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REVIEW

Management of patients with a failed kidney transplant: Dialysis reinitiation, immunosuppression weaning, and transplantectomy

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Abstract

The number of patients reinitiating dialysis after a failed transplant increases over time and has more than doubled between the year 1988 and 2010 (an increase from 2463 to 5588). More importantly, patients returning to dialysis have been shown to have a greater than three-fold increase in the annual adjusted mortality rates compared with those with a functioning graft. Continuation of immunosuppression to preserve residual graft function has been implicated to be a contributing factor, seemingly due to immunosuppression-associated cardiovascular and infectious complications and malignancy risk, among others. Nonetheless, maintenance low-dose immunosuppression has been suggested to confer survival benefit in patients returning to peritoneal dialysis. Whether early *vs* late reinitiation of dialysis or whether transplantectomy has an impact on patient survival remains poorly defined. Consensus guidelines for the management of a failed allograft are lacking. In this article, we present a literature overview on the ideal timing of dialysis reinitiation after graft loss, the management of immunosuppression after graft failure, and the risks and benefits of transplantectomy. The authors' perspectives on the management of this special patient population are also discussed.

Key words: Failed kidney transplant; Allosensitization; Immunosuppression weaning; Allograft nephrectomy; Transplantectomy; Dialysis reinitiation after transplant failure

Core tip: The number of patients with a failed allograft returning to dialysis increases over time. Studies suggest that such patients are at increased morbidity and mortality risks compared with their transplant-naïve, incident dialysis patients. This review provides a critical literature overview of the risks and benefits of early *vs* late dialysis re-initiation, immunosuppression weaning, and transplantectomy in patients with a failed allograft. Based on currently available literature, suggested guidelines for the management of this unique patient population are presented.

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INTRODUCTION

Retrospective analysis of the United States Renal Data System (USRDS) database showed that mortality in patients reinitiating dialysis after graft failure was primarily due to cardiac (36%) or infectious complications (17%)[1]. Continuation of immunosuppression has been suggested to play a causative role. Nevertheless, immunosuppression cessation is not without morbidity. Similarly, although transplantectomy would permit immunosuppression withdrawal, it may lead to other unfavorable outcomes.

Clinicians caring for patients with a recently failed allograft are generally faced with three important decisions: timing of dialysis reinitiation, immunosuppression management, and whether to perform transplantectomy[2]. When the cause of graft loss is due to primary nonfunction, arterial or venous thrombosis, hyperacute or early refractory acute rejection, most treating physicians advocate transplantectomy and immunosuppression cessation. In these circumstances, graft rupture or hemorrhage may occur if the graft is left *in situ*. However, when the allograft has been in place for more than 1-2 years, it is common practice to leave the failed allograft *in situ*. Nonetheless, a retained failed transplant has been suggested to be a source of a chronic inflammatory state, potentially leading to unfavorable outcomes. Immunosuppression management in such patients can be challenging. Although maintenance low dose-immunosuppression may preserve residual kidney function, circumvent graft intolerance syndrome, minimize allosensitization, and avoid overt acute rejection, long-term maintenance immunosuppression is not without adverse effects (Table 1). These may include immunosuppression-related malignancy and cardiovascular and metabolic complications. It is also noteworthy that although the number of patients reinitiating dialysis after a failed transplant has more than doubled in the last two decades, studies evaluating the optimal timing of dialysis reinitiation are lacking. A literature overview on the timing of dialysis reinitiation after graft failure, the potential beneficial and adverse effects of low-dose maintenance immunosuppression, and the risks and benefits of transplantectomy are presented.

TIMING OF DIALYSIS REINITIATION

Early studies in the mid 1970s to 1980s suggested that initiation of dialysis in end-stage kidney disease (ESKD) patients with a higher estimated glomerular filtration rate (eGFR) was associated with lower mortality[3]. However, these studies were subsequently criticized for small sample sizes and potential confounding factors. Over the past decade, several observational studies failed to demonstrate the survival benefits of early commencement of dialysis and such practice may even be associated with increased mortality risk[4]. A recent multicenter randomized controlled trial (the Initiating Dialysis Early and Late study) showed comparable mortality rates among early *vs* late dialysis initiation. Eight hundred and twenty eight patients with progressive stage V chronic kidney disease (CKD) (including patients with a failed transplant) were randomized to either start dialysis at eGFR of 10.0 to 14.0 mL/min (early-start group) or to continue routine medical management and start dialysis when eGFR reached 5.0 to 7.0 mL/min (late-start group)[5]. During a median follow up period of 3.59 years, mortality occurred in 37.6% and 36.6% of patients in the early- and late-start groups, respectively (HR 1.04, $P = 0.75$). The frequency of cardiovascular events, infections, or dialysis complications was comparable between the two groups. However, it is noteworthy that in the late-start group, nearly 76% of patients were started on dialysis when the estimated GFR was above the target 7.0 mL/min due to symptomatic uremia. It was thus concluded that planned early dialysis initiation in patients with stage V CKD provided no benefits in terms of survival or clinical outcomes. Similarly, a retrospective USRDS database study ($n = 310932$ patients who were started on dialysis between 2006 and 2008) showed no harmful or beneficial effects of early dialysis initiation on mortality (HR 1.025 per 1 mL/min per 1.73 m^2 for eGFR 5-14 1 mL/min per 1.73 m^2 and 0.973 per 1 mL/min per 1.73 m^2 for eGFR 14-20 mL/min per 1.73 m^2)[6].

Studies on the optimal timing of dialysis reinitiation after a failed transplant are limited. Current guidelines for transplant naïve patients with progressive CKD advocate late-start dialysis (defined as dialysis initiation at an eGFR between 6-9 mL/min). Results of two large registry studies suggested that early compared with late dialysis reinitiation in patients with failed kidney transplants may adversely impact survival. The USRDS registry study ($n = 4741$ patients followed for a median of 15 ± 11 mo after dialysis initiation) demonstrated that nonsurvivors had a significantly higher eGFR at dialysis initiation than their survivor counterparts (9.7 ± 4.8 *vs* 8.0 ± 3.7 mL/min per 1.73 m^2, respectively). Specifically, each 1 mL/min per m^2 higher eGFR at the time of dialysis reinitiation was found to be associated with a 4% higher mortality risk after dialysis reinitiation ($P < 0.01$)[1]. Nonetheless, it is speculated that the sickest patients tended to require commencement of dialysis at higher levels of residual kidney function. This confounding by indication was subsequently addressed in an analysis of the SRTR registry study using propensity score analysis. The study cohort consists of 747 failed kidney transplant patients who had reinitiated dialysis with eGFR < 15 mL/min. A propensity score for early

Table 1 Continuation of immunosuppression after a failed transplant

Potential beneficial effects	Potential adverse effects
Preservation of residual kidney function	Metabolic complications (diabetes, hypertension, dyslipidemia)
Decreased incidence of graft intolerance syndrome and the need for allograft nephrectomy	Steroid-associated adverse effects (*e.g.*, diabetes, cataracts, myopathy, and avascular necrosis among others)
Minimization of allosensitization	Cardiovascular complications
Avoidance of overt acute rejection	Increased susceptibility to infection
Prevention of adrenal insufficiency syndrome	Malignancy (especially skin cancers, Kaposi's sarcoma, non-Hodgkin's lymphoma, and lip cancers)
Prevention of reactivation of systemic disease (*e.g.*, systemic lupus erythematosus, vasculitis)	Costs (particularly when data supporting continued immunosuppression are lacking)

(eGFR > 10.5 mL/min per 1.73 m^2) *vs* late dialysis reinitiation was fitted by logistic regression. Peripheral vascular disease, diabetes mellitus, and male gender were associated with higher odds of early reinitiation of dialysis. In an unadjusted model, each 1 mL/min per 1.73 m^2 higher eGFR at dialysis reinitiation was associated with a 6% higher mortality risk. Such association was not observed in the fully adjusted model. However, there was a trend towards increased mortality risk in patients with a higher eGFR upon reinitiation of dialysis, particularly among the healthiest subgroups of patients identified by the propensity score, including female gender and younger subjects[7].

Whether early dialysis reinitiation in patients with failed transplants adversely impact outcomes is currently not known and warrants further studies. Based on available data, a number of investigators feel that reinitiation of dialysis based on eGFR alone is not justified and could be harmful in some cases[3]. Thus, as with transplant naïve patients, dialysis reinitiation in patients with graft failure may rely on eGFR as a rough guide that must be redefined by patients' comorbidities, nutritional status, and overall wellness.

IMMUNOSUPPRESSION MANAGEMENT

Consensus guidelines for the management of immunosuppression in patients with a failed allograft are lacking. Both continuation of low-dose immunosuppression and immunosuppression withdrawal have their inherent risks and benefits (Table 1)[2].

Continuation of immunosuppression: Potential beneficial effects

Preservation of residual kidney function: In the non-transplant settings, peritoneal dialysis (PD) and hemodialysis (HD) patients with preserved kidney function have been shown to have better survival rates compared with their oliguric or anuric counterparts[8,9]. Similar to the transplant naïve end stage kidney disease population, patient with a retained failed transplant and preserved residual graft function have been shown to have survival advantage over those who lost residual kidney function. A decision analytic model comparing continuation of immunosuppression with immunosuppressant withdrawal in patients returning to PD after graft failure suggests that continued immunosuppression may confer survival benefit over immunosuppressant cessation despite increased malignancy and infection risks (life expectancy: 5.8 years *vs* 5.3 years, respectively)[10]. The survival benefit was apparent even at marginal GFR (defined as an additional GFR of 1.48 mL/min), and incremental at increasing residual graft function. The study results suggest that the loss of residual kidney function may have an adverse impact on survival in patients reinitiating PD. Nevertheless, the study was not without shortcomings. The model hypothesized that continuation of maintenance immunosuppression would preserve residual kidney function, and the beneficial effects of residual graft function are similar to those of the native kidneys. Of interest, results of the USRDS registry analysis demonstrated that compared with hemodialysis, PD was associated with greater survival within the first year after dialysis initiation, but lower after 2 years[11]. It may be speculated that the early survival benefit of PD over HD was due to greater preservation of residual kidney function. Notably, the survival advantage of PD was not seen among patients who initiated PD at lower levels of eGFR. However, neither details on immunosuppression maintenance after graft failure nor data on differential rates of decline in residual kidney over time was provided. A case of well-preserved residual kidney function in a PD patient maintained on low dose dual immunosuppressive therapy after a failed allograft has been described. After return to PD, the patient continued to make 600-1200 cc of urine/day at one-year follow-up[12].

There is currently insufficient evidence to routinely recommend continued immunosuppression in patients returning to PD after graft loss.

Data for any potential survival benefits of continuation of maintenance immunosuppression among patients returning to HD are lacking.

Prevention of allosensitization: Immunosuppression withdrawal after kidney graft failure with or without transplantectomy has been shown to be an independent predictor of allosensitization[13,14]. In a single-center study consisting of 69 patients with confirmed alloantibody

negative at the time of graft loss, more than half (38/69) became sensitized over the following months or years. *De novo* class Ⅰ and/or class Ⅱ anti HLA antibodies (primarily of donor specificity) were detected only in patients whose immunosuppressants were discontinued after graft loss regardless of whether they had a nephrectomy or blood transfusion. Four of fifteen patients without nephrectomy or transfusion developed antibodies after cessation of immunosuppression. In contrast, none of the eleven patients who continued immunosuppressants developed antibodies, seven of whom had an allograft nephrectomy or blood transfusion[14]. In another study, *de novo* donor-specific antibodies (DSAs) appeared in nearly 48% of patients when immunosuppressive therapy was discontinued after graft loss. None of these patients had an allograft nephrectomy[15]. Of interest, it has been shown that a short exposure to the allograft is sufficient to stimulate the immune system and to induce alloantibody production[16]. In a small series of 32 patients who required transplantectomy after early graft loss, DSAs and non-DSA anti-HLA antibodies developed in > 50% of patients whose immunosuppressants were discontinued after transplantectomy (median time between transplantation and transplantectomy was 2.5 d). Histological analysis of explanted allografts showed no features of cellular or humoral rejection. There was no significant difference in the incidence of DSAs among patients receiving transfusions and those who did not[16].

Given current evidence, albeit scant of potential increased allosensitization with cessation of immunosuppression, it may be suggested that patients who are re-allograft transplant candidates be considered for continuation of maintenance therapy, particularly when living donation is a possibility. Whether patients with early graft loss requiring transplantectomy (particularly those anticipated to have a short wait time after early relisting) benefit from continuation of immunosuppression to minimize allosensitization warrant further investigation. In addition, the duration and intensity of maintenance immunosuppression remain to be defined.

Prevention of graft intolerance syndrome and transplantectomy: Graft intolerance syndrome refers to an immunologic intolerance to a retained failed graft, and commonly develops within the first year of dialysis reinitiation. Clinically, patients may present with graft enlargement or tenderness, gross hematuria, fevers, malaise, flu-like symptoms, or any constellation of signs and symptoms thereof. Graft intolerance syndrome may develop in 30% to 50% of patients despite various immunosuppression withdrawal protocols. Although such syndrome may be treated with a short course of high dose corticosteroid, symptom recurrence following immunosuppression weaning generally necessitates transplantectomy. In one single center study, immunosuppression weaning commonly led to symptomatic rejection with fever mimicking infection (93 of 186 study subjects were African Americans). A nearly 7-fold risk (P = 0.017) for admission within six months of graft failure with fever in the absence of infection was observed among African Americans who were tapered from immunosuppression. The majority of these patients ultimately required transplantectomy due to symptomatic rejection or fever of unknown etiology. Notably, fever resolved in all patients after transplantectomy[17].

In a single-center study consisting of 41 patients with graft loss occurring more than 6 mo after transplantation, the need for transplantectomy following immunosuppression weaning was found to correlate with the number of previous acute rejection episodes. In patients who had zero, one, or two or more rejection episodes, transplantectomy was required in 30%, 53% and 83%, respectively. It is suggested that gradual immunosuppression weaning or indefinite low-dose maintenance immunosuppression may prevent the need for transplantectomy[18].

Rapid steroid withdrawal may result in overt adrenal insufficiency variously manifested as hypotension, weakness, fevers, malaise, and weight loss, among others. In severe steroid withdrawal, patients may experience frequent hypotensive episodes during dialysis despite having volume overload[19]. When the graft is left *in situ* for more than one to two years, gradual immunosuppression weaning with close monitoring for clinically overt adrenal insufficiency or acute allograft rejection is advisable.

Continuation of immunosuppression: Potential adverse effects

Infectious, cardiovascular, and metabolic syndrome risks: While low-dose maintenance immunosuppression may be beneficial in preserving residual kidney function in patients who maintain good urine output, such practice is not without adverse effects. In a multi-center cohort study comprising 197 failed allografts in 177 transplant recipients whose allograft functioned for at least 3 mo, low-dose maintenance immunosuppression was associated with an increase in infectious- and cardiovascular disease-related morbidity and mortality[20]. The incidence of infectious complications per patient year was significantly higher in the immunosuppression continuation compared with that of immunosuppression withdrawal groups (1.7 *vs* 0.51, respectively, $P < 0.0001$). Similarly mortality associated with cardiovascular and infectious complications was higher among patients who continued immunosuppression compared with those whose immunosuppression was discontinued [Odd ratio (OR) of 4.9, 95%CI: 1.8-13.5 from cardiovascular disease and OR of 2.8, 95%CI: 1.1-7.0 from infectious complications]. Clinical acute rejection rates (graft tenderness and hematuria, in the absence or presence of non-infectious low-grade fever) were similar between the two groups (P = NS). Based on the study findings, the authors favored immunosuppression withdrawal over low-dose maintenance immunosuppression when patients returned to dialysis. In one single center study, an increase in infection-related complications was

observed among patients whose immunosuppression was weaned over a prolonged period (mean 14 ± 2 mo) compared with those whose immunosuppression was weaned over a shorter (mean 3 ± 1 mo) period (1.34 *vs* 0.87) infections per year, respectively[21]. Furthermore, the longer taper had no advantage over the shorter taper group in forestalling the need for transplantectomy. Mortality associated with disseminated histoplasmosis in a hemodialysis patient maintained on low-dose steroid and azathioprine after graft failure has been described[22].

