	6 mo	4 wk	1 mo
Time from donor to KT	58 d	60 d	55 d	75 d	48 d	60 d
With original donor						
LCM (%)	80/80/90	5/5/7	5/0/5	80/80/90	12/7/25	
FCM (MCS)	260/298	Neg/236	80/159	150/250	N/N	
DSA class 1 (MFI)	A1 = 8000 B57 = 6200	No DSA	B44 = 4188 A31 = 1100	A11 = 10000 B57 = 12645 BW4 = 12645	BW4 = 5000	
DSA class 2 (MFI)	DQ7 = 1600, DQ9 = 2200	DQ2 = 9000	No DSA	No DSA	DR13 = 7000	
With cross donor						
LCM (%)	Neg/neg/neg	15/15/20	Neg/neg	Neg/Neg/Neg	5/12/2010	Neg/Neg/Neg
FCM (MCS)	Neg/neg	Neg/200	Neg/neg	Neg/Neg	Neg/Neg	Neg/Neg
DSA class 1	No DSA	No DSA	No DSA	No DSA	No DSA	No DSA
DSA class 2	No DSA	No DSA	No DSA	No DSA	No DSA	No DSA
Desensitization	Yes	Yes	Yes	No	No	No
State	Gujarat	Rajasthan	Gujarat	Gujarat	Rajasthan	Rajasthan

CGN: Chronic glomerulonephritis; ADPKD: Autosomal dominant polycystic kidney disease; Neg: Negative; LCM: Lymphocyte cross match; FCM: Flow cross match; DSA: Donor specific antibody; MCS: Median channel shift; MFI: Mean fluorescent intensity; HT: Hypertension; DM: Diabetes mellitus; KT: Kidney transplant; KPD: Kidney paired donation.

Table 2 Desensitisation protocol and outcome

	Patient 1	Patient 2	Patient 3
Before desensitization with original donor			
LCM/DTT/AHG (%)	80/80/90	5/5/7	5/0/5
T/B FCM (MCS)	260/298	Neg/236	80/159
DSA class 1	A1 = 8000, B57 = 6200	No DSA	B44 = 4188, A31 1100
DSA class 2	DQ7 = 1600, DQ9 = 2200	DQ2 = 9000	No DSA
DP	8TPE + 8Bort/MP/IVIG + TAC/MMF	9TPE + 6Bort/MP/IVIG + R + TAC/MMF	4TPE + 4Bort/MP/IVIG + TAC/MMF
After desensitization with original donor			
LCM	80/80/90	17/15/20	5/0/7
FCM	254/333	Neg/504	Neg/129
DSA class 1	A1 = 2000, B57 = 5500	No DSA	B44 = 4622, A31 = 1400
DSA class 2	DQ7 = 2000, DQ9 = 2000	DQ2 = 12000	No DSA
Side effects	Tuberculosis	Graft nephrectomy	Tuberculosis

TPE: Plasmapheresis; Bort: Bortezomib; MP: Methyl prednisolone; IVIG: Immunoglobulin; R: Rituximab; TAC: Tacrolimus; MMF: Mycophenolate mofetyl; DP: Desensitization protocol; LCM: Lymphocyte cross match; FCM: Flow cross match; DSA: Donor specific antibody; Neg: Negative; MCS: Median channel shift; DTT: Dithiothreitol; AHG: Antihuman globulin test.

in the Indian setting due to shortage of surgical staff. Single center non-simultaneous KPD or multicenter simultaneous KPD are the two options to overcome this problem. The later way may involve donor travel or shipping of kidney. When cold ischemia time (CIT) is < 8 h, it has no impact on long term graft survival^[8,9]. Therefore, transport of living donor kidney may be a feasible alternative to donor travel in multicenter simultaneous KPD program where CIT < 8 h^[8,9]. Australian program preferred to ship donor kidneys rather than the donor despite prolonged CIT for interstate exchanges^[10].

In India, there is only one report of multicenter simultaneous KPD of 5 DRP^[11]. Therefore, single center non-simultaneous KT is the alternative way to overcome this problem. The practical solution to perform long chain KPD transplant is non-simultaneous KT after written informed consent of pairs and proper selection of patients and donors. There are two possible problems in doing non-simultaneous KT; donor renegeing and if recipient develops a medical issue, which could jeopardize the chain. We have done proper counseling for the pairs to avoid donor renegeing and one can have the option

Table 3 Human leukocyte antigen data of patients and donors

	A		B		Bw		Cw		DR B1		DR B3,4,5		DQ B1	
P1	2	29	44	-	4	-	7	15	4	15	51	53	4	6
D1	1	29	44	57	4	-	6	15	7	15	51	53	6	9
P2	2	33	40	44	4	6	7	15	10	15	51	-	5	6
D2	33	-	8	58	4	6	3	7	17	-	52	-	2	-
P3	2	11	35	52	4	6	12	-	4	15	51	53	6	8
D3	31	33	44	52	4	-	7	12	4	15	51	53	6	8
P4	24	32	18	35	6	-	4	7	11	15	51	52	6	7
D4	11	32	8	57	4	6	6	7	13	15	51	52	6	-
P5	24	33	8	35	6	-	4	7	17	-	52	-	2	-
D5	24	32	8	49	4	6	7	-	13	17	52	-	2	6
P6	11	24	13	56	4	6	3	4	15	17	51	52	2	5
D6	1	68	75	70	6	0	7	8	10	11	52	0	5	7

Table 4 Demographic of donor data

	Donor 1	Donor 2	Donor 3	Donor 4	Donor 5	Donor 6
Age (yr)	46	38	40	38	45	35
Gender	Male	Female	Female	Female	Female	Female
Weight (kg)	44	50	50	50	60	82
ABO group	A1	B	A	B	B	B
GFR (R/L)	54/61	50/50	47/55	55/55	56/50	52/49
Creatinine (mg/dL)	0.8	0.7	0.5	0.6	0.6	0.8
Renal vessel (R/L)	2A2V/1A1V	1A1V/1A1V	1A1V/1A1V	1A1V/2A1V	1A2V/1A1V	1A1V/1A1V
LDN side	L	L	L	L	L	L

GFR: Glomerular filtration rate; LDN: Laparoscopic donor nephrectomy; R: Right; L: Left; A: Artery; V: Vein.

Table 5 Surgical details and outcome

Patient	1	2	3	4	5	6
WIT (s)	113	120	137	97	136	85
CIT (min)	50	150	85	136	73	60
AT (min)	31	27	24	40	25	14
UO (mL)	1100	1200	600	700	1000	950
Robotic KT	Yes	Yes	Yes			
Open KT				Yes	Yes	Yes
LDN	Yes	Yes	Yes	Yes	Yes	Yes
KT date (2015)	17-Aug	21-Aug	17-Aug	18-Aug	18-Aug	7-Sep
Thymoglobulin (mg)	No	50	25	75	75	75
Creatinine (mg/dL)	0.7	0.7	1	0.7	1	1.1

WIT: Warm ischemia time; CIT: Cold ischemia time; AT: Anastomosis time; UO: Intra-operative urine output; LDN: Laparoscopic donor nephrectomy; KT: Kidney transplant.

of standard criteria young deceased donor KT without waiting time in case of donor renege. If donor of a patient donated kidney and that patient becomes untransplantable due to any reason (infection or heart disease) or patient expires, this would be quite unfair and may lead to legal and ethical problems. The morbidity and mortality on maintenance hemodialysis is high in the Indian scenario, and therefore, all the patients were kept in-house before KT.

Simultaneous vs non-simultaneous donor nephrectomy requirements

Simultaneous transplant surgery is an accepted standard practice in KPD and should be encouraged. Performing non-simultaneous surgeries in a closed loop KPD has

the risk of one donor renege and a recipient missing out on a kidney transplant, when their original donor has already donated a kidney. Non-simultaneous paired exchange transplants should be cautiously performed in carefully selected donors/recipient pairs with "due diligence" after legal permission from institutional ethical review board and written informed consent from the donors/recipients and proper counseling about risk and benefits is mandatory.

While non-simultaneous KPD chain transplantation is routine in North America and Europe, but uncommon in India. Apex Swap Transplant registry (ASTRA), Mumbai performed Indian first multicenter simultaneous KPD chain transplantation surgery of 5 DRP on the 25th of June 2013 in three hospitals in Mumbai with team work after

legal permission from the Maharashtra government^[11]. The waiting time between patient enrollment and transplantation was 2 years due to long time required in taking the legal permission and the second attempt^[11]. The first attempt resulted in failure and collapse of the chain due to the death of a patient. In the second attempt, they required 8 mo to rebuild a new chain (only one DRP was common). If the DPD transplant had not been carried out, some of them would have had to wait indefinitely and the others, for a long time. ASTRA has reported 30 two-way exchanges and 3 domino chain KT. This successful domino chain KT opens a new avenue to many more such dominos across the country giving an opportunity of getting a well-matched kidney to the patients who would otherwise land up on dialysis.

Advantages of single-center KPD program

Matching at the single-center KPD program would eliminate need of donor travel, donor separation from patient, close family members and familiar transplant team, increased cold ischemia time associated with transport of kidneys, the need for harmony and cooperation between different transplant centers, standardization of protocols between center for medical selection of donor and patients, privacy and legal concerns.

Advantages of KPD chain /DPD

Domino paired donation would facilitate more transplants by contributing to KPD programs than 2-way KPD. The advantages are expand blood type distribution of donors, increase number of transplants, better quality of transplants (HLA match, age of donor/recipient, waiting time, hard to transplant patients), and relaxes the reciprocity requirement of KPD. DPD would increase the number of transplants by about 20%, depending on the size of the pool^[12]. DPD is ideal in balancing the principles of utility and justice^[12,13]. A non-directed donor into non-simultaneous extended chain is not superior to DPD when segment lengths are limited to three^[14].

Even in KPD programs in which mathematical optimization are applied, more than 50% of the incompatible pairs in the pool remain unmatched. In many cases, pools of incompatible donor-recipient pairs have a high proportion of patients with blood types that are hard to match and those with HLA sensitization^[15].

In one South Korean center, 179 LDKT were performed, with 70 domino chains initiated by an altruistic living non-directed donor. One-year and 5-year patient and graft survival rates were 97.2% and 90.8%, and 98.3% and 87.7%, respectively, with a median follow-up of 46 mo. Multicenter domino KPD increases access to LDKT, with patients and graft survival rates similar to conventional KPD^[16].

Changing rules, saving lives - evolution of transplantation of human organs act and KPD

Long time is required to take legal permission from different state governments when 2 pairs are from different state. This is the biggest hurdle for multicenter KPD

expansion in India. Transplantation of Human Organs Act (THOA) and rules in India were amended in 2011 to promote organ transplantation, including KPD and KPD from near relatives is legal in India^[17]. According to THOA 2013, cases of swap donation referred to under subsection 3A of section 9 of the act shall be approved by authorization committee of hospital or district or state in which transplantation is proposed to be done and donation of organs shall be permissible only from near relatives of the swap recipients. This is new hope to reduce waiting time required to take legal permission from different state authorization committee. It will also increase the participation of compatible pairs to increase transplant quality like better HLA matching in spousal donors and young donor. In our study we have taken permission of 2 different state governments before KT.

Desensitization protocols vs KPD

The published literature on desensitization and outcome are from single-center studies with small sample size, short term follow-up and variable outcome^[18-20]. Multi-centric, prospective, randomized and controlled studies are needed for the evaluation of the efficacy and safety of desensitization protocols. Patients who underwent KT after desensitization therapy are often complicated by high rates of early acute humoral rejection and late antibody mediated injury due to low titers of DSA. Despite acceptable short-term patient and graft survivals, acute rejection rate and acute antibody-mediated rejection rate (36%, 28%, respectively), were significantly higher than in non-sensitized patients.

In our domino chain, 3 patients did not responded to desensitization protocols and 2 developed tuberculosis. Patients who are difficult in desensitization protocols (high PRA, high-strength DSA) and difficult in KPD [non-O donor (like AB), O recipient] can be considered for the combination of desensitization and KPD to increase match rate^[20]. CDC T and B cell cross-match-positive patients should not be considered for desensitization if they have more than three DSA and one DSA with MFI values > 5000.

In conclusion, we report first successful non-simultaneous single center 6-way kidney exchange transplantation from India. This procedure has the potential to expand the living donor pool and increases transplant opportunity for the sensitized patients.

COMMENTS

Background

Kidney paired donation (KPD) is routinely practiced in the Western countries, but is now being slowly introduced in India. The authors avoid desensitization protocols and ABO incompatible kidney transplantation (KT) due to high costs and increased risk of infections from intense immunosuppression.

Research frontiers

It is difficult to do simultaneous living donor KT beyond the 2-way exchange in the Indian setting due to shortage of surgical staff. Single center non-simultaneous KPD or multicenter simultaneous KPD are the two options to overcome this problem. This successful non-simultaneous single center 6-way

kidney exchange transplantation from India opens a new avenue to many more such KPD chain across the country giving an opportunity of getting a well-matched kidney to the patients who would otherwise land up on dialysis.

Innovations and breakthroughs

While non-simultaneous KPD chain transplantation is routine in North America and Europe, but uncommon in India.

Applications

Simultaneous transplant surgery is an accepted standard practice in kidney paired donation and should be encouraged. Non-simultaneous paired exchange transplants should be cautiously performed in carefully selected donors/recipient pairs. Counseling to understand the risks and benefits of this procedure is mandatory. Their experience will encourage other centers in India to undertake this practice.

Terminology

They report first non-simultaneous single center 6-way kidney exchange transplantation from India which has the potential to expand the living donor pool and increases kidney transplant opportunity for immunologically sensitized patients.

Peer-review

In general, the draft is interesting and had been good written.

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Urolithiasis in inflammatory bowel disease and bariatric surgery

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Abstract

AIM

To analyse current literature focusing on pathogenesis and therapeutic aspects of urolithiasis with inflammatory bowel disease (IBD) and following bariatric surgery.

METHODS

A systematic literature search was performed using PubMed, supplemented with additional references. Studies assessing the association of IBD or bariatric surgery with renal stones in both paediatric and adulthood were included.

RESULTS

Certain types of stones are seen more frequently with IBD. Hyperoxaluria and hypocitraturia are the main metabolic changes responsible for urolithiasis. The incidence of renal stones in malabsorptive types of bariatric surgery such as gastric bypass is high; this is not as common in modern restrictive surgical methods. Preventative methods and urine alkalinisation have been shown to be beneficial.

CONCLUSION

Both conditions are associated with renal stones. Patients' counselling and prevention strategies are the mainstay of urolithiasis management in these patients.

Key words: Kidney stones; Inflammatory bowel disease; Bariatric surgery; Crohn's; Ulcerative colitis; Urolithiasis

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Core tip: Urolithiasis continues to be a complication associated with inflammatory bowel disease and post bariatric surgery. Lowered urinary levels of anti-lithogenic substances (magnesium and citrate) have been suggested to be important in calculi development. Prevention is best achieved through dietary changes and targeted medical therapy.

Gkentzis A, Kimuli M, Cartledge J, Traxer O, Biyani CS. Urolithiasis in inflammatory bowel disease and bariatric surgery. *World J Nephrol* 2016; 5(6): 538-546 Available from: URL: <http://www.wjgnet.com/2220-6124/full/v5/i6/538.htm> DOI: <http://dx.doi.org/10.5527/wjn.v5.i6.538>

INTRODUCTION

Inflammatory bowel disease (IBD) is a condition which affects patients of all age groups and ethnicities. The two main entities are Crohn's disease (CD) and ulcerative colitis (UC). It is estimated that the incidence of each of these diseases is approximately 3-14/100000 patients/year respectively in the United States^[1]. The association of IBD with complications in the urinary tract has been reported in studies almost 40 years ago, with urolithiasis being a common manifestation^[2].

In recent years, the use of bariatric surgery for treatment of morbid obesity and its medical and functional consequences is rapidly rising up in the world's medical organisations agenda, as a consequence of social and lifestyle choices. In the United Kingdom almost 18000 operations have been done for this purpose in 2011-2013^[3] whereas in the United States, the incidence of bariatric surgery is estimated at 113000 cases per year^[4]. Although the metabolic syndrome (and obesity) is a known risk factor for urolithiasis^[5], those patients subjected to bariatric surgery appear to have an even higher risk of developing renal stone disease^[6].

As the exact association of urolithiasis with the above clinical entities has not been fully established, we sought to review the published data on this topic.

MATERIALS AND METHODS

Search strategy

Systematic search of the literature in PubMed using the combination of the words: "renal stones" or "kidney stones" or "urolithiasis" or "nephrolithiasis" and "inflammatory bowel disease" or "Crohn's" or "ulcerative colitis" or "short bowel" or "bariatric surgery".

Search was limited to English-language papers. Further references were identified from the reference list of retrieved articles.

Inclusion/exclusion criteria

Studies assessing the association of IBD or bariatric surgery with renal stones in both paediatric and adulthood were included. Case reports or studies including small number of patients (< 5) were excluded. Figure 1 summarises the search process. Out of the 62 studies assessed for eligibility, 10 were excluded: 3 were case reports, 5 included small number of patients, 1 referred to ankylosing spondylitis only rather than IBD and 1 referred to other urinary complications of IBD rather than renal stones.

RESULTS

The retrieved published studies were mainly one-arm, uncontrolled and observational. We separated the studies into those referring to IBD or bariatric surgery respectively.

IBD and renal stones

Incidence and clinical evaluation: No large epidemiological studies assessing the prevalence of IBD and urolithiasis co-existence were identified.

Small and historical studies initially reported this association along with other urogenital IBD complications (such as fistulation, intrinsic renal disease and obstructive uropathy)^[2,7]. From smaller studies it has been estimated that 9%-18% of adult IBD patients may be diagnosed with renal stones at some time in their life^[8,9]. In cases when the patients with IBD (Crohn's) had resection of the terminal ileum, the percentage of associated urolithiasis is much higher (reported up to 28%)^[9]; this is attributed to metabolic disturbances due to steatorrhoea and bile salt malabsorption. Interestingly, in patients with short bowel syndrome post several resections for complicated Crohn's, preservation of colon has been associated with higher incidence of renal stones compared with those who had a completion colectomy (24% vs 0% in a study of 52 patients)^[10]. In a study of UC patients who had a panproctocolectomy, the risk of renal stone disease was compared between conventional ileostomy and ileal J-pouch; the risks of forming uric acid stones were high for both ileostomy and J-pouch patients, but J-pouch reduced the risk of renal stones containing calcium (the relative probability of calcium stone formation was 0.58 in ileostomy group vs 0.18 in the J pouch group)^[11]. Another study on patients with ileal J-pouch post panproctocolectomy for UC showed that the presence of several extra-intestinal manifestations, no use of antibiotics and low serum bicarbonate level were the most important risk factors for the presence of concurrent urolithiasis; the overall incidence of the latter was 37% in this group^[12].