Similar to early reports, a recent study suggests that although immunosuppression weaning results in a higher risk of allosensitization, maintenance of immunosuppression other than low-dose steroid is associated with a greater incidence of infection and infection-related mortality[17]. In a single center consisting of 186 patients with failed kidney transplants, 44% were hospitalized with fever within six months of graft loss. The rates of hospitalization were comparable between patients who continued immunosuppression and those whose immunosuppression was tapered before hospitalization (45% *vs* 40%, respectively, *P* = NS). However, among febrile hospitalized patients, documented infections occurred in 88% of patients maintained on immunosuppressive therapy compared with 38% of those who had been weaned off of immunosuppression (defined as withdrawal of all immunosuppressive therapy with the exception of ⩽ 10 mg of prednisone daily). Notably, mortality risk was significantly higher in patients with documented infection, with dialysis catheter being the most common infectious source in both groups[17].

Adverse effects associated with long-term steroid use: Well-established adverse effects associated with long-term steroid use include avascular necrosis, osteoporosis, hyperglycemia, cataracts, myopathy and increased susceptibility to infections among others. Nevertheless, it is occasionally necessary to continue steroid to prevent flares of systemic disease such as vasculitis or systemic lupus erythematosus.

Malignancy: Recipients of organ transplants are at increased risk for developing certain neoplasms compared to that of the general population. Kidney transplant recipients receiving low-dose cyclosporine (CSA) was shown to have a significantly lower overall frequency of cancers ($P < 0.034$) and a lower incidence of virus-associated cancers ($P = 0.05$) compared with their normal-dose CSA counterparts[23]. Both the duration and intensity of immunosuppressive agents and their ability to foster the replication of oncogenic viruses have been implicated to play contributory roles in the carcinogenic process[2].

Studies evaluating reversal of cancer risk in patients reinitiating dialysis after graft loss is limited. However, it is noteworthy that studies in ESKD patients who received dialysis or a kidney transplant, and in HIV/AIDS subjects suggest that cancers may be classified into those that are related to ESKD, immune deficiency, non-immune deficiency, or uncertain status (Table 2)[24,25]. Although it is conceivable that immunosuppression cessation has no impact on risk reversal of various "non-immune deficiency-related" cancers, most treating physicians advocate rapid immunosuppression weaning or withdrawal in patients who had a history of cancer, irrespective of malignancy types. In cancers associated with immunosuppression, the risks of continued immunosuppression probably outweigh its benefits.

The Australia and New Zealand Dialysis and Transplantation Registry analysis demonstrated that among all cancers that occur at increased rates in kidney transplant recipients, the pattern of incidence after allograft loss was highly variable[26]. Nonetheless, the incidence of Kaposi's sarcoma and non-Hodgkin's lymphoma decreased markedly upon dialysis reinitiation and cessation of immunosuppression. The study also showed a significant decline in melanoma and lip cancer incidence. Of interest, risk reversal was commonly seen among infection-associated malignancies such as Kaposi's sarcoma with human herpes type 8 and non-Hodgkin's lymphoma with Epstein-Barr virus. The exact cause of increased risk of lip cancer in transplant patients has not been well-established. However, human papillomavirus has been implicated to play a causative role. Although an infectious source has not been identified in transplant patients with melanoma, the association between immunosuppression and its development in kidney transplant recipients has been well described[24].

Costs: Following graft loss and reinstitution of dialysis, the cost of low-dose maintenance immunosuppression should not be overlooked, particularly since data supporting continued immunosuppression are lacking. A typical immunosuppressive regimen consisting of low-dose prednisone and cyclosporine or tacrolimus (or mTOR inhibitors) costs more than two thousand United States dollars annually.

TRANSPLANTECTOMY

While practices differ among centers, most advocate transplantectomy in patients whose allograft failed within one to two years posttransplantation. However, no consensus exists on the timing and indications for transplantectomy when graft loss occurs more than 1-2 years after transplant.

The USRDS registry study demonstrated that transplantectomy was nearly twice as common in patients with early (< 12 mo) compared with late graft loss (⩾ 12 mo)[27]. However, whether transplantectomy was performed electively or for graft-related symptoms could not be determined from the study. A single-center study consisting of 34 pediatric kidney transplant

Table 2 Categorization of cancers in the end-stage kidney disease population

ESKD-related	Kidney
	Urinary tract
	Thyroid
	Myeloma
Immune-deficiency related	Hodgkin's lymphoma
	Non-Hodgkin's lymphoma
	Leukemia
	Melanoma of skin
	Kaposi's sarcoma
	Carcinoma of
	Lip
	Mouth, tongue, tonsil, oropharynx
	Esophagus
	Stomach
	Anus
	Liver
	Larynx
	Lung
	Cervix, uteri, vagina, vulva
	Penis
	Eye, squamous cell carcinoma only
Not-related to immune deficiency	Rectum
	Breast
	Ovary
	Prostate
Of uncertain status	All other cancers

ESKD: End-stage kidney disease.

recipients demonstrated that children with graft failure within one year of transplantation were four-fold more likely to require transplantectomy than those with graft loss after one year (P = 0.04)[28]. Fever, graft tenderness, and an elevated C-reactive protein were significantly more common in children who subsequently underwent transplantectomy than in those who did not. Of interest, one retrospective study suggested that transplantectomy may minimize allosensitization in patients with early (graft survival < 6 mo) but not late graft loss. Patients with early graft loss and nephrectomy demonstrated a decline in PRA at a median follow up of 47 mo (46% at the time of graft loss and 27% at last follow up, P = 0.02). In contrast, PRA remained elevated among those who had a nephrectomy after late graft loss[29]. It is suggested that the time of graft failure and subsequent allograft nephrectomy may play a contributory role in allosensitization.

In general, the decision to perform a failed graft nephrectomy requires careful consideration of potential risks and benefits.

Transplantectomy: Potential benefits

A retained failed allograft has been suggested to serve as a focus for a chronic inflammatory state. In one single-center study, patients with failed kidney transplants returning to hemodialysis were shown to exhibit worse anemia, erythropoietin resistance, and hypoalbuminemia, as well as worse C reactive protein (CRP), erythrocyte sedimentation rate (ESR), and ferritin profiles compared with their transplant naïve hemodialysis counterparts. Furthermore, amelioration of both clinical and laboratory parameters of the chronic inflammatory state was observed following transplantectomy. Although symptomatic patients undergoing transplantectomy had lower baseline hemoglobin and higher CRP, ESR, and ferritin compared with those with a retained graft, the former group of patients had a better hematologic and biochemical profile at 6 mo after transplantectomy compared with the latter[30].

It is noteworthy that hypoalbuminemia and high CRP have been shown to be markers for increased cardiovascular and global morbidity and mortality both in the general population and in ESKD patients on hemodialysis. Some centers favor transplantectomy in patients with biochemical indicators of chronic inflammation before the onset of overt clinical manifestations[31,32].

Retrospective study using the USRDS database (n = 10951 patients returning to long-term dialysis after a failed transplant) demonstrated that transplantectomy was associated with a 32% lower relative risk for all-cause mortality (adjusted HR = 0.68; 95%CI: 0.63 to 0.74)[32]. However, the study was not without shortcomings. Patients who had graft nephrectomy (n = 3451) were younger and in better health condition than their non-nephrectomized counterparts. It is also noteworthy that despite adjustment for confounding factors and likelihood of undergoing transplantectomy, limitations intrinsic to retrospective registry studies remain. In addition, in patients with the failed allograft left *in situ*, it is not known whether low-dose maintenance immunosuppression might be independently associated with increased infectious- and cardiovascular disease-related mortality.

Of interest, in a large retrospective studies consisting of more than 19000 patients with graft failure, transplantectomy in patients reinitiating dialysis was found to be associated with increased mortality among those with early graft loss [graft survival < 12 mo, HR 1.13 (95%CI: 1.01-1.26)] whereas among those with late graft loss (graft survival > 12 mo), transplantectomy was associated with decreased mortality rates (0.89 95%CI: 0.83-0.95)[27]. It is speculated that the association of transplantectomy and mortality risk in patients with early graft loss was due to graft-related symptoms rather than the nephrectomy procedure *per se*. Further studies are needed to determine whether transplantectomy after late graft loss confers a survival advantage over leaving the graft *in situ*.

Transplantectomy: Potential adverse effects

Leaving a failed allograft *in situ* may avoid potential morbidity and mortality associated with the surgical procedure. In addition, in patients with residual kidney function, a retained graft may allow more liberal fluid intake and improve patients' quality of life. In most series reported, transplantectomy-associated morbidity

occurred in 17%-60% and mortality in 1.5% to 14% of patients[33]. The wide variation in the mortality rates reported may be due in part to the timing of surgery, the indication for graft nephrectomy, the patients' condition at the time of surgery, the surgical techniques, and individual centers' practice and experiences[31]. Symptomatic patients who need urgent transplantectomy are more likely to have worse outcomes than those undergoing elective transplantectomy. In one study, patients who underwent graft nephrectomy under suboptimal medical conditions (severe rejection or graft sepsis, hemorrhage from anastomotic suture line), a mortality rate of up to 39% has been reported[34].

Allograft nephrectomy has been shown to be associated with allosensitization, potentially resulting in prolonged wait times for a crossmatch negative kidney in re-allograft candidates. It is speculated that a retained allograft may serve as an antibody sponge, or alternatively, rapid immunosuppression weaning after transplantectomy may promote antibody-mediated allosensitization against the allograft. In one single-center study, *de novo* donor-specific antibodies (DSAs, tested *via* Luminex single-antigen assay) were detected as soon as five days after transplantectomy, suggesting that the antibodies were preformed[15]. Furthermore, the median fluorescence intensity (MFI) of alloantibodies remained stable or declined during follow up. It was hypothesized that if DSAs had appeared because of injury caused by graft nephrectomy, the MFI would have increased during follow up. Whether the detection of preformed DSAs after graft nephrectomy may have important implications in identifying unacceptable antigens for patients awaiting a repeat transplant remains to be studied.

Although post allograft nephrectomy rise in PRAs or DSAs may reflect preformed antibodies, it is also tempted to speculate that transplantectomy may stimulate pro-inflammatory cytokine production and upregulation of HLA alloantibodies. Alternatively, sensitization may occur due to the persistence of antigen-presenting cells or residual donor tissues and vessels[16].

The mechanism(s) or predominant mechanism of *de novo* development of anti-HLA alloantibodies after graft nephrectomy is currently not fully understood. Nonetheless, there has been ample literature showing that transplantectomy leads to an increase in class Ⅰ and class Ⅱ PRA, and DSA and non-DSAs to variable extent[13,16,35-38]. Whether immunosuppression weaning over a prolonged period after graft nephrectomy may reduce the risk of *de novo* anti-HLA alloantibodies development is unknown and warrants further exploration. Prospective studies to assess the potential mechanism(s) of allosensitization after transplantectomy and the impact of such procedure on graft and patient survival as well as on acute rejection rates after a repeat transplant are needed.

Impact of transplantectomy on a repeat transplant

The literature on the impact of transplantectomy on the outcomes of retransplantation have yielded variable and even contradictory results. Selected studies are discussed.

Studies indicating an adverse impact of transplantectomy on various clinical outcomes of a repeat transplant: Early single-center study demonstrated that transplantectomy was associated with a significant increase in PRA levels and a higher incidence of delayed graft function in a repeat transplant[39]. A trend for reduced graft survival was observed among patients whose first grafts failed within the first post-transplant year. However, transplant nephrectomy had no impact on the incidence of acute rejection or renal function of a repeat graft at 3-year follow-up.

In a retrospective study consisting of 192 recipients of a reallograft transplant, nephrectomy of the primary failed graft was shown to have an adverse impact on reallograft transplantation (P = 0.0003)[40]. Multivariate analysis demonstrated a significant relationship between survival of the primary allograft and repeat transplant outcomes. Subgroup analysis performed in patients whose graft functioned more than 6 mo (n = 90) similarly demonstrated that nephrectomy of the failed graft is a risk factor for worse retransplantation outcomes. Other identified risk factors included advanced donor age, longer time interval from transplantectomy to reallograft transplantation, and the lack of induction with Minnesota antilymphocyte globulin.

In a retrospective study comprising 121 patients who had a nephrectomy and 45 who did not undergo nephrectomy prior to repeat transplantation, pre-transplant graft nephrectomy and panel reactive antibody levels greater than 70% were found to be independent risk factors for graft failure after a repeat transplant[41]. Subgroup analysis showed that pretransplant graft nephrectomy adversely affected survival of a subsequent graft among high risk patients defined as those with multiple transplants (≥ 2 transplants) and those who received an allograft from an older donor (> 65 years of age), as well as among European Senior Program patients. However, in the subgroup of patients without "high risk" factors, nephrectomy of a previous graft had no impact on delayed graft function, or graft or patient survival rates after a repeat transplant. Nonetheless, pretransplant nephrectomy was associated with increased rejection rates presumably due to elevated PRA levels.

Studies suggesting a neutral impact of transplantectomy on various outcomes of a repeat transplant: In a retrospective analysis to evaluate graft survival in patients who underwent transplantectomy prior to reallograft transplantation (n = 68) compared with those who did not (n = 21), nephrectomy of a failed graft was found to have no significant impact on the survival

Table 3 Transplantectomy: Potential risks and benefits and impact on a repeat transplant

	Comments
Potential benefits	
A failing graft is a focus of a chronic inflammatory state	
May reduce mortality rates	Variable results, further studies are needed
Potential adverse effects	
Residual kidney function may allow less stringent fluid restriction	
Surgery-related morbidity and mortality	Morbidity 17%-60% in most series reported Mortality 1.5%-14% in most series reported
Allosensitization and the potential for future prolonged wait-times for a compatible crossmatch kidney	
Impact on a repeat transplant	
Mixed reports due to potential confounding factors	
Differences among studies in:	
Immunosuppression withdrawal protocols	
Recipient and donor demographics	
Era of transplantation	
Indications for transplantectomy	
Time on dialysis prior to a repeat transplant	
Causes of prior graft loss	
Allosensitization associated with blood transfusion	
Pre-existing DSA with or without complement-fixing DSA (see text)	
HLA matching of subsequent graft	
Donor type (living *vs* deceased)	
Others	

DSA: Donor-specific antibody.

of a future allograft[42]. Five-year actuarial patient survival were 94.1% and 87.5%, respectively (P = 0.69). PRA levels at the time of retransplantation were comparable between the two groups (37% *vs* 29%, respectively). Multivariate analysis showed a negative impact of PRA levels on graft survival independent of transplantectomy (P = 0.04).

One single-center retrospective study demonstrated that dialysis time was significantly longer in patients who had a graft nephrectomy than those who did not, presumably due to higher PRA levels in the nephrectomy group, making it difficult to obtain a negative crossmatch donor kidney. Nonetheless, acute rejection episodes and one-, five-, and ten-year graft survival rates were not different between the nephrectomy and no nephrectomy group[43]. Univariate analysis demonstrated that PRA levels and the number of acute rejection episodes had no significant impact on graft or patient survival, whether or not the patient had transplantectomy (P = 0.3 for both).