A study from Japan assessed the risk factors for developing urolithiasis in patients with CD. Renal stones were more frequent in men. The mean time from Crohn's diagnosis to diagnosis of calculi was 8.8 years (range 0 to 22 years). The commonest type of stone was calcium oxalate. The probability of developing calculi was approximately eight times higher for patients with a urine pH of ≤ 6.0 than for those with a urine pH of ≥ 6.5 ^[13].

Renal stone disease appears to be less common in the paediatric population with IBD. A report from a national IBD registry in Italy estimated its incidence to be at 0.37%^[14].

In a study when computerised tomography was used to diagnose asymptomatic extra-intestinal manifestations of CD, 4% of patients were found to have concomitant urolithiasis^[15].

With regards to patients' awareness, only 12% of those included in a cross-sectional survey in Canada

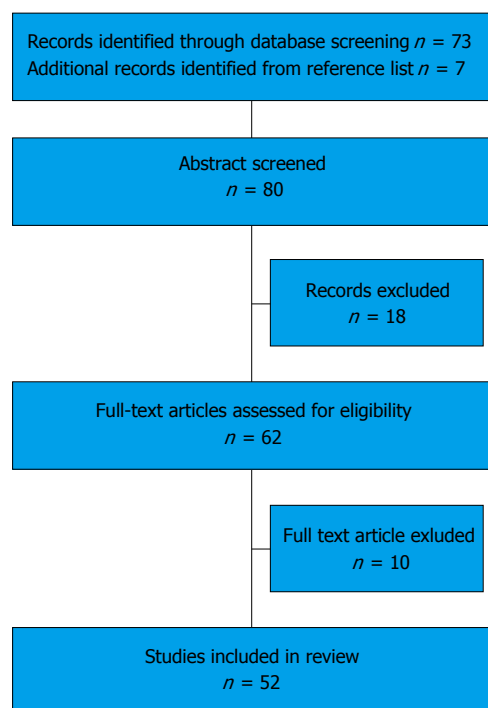


Figure 1 Study selection flowchart.

reported that they had been aware of the IBD-nephrolithiasis association. They had obtained their information from their gastroenterologist or the internet and they had much better knowledge on other potential extra-intestinal IBD manifestations such as risk of colon cancer (75%), arthritis (77%) and dermatological manifestations (49%)^[16].

An interesting observation on patients with IBD and urolithiasis was made in a large study (14352 patients) from emergency departments in the United States. IBD patients with urolithiasis presented with infections, sepsis and renal failure more frequently than non-IBD patients [infections (10.4% vs 9.1%; $P < 0.001$), sepsis (0.6% vs 0.2%; $P < 0.001$), and end-organ failure (6.3% vs 1.6%; $P < 0.001$) respectively]. This was due to the increased occurrence and severity of infected urolithiasis in this group as well as the fact that the urolithiasis was noted to occur more commonly in older patients (with IBD) compared with non-IBD controls^[17]. In another study it was noted that recurrent urolithiasis and surgical interventions for its treatment, along with bowel resections are the two independent risk factors for the development of chronic kidney disease in IBD patients; this is generally a rare phenomenon particularly in UC^[18].

Pathogenesis: CD and UC are characterized by recurrent inflammatory involvement of different intestinal segments resulting in malabsorption of bile salts and fatty acids^[19]. This causes increased oxalate absorption by increasing oxalate solubility in the intestinal lumen and permeability of the colonic mucosa^[20]; this secondary hyperoxaluria is associated with oxalate renal stones^[9,21]. In addition to that, the loss of bicarbonate in

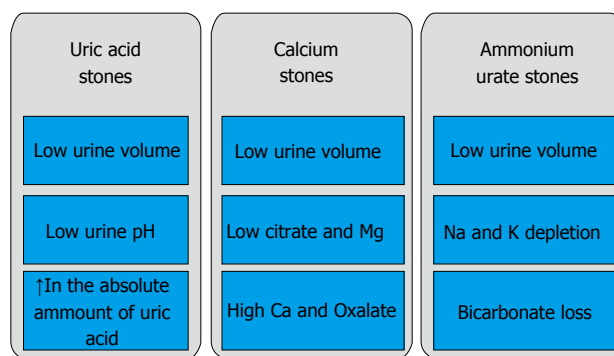


Figure 2 Risk factors for stones in patients with bowel disease.

the liquid stool and the dehydration cause acidosis and subsequent reduced citrate excretion. This can lead to uric acid or mixed stones^[21]. Factors such as decreased urinary excretion of other inhibitors of crystallisation (magnesium), dietary parameters and medications (such as aminosaliclates)^[22], can also play a role in stone formation in these patients^[23] (Figure 2).

In a study including both paediatric and adult patients with CD, the patients' 24 h urine oxalate levels and intestinal oxalate absorption were assessed vs the relevant parameters in healthy individuals. The intestinal absorption of oxalate was significantly higher in CD patients; (0.92 ± 0.57 mmol/1.73 m² per 24 h) compared with those without the disease (0.53 ± 0.13 mmol/1.73 m² per 24 h) respectively ($P < 0.05$); this also correlated with hyperoxaluria and risk of developing urolithiasis^[24].

Kumar *et al*^[25] examined the association of intestinal oxalate degrading bacteria *Oxalobacter formigenes* with the development of hyperoxaluria in IBD. The investigators studied stool samples of IBD patients and controls respectively for the presence of *O. formigenes* using polymerase chain reaction. Only 10.4% of patients with IBD compared to 56% of controls had positive for *O. formigenes* stool samples. Patients without *O. formigenes* had higher urinary oxalate than those with it. They were also more likely to develop renal stones. In this study there was no significant difference in the excretion of citrate. However, in those patients who had hypermagnesiuria (another stone inhibitor) the formation of stones was not common, irrespective of the IBD status. A recent study suggested that oxalate is not only absorbed by the gastrointestinal (GI) tract in different concentrations depending on its permeability, but can also be secreted by the small intestine^[26]. This is an interesting observation which provides some evidence that perhaps diseased GI tract can influence the oxalate metabolism and result in hyperoxaluria and subsequent urolithiasis.

Contrary to the above, a study suggested that the formation of renal stones in IBD patients is more likely related to low urinary concentration of magnesium and citrate relative to calcium than the hyperoxaluria^[27]. However, only 40 patients with IBD and 17 controls were

included in the study with 2 IBD patients developing renal stones.

Evan *et al*^[28] analysed renal biopsies of IBD patients with urolithiasis. The histopathological abnormalities seen, included moderate glomerular sclerosis, tubular atrophy, and interstitial fibrosis. Randall's plaque was abundant, with calcium oxalate stone overgrowth; as the authors suggested this was similar to that seen in idiopathic calcium oxalate stone formers, and primary hyperparathyroidism. Abundant plaque was compatible with low urine volume and acidic pH.

Interestingly a recent study provided early evidence of genetic background as an independent risk factor for urolithiasis in IBD patients alongside known mechanisms such as malabsorption and medication^[29].

Another risk factor for urolithiasis in IBD patients with intestine failure (short bowel) is the use of vitamin C in parenteral nutrition. This has been shown to increase urinary oxalate excretion and subsequently increase the risk of urinary stone formation^[30].

Ileostomy: The formation of ileostomy post bowel resection is an independent risk factor for the formation of renal stones^[13]. The relative dehydration from liquid stool, and the acidic PH from bicarbonate loss are responsible for this. In this acidic environment, the urinary citrate level excretion reduces. The stones most commonly seen in these patients contain uric acid or are of mixed composition^[21]. In addition, the risk of calcium containing stones also increases with ileostomy. In a retrospective study comparing IBD patients with ileostomy vs IBD patients with J-pouch vs controls, the relative risk of calcium containing stones formation was significantly higher (0.58 vs 0.18) in those with ileostomy^[11].

Treatment: In our review, no study directly assessing the *in vivo* effect of a treatment to reduce urolithiasis in IBD patients was identified.

In a study using computer modelling, simulated urine situations based on reported composition values of IBD patients were compared with the relevant ones from normal individuals. In this model, supplementation of calcium would reduce the urinary calcium oxalate supersaturation in IBD patients as their hyperoxaluria is much more prominent compared with non-IBD stone formers. The authors suggested that calcium supplements can help reduce stone risk in those patients, but initial efforts should be directed towards reducing urinary oxalate by reducing dietary oxalate. Citrate therapy that increases both urine pH and urinary citrate could also provide an additional therapeutic benefit^[31].

Several authors have advocated preventative strategies. These include close monitoring of IBD patients with urinalysis and imaging of the upper tracts at regular intervals^[13] as well as early surgical intervention even in cases of small renal stone burden^[17]. Also the avoidance of low urinary citrate and magnesium levels could prevent

the stone formation in these patients^[25,27,32]. In UC patients who had panproctocolectomy and formation of ileoanal pouch, close monitoring for renal stone formation and administration of prophylactic oral bicarbonate has been suggested^[12]. Urinary alkalisation along with increased hydration is also advocated in IBD patients receiving aminosalicylates^[22].