Differential impact of transplantectomy on the outcomes of a future allograft: Retrospective study using the USRDS database (n = 19107 patients returning to dialysis after first graft failure) demonstrated that transplantectomy after early graft loss (graft survival of less than twelve months) was associated with a lower risk of repeat graft failure, whereas transplantectomy for late graft loss (graft survival of $\geqslant$ 12 mo) may be deleterious to repeat transplant outcomes[27]. However, further analysis demonstrated that the protective effect of transplantectomy among those with early graft loss was due to a decrease in death with a functioning graft rather than an improvement in death-censored graft survival. It is speculated that there is a complex association between a retained failed graft and cardiovascular disease. In contrast to early graft loss, leaving the graft *in situ* in patients with late graft loss was shown have some protective effect on a repeat transplant, possibly related to development of tolerance and acceptance of a repeat transplant in the presence of donor antigen. Alternatively, it is suggested that if symptomatic immunological responses prompted a transplantectomy, then primary graft nephrectomy is simply a marker of high immunological risk for repeat transplant failure.

The potential risks and benefits of nephrectomy of a failed graft and its impact on a repeat transplant are summarized in Table 3.

Transplantectomy in patients with graft loss due to BK nephropathy: While some centers advocate graft nephrectomy prior to repeat transplant in patients with graft loss due to BK nephropathy (BKN), re-allograft transplant can be safely performed without original allograft nephrectomy but preferably following BK viral clearance[44]. Nonetheless, successful re-allograft transplant in the setting of severe viremia without concomitant nephrectomy of the allograft in a patient with graft failure due to BKN can be achieved. The patient is a 65-year-old woman who underwent urgent combined liver and repeat kidney allograft transplant due to fulminant hepatic failure and kidney graft failure due to BKN. She received no induction therapy and was maintained on low-dose tacrolimus and prednisone dual therapy. At the time of transplant, plasma BK PCR was 946000 copies/mL. Three months after transplant

plasma BK was undetectable and remained undetectable at 15 mo follow-up (unpublished observation).

Impact of transplantectomy on future retransplantation: The authors' perspectives

The variable and even conflicting results on the impact of transplantectomy on future reallograft transplantation may reflect a multitude of potential contributing factors including but not limited to institution dependent practice on indications for nephrectomy following a failed graft, differences in study design and immunosuppressive withdrawal protocols, donor and recipient demographics, recipient comorbid conditions, era of transplantation, time on dialysis prior to a repeat transplant, the causes of prior graft loss, donor type (living *vs* deceased), quality and HLA-matching of subsequent allograft, alllosensitization associated with blood transfusion, and pre-existing DSA with or without complement-fixing DSA at the time of transplantation, among others. Recent studies have shown that DSA with the ability to bind to C1q and activate complement are associated with greater risk of acute rejection and graft loss than non-complement fixing DSA[45].

While it remains unclear whether transplantectomy after late graft failure has a salutary or harmful effect on a repeat transplant, graft intolerance syndrome refractory to medical treatment is an indication for transplantectomy. In patients with multiple retained failed allografts, graft nephrectomy prior to retransplantation may also be inevitable. Monitoring PRA levels and HLA class Ⅰ/Ⅱ alloantibodies (using Luminex single-antigen assays) prior to and after graft nephrectomy as well as before retransplantation may be invaluable in guiding immunosuppression in re-allograft transplant recipients. In recent years various desensitization protocols have allowed highly sensitized patients to undergo successful retransplantation. Although no consensus exists, graft nephrectomy in patients with erythropoietin resistance and refractory anemia or hypoalbuminemia attributed to the failed allograft may be justifiable. Nonetheless, the decision to perform transplantectomy should be individualized. Effort to reduce cardiovascular and infectious complications undoubtedly improves clinical outcomes after reallograft transplantation whether or not nephrectomy is performed.

MANAGEMENT OF PATIENTS WITH A FAILED KIDNEY TRANSPLANT: THE AUTHORS'OPINION

Clinical studies to support or refute early *vs* late reinitiation of dialysis in patients with a failed kidney transplant are currently lacking. In the authors' opinion, reinitiation of dialysis should not be based solely on an absolute level of residual kidney function. Nonetheless, dialysis reinitiation when eGFR reaches 6-9 mL/min or less seems reasonable. In patients with higher level of residual kidney function, dialysis reinitiation should be based on clinical or laboratory parameters or both. Similar to the nontransplant settings, clinical indications may include symptomatic uremia, volume overload or hyperkalemia refractory to medical treatment, or malnourishment among others. In patients with a failed transplant and significant comorbid conditions such as long-standing diabetes with its associated micro- and macrovascular complications, or infectious or urological complications, weaning of immunosuppression and early return to dialysis seem justifiable.

Although evidence-based recommendations are lacking, continuation of low-dose immunosuppression seems appropriate in pre-dialysis patients and in those with symptomatic rejection to serve as a bridge to allograft nephrectomy. Maintenance low-dose immunosuppression may also be beneficial in patients with anticipated living donor re-allograft transplant or those with residual urine output greater than 0.5 to 1 liter a day. Nevertheless, in the latter group, immunosuppression withdrawal should be considered in high risk patients or those with significant comorbid conditions. These include older age, obesity, diabetes mellitus, neurogenic bladder, recurrent episodes of urinary tract infections or urosepsis, or history of cancers, among others. Proposed algorithm for the management of immunosuppression after allograft loss is shown in Figure 1. Although immunosuppression withdrawal protocols differ among centers, most clinicians advocate immediate discontinuation of antimetabolite (mycophenolate mofetil/mycophenolic acid or azathioprine). Cyclosporine or tacrolimus is generally weaned over several weeks and prednisone over three to six months. At the authors' institution, antimetabolite is discontinued upon return to dialysis, calcineurin inhibitors are weaned over four to six weeks, and prednisone dose is decreased by 1 mg/month until discontinued. Proposed immunosuppression weaning protocols are shown in Table 4.

Transplantectomy is usually performed and immunosuppression rapidly tapered when graft loss occurs within one year after transplant. Whether patients with early graft loss requiring transplantectomy (particularly those with a live donor and those anticipated to have a relatively short wait time after early relisting) benefit from continuation of immunosuppression to minimize allosensitization warrant further exploration. In addition, the duration and intensity of maintenance immunosuppression remain to be defined. At the authors' institution, the graft is usually left in place when graft loss occurs more than one year after transplant. Transplantectomy is generally performed in patients with graft intolerance syndrome or those requiring space for retransplantation. In patients with clinical signs or symptoms suggestive of a chronic inflammatory state, transplantectomy may be considered at the discretion of the treating physician. More importantly, community nephrologists should remain vigilant to the early

Table 4 Suggested immunosuppression withdrawal protocols based on maintenance therapy

CNI + antimetabolite[a] + prednisone	CNI + mTOR inh + prednisone	mTOR inh + prednisone
Discontinue antimetabolite at initiation of dialysis	Discontinue mTOR inh at initiation of dialysis	Taper mTOR inh over 4-6 wk[b]
Taper CNI over 4-6 wk[b]	Taper CNI over 4-6 wk[b]	Maintain same steroid dose at initiation of dialysis x 2-4 wk, then taper by 1 mg/mo (starting from 5 mg daily) until off
Maintain same steroid dose at initiation of dialysis x 2-4 wk, then taper by 1 mg/mo (starting from 5 mg daily) until off	Maintain same steroid dose at initiation of dialysis x 2-4 wk, then taper by 1 mg/mo (starting from 5 mg daily) until off	

[a]Mycophenolate Mofetil (Cellcept®) or Mycophenolic Acid (Myfortic®) or Azathioprine (Imuran®); [b]Taper can be done over a shorter period in slow chronic progressive graft failure but over a longer period when graft failure occurred following recent acute rejection episodes. CNI: Calcineurin inhibitor; mTOR inh: Mammalian target of rapamycin inhibitor.

Table 5 Absolute and relative indications for transplantectomy

Absolute indications (commonly accepted)	Relative indications (controversial)
Primary nonfunction	The presence of hematologic or biochemical markers of the chronic inflammatory state
Hyperacute rejection	Erythropoietin resistance anemia
Early recalcitrant acute rejection	Elevated ferritin level
Early graft loss (generally defined as graft loss within the first year)	Elevated C reactive protein
Arterial or venous thrombosis	Elevated erythrocyte sedimentation rate
Graft intolerance syndrome	Low prealbumin/albumin
Recurrent urinary tract infections or sepsis/urosepsis	Graft loss due to BK nephropathy and high level BK viremia (see text)
Multiple retained failed transplants prior to a repeat transplant	

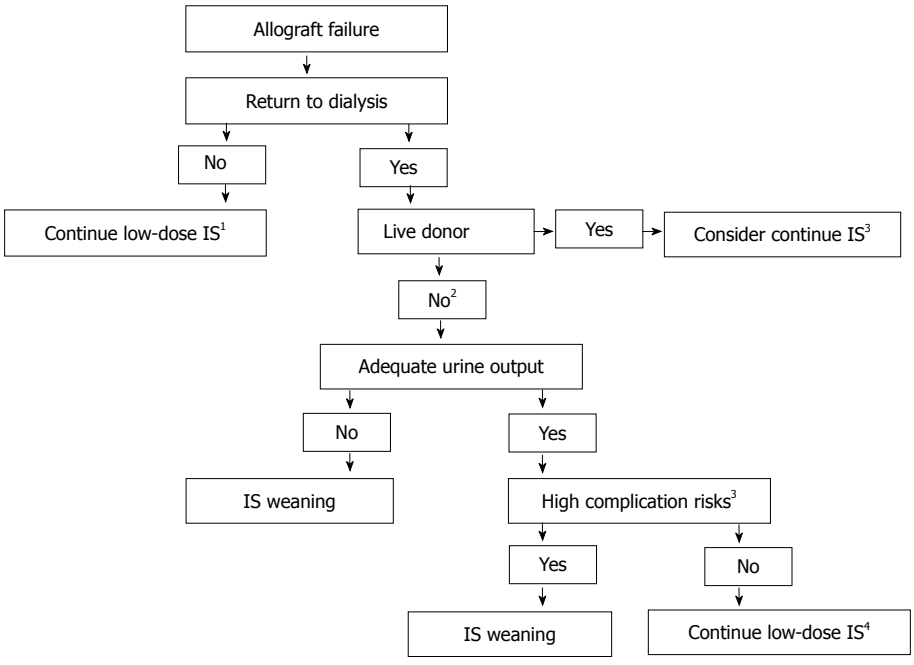

Figure 1 Suggested algorithm for the management of immunosuppression after allograft failure. [1]Continue antimetabolite and low-dose prednisone (usually 5 mg daily), calcineurin inhibitor dose reduction (or mTOR inh dose reduction if used as based-therapy); [2]No live donor or not a re-allograft candidate; [3]See text; [4]Usually prednisone 5 mg daily ± low-dose calcineurin inhibitor (or low-dose mTOR inh if used as based-therapy). IS: Immunosuppression; mTOR inh: Mammalian target of rapamycin inhibitor; ± with or without.

recognition of signs and symptoms of an infected or acutely rejecting allograft for early medical treatment and prevention of emergent transplantectomy given the increased morbidity and mortality associated with the latter. In patients with graft failure due to recent episodes of late acute rejection, gradual weaning of immunosupression is advisable to prevent graft intolerance syndrome and obviate the need to perform urgent transplantectomy. In patients with graft loss due to BK nephropathy, repeat transplant can be safely

performed without prior graft nephrectomy but preferably following BK viral clearance. Suggested absolute and relative indications for graft nephrectomy are shown in Table 5.