Bariatric surgery and renal stones

Incidence: Bariatric operations have traditionally been divided into three groups: (1) restrictive, *i.e.*, procedures that produce weight loss solely by limiting intake (gastric banding, gastric sleeve); (2) malabsorptive, *i.e.*, operations that induce weight loss totally by interference with digestion and absorption (intestinal bypass); and (3) mixed, *i.e.*, procedures that limit intake and produce malabsorption (gastric bypass, duodenal switch)^[33].

The most commonly performed bariatric operation is Roux-en-Y gastric bypass (RYGB)^[3]. It belongs to the "mixed group" in the bariatric operations classification. Nelson *et al*^[34] first described the association of gastric bypass surgery with the formation of calcium oxalate renal stones. Matlaga *et al*^[35] reviewed the insurance claims and records of 4639 patients who had RYGB for obesity and compared them with the relevant ones from other 4639 obese patients over a four-year period. Urolithiasis was diagnosed in 7.65% (355 of 4639) of RYGB patients compared to 4.63% (215 of 4639) of patients in the control group ($P < 0.0001$). The patients in the treatment group more commonly underwent shock wave lithotripsy and ureteroscopy, making the RYGB surgery a significant predictor of diagnosis and requirement for treatment of urolithiasis in the post-operative period. In a retrospective study of 972 patients who underwent RYGB, 3.2% developed *de-novo* urolithiasis post-operatively (mean FU was 2.8 years); in those individuals who had renal stones pre-operatively (8.8%), the recurrence of the disease was even more common (32%)^[36]. The authors concluded that although the RYGB had a significant influence on the development of stones, the combination of pre-operative stone history with this type of bariatric surgery is the most important risk factor for recurrent urolithiasis. Other reviewers have come to the same conclusions^[6]. In a large, prospective study of 151 patients undergoing laparoscopic RYGB, Valezi *et al*^[37] reported *de novo* stone incidence of 8% at 12 mo post-surgery. The relative risk for development of stones increased by approximately 70%^[37]. In another study of patients who had RYGB for gastric cancer, the investigators assessed the incidence of renal stones post-operatively. Computed tomography scans from gastric cancer patients who had either distal gastrectomy with Billroth I anastomosis/RYGB vs total gastrectomy with RYGB reconstruction were reviewed. Patients with total gastrectomy were more likely to have renal stones (25%) than patients with some remaining stomach (7%). The authors hypothesised that total gastrectomy may lead to more fat malabsorption than partial gastrectomy,

perhaps exacerbating hyperoxaluria^[38]. Although these procedures were not directly done for the purpose of weight loss, the study adds some evidence that the malabsorptive procedures are associated with increased incidence of urolithiasis; in particular, in this study the extent of resection, and not just the bypass itself, was an independent factor for the development of renal stones.

A relative historical bariatric operation, jejunio-ileal bypass, has also been implicated with significant risk of post-operative urolithiasis. In a retrospective study of 56 patients who had undergone this surgery because of morbid obesity, twenty-two (39.3%) were found to have renal calculi in a 16-year mean follow-up^[39]. Interestingly, patients who had surgery for reversal of this bypass continued to have high incidence of renal stones despite the decrease in their 24-h urinary oxalate levels^[40]. The authors of this study concluded that the persistence of low citrate in the urine is implicated on this phenomenon.

Sleeve gastrectomy and gastric banding are the most commonly done bariatric operations of the restrictive type. Patients who undergo these procedures generally appear to have less likelihood of developing renal stones post-operatively. Chen *et al*^[41] reported an approximate stone incidence rate of 1% in a retrospective review of 332 cases of gastric banding and 85 cases of sleeve gastrectomy respectively; they estimated a person-time incidence rate of 3.40 stone diagnoses per 1000 person-years for gastric banding and 5.25 stone diagnoses per 1000 person-years for sleeve gastrectomy respectively^[41]. Semins *et al*^[42] reported no association of gastric banding surgery with increased risk of urolithiasis or renal stone intervention post-operatively. In their case-control study, the authors retrospectively reviewed the insurance claims of 201 morbidly obese patients who had gastric banding surgery and 201 morbidly obese controls through a national database. The incidence of new renal stones was 1.5% in the operated individuals and 6% in the controls; this was the first study which provided evidence that the outcome of restrictive bariatric surgery on the development of urolithiasis is different compared to malabsorptive or mixed procedures.

Pathophysiology: Bariatric surgery has been associated with several metabolic and urinary changes first described in the 1980s^[43]. The most common one is the development of hyperoxaluria, first seen post jejunio-ileal bypass surgery.

In a prospective study of 21 patients undergoing RYGB, 24-h urine specimens were analysed pre- and post-operatively^[44]; urinary oxalate excretion increased significantly after RYGB (33 ± 9 mg/d pre-operatively vs 63 ± 29 mg/d post-operatively). *De novo* hyperoxaluria developed in 11 (52%) patients. There was also significant increase of patients with hypocitraturia (from 10% at baseline to 48%) at 2 years. Wu *et al*^[45] performed a similar study; 38 patients undergoing RYGB had serum and urine chemistry assessed preoperatively and 6 mo post-operatively. Urinary changes known to increase the risk of urolithiasis were found: Decrease in

total urine volume (from 2 L/d to 1.5 L/d respectively), increase in calcium (from 139 mg/d to 182 mg/d respectively), and increase in oxalate (from 38 mg/d to 48 mg/d respectively); all these changes were statistically significant. Their conclusion was that this study provided evidence that renal stones post RYGB is caused by many biochemical factors, with hyperoxaluria being the main one^[45].

Similar conclusions were drawn by Patel *et al*^[46] in their case-control study of 58 patients who underwent RYGB (52) or duodenal switch (6) procedures. In this study 67.2% of patients who had bariatric surgery developed hyperoxaluria (in their 24-h urine specimens 6 mo post-operatively) compared to almost 34.1% of non-stone formers (controls) and 37% of stone formers with no history of bariatric surgery respectively. Hyperoxaluria and hypocitraturia were also demonstrated in post RYGB patients in a cross-sectional study by Maalouf *et al*^[47]. They also pointed out that in their study the urinary calcium excretion decreased which may be counteracting the effects of the above significant risk factors. Asplin *et al*^[48] provided evidence that hyperoxaluria is a common metabolic finding in patients post RYGB with calcium oxalate being the main driving force for the development of kidney stones; the levels of urinary oxalate were not as high as they found in the jejunioileal bypass cohort^[48].

The hyperoxaluria in post bariatric surgery patients is believed to be caused by increased intestinal oxalate absorption due to the relative fat malabsorption, particularly in the bypass operations. As fat is malabsorbed, fat-soluble vitamins and calcium ions are saponified by intraluminal free fatty acids, leading to steatorrhea. The calcium is less available in the intestinal lumen, which subsequently decreases binding with oxalate. When oxalate is free, it gets absorbed by the intestine and then majority is excreted in the kidney. It may then precipitate with urinary calcium to form insoluble crystals and eventually kidney stones. In addition to the above, permeability of the intestine to oxalate is increased by exposure to unconjugated bile salts and long chain fatty acids, both of which are increased in the GI tract of RYGB patients^[49,50].

A study by Froeder *et al*^[51] used the oxalate load test as a direct assessment of intestinal oxalate absorption in patients post gastric bypass surgery. The mean oxaluric response to this load was markedly elevated in post-bariatric surgery patients, suggesting that increased intestinal absorption of dietary oxalate is a predisposing mechanism for enteric hyperoxaluria^[51].

In the restrictive group of bariatric operations (such as gastric banding and sleeve gastrectomy) the metabolic changes are not as common as they are in the malabsorption group. Studies comparing the metabolic profile of patients' groups who had one procedure from each group concluded that urinary oxalate excretion of the "restrictive" cohorts was significantly less than the "RYGB" cohorts and not significantly different from that of the normal subjects and routine stone-formers^[52-55]. This difference is likely related to the lower supersaturation

Table 1 Prevention and treatment recommendations in urolithiasis associated with inflammatory bowel disease

IBD and stones treatment	
All patients	Counselling about IBD association with renal stones Increased hydration Monitor with regular urinalysis and imaging of upper tracts Early surgical intervention even in small renal stone burden
Established hyperoxaluria or calcium oxalate stones	Reduction in dietary oxalate Calcium supplements
Uric acid stones	Urinary alkalinisation Citrate supplementation
Panproctocolectomy	Consideration for oral bicarbonate supplementation

IBD: Inflammatory bowel disease.

of calcium oxalate, predominantly due to higher urinary volume and lower urinary calcium excretion in restrictive procedures compared to RYGB^[56].

Treatment: Patients submitted to bariatric surgery are at risk of nephrolithiasis and nephropathy. Accurate stone screening, careful monitoring of renal function and diet counselling are strongly encouraged in these patients^[6,45]. Close monitoring with renal tract imaging/screening is advocated mainly for patients who undergo gastric bypass surgery and have a previous history of urolithiasis^[36].

Strategies to prevent stones after bariatric surgery are similar to those recommended to all stone formers. They include increased daily fluid intake to achieve urine volumes higher than 2 L/d and low oxalate (< 100 mg/d) intake. Additionally, low sodium and animal protein intake are indicated^[49]. Bariatric stone formers should particularly reduce daily fat intake to minimise enteric oxalate absorption and consider calcium supplementation^[56]. Citrate salts, like potassium citrate, can be used to correct metabolic acidosis and hypocitraturia^[35,44].