REFERENCES

1 **Gill JS**, Abichandani R, Kausz AT, Pereira BJ. Mortality after kidney transplant failure: the impact of non-immunologic factors. *Kidney Int* 2002; **62**: 1875-1883 [PMID: 12371992]
2 **Pham PT**, Pham PC. Immunosuppressive management of dialysis patients with recently failed transplants. *Semin Dial* 2011; **24**: 307-313 [PMID: 21564300]
3 **Molnar MZ**, Ichii H, Lineen J, Foster CE, Mathe Z, Schiff J, Kim SJ, Pahl MV, Amin AN, Kalantar-Zadeh K, Kovesdy CP. Timing of return to dialysis in patients with failing kidney transplants. *Semin Dial* 2013; **26**: 667-674 [PMID: 24016076]
4 **Beddhu S**, Samore MH, Roberts MS, Stoddard GJ, Ramkumar N, Pappas LM, Cheung AK. Impact of timing of initiation of dialysis on mortality. *J Am Soc Nephrol* 2003; **14**: 2305-2312 [PMID: 12937307]
5 **Cooper BA**, Branley P, Bulfone L, Collins JF, Craig JC, Fraenkel MB, Harris A, Johnson DW, Kesselhut J, Li JJ, Luxton G, Pilmore A, Tiller DJ, Harris DC, Pollock CA. A randomized, controlled trial of early versus late initiation of dialysis. *N Engl J Med* 2010; **363**: 609-619 [PMID: 20581422]
6 **Scialla JJ**, Liu J, Crews DC, Guo H, Bandeen-Roche K, Ephraim PL, Tangri N, Sozio SM, Shafi T, Miskulin DC, Michels WM, Jaar BG, Wu AW, Powe NR, Boulware LE. An instrumental variable approach finds no associated harm or benefit with early dialysis initiation in the United States. *Kidney Int* 2014; **86**: 798-809 [PMID: 24786707]
7 **Molnar MZ**, Streja E, Kovesdy CP, Hoshino J, Hatamizadeh P, Glassock RJ, Ojo AO, Kalantar-Zadeh K. Estimated glomerular filtration rate at reinitiation of dialysis and mortality in failed kidney transplant recipients. *Nephrol Dial Transplant* 2012; **27**: 2913-2921 [PMID: 22523118]
8 **Shemin D**, Bostom AG, Laliberty P, Dworkin LD. Residual renal function and mortality risk in hemodialysis patients. *Am J Kidney Dis* 2001; **38**: 85-90 [PMID: 11431186]
9 **Bargman JM**, Thorpe KE, Churchill DN. Relative contribution of residual renal function and peritoneal clearance to adequacy of dialysis: a reanalysis of the CANUSA study. *J Am Soc Nephrol* 2001; **12**: 2158-2162 [PMID: 11562415]
10 **Jassal SV**, Lok CE, Walele A, Bargman JM. Continued transplant immunosuppression may prolong survival after return to peritoneal dialysis: results of a decision analysis. *Am J Kidney Dis* 2002; **40**: 178-183 [PMID: 12087576]
11 **Perl J**, Dong J, Rose C, Jassal SV, Gill JS. Is dialysis modality a factor in the survival of patients initiating dialysis after kidney transplant failure? *Perit Dial Int* 2013; **33**: 618-628 [PMID: 24084843]
12 **Elmahi N**, Csongrádi E, Kokko K, Lewin JR, Davison J, Fülöp T. Residual renal function in peritoneal dialysis with failed allograft and minimum immunosuppression. *World J Transplant* 2013; **3**: 26-29 [PMID: 24175204]
13 **Scornik JC**, Kriesche HU. Human leukocyte antigen sensitization after transplant loss: timing of antibody detection and implications for prevention. *Hum Immunol* 2011; **72**: 398-401 [PMID: 21354453]
14 **Augustine JJ**, Woodside KJ, Padiyar A, Sanchez EQ, Hricik DE, Schulak JA. Independent of nephrectomy, weaning immunosuppression leads to late sensitization after kidney transplant failure. *Transplantation* 2012; **94**: 738-743 [PMID: 22955228]
15 **Del Bello A**, Congy-Jolivet N, Sallusto F, Guilbeau-Frugier C, Cardeau-Desangles I, Fort M, Esposito L, Guitard J, Cointault O, Lavayssière L, Nogier MB, Blancher A, Rostaing L, Kamar N. Donor-specific antibodies after ceasing immunosuppressive therapy, with or without an allograft nephrectomy. *Clin J Am Soc Nephrol* 2012; **7**: 1310-1319 [PMID: 22626959]
16 **Del Bello A**, Congy N, Sallusto F, Cardeau-Desangles I, Fort M, Esposito L, Guitard J, Cointault O, Lavayssière L, Nogier MB, Game X, Blancher A, Rostaing L, Kamar N. Anti-human leukocyte antigen immunization after early allograft nephrectomy. *Transplantation* 2012; **93**: 936-941 [PMID: 22361471]
17 **Woodside KJ**, Schirm ZW, Noon KA, Huml AM, Padiyar A, Sanchez EQ, Sarabu N, Hricik DE, Schulak JA, Augustine JJ. Fever, infection, and rejection after kidney transplant failure. *Transplantation* 2014; **97**: 648-653 [PMID: 24637864]
18 **Madore F**, Hébert MJ, Leblanc M, Girard R, Bastien E, Morin M, Beaudry C, Boucher A, Dandavino R. Determinants of late allograft nephrectomy. *Clin Nephrol* 1995; **44**: 284-289 [PMID: 8605707]
19 **Verresen L**, Vanrenterghem Y, Waer M, Hauglustaine D, Michielsen P. Corticosteroid withdrawal syndrome in dialysis patients. *Nephrol Dial Transplant* 1988; **3**: 476-477 [PMID: 3140138]
20 **Smak Gregoor PJ**, Zietse R, van Saase JL, op de Hoek CT, IJzermans JN, Lavrijssen AT, de Jong GM, Kramer P, Weimar W. Immunosuppression should be stopped in patients with renal allograft failure. *Clin Transplant* 2001; **15**: 397-401 [PMID: 11737116]
21 **Kiberd BA**, Belitsky P. The fate of the failed renal transplant. *Transplantation* 1995; **59**: 645-647 [PMID: 7878774]
22 **Smak Gregoor PJ**, van Saase JL, vd Ingh HF, Weimar W, Kramer P. Disseminated histoplasmosis in a haemodialysis patient on immunosuppression after graft failure. *Nephrol Dial Transplant* 1996; **11**: 542-544 [PMID: 8671831]
23 **Dantal J**, Hourmant M, Cantarovich D, Giral M, Blancho G, Dreno B, Soulillou JP. Effect of long-term immunosuppression in kidney-graft recipients on cancer incidence: randomised comparison of two cyclosporin regimens. *Lancet* 1998; **351**: 623-628 [PMID: 9500317]
24 **Vajdic CM**, van Leeuwen MT, Webster AC, McCredie MR, Stewart JH, Chapman JR, Amin J, McDonald SP, Grulich AE. Cutaneous melanoma is related to immune suppression in kidney transplant recipients. *Cancer Epidemiol Biomarkers Prev* 2009; **18**: 2297-2303 [PMID: 19622722]
25 **Stewart JH**, Vajdic CM, van Leeuwen MT, Amin J, Webster AC, Chapman JR, McDonald SP, Grulich AE, McCredie MR. The pattern of excess cancer in dialysis and transplantation. *Nephrol Dial Transplant* 2009; **24**: 3225-3231 [PMID: 19589786]
26 **van Leeuwen MT**, Webster AC, McCredie MR, Stewart JH, McDonald SP, Amin J, Kaldor JM, Chapman JR, Vajdic CM, Grulich AE. Effect of reduced immunosuppression after kidney transplant failure on risk of cancer: population based retrospective cohort study. *BMJ* 2010; **340**: c570 [PMID: 20150194]
27 **Johnston O**, Rose C, Landsberg D, Gourlay WA, Gill JS. Nephrectomy after transplant failure: current practice and outcomes. *Am J Transplant* 2007; **7**: 1961-1967 [PMID: 17617860]
28 **Minson S**, Muñoz M, Vergara I, Mraz M, Vaughan R, Rees L, Olsburgh J, Calder F, Shroff R. Nephrectomy for the failed renal allograft in children: predictors and outcomes. *Pediatr Nephrol* 2013; **28**: 1299-1305 [PMID: 23605376]
29 **Sener A**, Khakhar AK, Nguan CY, House AA, Jevnikar AM, Luke PP. Early but not late allograft nephrectomy reduces allosensitization after transplant failure. *Can Urol Assoc J* 2011; **5**: E142-E147 [PMID: 21388588]
30 **López-Gómez JM**, Pérez-Flores I, Jofré R, Carretero D, Rodríguez-Benitez P, Villaverde M, Pérez-García R, Nassar GM, Niembro E, Ayus JC. Presence of a failed kidney transplant in patients who are on hemodialysis is associated with chronic inflammatory state and erythropoietin resistance. *J Am Soc Nephrol* 2004; **15**: 2494-2501 [PMID: 15340000]
31 **Ayus JC**, Achinger SG. At the peril of dialysis patients: ignoring the failed transplant. *Semin Dial* 2005; **18**: 180-184 [PMID: 15934958]
32 **Ayus JC**, Achinger SG, Lee S, Sayegh MH, Go AS. Transplant nephrectomy improves survival following a failed renal allograft. *J Am Soc Nephrol* 2010; **21**: 374-380 [PMID: 19875809]
33 **Akoh JA**. Transplant nephrectomy. *World J Transplant* 2011; **1**: 4-12 [PMID: 24175187]
34 **Sharma DK**, Pandey AP, Nath V, Gopalakrishnan G. Allograft nephrectomy--a 16-year experience. *Br J Urol* 1989; **64**: 122-124 [PMID: 2670045]
35 **Knight MG**, Tiong HY, Li J, Pidwell D, Goldfarb D. Transplant ne-

phrectomy after allograft failure is associated with allosensitization. *Urology* 2011; **78**: 314-318 [PMID: 21676445]
36 **van den Berg-Loonen EM**, Billen EV, Voorter CE, van Heurn LW, Claas FH, van Hooff JP, Christiaans MH. Clinical relevance of pretransplant donor-directed antibodies detected by single antigen beads in highly sensitized renal transplant patients. *Transplantation* 2008; **85**: 1086-1090 [PMID: 18431226]
37 **Marrari M**, Duquesnoy RJ. Detection of donor-specific HLA antibodies before and after removal of a rejected kidney transplant. *Transpl Immunol* 2010; **22**: 105-109 [PMID: 20035868]
38 **Adeyi OA**, Girnita AL, Howe J, Marrari M, Awadalla Y, Askar M, Martell J, Zeevi A, Shapiro R, Nalesnik M, Randhawa P, Demetris AJ, Duquesnoy RJ. Serum analysis after transplant nephrectomy reveals restricted antibody specificity patterns against structurally defined HLA class I mismatches. *Transpl Immunol* 2005; **14**: 53-62 [PMID: 15814283]
39 **Sumrani N**, Delaney V, Hong JH, Daskalakis P, Sommer BG. The influence of nephrectomy of the primary allograft on retransplant graft outcome in the cyclosporine era. *Transplantation* 1992; **53**: 52-55 [PMID: 1733085]
40 **Abouljoud MS**, Deierhoi MH, Hudson SL, Diethelm AG. Risk factors affecting second renal transplant outcome, with special reference to primary allograft nephrectomy. *Transplantation* 1995; **60**: 138-144 [PMID: 7624955]
41 **Schleicher C**, Wolters H, Kebschull L, Anthoni C, Suwelack B, Senninger N, Palmes D, Mersfeld B. Impact of failed allograft nephrectomy on initial function and graft survival after kidney retransplantation. *Transpl Int* 2011; **24**: 284-291 [PMID: 21155899]
42 **Ahmad N**, Ahmed K, Mamode N. Does nephrectomy of failed allograft influence graft survival after re-transplantation? *Nephrol Dial Transplant* 2009; **24**: 639-642 [PMID: 18852188]
43 **Surga N**, Viart L, Wetzstein M, Mazouz H, Collon S, Tillou X. Impact of renal graft nephrectomy on second kidney transplant survival. *Int Urol Nephrol* 2013; **45**: 87-92 [PMID: 23328966]
44 **Geetha D**, Sozio SM, Ghanta M, Josephson M, Shapiro R, Dadhania D, Hariharan S. Results of repeat renal transplantation after graft loss from BK virus nephropathy. *Transplantation* 2011; **92**: 781-786 [PMID: 21836535]
45 **Sutherland SM**, Chen G, Sequeira FA, Lou CD, Alexander SR, Tyan DB. Complement-fixing donor-specific antibodies identified by a novel C1q assay are associated with allograft loss. *Pediatr Transplant* 2012; **16**: 12-17 [PMID: 22093755]

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REVIEW

Novel biomarkers of acute kidney injury: Evaluation and evidence in urologic surgery

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Abstract

Patients undergoing urologic surgery are at risk of acute kidney injury (AKI) and consequently long-term deterioration in renal function. AKI is further associated with significantly higher odds of perioperative complications, prolonged hospital stay, higher mortality and costs. Therefore, better awareness and detection of AKI, as well as identification of AKI determinants in the urological surgery setting is warranted to pre-empt and mitigate further deterioration of renal function in patients at special risk. New consensus criteria provide precise definitions of diagnosis and description of the severity of AKI. However, they rely on serum creatinine (SCr), which is known to be an inaccurate marker of early changes in renal function. Therefore, several new urinary and serum biomarkers promise to address the gap associated with the use of SCr. Novel biomarkers may complement SCr measurement or most likely improve the diagnostic accuracy of AKI when used in combinations. However, novel biomarkers have to prove their clinical applicability, accuracy, and cost effectiveness prior to implementation into clinical practice. Most preferably, novel biomarkers should help to positively improve a patient's long-term renal functional outcomes. The purpose of this review is to discuss currently available biomarkers and to review their clinical evidence within urologic surgery settings.

Key words: Acute kidney injury; Urology; Outcome; Renal function; Biomarker; Surgery

Core tip: Patients undergoing renal surgery represent a unique population at risk of acute kidney injury (AKI). AKI is known to be associated with adverse perioperative

outcomes. Therefore, efforts are warranted to promote awareness for AKI. Novel biomarkers promise to improve early and accurate detection of AKI, which may help to provide better patients' outcomes. However, these biomarkers still have to prove their clinical effectiveness prior to their implementation into urologic surgery settings.

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INTRODUCTION

Urologic patients are at risk of acute kidney injury (AKI)[1-3]. A recent study evaluating procedure-dependent incidence of AKI in patients undergoing urologic surgery found that AKI was most frequently associated with partial/radical nephrectomy and nephroureterectomy (43.1%), transurethral resection of bladder tumor (15.3%), cystoprostatectomy (3.6%), ureteroscopic lithotripsy (3.6%), transurethral resection of the prostate (2.2%), radical prostatectomy (1.5%) and JJ-stent insertion (1.5%)[4].

Potentially reversible causes of AKI related to urologic surgery may be of pre- (*e.g.*, postoperative bleeding, sepsis) or post-renal (*e.g.*, urinary obstruction, dislocation of ureteric stent, anastomotic leak) origin. However, AKI observed in renal surgery patients is largely related to direct renal damage, resulting in a potentially irreversible decline of renal function. Although partial nephrectomy for renal cell carcinoma aims to preserve renal function, AKI following the direct removal of renal parenchyma and damage of the remaining tissue from hyperfiltration or ischemia is a commonly observed adverse event in these patients[5,6]. Besides the volume of preserved renal parenchyma, type and duration of ischemia during partial nephrectomy remain the most important modifiable factors for renal functional outcome[7].

Ischemic renal injury leads to a robust inflammatory response within the kidney, but also extrarenal manifestations have been observed[8-10]. Furthermore, the impact of renal ischemia-reperfusion injury on tumor propagation, malignant progression, and resistance to therapy is a topic of current investigations[11,12]. In addition, there is evidence demonstrating an impact of postoperative AKI on adverse surgical outcomes[13]. Indeed, AKI is associated with higher complication rates, longer hospital stays, increased mortality, and therefore greater utilization of health care resources and associated costs[14,15]. As patients undergoing urologic oncologic surgery often present with (unknown) pre-existing chronic kidney disease (CKD) at the time of surgery[16,17] an additional perioperative episode of AKI may contribute to worse renal recovery, long-term renal function deterioration and progression of CKD[3,18]. Consequently, urologists need to seek out the risk factors for AKI, identify the present signs and foresee its impact on the perioperative outcome of their patients[13].

While there are excellent reviews highlighting the most promising urinary and serum biomarkers of AKI[19,20], the purpose of this review is to discuss currently available biomarkers and to review their clinical evidence within urologic surgery settings.

DATA ACQUISITION

A non-systematic PubMed/Medline literature search was performed to identify original articles, review articles, and editorials evaluating AKI biomarkers in urologic surgery using the keywords "acute kidney injury, biomarkers, surgery, urology," of the last 3 years (May 30, 2001 to July 31, 2014). The literature search was restricted to English language and availability of full text.

RESULTS

Definition and diagnosis of acute kidney injury

Due to a lack of consensus on the definition of acute renal failure, a wide variation exists in estimates of disease prevalence and mortality[15]. Currently, "AKI" is defined as an abrupt deterioration of kidney function and includes a spectrum ranging from minor renal functional impairment to acute renal failure requiring renal replacement therapy. The Risk, Injury, Failure, Loss, and End-stage kidney disease (RIFLE) staging criteria was the first consensus definition for AKI[21], followed by the Acute Kidney Injury Network (AKIN) classification, which defines AKI as an absolute increase in the serum creatinine (SCr) concentration of $\geqslant$ 0.3 mg/dL from baseline within 48 h[22]. More recently, the Kidney Disease/Improving Global Outcomes (KDIGO) group revised the definition of AKI, retaining AKIN staging criteria by classifying patients according to changes in SCr and urine output[23]. RIFLE, AKIN and KDIGO definitions have emphasized on the non-negligible incidence of AKI and its long-term adverse outcomes[21-23].

Biomarkers of acute kidney injury

Serum creatinine: SCr, is the gold-standard marker for renal function. However, SCr concentrations can be affected by age, gender, and racial differences of body mass as well as dietary factors and volume status[24]. In general, equations that estimate renal function, such as the Modification of Diet in Renal Disease or the Chronic Kidney Disease Epidemiology Collaboration equations[25,26], attempt to overcome the relative inaccuracy of SCr by including these patient characteristics to estimate glomerular filtration rate

Table 1 Baseline reference values of novel biomarkers of acute kidney injury obtained from different studies

Biomarker	Injury	Source	Test	Unit	Healthy controls (range)
Cystatin C	Proximal tubule injury	Serum	Nephelometric immunoassay/ELISA	mg/L	0.53-0.95[38] 0. 85 ± 0.21[39]
		Urine	Nephelometric immunoassay/ELISA	mg/L	0.05[1]-0.28[37] 0.02-0.11[96]
NGAL	Ischemia and nephrotoxins	Serum	ELISA	ng/mL	86.3 ± 43.0 (men)[54] 88.9 ± 38.2 (women)[54] 56.71 ± 17.57[39] 1.7 ± 0.5[55] 0.4-100[56]
		Urine	ELISA	ng/mL	5.7-17.7[55] 11.94 ± 8.09[39] 0.8-28.9 (men)[96] 1.9-316.7 (women)[96]
KIM-1	Ischemia and nephrotoxins	Urine	ELISA	pg/mL	59-2146[70] 395.1 ± 398.8[39] 31.0-1000.0[56] 31.0-1736.5[96]
IL-18	Toxic, delayed graft function	Urine	ELISA	pg/mL	1.4-1.8[80] 3.0-108.6[96] 6.2-311.1[96]
L-FABP	Ischemia and nephrotoxins	Urine	ELISA	ng/mL μg/gCr	3-400[83] 5.67 (2.74-8.21)[87]
NAG	Tubule injury	Urine	Colorimetry	U/g	0.75-0.90 U/g[95] 1.06 ± 0.1 U/g (children)[90]

[1]Lower reference values are not presented due to the detection limit of 0.05 mg/L. NGAL: Neutrophil gelatinase-associated lipocalin; KIM-1: Kidney injury molecule-1; IL-18: Interleukin-18; L-FABP: Liver-type fatty acid binding protein; NAG: N-acetyl-β-D-glucosaminidase; ELISA: Enzyme-linked immunosorbent assay; U: Unit.

(GFR)[27]. Nonetheless SCr is primarily a marker of glomerular function, and SCr-based measurements may be inaccurate in detecting an abrupt decline in renal function, as the functional reserve of the remaining healthy nephrons prevents a significant rise in SCr until 50% of nephrons are lost[28,29]. Furthermore, the early phase of AKI is accompanied with few symptoms or may even be asymptomatic. Thus, it is critical to note that even if the SCr-based estimation of renal function is "normal", loss of renal reserve may already have begun.

Consequently, recent research has focused on novel biomarkers that are directly related to the underlying renal injury and may diagnose AKI more expeditiously and accurately, while concurrently predicting its severity[30,31]. Most perioperative studies on AKI have been performed in the setting of cardiac surgery. However, as the awareness of AKI is increasing, other surgical specialties are evaluating this adverse outcome as well[32,33]. Additional biomarkers of AKI to rely on would be preferable especially in urologic high-risk patients (*e.g.*, renal surgery, pre-existing CKD). In fact, several promising serum and urinary biomarkers are now available including serum and urinary Cystatin C (sCysC and uCysC), neutrophil gelatinase-associated lipocalin (sNGAL and uNGAL), and urinary Kidney Injury Molecule 1 (uKIM-1), Interleukin-18 (uIL-18), Liver-type fatty acid binding protein (uL-FABP) and N-acetyl-β-D-glucosaminidase (uNAG)[34]. However, these biomarkers are still under investigation: baseline values are often obtained from healthy volunteers and optimal cut-off values to define AKI need to be determined (Table 1). Some of these biomarkers already demonstrated additional prognostic value in the urologic surgery setting (Table 2), whereas others have yet to prove their clinical utility.

Novel biomarkers of acute kidney injury

Serum and urinary cystatin C: CysC is a low-molecular weight protein that is freely filtered across the glomerular membrane and in consequence less reliant on age, sex, race and muscle mass, compared to SCr[35]. Moreover, although CyC is not normally detected in the urine, it has been found in the urine of patients with tubular disease, suggesting its putative role as a marker of renal tubular damage[36]. Nephelometric measurements of CysC have upper reference values of 0.28 mg/L[37] in the urine and range between 0.53-0.95 mg/L in the serum of healthy individuals[38,39].

CysC has been proposed as a complementary or possibly marker of baseline renal function[35,40]. Although sCysC measurement is currently 10 times more expensive than SCr, it is implemented in routine renal function measurement of pediatric patients and used to monitor kidney transplant patients[41-43]. Furthermore, there is evidence suggesting that an elevation of sCysC predates minor decreases in GFR 1 to 2 d prior to symptoms, SCr elevation and/or renal function decline[40,44,45]. Early elevations of uCysC levels were significant predictors of AKI after elective cardiac surgery[46], and are correlated

Table 2 Biomarkers of acute kidney injury evaluated within urologic surgery settings

Ref.	Biomarker	Source	Cohort	Surgical setting	Outcome	Comparison	Time
Langetepe *et al*[65]	CysC, NGAL, KIM-1 Cr	Urine Serum	31 RCC patients	PN, RN	Increased values of CysC, NGAL, KIM-1 NGAL significant correlation to Cr No advantage for earlier detection of renal injury	Pre-/postoperative	24 h after surgery
Sprenkle *et al*[63]	NGAL	Urine	PN: 88 patients, RN: 32 patients, thoracic surgery: 42 patients	PN, RN (warm or cold ischemia)	No association between postoperative NGAL and any AKI AKI was not significantly associated with increased NGAL in PN patients No correlation with ischemia time Patients with eGFR < 60 mL/min per 1.73 m^2 had higher NGAL postoperatively than those with an eGFR > 60 mL/min per 1.73 m^2	PN/RN /thoracic surgery patients	4, 8, 12, 24 h post surgery
Parekh *et al*[62]	Cr, NGAL, CysC NGAL, LFABP, NAG, KIM-1, IL-18	Serum Urine, (renal biopsy)	20 patients with renal mass	PN (warm or cold ischemia)	Cr was significantly increased at 24 h CysC was not significantly changed at 2 or 24 h Significant increases serum NGAL at 2 and 24 h, increase of NGAL with increased ischemia time, no relation to peak Cr or morphology-score Early increases of L-FABP Early increase of NAG Increased NGAL at all times KIM-1maximally increased at 24 h IL-18 was increased at all time points	Correlation to renal biopsies (pre-, intra. postoperative)	2 or 24 h after surgery
Schmid *et al*[50]	Cr, CysC	Serum	31 RCC patients	PN, RN	Postoperative Cysc and Cr elevations similarly predict renal function deterioration CysC-based GFR appears superior to eGFR in "Cr-blind" area	Pre-/postoperative, 1 yr follow up	24 h, 1 yr after surgery
Xue *et al*[76]	Cr NGAL, KIM-1	Serum Urine	90 patients with obstructive uropathy	NA	KIM-1 and NGAL good accuracy for detecting AKI KIM-1 predicts the renal outcome 72 h postoperatively	Pre-/postoperative	4, 8, 12, 24, 48, 72 h after surgery
Cost *et al*[66]	NGAL	Urine (bladder and renal pelvis)	61 pediatric patients with ureteropelvic junction obstruction	Pyeloplasty	Significantly increased bladder NGAL Inverse correlation of bladder and renal pelvic NGAL levels with the differential renal function of the affected kidney	Healthy children	Intraoperative
Zekey *et al*[64]	Cr NGAL	Serum Urine	40 patients with kidney stones	SWL	No statistical Cr and urine NGAL levels	Before/after intervention	day 1, 2, 7 after intervention
Fahmy *et al*[74]	KIM-1, NAG	Urine	60 patients with kidney stones (50 SWL, 10 URS)	SWL, URS	KIM-1 values were increased in patients with kidney stones when compared with volunteers KIM-1 and NAG levels significantly increased post-SWL Poor kidney function was significantly associated with increased KIM-1 and NAG baseline and post-SWL No significant change in urinary KIM-1 and NAG concentrations before and after URS	Volunteers without kidney stones	2-3 h after intervention
Ng *et al*[82]	IL-18, NAG	Urine	206 patinets with renal stones	SWL	Increased IL-18 and NAG I slower shock wave delivery group	60 *vs* 120 shock waves/min	After intervention
Hatipoğlu *et al*[75]	KIM-1 (free radical production)	Urine	30 patients with kidney stones	SWL	Significant increase of KIM-1	Pre-/postoperative	2 h after intervention

PN: Partial nephrectomy; RN: Radical nephrectomy; NGAL: Neutrophil gelatinase-associated lipocalin; KIM-1: Kidney injury molecule-1; URS: Ureterorenoscopy; SWL: Shockwave lithotripsy; Cr: Creatinine; CysC: Cystatin C; LFABP: Liver fatty acid–binding protein; NAG: Nacetyl-b-D-glucosaminidase; eGFR: Estimated glomerular filtration rate; RCC: Renal cell carcinoma; NA: Not available.

with the need for renal replacement therapy in patients with acute tubular necrosis[47]. However, other studies were not able to corroborate these findings[37] and suggest that sCysC is unreliable in the context of

postrenal obstruction[48]. Yet, uCysC was shown to be independently associated with mortality in critically ill patients with AKI[49].

In patients undergoing partial or radical nephrectomy, elevations of both SCr and sCysC on postoperative day one predicted renal function deterioration one year after surgery, while sCysC correlated better to renal function estimates compared to SCr in the "SCr-blind" area[50].

Serum and urinary NGAL: Production of NGAL, a lipocalin protein involved in innate immunity by binding iron to limit bacterial growth[51], is upregulated following renal injury, and consequently detectable in serum and urine hours prior to functional changes[52,53]. sNGAL values in healthy individuals should be around 86.3 ng/mL in men and 88.9 ng/mL in women[39,54-56], but may increase > 10-fold in serum and > 100-fold in urine following an acute injury[57].

A meta-analysis of 19 observational studies including 2500 patients was performed to estimate the diagnostic and prognostic accuracy of NGAL for AKI detection and to establish the role of urinary and serum NGAL in the context of AKI[58]. Xin *et al*[59] showed that for patients undergoing cardiac surgery, an increase of sNGAL was not temporally different to the rise of SCr within 48 h after AKI, however uNGAL (and IL-18) significantly increased to a peak of 400 ng/mL within 2-4 h of AKI.

Induction of unilateral renal ischemia in animal models results in physiological changes of the ischemic and contralateral kidney, with a corresponding increase of uNGAL and decrease of renal function[60,61]. Parekh *et al*[62] studied the renal response to > 30 min of warm or cold clamp ischemia in patients undergoing partial nephrectomy and observed significant increases in sNGAL 2 and 24 h after surgery. While levels of all urinary biomarkers studied (NGAL, KIM-1, IL-18, NAG, L-FABP) increased 2 and/or 24 h after surgery, sCysC levels did not change significantly (SWL)[62]. Conversely, Sprenkle *et al*[63] did not observe increased uNGAL in partial nephrectomy patients within 24 h after surgery. Accordingly, no statistically significant change of uNGAL levels was observed in 40 nephrolithiasis patients treated with shock-wave lithotripsy[64]. Yet, our own data showed increased levels of uNGAL, KIM-1 and uCysC in 31 patients 24 h after partial or radical nephrectomy, but only uNGAL was correlated with SCr-based measurement of renal function[65]. Increased levels of uNGAL have also been obtained from bladder urine in children with ureteropelvic junction obstruction undergoing unilateral pyeloplasty[66]. Finally, uNGAL may serve as an early indicator for cisplatin nephrotoxicity[67], which may be useful for patients with muscle-invasive bladder undergoing neoadjuvant chemotherapy prior to radical cystectomy.

Urinary KIM-1: KIM-1 is a transmembrane glycoprotein undetectable in healthy kidney tissue, but it represents the most upregulated protein in proximal tubular cells after ischemic or nephrotoxic injury[68]. KIM-1 can be immediately detected in the urine following injury[69,70]. A strong correlation between immunohistochemical KIM-1 expression and tubular cell injury was shown in renal allograft biopsies of patients with active antibody-mediated transplant rejection[71], suggesting that KIM-1 is a reliable marker for tubular epithelial injury prior to elevated blood biochemical indexes and morphological changes. In addition, children with AKI following cardiac surgery demonstrated elevated uKIM-1 levels 12 h after surgery[72]. KIM-1 is measured in the urine by means of enzyme-linked immunosorbent assay, with normal values ranging between 59-2146 pg/mL in the healthy population[70,73].

A significant increase of uKIM-1 levels 2-3 h after SWL treatment[74,75] suggests direct ischemic damage and the release of free radicals. Both uKIM-1 and uNGAL demonstrated accuracy in detecting AKI among patients undergoing surgery for obstructive nephropathy; furthermore they might play a potential role in predicting postoperative renal recovery and long-term renal outcome[76,77].

Urinary IL-18: IL-18 is a pro-inflammatory cytokine that is activated in proximal tubule cells and excreted in the urine following a kidney injury. Increased expression of *IL-18* genes has been demonstrated after renal ischemic injury[78]. Animal models revealed that *IL-18* stimulates a positive feedback *via* IL-18 receptor during renal obstruction, which further stimulates IL-18 production and gene expression[79].

Initially described in the pediatric cardiac surgery setting, IL-18 the urine increased 6 h in after surgery, whereas SCr did not reveal AKI until 48-72 h after surgery[55]. Moreover, uIL-18 also increased significantly in adults and peaked at 600 pg/mL within 2-4 h after AKI[59]. Another study demonstrated an increase from 1.4 pg/mL to a peak of 234 pg/mL (about 25-fold) 12 h after cardiopulmonary bypass surgery in patients presenting AKI[55]. In patients with respiratory distress syndrome experiencing AKI, median uIL-18 was 104 pg/mL (range: 0 to 955 pg/mL), compared to 0 (range: 0 to 173 pg/mL) in control patients; IL-18 levels of > 100 pg/mL were associated with a 6.5-fold higher risk of AKI 24 h after hospitalization. Furthermore, higher level of uIL-18 (and serum IL-18) in ICU patients developing (dialysis-dependent) AKI was independently associated with mortality[80,81].

Finally, patients undergoing SWL showed a significant increase of uIL-18 (and uNAG) when treated with slower shock waves[82].

Urinary L-FABP: L-FABP is a 14-kDa protein expressed in proximal tubular epithelial cells. The urine of healthy individuals contains approximately 16 ng/mL L-FABP[83]. The gene responsible for L-FABP is associated with hypoxic stress. L-FABP binds unsaturated fatty acids and lipid peroxidation products during tissue injury from hypoxia[84]. Urinary excretion of L-FABP thus reflects stress within proximal tubular epithelial cells,

and its correlation with renal function deterioration has been reported with AKI following contrast nephropathy and cardiac surgery[83,85]. Although uL-FABP may be a promising biomarker for early detection of AKI, as demonstrated in an animal model of ischemia-reperfusion[86], or for prediction of the need for dialysis and in-hospital mortality[87], its value in urologic surgery warrants further investigation.

NAG: NAG, a tubular lysosomal brush border enzyme, is released into the urine following (reversible) renal proximal tubule injury. NAG is elevated in the urine of children with chronic renal obstruction[88], regardless of the grade of hydronephrosis[89], and following AKI[90,91]. Rat models with isolated blunt renal trauma showed increased uNAG in the early stage after injury[92]. However, the clinical utility of NAG remains limited as the urinary excretion of this enzyme is also increased in glomerular diseases such as diabetic nephropathy[93]. The combination of urinary L-FABP (high sensitivity) and uNAG or uNGAL (high specificity) may enhance the detection of early postoperative AKI in patients undergoing cardiac surgery[94,95].

CONCLUSION

A plethora of novel biomarkers for AKI have recently been described. Whereas sCysC, uCysC, sNGAL, uNGAL, uKIM-1 and uNAG have shown promise, we did not find convincing evidence for uIL-18 and uL-FABP. However, from a clinical perspective current use of these biomarkers in the urologic surgery setting is rare. Notable reasons behind this are the limited availability of assays, additional cost and the (currently) poor sensitivity and specificity demonstrated in urologic patients. Consequently, until now none of these biomarkers has been able to allow early detection of AKI in a way that would positively improve a patient's long-term outcomes and justify a regular implementation in specific urologic surgery settings. SCr remains the mainstay for evaluation of kidney function in urologic surgical patients. However, novel biomarkers may complement SCr measurement to indicate the need for urgent drainage or initiation of renoprotective measures. Moreover, it is likely that a combined use of these novel biomarkers will be needed to improve the diagnostic accuracy of AKI. Multiplex assays for simultaneous quantification of several biomarkers promise to overcome the flaws of single marker use and demonstrate the advantage of combinations reflecting different aspects of renal injury[96]. While these assays are currently more expensive compared to traditional SCr measurement, the hope is that the incremental diagnostic accuracy would offset costs by mitigating costly associated complications of AKI.