A phase II randomised study has assessed the use of potassium calcium citrate (PCC) in post RYGB patients^[57]. PCC significantly increased citrate, and pH. The urinary saturation of uric acid decreased significantly. Also calcium oxalate agglomeration was significantly inhibited by PCC. As it has not yet been tested in phase III trials it is not available for clinical use.

DISCUSSION

The prevalence of symptomatic nephrolithiasis is higher in patients with IBD and after bariatric surgery. It is prudent to understand the pathophysiology of this condition as well as the basics of prevention. IBD commonly results in impaired intestinal function and in a malabsorptive state. Similarly, bariatric surgery reforms the normal GI track with subsequent metabolic disturbances. Both these entities have been associated with the development of urolithiasis. The malabsorption state may result in increased absorption of oxalate or decreased absorption of citrate, magnesium, bicarbonate, water, potassium leading to increase in supersaturation state.

Urate stones form due to loss of intestinal fluid and bicarbonate which lead to concentrated acidic urine. This increases the precipitation of urate. In addition, inhibitors of crystallization (*e.g.*, citrate) are lost with diarrhoea. Management of uric acid stones includes treatment of diarrhoea, alkalinising the urine and increasing fluid intake. If stones recur allopurinol is of great help but it interacts with azathioprine.

Calcium oxalate stones are related to increased urinary oxalate excretion caused by increased intestinal absorption known as enteric hyperoxaluria. Enteric hyperoxaluria is firstly related to bile salt malabsorption in the diseased or resected distal ileum. Malabsorbed fats bind intraluminal calcium, decreasing the amount of calcium available for oxalate resulting in increased oxalate absorption. Increased colonic permeability to oxalate caused by the malabsorbed bile salts and fatty acids can also cause hyperoxaluria. Hyperoxaluria may be seen in patients on long-term parenteral nutrition and with minimal oral intake. Risk of calcium oxalate stones can be reduced by managing enteric hyperoxaluria, increasing oral fluid intake, a low fat and oxalate diet, calcium supplementation, oxalate synthesis can be reduced by cholestyramine and pyridoxine (B6). In case of recurrence, alkalinisation of urine, citrate and Mg supplementation can be used (Tables 1 and 2).

This review highlights the lack of good quality studies reported in the literature. Majority of studies are retrospective and with small numbers therefore a prospective database is required to capture all information and develop a better understanding of urolithiasis in this group of patients. We suggest a complete blood and urine metabolic work up according to European guidelines at baseline should be considered and 2 yearly thereafter along with routine tests to monitor IBD. This will allow us to gather evidence to recognise lithogenic factors. In addition, a renal USS at diagnosis and 2 yearly thereafter should be considered for early diagnosis. Furthermore, early diagnosis may help to establish a proper correlation between severity of IBD and urinary metabolic parameter.

IBD and bariatric surgery are closely associated with the development of urolithiasis. The mechanism of this relation is multifactorial but has mainly related to the

Table 2 Prevention and treatment recommendations in urolithiasis associated with bariatric surgery

Bariatric surgery and stones treatment	
All patients	Counselling about bariatric surgery association with renal stones
	Regular upper tract imaging (in gastric bypass)
	Increased hydration
	Focused dietary advice
	Low oxalate
	Low sodium
	Low animal protein
	Low fat
	Citrate supplementation if evidence of hypocitraturia

increased absorption of oxalate in the intestine which is usually increased in those conditions and also other metabolic disturbances found in these entities. The patients should be informed of this association and follow the appropriate preventative strategies to reduce the risk of renal stones.

COMMENTS

Background

The intestinal malabsorption from inflammatory bowel disease (IBD) and bariatric surgery commonly leads to urolithiasis. In this review, the known evidence in pathogenesis and prevention/treatment of this entity is analysed. The increased absorption of oxalate or reduced absorption of citrate, magnesium, bicarbonate, water and potassium result in supersaturation and formation of renal stones. Counselling about IBD/bariatric surgery association with renal stones, increased hydration, monitoring with regular urinalysis and imaging of upper tracts as well as early surgical intervention even in small renal stone burden are required to reduce the morbidity of urolithiasis in these patients. A summary of lithogenic risk factors and recommendations for prevention is included.

Research frontiers

Potential future studies could focus more on IBD/bariatric surgery association with urolithiasis epidemiology with better data capture and analysis. There is also a field to explore the most efficient investigations' protocol for diagnosis and monitoring in these patients' groups.

Innovations and breakthroughs

This review emphasizes the lack of good quality studies in the field of urolithiasis in patients with IBD or following bariatric surgery. It will assist the readers to understand its pathogenesis and also the ways to prevent it. It will also increase the awareness of this association in clinicians (such as General Practitioners, Physicians, Gastroenterologists and Gastrointestinal Surgeons) as well as the patients with IBD or following bariatric surgery.

Applications

The authors suggest a complete blood and urine metabolic work up (according to European guidelines) at baseline. Repeated tests should be considered 2 yearly thereafter along with routine tests to monitor IBD. In addition, a renal USS at diagnosis of IBD or following bariatric surgery and 2 yearly thereafter should also be considered for early diagnosis and appropriate monitoring respectively.

Terminology

Crohn's disease is a chronic or long lasting disease that causes inflammation in the gastrointestinal tract. Most commonly, Crohn's affects the small intestine and the beginning of the large intestine. Duodenal switch procedure involves a partial gastrectomy (reducing stomach along greater curvature) effectively restricting its capacity while maintaining its normal function. Gastric banding

is an adjustable gastric band, frequently called a lap-band (when done laparoscopically). A band is an inflatable silicone device placed around the top portion of the stomach to treat obesity, intended to slow utilisation of food and thus reduce the amount of food consumed. Gastric bypass surgery is a surgical procedure in which the stomach is divided into a small upper pouch and a much larger lower remnant pouch and then the small intestine is repositioned to connect to both. Jejunioileal bypass surgery is the most effective surgical intervention for achieving and maintaining weight loss. Typically, 35 centimeters of proximal jejunum is anastomosed, end-to-side or end-to-end, to the terminal 10 centimeters of ileum. Ileostomy a surgical operation in which a damaged part is removed from the ileum and the cut end diverted to an artificial opening in the abdominal wall. Ileal J pouch, the ileal pouch-anal anastomosis is a surgical procedure that is used to restore gastrointestinal continuity after surgical removal of the colon and rectum. A J pouch or an internal pouch, the procedure involves the creation of a pouch of small intestine to recreate the removed rectum. Panproctocolectomy is the removal of the entire colon, rectum and anal canal. This type of stoma is permanent. Roux-en-Y gastric bypass is a surgical procedure which may be done for severe obesity. The procedure involves cutting the stomach in two to create a pouch out of the smaller proximal portion of the stomach, attaching it to the small intestine, bypassing a large part of the stomach and all of the duodenum. Ulcerative colitis is a long-term inflammatory bowel disease. It causes swelling and ulcerations of the colon and rectum.

Peer-review

This is an excellent paper.

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Antineutrophil cytoplasmic antibodies crescentic allograft glomerulonephritis after sofosbuvir therapy

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Abstract

Antineutrophil cytoplasmic antibodies (ANCA) are well known to be associated with several types of vasculitis, including pauci-immune crescentic glomerulonephritis, a form of rapid progressive glomerular nephritis (RPGN). ANCA vasculitis has also been reported after administration of propylthiouracil, hydralazine, cocaine (adulterated with levamisole), allopurinol, penicillamine and few other drugs. All previously reported cases of drug-associated ANCA glomerulonephritis were in native kidneys. Sofosbuvir is a new and effective drug for hepatitis C virus infection. Here, we report a case of ANCA vasculitis and RPGN following sofosbuvir administration in a kidney transplant recipient. It also represents the first case of drug-associated ANCA vasculitis in a transplanted kidney. Further drug monitoring is necessary to elucidate the degree of association and possible causal effect of sofosbuvir and perinuclear ANCA vasculitis.

Key words: Crescentic glomerulonephritis; Vasculitis; Antineutrophil cytoplasmic antibody; Sofosbuvir; Kidney transplant

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Core tip: Antineutrophil cytoplasmic antibodies (ANCA) vasculitis is well known to be associated with several drugs, including propylthiouracil, hydralazine, cocaine, levamisole and others. All previous cases of drug-associated ANCA glomerulonephritis were in native kidneys. Here, we report a case of ANCA vasculitis following sofosbuvir administration in a kidney transplant recipient. It is also the first case of drug-associated ANCA vasculitis in a transplanted kidney. Further drug monitoring is necessary to elucidate the degree of association and possible causal effect of sofosbuvir and perinuclear ANCA vasculitis.

Gadde B, Lee B, Kidd L, Zhang R. Antineutrophil cytoplasmic

antibodies crescentic allograft glomerulonephritis after sofosbuvir therapy. *World J Nephrol* 2016; 5(6): 547-550 Available from: URL: <http://www.wjgnet.com/2220-6124/full/v5/i6/547.htm> DOI: <http://dx.doi.org/10.5527/wjn.v5.i6.547>

INTRODUCTION

Antibodies directed against neutrophil cytoplasmic antigens were first described in patients with pauci-immune glomerulonephritis in 1982^[1]. Antineutrophil cytoplasmic antibodies (ANCA) are associated with cases of small vessel vasculitis in granulomatosis with polyangiitis (Wegener's), microscopic polyangiitis, Churg-Strauss syndrome, "renal-limited" rapidly progressive glomerular nephritis (RPGN), and certain drug-induced vasculitis syndromes^[2]. In these conditions, ANCA consistently have specificities for either PR3 or MPO, but almost never for both. We present a case of crescentic allograft glomerular nephritis after sofosbuvir therapy.