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REFERENCES

1 **Wang SJ**, Mu XN, Zhang LY, Liu QY, Jin XB. The incidence and clinical features of acute kidney injury secondary to ureteral calculi. *Urol Res* 2012; **40**: 345-348 [PMID: 21853241 DOI: 10.1007/s00240-011-0414-6]

2 **Kim MJ**, Bachmann A, Mihatsch MJ, Ruszat R, Sulser T, Mayr M. Acute renal failure after continuous flow irrigation in patients treated with potassium-titanyl-phosphate laser vaporization of prostate. *Am J Kidney Dis* 2008; **51**: e19-e24 [PMID: 18371525 DOI: 10.1053/j.ajkd.2007.11.031]

3 **Cho A**, Lee JE, Kwon GY, Huh W, Lee HM, Kim YG, Kim DJ, Oh HY, Choi HY. Post-operative acute kidney injury in patients with renal cell carcinoma is a potent risk factor for new-onset chronic kidney disease after radical nephrectomy. *Nephrol Dial Transplant* 2011; **26**: 3496-3501 [PMID: 21406544 DOI: 10.1093/ndt/gfr094]

4 **Caddeo G**, Williams ST, McIntyre CW, Selby NM. Acute kidney injury in urology patients: incidence, causes and outcomes. *Nephrourol Mon* 2013; **5**: 955-961 [PMID: 24693501 DOI: 10.5812/numonthly.12721]

5 **Brenner BM**, Lawler EV, Mackenzie HS. The hyperfiltration theory: a paradigm shift in nephrology. *Kidney Int* 1996; **49**: 1774-1777 [PMID: 8743495]

6 **Lane BR**, Babineau DC, Poggio ED, Weight CJ, Larson BT, Gill IS, Novick AC. Factors predicting renal functional outcome after partial nephrectomy. *J Urol* 2008; **180**: 2363-2368; discussion 2363-2368 [PMID: 18930264 DOI: 10.1016/j.juro.2008.08.036]

7 **Becker F**, Van Poppel H, Hakenberg OW, Stief C, Gill I, Guazzoni G, Montorsi F, Russo P, Stöckle M. Assessing the impact of ischaemia time during partial nephrectomy. *Eur Urol* 2009; **56**: 625-634 [PMID: 19656615 DOI: 10.1016/j.eururo.2009.07.016]

8 **Bonventre JV**, Zuk A. Ischemic acute renal failure: an inflammatory disease? *Kidney Int* 2004; **66**: 480-485 [PMID: 15253693 DOI: 10.1111/j.1523-1755.2004.761_2.x]

9 **Hoke TS**, Douglas IS, Klein CL, He Z, Fang W, Thurman JM, Tao Y, Dursun B, Voelkel NF, Edelstein CL, Faubel S. Acute renal failure after bilateral nephrectomy is associated with cytokine-mediated pulmonary injury. *J Am Soc Nephrol* 2007; **18**: 155-164 [PMID: 17167117 DOI: 10.1681/ASN.2006050494]

10 **Kramer AA**, Postler G, Salhab KF, Mendez C, Carey LC, Rabb H. Renal ischemia/reperfusion leads to macrophage-mediated increase in pulmonary vascular permeability. *Kidney Int* 1999; **55**: 2362-2367 [PMID: 10354283 DOI: 10.1046/j.1523-1755.1999.00460.x]

11 **Höckel M**, Vaupel P. Tumor hypoxia: definitions and current clinical, biologic, and molecular aspects. *J Natl Cancer Inst* 2001; **93**: 266-276 [PMID: 11181773]

12 **Skolarikos AA**, Papatsoris AG, Alivizatos G, Deliveliotis C. Molecular pathogenetics of renal cancer. *Am J Nephrol* 2006; **26**: 218-231 [PMID: 16733347 DOI: 10.1159/000093631]

13 **Schmid M**, Abd-El-Barr AE, Gandaglia G, Sood A, Olugbade K, Ruhotina N, Sammon JD, Varda B, Chang SL, Kibel AS, Chun FK, Menon M, Fisch M, Trinh QD. Predictors of 30-day acute kidney injury following radical and partial nephrectomy for renal cell carcinoma. *Urol Oncol* 2014; **32**: 1259-1266 [PMID: 25129142 DOI: 10.1016/j.urolonc.2014.05.002]

14 **Chertow GM**, Soroko SH, Paganini EP, Cho KC, Himmelfarb J, Ikizler TA, Mehta RL. Mortality after acute renal failure: models for prognostic stratification and risk adjustment. *Kidney Int* 2006; **70**: 1120-1126 [PMID: 16850028 DOI: 10.1038/sj.ki.5001579]

15 **Lameire NH**, Bagga A, Cruz D, De Maeseneer J, Endre Z, Kellum JA, Liu KD, Mehta RL, Pannu N, Van Biesen W, Vanholder R. Acute kidney injury: an increasing global concern. *Lancet* 2013; **382**: 170-179 [PMID: 23727171 DOI: 10.1016/S0140-6736(13)60647-9]

16 **Lane BR**, Poggio ED, Herts BR, Novick AC, Campbell SC. Renal function assessment in the era of chronic kidney disease: renewed emphasis on renal function centered patient care. *J Urol* 2009; **182**:

435-443; discussion 443-444 [PMID: 19524967 DOI: 10.1016/j.juro.2009.04.004]
17 **Schmid M**, Ravi P, Abd-El-Barr AE, Klap J, Sammon JD, Chang SL, Menon M, Kibel AS, Fisch M, Trinh QD. Chronic kidney disease and perioperative outcomes in urological oncological surgery. *Int J Urol* 2014; **21**: 1245-1252 [PMID: 25041641 DOI: 10.1111/iju.12563]
18 **Ali T**, Khan I, Simpson W, Prescott G, Townend J, Smith W, Macleod A. Incidence and outcomes in acute kidney injury: a comprehensive population-based study. *J Am Soc Nephrol* 2007; **18**: 1292-1298 [PMID: 17314324 DOI: 10.1681/ASN.2006070756]
19 **Lane BR**. Molecular markers of kidney injury. *Urol Oncol* 2013; **31**: 682-685 [PMID: 21723753 DOI: 10.1016/j.urolonc.2011.05.007]
20 **Slocum JL**, Heung M, Pennathur S. Marking renal injury: can we move beyond serum creatinine? *Transl Res* 2012; **159**: 277-289 [PMID: 22424431 DOI: 10.1016/j.trsl.2012.01.014]
21 **Bellomo R**, Ronco C, Kellum JA, Mehta RL, Palevsky P. Acute renal failure - definition, outcome measures, animal models, fluid therapy and information technology needs: the Second International Consensus Conference of the Acute Dialysis Quality Initiative (ADQI) Group. *Crit Care* 2004; **8**: R204-R212 [PMID: 15312219 DOI: 10.1186/cc2872]
22 **Mehta RL**, Kellum JA, Shah SV, Molitoris BA, Ronco C, Warnock DG, Levin A. Acute Kidney Injury Network: report of an initiative to improve outcomes in acute kidney injury. *Crit Care* 2007; **11**: R31 [PMID: 17331245 DOI: 10.1186/cc5713]
23 **KDIGO AKI Working Group**. KDIGO clinical practice guideline for acute kidney injury. *Kidney Int Suppl* 2012; **2**: 1-138
24 **Michels WM**, Grootendorst DC, Verduijn M, Elliott EG, Dekker FW, Krediet RT. Performance of the Cockcroft-Gault, MDRD, and new CKD-EPI formulas in relation to GFR, age, and body size. *Clin J Am Soc Nephrol* 2010; **5**: 1003-1009 [PMID: 20299365 DOI: 10.2215/CJN.06870909]
25 **Levey AS**, Coresh J, Greene T, Marsh J, Stevens LA, Kusek JW, Van Lente F. Expressing the Modification of Diet in Renal Disease Study equation for estimating glomerular filtration rate with standardized serum creatinine values. *Clin Chem* 2007; **53**: 766-772 [PMID: 17332152 DOI: 10.1373/clinchem.2006.077180]
26 **Levey AS**, Stevens LA, Schmid CH, Zhang YL, Castro AF, Feldman HI, Kusek JW, Eggers P, Van Lente F, Greene T, Coresh J. A new equation to estimate glomerular filtration rate. *Ann Intern Med* 2009; **150**: 604-612 [PMID: 19414839 DOI: 10.7326/0003-4819-150-9-200905050-00006]
27 **Levey AS**, Bosch JP, Lewis JB, Greene T, Rogers N, Roth D. A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation. Modification of Diet in Renal Disease Study Group. *Ann Intern Med* 1999; **130**: 461-470 [PMID: 10075613 DOI: 10.7326/0003-4819-130-6-199903160-00002]
28 **Hayslett JP**. Functional adaptation to reduction in renal mass. *Physiol Rev* 1979; **59**: 137-164 [PMID: 220646]
29 **Hostetter TH**, Olson JL, Rennke HG, Venkatachalam MA, Brenner BM. Hyperfiltration in remnant nephrons: a potentially adverse response to renal ablation. *J Am Soc Nephrol* 2001; **12**: 1315-1325 [PMID: 11373357]
30 **Coca SG**, Yalavarthy R, Concato J, Parikh CR. Biomarkers for the diagnosis and risk stratification of acute kidney injury: a systematic review. *Kidney Int* 2008; **73**: 1008-1016 [PMID: 18094679 DOI: 10.1038/sj.ki.5002729]
31 **Waikar SS**, Bonventre JV. Biomarkers for the diagnosis of acute kidney injury. *Nephron Clin Pract* 2008; **109**: c192-c197 [PMID: 18802367 DOI: 10.1159/000142928]
32 **Dedeoglu B**, de Geus HR, Fortrie G, Betjes MG. Novel biomarkers for the prediction of acute kidney injury in patients undergoing liver transplantation. *Biomark Med* 2013; **7**: 947-957 [PMID: 24266830 DOI: 10.2217/bmm.13.91]
33 **Sung WC**, Yu HP, Tsai YF, Chung PC, Lin CC, Lee WC. The ratio of plasma interleukin-18 is a sensitive biomarker for acute kidney injury after liver transplantation. *Transplant Proc* 2014; **46**: 816-817 [PMID: 24767355 DOI: 10.1016/j.transproceed.2013.09.055]
34 **Thomas AA**, Demirjian S, Lane BR, Simmons MN, Goldfarb DA, Subramanian VS, Campbell SC. Acute kidney injury: novel biomarkers and potential utility for patient care in urology. *Urology* 2011; **77**: 5-11 [PMID: 20599252 DOI: 10.1016/j.urology.2010.05.004]
35 **Roos JF**, Doust J, Tett SE, Kirkpatrick CM. Diagnostic accuracy of cystatin C compared to serum creatinine for the estimation of renal dysfunction in adults and children--a meta-analysis. *Clin Biochem* 2007; **40**: 383-391 [PMID: 17316593 DOI: 10.1016/j.clinbiochem.2006.10.026]
36 **Conti M**, Moutereau S, Zater M, Lallali K, Durrbach A, Manivet P, Eschwège P, Loric S. Urinary cystatin C as a specific marker of tubular dysfunction. *Clin Chem Lab Med* 2006; **44**: 288-291 [PMID: 16519600 DOI: 10.1515/CCLM.2006.050]
37 **Royakkers AA**, van Suijlen JD, Hofstra LS, Kuiper MA, Bouman CS, Spronk PE, Schultz MJ. Serum cystatin C-A useful endogenous marker of renal function in intensive care unit patients at risk for or with acute renal failure? *Curr Med Chem* 2007; **14**: 2314-2317 [PMID: 17896979]
38 **Croda-Todd MT**, Soto-Montano XJ, Hernández-Cancino PA, Juárez-Aguilar E. Adult cystatin C reference intervals determined by nephelometric immunoassay. *Clin Biochem* 2007; **40**: 1084-1087 [PMID: 17624320 DOI: 10.1016/j.clinbiochem.2007.05.011]
39 **Matys U**, Bachorzewska-Gajewska H, Malyszko J, Dobrzycki S. Assessment of kidney function in diabetic patients. Is there a role for new biomarkers NGAL, cystatin C and KIM-1? *Adv Med Sci* 2013; **58**: 353-361 [PMID: 24384771 DOI: 10.2478/v10039-012-0077-8]
40 **Dharnidharka VR**, Kwon C, Stevens G. Serum cystatin C is superior to serum creatinine as a marker of kidney function: a meta-analysis. *Am J Kidney Dis* 2002; **40**: 221-226 [PMID: 12148093 DOI: 10.1053/ajkd.2002.34487]
41 **Le Bricon T**, Thervet E, Benlakehal M, Bousquet B, Legendre C, Erlich D. Changes in plasma cystatin C after renal transplantation and acute rejection in adults. *Clin Chem* 1999; **45**: 2243-2249 [PMID: 10585359]
42 **Ayub S**, Zafar MN, Aziz T, Iqbal T, Khan S, Rizvi SA. Evaluation of renal function by cystatin C in renal transplant recipients. *Exp Clin Transplant* 2014; **12**: 37-40 [PMID: 24471722 DOI: 10.6002/ect.2013.0202]
43 **Fox JA**, Dudley AG, Bates C, Cannon GM. Cystatin C as a marker of early renal insufficiency in children with congenital neuropathic bladder. *J Urol* 2014; **191**: 1602-1607 [PMID: 24679869 DOI: 10.1016/j.juro.2013.09.093]
44 **Herget-Rosenthal S**, Marggraf G, Hüsing J, Göring F, Pietruck F, Janssen O, Philipp T, Kribben A. Early detection of acute renal failure by serum cystatin C. *Kidney Int* 2004; **66**: 1115-1122 [PMID: 15327406 DOI: 10.1111/j.1523-1755.2004.00861.x]
45 **Pirgakis KM**, Makris K, Dalainas I, Lazaris AM, Maltezos CK, Liapis CD. Urinary cystatin C as an early biomarker of acute kidney injury after open and endovascular abdominal aortic aneurysm repair. *Ann Vasc Surg* 2014; **28**: 1649-1658 [PMID: 24858592 DOI: 10.1016/j.avsg.2014.04.006]
46 **Koyner JL**, Bennett MR, Worcester EM, Ma Q, Raman J, Jeevanandam V, Kasza KE, O'Connor MF, Konczal DJ, Trevino S, Devarajan P, Murray PT. Urinary cystatin C as an early biomarker of acute kidney injury following adult cardiothoracic surgery. *Kidney Int* 2008; **74**: 1059-1069 [PMID: 18650797 DOI: 10.1038/ki.2008.341]
47 **Herget-Rosenthal S**, Poppen D, Hüsing J, Marggraf G, Pietruck F, Jakob HG, Philipp T, Kribben A. Prognostic value of tubular proteinuria and enzymuria in nonoliguric acute tubular necrosis. *Clin Chem* 2004; **50**: 552-558 [PMID: 14709451 DOI: 10.1373/clinchem.2003.027763]
48 **Hasslacher J**, Lehner GF, Joannidis M. Insufficient performance of serum cystatin C as a biomarker for acute kidney injury of postrenal etiology. *Intensive Care Med* 2012; **38**: 170-171 [PMID: 21965103 DOI: 10.1007/s00134-011-2384-0]
49 **Nejat M**, Pickering JW, Walker RJ, Westhuyzen J, Shaw GM, Frampton CM, Endre ZH. Urinary cystatin C is diagnostic of acute kidney injury and sepsis, and predicts mortality in the intensive care unit. *Crit Care* 2010; **14**: R85 [PMID: 20459852 DOI: 10.1186/cc9014]

50 **Schmid M**, Schwaiger B, Tenschert W, Shaffu M, Thabaz R, Langetepe J, Ahyai S, Eichelberg C, Fisch M, Chun F. Surveillance of renal function after (partial) tumor nephrectomy by Cystatin C-measurement. *J Urol* 2013; **189**: Supplement e1-e952

51 **Yang J**, Goetz D, Li JY, Wang W, Mori K, Setlik D, Du T, Erdjument-Bromage H, Tempst P, Strong R, Barasch J. An iron delivery pathway mediated by a lipocalin. *Mol Cell* 2002; **10**: 1045-1056 [PMID: 12453413 DOI: 10.1016/S1097-2765(02)00710-4]

52 **Mishra J**, Ma Q, Prada A, Mitsnefes M, Zahedi K, Yang J, Barasch J, Devarajan P. Identification of neutrophil gelatinase-associated lipocalin as a novel early urinary biomarker for ischemic renal injury. *J Am Soc Nephrol* 2003; **14**: 2534-2543 [PMID: 14514731 DOI: 10.1097/01.ASN.0000088027.54400.C6]

53 **Borregaard N**, Cowland JB. Neutrophil gelatinase-associated lipocalin, a siderophore-binding eukaryotic protein. *Biometals* 2006; **19**: 211-215 [PMID: 16718606 DOI: 10.1007/s10534-005-3251-7]

54 **Stejskal D**, Karpísek M, Humenanska V, Hanulova Z, Stejskal P, Kusnierova P, Petzel M. Lipocalin-2: development, analytical characterization, and clinical testing of a new ELISA. *Horm Metab Res* 2008; **40**: 381-385 [PMID: 18393169 DOI: 10.1055/s-2008-1062746]

55 **Parikh CR**, Mishra J, Thiessen-Philbrook H, Dursun B, Ma Q, Kelly C, Dent C, Devarajan P, Edelstein CL. Urinary IL-18 is an early predictive biomarker of acute kidney injury after cardiac surgery. *Kidney Int* 2006; **70**: 199-203 [PMID: 16710348 DOI: 10.1038/sj.ki.5001527]

56 TECOmedical Group, Switzerland. Novel kidney injury biomarkers. Available from: URL: http: //www.tecomedical.com

57 **Mori K**, Lee HT, Rapoport D, Drexler IR, Foster K, Yang J, Schmidt-Ott KM, Chen X, Li JY, Weiss S, Mishra J, Cheema FH, Markowitz G, Suganami T, Sawai K, Mukoyama M, Kunis C, D' Agati V, Devarajan P, Barasch J. Endocytic delivery of lipocalin-siderophore-iron complex rescues the kidney from ischemia-reperfusion injury. *J Clin Invest* 2005; **115**: 610-621 [PMID: 15711640 DOI: 10.1172/JCI23056]

58 **Haase M**, Bellomo R, Devarajan P, Schlattmann P, Haase-Fielitz A. Accuracy of neutrophil gelatinase-associated lipocalin (NGAL) in diagnosis and prognosis in acute kidney injury: a systematic review and meta-analysis. *Am J Kidney Dis* 2009; **54**: 1012-1024 [PMID: 19850388 DOI: 10.1053/j.ajkd.2009.07.020]

59 **Xin C**, Yulong X, Yu C, Changchun C, Feng Z, Xinwei M. Urine neutrophil gelatinase-associated lipocalin and interleukin-18 predict acute kidney injury after cardiac surgery. *Ren Fail* 2008; **30**: 904-913 [PMID: 18925531 DOI: 10.1080/08860220802359089]

60 **Silberstein JL**, Sprenkle PC, Su D, Power NE, Tarin TV, Ezell P, Sjoberg DD, Feifer A, Fleisher M, Russo P, Touijer KA. Neutrophil gelatinase-associated lipocalin (NGAL) levels in response to unilateral renal ischaemia in a novel pilot two-kidney porcine model. *BJU Int* 2013; **112**: 517-525 [PMID: 23510358 DOI: 10.1111/bju.12066]

61 **Woodson BW**, Wang L, Mandava S, Lee BR. Urinary cystatin C and NGAL as early biomarkers for assessment of renal ischemia-reperfusion injury: a serum marker to replace creatinine? *J Endourol* 2013; **27**: 1510-1515 [PMID: 24266750 DOI: 10.1089/end.2013.0198]

62 **Parekh DJ**, Weinberg JM, Ercole B, Torkko KC, Hilton W, Bennett M, Devarajan P, Venkatachalam MA. Tolerance of the human kidney to isolated controlled ischemia. *J Am Soc Nephrol* 2013; **24**: 506-517 [PMID: 23411786 DOI: 10.1681/ASN.2012080786]

63 **Sprenkle PC**, Wren J, Maschino AC, Feifer A, Power N, Ghoneim T, Sternberg I, Fleisher M, Russo P. Urine neutrophil gelatinase-associated lipocalin as a marker of acute kidney injury after kidney surgery. *J Urol* 2013; **190**: 159-164 [PMID: 23391468 DOI: 10.1016/j.juro.2013.01.101]

64 **Zekey F**, Senkul T, Ates F, Soydan H, Yilmaz O, Baykal K. Evaluation of the impact of shock wave lithotripsy on kidneys using a new marker: how do neutrophil gelatinese-associated lypocalin values change after shock wave lithotripsy? *Urology* 2012; **80**: 267-272 [PMID: 22503759 DOI: 10.1016/j.urology.2012.02.015]

65 **Langetepe J**, Schmid M, Schwaiger B, Tahbaz R, Ahyai S, Eichelberg C, Fisch M, Chun F. Serum and urine biomarker profile of kidney injury after (partial) tumor nephrectomy. 2014. Available from: URL: http: //www.uroweb.org/events/abstracts-online/?AID=51341

66 **Cost NG**, Noh PH, Devarajan P, Ivancic V, Reddy PP, Minevich E, Bennett M, Haffner C, Schulte M, DeFoor WR. Urinary NGAL levels correlate with differential renal function in patients with ureteropelvic junction obstruction undergoing pyeloplasty. *J Urol* 2013; **190**: 1462-1467 [PMID: 23791906 DOI: 10.1016/j.juro.2013.05.003]

67 **Mishra J**, Mori K, Ma Q, Kelly C, Barasch J, Devarajan P. Neutrophil gelatinase-associated lipocalin: a novel early urinary biomarker for cisplatin nephrotoxicity. *Am J Nephrol* 2004; **24**: 307-315 [PMID: 15148457 DOI: 10.1159/000078452]

68 **Bonventre JV**. Kidney injury molecule-1: a translational journey. *Trans Am Clin Climatol Assoc* 2014; **125**: 293-299; discussion 299 [PMID: 25125746]

69 **Devarajan P**. Biomarkers for the early detection of acute kidney injury. *Curr Opin Pediatr* 2011; **23**: 194-200 [PMID: 21252674 DOI: 10.1097/MOP.0b013e328343f4dd]

70 **Vaidya VS**, Ramirez V, Ichimura T, Bobadilla NA, Bonventre JV. Urinary kidney injury molecule-1: a sensitive quantitative biomarker for early detection of kidney tubular injury. *Am J Physiol Renal Physiol* 2006; **290**: F517-F529 [PMID: 16174863 DOI: 10.1152/ajprenal.00291.2005]

71 **Song L**, Xue L, Yu J, Zhao J, Zhang W, Fu Y. Kidney injury molecule-1 expression is closely associated with renal allograft damage. *Bosn J Basic Med Sci* 2013; **13**: 170-174 [PMID: 23988168]

72 **Han WK**, Waikar SS, Johnson A, Betensky RA, Dent CL, Devarajan P, Bonventre JV. Urinary biomarkers in the early diagnosis of acute kidney injury. *Kidney Int* 2008; **73**: 863-869 [PMID: 18059454 DOI: 10.1038/sj.ki.5002715]

73 **Chaturvedi S**, Farmer T, Kapke GF. Assay validation for KIM-1: human urinary renal dysfunction biomarker. *Int J Biol Sci* 2009; **5**: 128-134 [PMID: 19173034]

74 **Fahmy N**, Sener A, Sabbisetti V, Nott L, Lang RM, Welk BK, Méndez-Probst CE, MacPhee RA, VanEerdewijk S, Cadieux PA, Bonventre JV, Razvi H. Urinary expression of novel tissue markers of kidney injury after ureteroscopy, shockwave lithotripsy, and in normal healthy controls. *J Endourol* 2013; **27**: 1455-1462 [PMID: 24180435 DOI: 10.1089/end.2013.0188]

75 **Hatipoğlu NK**, Evliyaoğlu O, Işık B, Bodakçi MN, Bozkurt Y, Sancaktutar AA, Söylemez H, Atar M, Penbegül N, Yünce M, Dağgulli M. Antioxidant signal and kidney injury molecule-1 levels in shockwave lithotripsy induced kidney injury. *J Endourol* 2014; **28**: 224-228 [PMID: 24044353 DOI: 10.1089/end.2013.0535]

76 **Xue W**, Xie Y, Wang Q, Xu W, Mou S, Ni Z. Diagnostic performance of urinary kidney injury molecule-1 and neutrophil gelatinase-associated lipocalin for acute kidney injury in an obstructive nephropathy patient. *Nephrology* (Carlton) 2014; **19**: 186-194 [PMID: 24165570 DOI: 10.1111/nep.12173]

77 **Lucarelli G**, Mancini V, Galleggiante V, Rutigliano M, Vavallo A, Battaglia M, Ditonno P. Emerging urinary markers of renal injury in obstructive nephropathy. *Biomed Res Int* 2014; **2014**: 303298 [PMID: 25101270 DOI: 10.1155/2014/303298]

78 **Supavekin S**, Zhang W, Kucherlapati R, Kaskel FJ, Moore LC, Devarajan P. Differential gene expression following early renal ischemia/reperfusion. *Kidney Int* 2003; **63**: 1714-1724 [PMID: 12675847 DOI: 10.1046/j.1523-1755.2003.00928.x]

79 **VanderBrink BA**, Asanuma H, Hile K, Zhang H, Rink RC, Meldrum KK. Interleukin-18 stimulates a positive feedback loop during renal obstruction via interleukin-18 receptor. *J Urol* 2011; **186**: 1502-1508 [PMID: 21855933 DOI: 10.1016/j.juro.2011.05.046]

80 **Parikh CR**, Abraham E, Ancukiewicz M, Edelstein CL. Urine IL-18 is an early diagnostic marker for acute kidney injury and predicts mortality in the intensive care unit. *J Am Soc Nephrol* 2005; **16**: 3046-3052 [PMID: 16148039 DOI: 10.1681/ASN.2005030236]

81 **Lin CY**, Chang CH, Fan PC, Tian YC, Chang MY, Jenq CC, Hung CC, Fang JT, Yang CW, Chen YC. Serum interleukin-18 at commencement of renal replacement therapy predicts short-term prognosis in critically ill patients with acute kidney injury. *PLoS One* 2013; **8**: e66028 [PMID: 23741523 DOI: 10.1371/journal.pone.0066028]

82 **Ng CF**, Lo AK, Lee KW, Wong KT, Chung WY, Gohel D. A pro-

spective, randomized study of the clinical effects of shock wave delivery for unilateral kidney stones: 60 versus 120 shocks per minute. *J Urol* 2012; **188**: 837-842 [PMID: 22819406 DOI: 10.1016/j.juro.2012.05.009]

83 **Matsui K**, Kamijo-Ikemori A, Sugaya T, Yasuda T, Kimura K. Usefulness of urinary biomarkers in early detection of acute kidney injury after cardiac surgery in adults. *Circ J* 2012; **76**: 213-220 [PMID: 22094907]

84 **Bennaars-Eiden A**, Higgins L, Hertzel AV, Kapphahn RJ, Ferrington DA, Bernlohr DA. Covalent modification of epithelial fatty acid-binding protein by 4-hydroxynonenal in vitro and in vivo. Evidence for a role in antioxidant biology. *J Biol Chem* 2002; **277**: 50693-50702 [PMID: 12386159 DOI: 10.1074/jbc.M209493200]

85 **Manabe K**, Kamihata H, Motohiro M, Senoo T, Yoshida S, Iwasaka T. Urinary liver-type fatty acid-binding protein level as a predictive biomarker of contrast-induced acute kidney injury. *Eur J Clin Invest* 2012; **42**: 557-563 [PMID: 22070248 DOI: 10.1111/j.1365-2362.2011.02620.x]

86 **Yamamoto T**, Noiri E, Ono Y, Doi K, Negishi K, Kamijo A, Kimura K, Fujita T, Kinukawa T, Taniguchi H, Nakamura K, Goto M, Shinozaki N, Ohshima S, Sugaya T. Renal L-type fatty acid-binding protein in acute ischemic injury. *J Am Soc Nephrol* 2007; **18**: 2894-2902 [PMID: 17942962 DOI: 10.1681/ASN.2007010097]

87 **Susantitaphong P**, Siribamrungwong M, Doi K, Noiri E, Terrin N, Jaber BL. Performance of urinary liver-type fatty acid-binding protein in acute kidney injury: a meta-analysis. *Am J Kidney Dis* 2013; **61**: 430-439 [PMID: 23228945 DOI: 10.1053/j.ajkd.2012.10.016]

88 **Carr MC**, Peters CA, Retik AB, Mandell J. Urinary levels of the renal tubular enzyme N-acetyl-beta-D-glucosaminidase in unilateral obstructive uropathy. *J Urol* 1994; **151**: 442-445 [PMID: 8283554]

89 **Skalova S**, Rejtar P, Kutilek S. Increased urinary N-acetyl-beta-D-glucosaminidase activity in children with hydronephrosis. *Int Braz J Urol* 2007; **33**: 80-83; discussion 84-86 [PMID: 17335604 DOI: 10.1590/S1677-55382007000100014]

90 **Bernard AM**, Vyskocil AA, Mahieu P, Lauwerys RR. Assessment of urinary retinol-binding protein as an index of proximal tubular injury. *Clin Chem* 1987; **33**: 775-779 [PMID: 3297418]

91 **Liangos O**, Perianayagam MC, Vaidya VS, Han WK, Wald R, Tighiouart H, MacKinnon RW, Li L, Balakrishnan VS, Pereira BJ, Bonventre JV, Jaber BL. Urinary N-acetyl-beta-(D)-glucosaminidase activity and kidney injury molecule-1 level are associated with adverse outcomes in acute renal failure. *J Am Soc Nephrol* 2007; **18**: 904-912 [PMID: 17267747 DOI: 10.1681/ASN.2006030221]

92 **Hanbeyoğlu A**, Kazez A, Ustündağ B, Akpolat N. Determination of urinary N-acetyl-ß-D glucosaminidase (NAG) levels in experimental blunt renal trauma. *Ulus Travma Acil Cerrahi Derg* 2011; **17**: 475-481 [PMID: 22289997 DOI: 10.5505/tjtes.2011.57973]

93 **Marchewka Z**, Kuźniar J, Długosz A. Enzymuria and beta2-mikroglobulinuria in the assessment of the influence of proteinuria on the progression of glomerulopathies. *Int Urol Nephrol* 2001; **33**: 673-676 [PMID: 12452627]

94 **Katagiri D**, Doi K, Honda K, Negishi K, Fujita T, Hisagi M, Ono M, Matsubara T, Yahagi N, Iwagami M, Ohtake T, Kobayashi S, Sugaya T, Noiri E. Combination of two urinary biomarkers predicts acute kidney injury after adult cardiac surgery. *Ann Thorac Surg* 2012; **93**: 577-583 [PMID: 22269724 DOI: 10.1016/j.athoracsur.2011.10.048]

95 **Liu S**, Che M, Xue S, Xie B, Zhu M, Lu R, Zhang W, Qian J, Yan Y. Urinary L-FABP and its combination with urinary NGAL in early diagnosis of acute kidney injury after cardiac surgery in adult patients. *Biomarkers* 2013; **18**: 95-101 [PMID: 23167703 DOI: 10.3109/1354750X.2012.740687]

96 **Zhang X,** Gibson B, Mori R, Snow-Lisy D, Yamaguchi Y, Campbell SC, Simmons MN, Daly TM. Analytical and biological validation of a multiplex immunoassay for acute kidney injury biomarkers. *Clin Chim Acta* 2013; **415**: 88-93 [PMID: 23041213 DOI: 10.1016/j.cca.2012.09.022]

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REVIEW

Complement involvement in kidney diseases: From physiopathology to therapeutical targeting

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Abstract

Complement cascade is involved in several renal diseases and in renal transplantation. The different components of the complement cascade might represent an optimal target for innovative therapies. In the first section of the paper the authors review the physiopathology of complement involvement in renal diseases and transplantation. In some cases this led to a reclassification of renal diseases moving from a histopathological to a physiopathological classification. The principal issues afforded are: renal diseases with complement over activation, renal diseases with complement dysregulation, progression of renal diseases and renal transplantation. In the second section the authors discuss the several complement components that could represent a therapeutic target. Even if only the anti C5 monoclonal antibody is on the market, many targets as C1, C3, C5a and C5aR are the object of national or international trials. In addition, many molecules proved to be effective *in vitro* or in preclinical trials and are waiting to move to human trials in the future.