Sofosbuvir is a non-structural protein 5B RNA-dependent RNA polymerase inhibitor indicated for the treatment of chronic hepatitis C virus (HCV) infection as part of a combination antiviral treatment regimen^[3]. Sofosbuvir's efficacy has been established in subjects with HCV genotype 1, 2, 3 or 4 infections, including those with hepatocellular carcinoma meeting Milan criteria (awaiting liver transplantation) and those with HCV and human immunodeficiency virus-1 co-infection^[3]. The most common adverse reactions reported in clinical trials are that of fatigue, headache, nausea, insomnia, and anemia. Less common adverse reactions include pancytopenia and severe depression^[3]. Here, we report the first case of ANCA vasculitis and RPGN following sofosbuvir administration in a kidney transplant recipient.

CASE REPORT

Our patient is a middle aged gentleman with end-stage renal disease. The cause of his kidney failure was thought to be secondary to long-standing hypertension. He also had chronic HCV infection (treatment naïve) due to remote history of unprotected sexual activities. He underwent a living unrelated donor kidney transplant through our paired exchange program in June 2013. The patient received one dose of alemtuzumab (30 mg) induction, and tacrolimus and mycophenolic acid maintenance without steroid. His serum creatinine (sCr) nadir was 1.4 mg/dL 1 wk after transplant. Twelve weeks post-transplant, sCr was elevated to 2.0 mg/dL. A kidney biopsy was performed. There was acute tubular necrosis and tubular isometric vacuolization. Tacrolimus renal toxicity was suspected, and it was replaced with cyclosporine. Subsequently, his sCr stabilized at 1.6 mg/dL.

The patient had HCV genotype 1a infection with a viral load of 937000 IU/mL prior to transplantation. Liver function tests were normal and liver biopsy revealed stage 0 fibrosis. Thirty-two weeks after kidney transplantation, elevated liver enzymes were noted:

AST 47 U/L (0-35 U/L) and ALT 63 U/L (0-55U/L). Liver biopsy revealed chronic hepatitis C changes and early focal bridging fibrosis. At week 40 after transplant, he was started with combination of ribavirin (400 mg/d) and sofosbuvir (400 mg/d). Between 6 to 10 wk of HCV treatment, he developed severe anemia that required blood transfusion for several times, despite of high dose of erythropoietin treatment. At 12 wk of HCV treatment, the sCr increased to 3.7 mg/dL. Urine analysis had 30 mg/dL of protein and 131 red blood cells/high powered field (RBCs/HPF), and spot urine protein-to-creatinine ratio was 0.6. An allograft biopsy showed the presence of thrombotic microangiopathy. The viral load assays by PCR for HCV, cytomegalovirus, Epstein-Barr virus, and BK polyoma were all negative. Microbacterial cultures and drug screens were all negative. The patient had no detectable donor-specific antibodies. At this time, sofosbuvir, ribavirin, and cyclosporine were discontinued. He was started on prednisone 10 mg/d and continued on mycophenolic acid. His sCr stabilized at 2.4 mg/dL.

Our patient suddenly developed hemoptysis and was admitted to an outside hospital at 57 wk after transplant (17 wk since HCV treatment). A high resolution chest tomography of chest found bilateral peri-hilar ground glass opacities. A bronchoalveolar lavage with biopsy was performed and it revealed hemosiderin-laden macrophages and few acute and chronic inflammatory cells with focal mild interstitial fibrosis. He was empirically treated with amoxicillin for 2 wk and hemoptysis resolved at 1 wk of this treatment.

About 70 wk after transplant (30 wk after initiation of sofosbuvir and ribavirin treatment), he returned to transplant clinic with a sCr of 4.3 mg/dL and urine protein-to-creatinine ratio of 5. Urine analysis showed more than 500 mg/dL of protein, 70 RBCs/HPF. A third allograft kidney biopsy was performed and showed cellular crescents in glomeruli with pauci-immune immunofluorescent staining. Peritubular capillaries were negative for C4d staining. Electron microscopy showed normal glomerular basement membrane thickness, without electron dense deposits. A p-ANCA titer was positive at 1:320 dilution and myeloperoxidase antibody was > 100 U/mL. Other tests, including c-ANCA, ANA profile, donor-specific antibodies, HCV viral load, rheumatoid factor, cryoglobulin and drug screens were all negative. Complement factors 3 and 4 were normal. The patient was diagnosed with perinuclear ANCA (p-ANCA) associated crescentic glomerulonephritis in the transplanted allograft.

Our patient was initially treated with methyl prednisolone 500 mg IV daily for 3 doses, then prednisone 60 mg daily as well as cyclophosphamide 200 mg daily. However, his renal function continued deterioration with sCr of 6.4 mg/dL after 1 wk of this combination therapy. Therefore, daily plasmapheresis was added for a total of 7 treatments. His renal function continued to decline and hemodialysis was started shortly. The dose of cyclophosphamide was reduced as he developed neutropenia and painful stomatitis 2 wk later. A course

of rituximab (750 mg weekly for 4 doses) was added. Unfortunately, he remained dialysis-dependent despite of our comprehensive efforts in treating his ANCA associated RPGN.

DISCUSSION

Our patient had a complicated course after kidney transplant with 3 significant events, for which kidney biopsies found 3 different pathologies. We believe these events may not be pathophysiologically related. The first episode of graft dysfunction at 12 wk post-transplant was consistent with typical tubular toxicity of tacrolimus. After the initiation of sofosbuvir and ribavirin therapy for HCV, he experienced the common side effect of severe anemia from this therapy^[4]. Later, the worsening renal function with biopsy-confirmed thrombotic microangiopathy led us to stop his HCV treatment, although the concurrent cyclosporine was implicated as the likely cause. Obviously, we cannot determine which one of these 3 drugs (sofosbuvir, ribavirin or cyclosporine) was responsible for the development of thrombotic microangiopathy. The most significant and interesting event was the diagnosis of ANCA-associated crescentic glomerulonephritis 30 wk after the initiation of sofosbuvir and ribavirin therapy. This was preceded with acute pulmonary hemorrhage when he was admitted to an outside facility. It is possible that our patient might have had a pulmonary involvement of ANCA vasculitis that was not diagnosed at that time.

To induce remission of new-onset organ-threatening or life-threatening ANCA-vasculitis, the combination of high dose of steroids with either cyclophosphamide or rituximab is indicated^[5]. In addition, plasma exchange is also suggested for patients suffered from severe alveolar hemorrhage or RPGN with sCr level of > 5.7 mg/dL^[5]. Our patient was initially treated with pulse steroids and cyclophosphamide. However, his renal function continued deterioration and a course of plasmapheresis were then added into his treatment. Due to the severe side effects of neutropenia and painful stomatitis, we reduced the dose of cyclophosphamide to half, but added rituximab with the hope to rescue his renal function. Unfortunately, he remained dialysis-dependent despite of our comprehensive efforts in treating his RPGN.

ANCA vasculitis has been reported after administration of propylthiouracil, hydralazine, cocaine, levamisole, allopurinol, penicillamine and possibly other drugs^[2,6-8]. None of these medications were used and all drug screening tests were negative in our case. However, our patient was treated with sofosbuvir and ribavirin for his HCV infection before p-ANCA vasculitis developed. This suggests sofosbuvir and/or ribavirin as the possible cause. Ribavirin was first approved by Food and Drug Administration (FDA) on June 3, 1998 for HCV combination treatment. To our knowledge, there is no reported case of ANCA vasculitis associated with ribavirin yet in literature. Sofosbuvir was approved by FDA on December 6, 2013 for HCV treatment. As a new agent on the market, we suspect sofosbuvir as the possible cause of ANCA vasculitis

in our patient. Also, this case is particularly interesting in that p-ANCA vasculitis was present in the transplanted kidney as a pauci-immune crescentic glomerulonephritis. All previously reported cases of drug-associated ANCA glomerulonephritis were in native kidneys. Therefore, our case also represents the first case of drug-associated ANCA vasculitis in a transplanted kidney.

Sofosbuvir is part of a very effective and contemporary therapy for HCV infection. There will be increasing number of patients worldwide treated with this regimen or similar in the coming years. We wish our case provides a timely alert. Further drug monitoring is necessary to elucidate the degree of association and possible causal effect of sofosbuvir and p-ANCA vasculitis.

COMMENTS

Case characteristics

A middle aged gentleman developed hemoptysis 57 wk after a living unrelated donor kidney transplant, then acute renal injury.

Clinical diagnosis

Active urine sediments with hematuria and proteinuria.

Clinical diagnosis

Acute allograft rejection, BK virus nephropathy, cytomegalovirus infection, *de novo* nephritis.

Laboratory diagnosis

Plasma perinuclear antineutrophil cytoplasmic antibodies (p-ANCA) titer was positive at 1:320 dilution and myeloperoxidase antibody was > 100 U/mL.

Pathological diagnosis

Kidney biopsy showed cellular crescents in glomeruli with pauci-immune immunofluorescent staining.

Treatment

Initially treated with pulse steroids and cyclophosphamide, then plasmapheresis and rituximab were added.

Related reports

Several drugs have been reported to be associated with ANCA glomerulonephritis, and all of them were in native kidneys. This case suggests sofosbuvir as a new cause of ANCA vasculitis. It also represents the first case of possible drug-associated ANCA vasculitis in a transplanted kidney.

Term explanation

Drug-associated ANCA vasculitis not only occurs in native kidneys, it may also attack a transplanted kidney.

Experiences and lessons

Future drug monitoring is necessary to elucidate the degree of association and possible causal effect of sofosbuvir and p-ANCA vasculitis, including the kidney transplant recipients.

Peer-review

This is a well written paper of interest to readership of the journal.

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New *SLC12A3* disease causative mutation of Gitelman's syndrome

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Abstract

Gitelman's syndrome (GS) is a salt-losing tubulopathy with an autosomal recessive inheritance caused by mutations of *SLC12A3*, which encodes for the thiazide-sensitive NaCl cotransporter. In this study we report a new mutation of *SLC12A3* found in two brothers affected by GS. Hypokalemia, hypocalciuria and hyperreninemia were present in both patients while hypomagnesemia was detected only in one. Both patients are compound heterozygotes carrying one well known GS associated mutation (c.2581 C > T) and a new one (c.283delC) in *SLC12A3* gene. The new mutation results in a possible frame-shift with a premature stop-codon (pGln95ArgfsX19). The parents of the patients, heterozygous carriers of the mutations found in *SLC12A3*, have no disease associated phenotype. Therefore, the new mutation is causative of GS.

Key words: Gitelman's syndrome; Thiazide-sensitive NaCl cotransporter; Frame-shift mutation; Tubulopathy; *SLC12A3* gene

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Core tip: Mutations of *SLC12A3* gene coding for the thiazide-sensitive NaCl cotransporter are causative of Gitelman's syndrome (GS), a tubulopathy transmitted by an autosomal recessive mechanism. This study identifies a new mutation of *SLC12A3* in two cases of GS. Genetic

evidence that this mutation is a disease causative of GS was also reported.

Grillone T, Menniti M, Bombardiere F, Vismara MFM, Belviso S, Fabiani F, Perrotti N, Iuliano R, Colao E. New *SLC12A3* disease causative mutation of Gitelman's syndrome. *World J Nephrol* 2016; 5(6): 551-555 Available from: URL: <http://www.wjgnet.com/2220-6124/full/v5/i6/551.htm> DOI: <http://dx.doi.org/10.5527/wjn.v5.i6.551>

INTRODUCTION

Gitelman's syndrome (GS) is an autosomal recessive salt-wasting disease prevalently caused by mutations occurring in the *SLC12A3* gene coding for the thiazide-sensitive NaCl cotransporter (NCC)^[1]. This disorder is a tubulopathy characterized by hypokalemic metabolic alkalosis, hypocalciuria and hypomagnesemia. Patients affected by GS often experience muscle pain after physical exercise, other clinical signs are weakness, thirst, nocturia, paresthesia, tetany. Symptoms are essentially due to compensation effects determined by sodium salt-wasting that occurs because of inefficient re-absorption of sodium in distal convolute tubule^[2,3].

The symptoms of GS are similar but less severe than those characterizing Bartter's syndrome, a different channelopathy with salt-wasting and hypotension. Differential diagnosis between GS and Bartter's syndrome, can be made by the demonstration of hypomagnesemia, hypocalciuria and delayed presentation of symptoms in GS^[4]. The prevalence of GS is around 1:40000^[2].

Although the majority of mutations in GS patients were found in *SLC12A3*, in a small percentage of subjects mutations of *CLCNKB* gene, coding for the chloride channel Kb, were demonstrated to be causative of the disease^[5]. In addition, a relevant percentage of GS clinical cases are heterozygous carriers or do not show any mutations in *SLC12A3*^[6]. Therefore, the disease causative mutations are not always well characterized and even rare polymorphisms of *SLC12A3* have, so far, uncertain classification^[6,7]. Mutations of *SLC12A3* span along the entire coding sequence^[8] and the description of new mutations are extremely useful to assess the risk of GS transmission.

In this study we describe a novel frame-shift mutation in *SLC12A3* associated with two cases of GS and we bring genetic evidence that the frame-shift mutation is a novel causative mutation in GS.

CASE REPORT

Probands are two brothers of 24 and 20 years, the younger one was admitted to the hospital after an episode of paresthesia after intense physical exercise. Laboratory tests showed a severe hypokalemia (1.5 mEq/L normal range 3.5-5.0 mEq/L) that required

Table 1 Biochemical characteristics of the two Gitelman's syndrome affected patients

Variable	Patient 1	Patient 2	Reference values
Blood tests			
Creatinine (mg/dL)	1.11	0.93	0.7-1.2
Glucose (mg/dL)	104	97	75-110
Sodium (mEq/L)	141	140	135-145
Potassium (mEq/L)	3.2 ¹	3.0 ¹	3.5-5.0
Chloride (mEq/L)	98	95 ¹	97-108
Calcium (mg/dL)	9.7	10.4 ¹	8.6-10.2
Phosphate (mg/dL)	4.1	3.4	2.5-4.5
Magnesium (mEq/L)	1.5 ¹	1.9	1.6-2.6
Aldosterone (pg/mL)	315 ¹	271	35-300
Renin (pg/mL)	59.7 ¹	53.4 ¹	3.2-32.6
Urine tests			
FEMg (%)	2.6	4.1 ¹	0.5-4.0
FEK (%)	23.7 ¹	17.7 ¹	< 10
Sodium (mEq/24 h)	200	247.5 ¹	40-220
Potassium (mEq/24 h)	104.8	87.2	25-125
Calcium (mg/24 h)	84 ¹	85.5 ¹	100-300
Phosphate (g/24 h)	1.15	1.01	0.4-1.3
Magnesium (mg/24 h)	59 ¹	128.5 ¹	73-122
Calcium/creatinine (mg/mg)	0.055	0.056	< 0.2

¹Abnormal values. FEMg: Fractional excretion of magnesium; FEK: Fractional excretion of potassium.

intravenous potassium supplementation. Subsequent laboratory tests confirmed the persistence of hypokalemia, hypomagnesemia and hypocalciuria (Table 1) that was corrected by therapy based on potassium and magnesium supplements with significant clinical improvements. After this episode, the older brother, although asymptomatic, underwent laboratory tests that showed hypokalemia and hypocalciuria (Table 1), and required potassium and magnesium supplements. Clinical findings were compatible with a diagnosis of GS. Blood samples of the two brothers and their parents were then collected for genetic analysis after informed consent was obtained.

Total DNA was extracted from whole blood by commercial kit (Nuclear Laser), following manufacturer's instructions. All the exons and flanking sequences of *SLC12A3* gene were amplified by PCR using primers and experimental conditions published by Vargas-Poussou *et al.*^[6]. Amplified DNA fragments were checked on agarose gel electrophoresis then purified and bidirectionally sequenced with ABI BigDye terminator cycle sequencing kit on a 310 ABI PRISM Genetic Analyzer (Applied Biosystems). NM_000339.2 was used as reference sequence. The Human Splicing Finder web source was used to predict a possible change in the *SLC12A3* splicing^[9].

We present a case of two brothers both diagnosed with GS, who were reported for genetic counseling to the unit of medical genetics of our university hospital. Biochemical tests were in agreement with the diagnosis, with the exception of blood magnesium which was in the normal range in one of the patients. Hypomagnesemia is not constant in GS, in fact magnesium levels have been

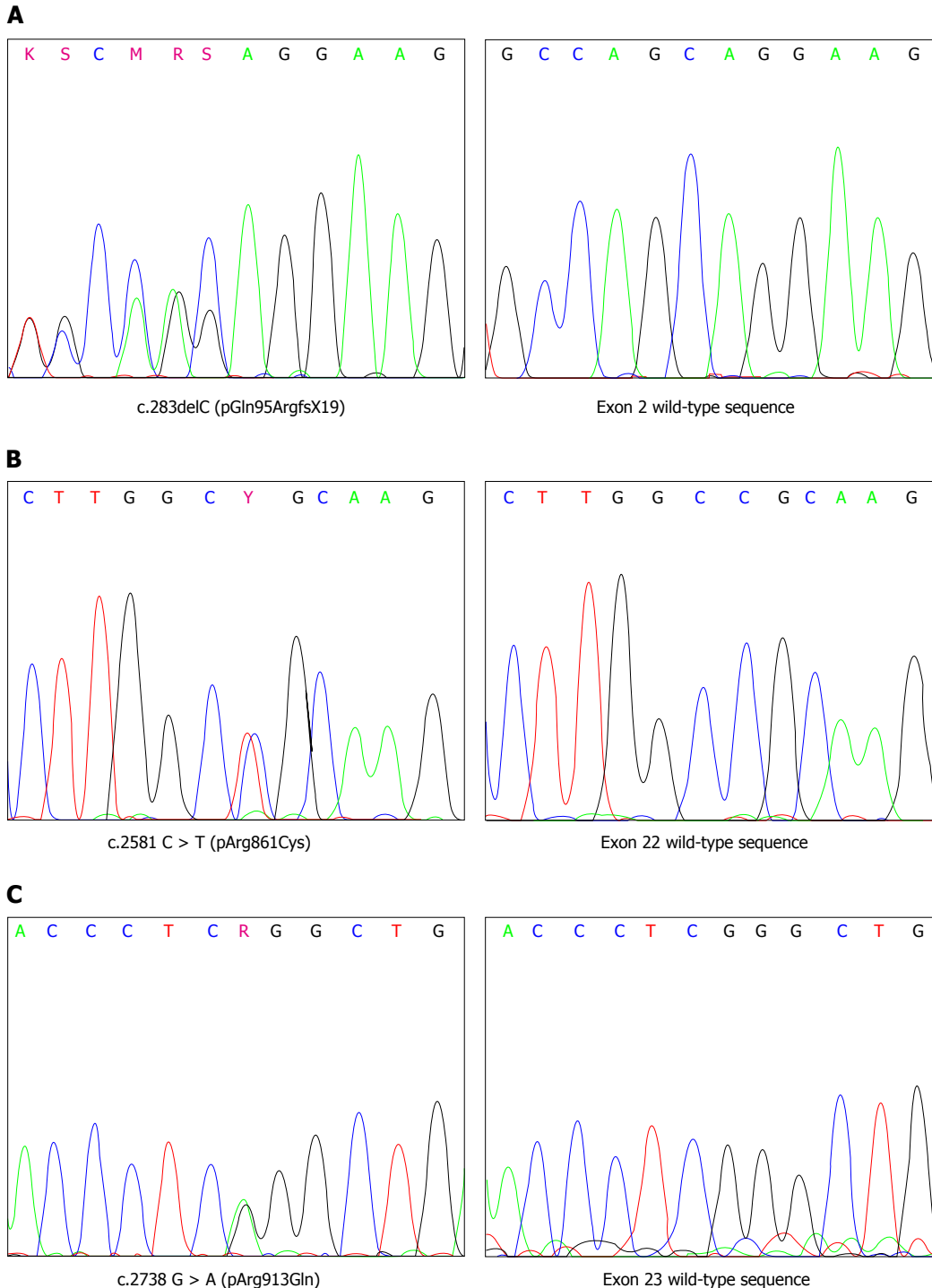


Figure 1 Electropherograms showing DNA sequences of exon 2 (A), exon 22 (B) and exon 23 (C), in the regions containing the variations detected.

reported normal in some GS patients^[10].

Sequence analysis of *SLC12A3* revealed that both the siblings, were compound heterozygotes for a so far undescribed one base-pair deletion c.283delC in exon 2 and for the already known mutation c.2581 C > T (pArg861Cys) in exon 22 associated with GS (Figure 1). In addition, both the siblings were heterozygous for the c.2738 G > A (pArg913Gln) polymorphism which has been associated with GS (Figure 1).

Both the parents of the two siblings were reported

normokalemic and had no clinical or biochemical signs of GS suggesting that they could be heterozygous carriers of the GS associated mutations found in their siblings.

To define whether the mutations and the polymorphism detected in the patients were positioned in cis or in trans, exon 2, exon 22 and exon 23 were sequenced in the parents. The analysis revealed that the mother was heterozygous for the frame-shift mutation in exon 2 whereas the father was heterozygous for the mutation in exon 22 and the polymorphism in exon 23, demonstrating

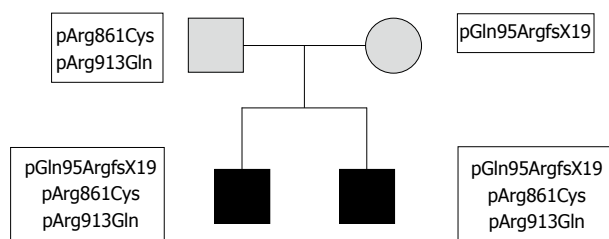


Figure 2 Pedigree of family. Gitelman's syndrome affected patients are colored in black, heterozygous carriers are filled in gray. Mutations and polymorphisms of subjects are reported close to their respective symbols.

that both parents were indeed heterozygous carriers and their siblings are compound heterozygotes for the mutations found in *SLC12A3* gene (Figure 2).

Since mutation in exon 2 segregates independently from mutation in exon 22 and polymorphisms in exon 23, we conclude that the mutation in exon 2 is likely to be causative of disease.

The micro-deletion c.283delC affects the first base of exon 2. Human Splicing Finder web source was used to predict the possible consequences of the mutation in the splicing process. The score of mutant acceptor site resulted even higher than that of the wild-type site (82.02 vs 80.82, +1.49%) strongly arguing against a change of splicing site recognition. In fact, the mutation leaves unaltered the last two bases (AG) of intron 1 and modifies the first two bases of exon 2 (AG instead of CA) generating an even better consensus sequence for an acceptor splice site^[11]. Thus, c.283delC probably results in a frame-shift that introduces a premature stop-codon nineteen codons downstream of the mutation (pGln95ArgfsX19).

DISCUSSION

GS is a recessive genetic disorder caused mainly by mutations occurring in *SLC12A3* gene. The mutations impair the activity of thiazide-sensitive sodium-chloride cotransporter encoded by *SLC12A3* and could be classified in five different classes according to their effect on the protein^[12].

The class 1 of mutant impairs protein synthesis. In this class are included mutations that generate a premature termination of the translation (nonsense, frame-shift and splice site mutations) and mutations located in the promoter that may affect gene expression^[8]. Large deletions including one or more exons of *SLC12A3* have also been detected^[6], the majority of these mutations should be included in the class 1. Mutations that generate premature stop codons in the translation produce a truncated inactive protein. In some cases, a nonsense mediated decay mechanism, for the messenger RNA harboring a premature stop-codon, has been suggested^[13].

Missense mutations belong to different classes of mutations since they can impair protein processing (class 2), impair the correct positioning into the plasma membrane (class 3), reduce the ion transport activity

(class 4) or affect the stability of the protein inducing an accelerated degradation (class 5), of the *SLC12A3* protein product.

Class 2 mutants are represented by N-glycosylation deficient proteins such as T392I or R399C^[8,13]. N-glycosylation in N404 and N424 residues is required for the correct processing of NCC^[14] and it was impaired by this class of mutations. The lack of proper N-glycosylation causes the deficiency of protein into plasma membrane.

Class 3 mutants, such as N442S and Q1030R result in proteins that could be functional but have an impaired insertion into the plasma membrane^[8]. In this case, the trafficking of the cotransporter is affected because of the intrinsic nature of the mutant protein itself or as consequence of the loss of interaction with other proteins that have a role in the regulation of NCC trafficking^[8].

Class 4 mutants, such as A588V and P751L^[8,13], are proteins that show a reduced Na⁺ uptake. Although these mutant proteins are fully glycosylated and correctly positioned into the plasma membrane, they are deficient in their functional role. Depending on the position of mutated residue in the primary sequence of NCC, decreased ion affinity or impaired regulation was hypothesized as potential mechanisms of reduced cotransporter activity^[8,13].

The presence of a class 5 of mutants (accelerated degradation) was postulated but clear examples of this kind of mutants remain to be demonstrated.

We present the case of two siblings, compound heterozygous, carrying mutations of *SLC12A3*. One of these mutations, inherited from the father, is the missense pArg861Cys which was clearly associated with GS^[6]. Although this is a well-known mutation, its effect on the NCCT co-transporter has never been characterized. Moreover, both patients inherited from their father also a polymorphic variation, which has been in some cases associated with GS, although with a less clear involvement in the pathology^[6].

The second mutation carried by our patients, inherited from the mother, is a deletion of the first nucleotide of the second exon of *SLC12A3*. It has never been reported so far. Since the new mutation does not seem to affect the splicing site acceptor recognition, it could result in a frame-shift with a premature stop of the translation and a clear loss-of-function. However, the possibility of skipping of exon 2 cannot be completely discarded, since that mechanism has been experimentally verified for the mutation c.1926delC located at the 5' end of exon 16 of *SLC12A3*^[15].

A clear genotype/phenotype correlation is not possible in GS probably because many other genetic factors, different from the *SLC12A3* causative mutations, can influence the severity of the pathology. Interestingly our patients share an identical *SLC12A3* genotype and show a similar disease associated phenotype. The definition of *SLC12A3* genotype may be a useful tool in the clinical characterization of GS and in genetic counseling of salt losing tubulopathies.

COMMENTS

Case characteristics

Two brothers, the youngest one had an episode of paresthesia after intense physical exercise.

Clinical diagnosis

Muscular weakness related to a suspected Gitelman's syndrome (GS), a genetic disease with autosomal recessive inheritance.

Differential diagnosis

Although less severe, GS can be confused with Bartter's syndrome.

Laboratory diagnosis

Hypokalemia, hypomagnesemia and hypocalciuria. In both brothers two point mutations of *SLC12A3*, a gene associated with GS, were detected. One of this mutation was characterized for the first time. Parents, who are healthy, carried just one mutation of *SLC12A3*.

Treatment

Supplementation of potassium and magnesium.

Term explanation

GS: GS; NCC: Thiazide-sensitive NaCl cotransporter.

Experiences and lessons

Although GS could be indolent, symptoms can be experienced after intense physical exercise. Thus, a clinical diagnosis confirmed by a genetic test is very important.

Peer-review

The authors described brothers with Gitelman syndrome who had a new mutation of *SLC12A3*. The paper is well-written and interesting findings.

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