Key words: Complement cascade; Complement and glomerulopathies; Eculizumab; Targeting complement; Complement and renal transplantation

Core tip: Our therapeutical armamentarium is to date limited in many kidney diseases and in several aspects of renal transplantation. The findings that complement cascade is involved in many kidney diseases and in renal transplantation offer the availability of new therapeutical targets basing on the pathogenesis. The anti C5 monoclonal antibody, eculizumab, is now used to treat the atypical hemolytic uremic syndrome (aHUS), but 24 trials are ongoing in different renal diseases and in renal transplantation. Other targets as C1, C3, C5a, and C5aR are innovative treatments for diseases as aHUS, membranoproliferative glomerulonephritis, ischemia-reperfusion injury, and objects of ongoing trials.

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INTRODUCTION

The complement system serves as first line defense against invading pathogens and is a component of the innate immune system[1,2]. The complement system represents a link between the innate and adaptive immunity. In addition, several studies examined the cross-talk between complement and toll-like receptors, another component of the innate immune system and the complement system[3]. The complement system is composed of three distinct activation pathways: classic pathway (CP), alternative pathway (AP), and mannose-binding lectin pathway (LP). Any pathway activates the complement cascade generating C3-convertase which cleaves C3 into C3a and C3b[4]. In normal conditions a small amount of C3-convertase is activated by the AP and is necessary to have regulators to prevent complement attack on healthy self cells[5] (Figure 1). This regulation is provided by a combination of plasma and cell surface inhibitory proteins. Fluid phase regulators include C1-inhibitor (C1-INH) that prevents the auto activation of the initial complex of the CP, the decay-accelerating factor that binds to C4b, and acts as co-factor for factor I (CFI) cleavage of C4b opsonin. Another regulator for AP is factor H (CFH), which also acts as a co-factor for CFI in the inactivation of C3b. Other regulators are Clusterin and Vitronectin that inhibit the insertion of terminal complexes into the cell membranes. Finally, carboxypeptidase N acts as anaphylatoxin inhibitor.

Finally, cell surface regulatory proteins, including regulators complement receptor 1 (CR1) (C3b receptor), membrane co-factor protein (MCP, CD46), and decay-accelerating factor (DAF, CD55) act inhibiting the C3 and C5 convertases activity. Complement-mediated injury is the result of the prevalence of the activating factor over the complement regulators[6,7].

Independently from complement involvement, serum complements levels may are low or normal. The pathogenesis of hypocomplementemia is related to the high consumption rate due to immune deposits, but other factors are the presence of hereditary complement deficiency and the presence of circulating factors that promote complement activation and consumption. When the CP is activated both C3 and C4 may be low. C3 levels low alone may be expression of the activation of the AP.

In kidney diseases and in kidney transplantation, the complement cascade is frequently involved and might represent a first line therapeutic strategy.

RESEARCH

We have analyzed the available data on complement and renal diseases and renal transplantation by careful revision of the currently available data. Literature research was performed using PubMed (NCBI/NIH) under employment of the search terms "complement cascade", "complement and glomerulopathies", "dense deposit disease", "membranoproliferative glomerulonephritis", "C3 glomerulonephritis", "complement and renal transplantation", "targeting complement", "eculizumab". Studies currently under way were sought for in "clinicaltrials.gov" and the European EUDRACT register. The papers published in the last three years on international journals on transplantation and kidney disease were carefully examined. Almost 160 papers were selected for this review.

PHYSIOPATHOLOGY OF COMPLEMENT INVOLVEMENT IN KIDNEY DISEASES

There are 2 broad categories of kidney diseases in which the complement system has a pathogenic role. The first is associated with complement over activation, and the second with complement dysregulation. Moreover the complement system is frequently involved in the kidney injuries after kidney transplantation[8] and in the progression of kidney diseases. The principal complement abnormalities leading to renal diseases are summarized in Table 1[9].

OVERACTIVATION OF COMPLEMENT

Lupus nephritis

Deficiencies in the early components of the CP including C1q, C2 and C4 are associated with the development of systemic lupus erythematosus (SLE)[10]. Familial C1q deficiency has been found to be a relevant genetic risk factor for the development of SLE, indeed C1q deficiency results in impaired phagocytosis[11]. The apoptotic cells are not immunologically benign and the reduced phagocytic clearance of these cells increases the likelihood that auto antigens are presented to lymphocytes and induce the development of the autoimmunity. Thirty percent of patients with lupus nephritis have C1q antibodies[12]. The products of C5 metabolism may also contribute directly to glomerular injury and in studies of murine models of lupus nephritis, a monoclonal antibody that blocked C5 cleavage significantly ameliorated the glomerulonephritis and prolonged survival[13].

Anti glomerular basement membrane glomerulonephritis

The complement system is involved in anti glomerular basement membrane glomerulonephritis either through CP and enhancing the inflammatory response through C5a activation[14] and/or cell lysis effect of C5b-9[15].

Antineutrophil cytoplasmic antibody associated vasculitis

Several authors[16,17] documented the involvement of complement in antineutrophil cytoplasmic antibody (ANCA) glomerulonephritis. Chen *et al*[16] documented C3 deposits in the glomeruli of patients with high levels of proteinuria and poor renal function. C5-9, C3d and complement factor B (CFB) were also reported in

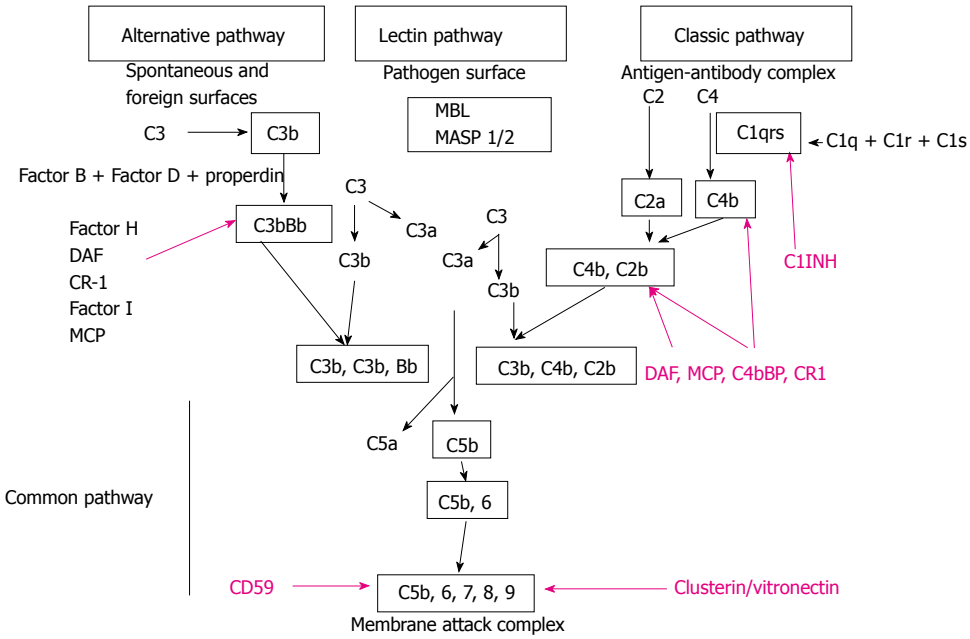

Figure 1 Representation of the classical, lectin and alternative pathways of complement activation, including regulatory molecules (purple). MBL: Mannose binding lectin; MASP 1/2: Mannan-binding lectin-associated serine protease-1; C1INH: C1 inhibitor; DAF: Decay accelerating factor; CR-1: Complement receptor 1; MCP: Membrane co-factor protein.

Table 1 Representative abnormalities in complement leading to renal disease

Components/related molecules	Diseases
Complement C3	C3 glomerulopathy (DDD), aHUS
Factor H	C3 glomerulopathy (DDD/C3GN), aHUS
Factor I	C3 glomerulopathy (C3GN), aHUS
MCP	aHUS
Factor B	aHUS
CFHR5	Familial C3 glomerulopathy (CFHR5 nepropathy)
CFHR3-1	Familial C3 glomerulopathy
CFHR1/3	IgA nephropathy, aHUS
Factor B autoantibody	C3 glomerulopathy (DDD)
Factor H autoantibody	C3 glomerulopathy (DDD/C3GN)
Bb (activated factor B)	HUS, ANCA-associated vasculitis
C3Nef	C3 glomerulopathy (DDD, C3GN)
Soluble C5b-9	HUS, TTP, ANCA-associated vasculitis
C3a	ANCA-associated vasculitis, TTP
C5a	ANCA-associated vasculitis
C1q/C1qR	C1q nephropathy
Properdin	TI injury due to massive proteinuria
C5	ANCA-associated vasculitis
Factor B	ANCA-associated vasculitis
CRaR	TI inflammation, IRI
C5aR	IRI
Factor H	IRI
C5b-9	IRI
CD59	IRI

DDD: Dense deposit disease; aHUS: Atypical hemolytic uremic syndrome; C3GN: C3 glomerulonephritis; MCP: Membrane co-factor protein; CFHR5: Complement factor H-related protein; ANCA: Anti neutrophil cytoplasmic antibody; C3Nef: C3 nephritic factor; TTP: Thrombotic thrombocytopenic purpura; TI: Tubulo-interstitial; IRI: Ischemia-reperfusion-injury.

biopsies from patients with myeloperoxidase (MPO)-ANCA-associated pauci-immune glomerulonephritis. Xing *et al*[17], from the same group, observed that C4d was negative in biopsies of patients with MPO-ANCA glomerulonephritis. These studies suggest that this model of glomerulonephritis requires the activation of the AP, not the CP or the LP. Further studies in patients with active ANCA associated vasculitis documented high levels of C3a, C5a, soluble C5b-9 and Bb[18]. Recently other authors documented[19] that the C5a specific receptor

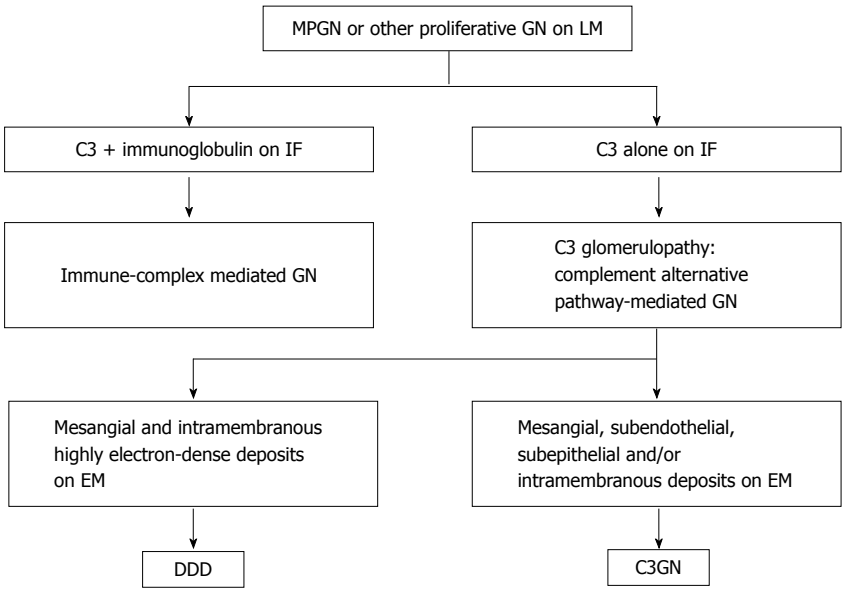

Figure 2 Reclassification of membrano-proliferative glomerulonephritis. C3GN: C3 glomerulonephritis; DDD: Dense deposit disease; EM: Electron microscopy; GN: Glomerulonephritis; IF: Immunofluorescence; LM: Light microscopy; MPGN: Membrano-proliferative glomerulonephritis.

(C5aR) expressed on neutrophils is involved in the pathogenesis of ANCA-induced glomerulonephritis (GN). Therefore targeting the C5a-C5aR receptor interaction in such patients might represent a therapeutical strategy[20]. A clinical trial to evaluate the safety and efficacy of an inhibitor of the C5a receptor (CCX168) is ongoing and an interim analysis reported promising results[21].

Membranous nephropathy

Several studies have identified that autologous antigens are the target of antibody response in idiopathic membranous nephropathy (MN). According to the latest studies[22] M-type phospholipase A2 receptor (PLA2R) located on podocytes has been identified as the target antigen in idiopathic MN. The predominant anti PLA2R IgG subclass activates the alternative or the mannose binding lectin (MBL) pathway[23]. This is confirmed by some studies documenting glomerular MBL and C4b deposition in MN[24,25].

In human secondary MN, C1q, C3, C4, CFB, MBL, and C5b-9 typically are present and co-deposited with IgG, suggesting that the LP and the AP could play the relevant role[26,27].

C1Q nephropathy

C1q nephropathy is characterized by the presence of conspicuous C1q immune deposits in glomeruli with no evidence of SLE MPGN type Ⅰ. C1q nephropathy is characterized by the C1q binding to poly-anionic substances (DNA, RNA, viral proteins) or to C1q receptors, according some authors C1q nephropathy has been thought to be a subgroup of primary focal segmental glomerular sclerosis[28].

IgA nephropathy

Two distinct mechanisms of complement activation are involved in IgA nephropathy. The AP is the key pathway in 75% of cases[29,30]. In 25% of biopsy specimens, the presence of glomerular IgA1 and C3 is associated with MBL and MBL-associated serine protease 1 (MASP-1) deposition. MBL binds to the abnormally galactosylated region of the IgA1 through its carbohydrate binding domain resulting in complement catabolism through the lectin binding pathway. The presence of MBL and MASP-1 is associated with disease severity and poor histological prognostic features[31].

Immune complexes-associated membranoproliferative glomerulonephritis

In the past on immunopathological basis the MPGN was classified, on the basis of immunopathological findings, into three subtypes: MPGN type Ⅰ, Ⅱ [also known as dense deposit disease (DDD), and Ⅲ]. Recently, the classification of MPGN has been completely reviewed by Bomback *et al*[32] on pathogenetic basis[33]. This led to a new understanding of the pathogenesis and to a reclassification of MPGN (Figure 2). The former MPGN type Ⅰ and Ⅲ belong to the chapter of complement over activation, while C3GN and the DDD will be described in the chapter of complement dysregulation.

In immune complex associated MPGN, the CP is activated by antibodies. Monoclonal antibodies or immune complexes precipitate catabolism, resulting in the chemo attraction of leukocytes and the direct cell injury by the MAC. The leukocyte generation of cytokines and proteases stimulate the mesangial cell proliferation and the matrix expansion[34].

DYSREGULATION OF COMPLEMENT

Atypical hemolytic uremic syndrome

The dysregulation of the AP cascade due to acquired or genetic factors leads to defective complement control that may cause a range of complement associated glomerulopathies (Figure 3). The better known among them is the atypical HUS (aHUS). aHUS classically is a triad of microangiopathic hemolytic anemia, acute kidney injury and thrombocytopenia caused by failure

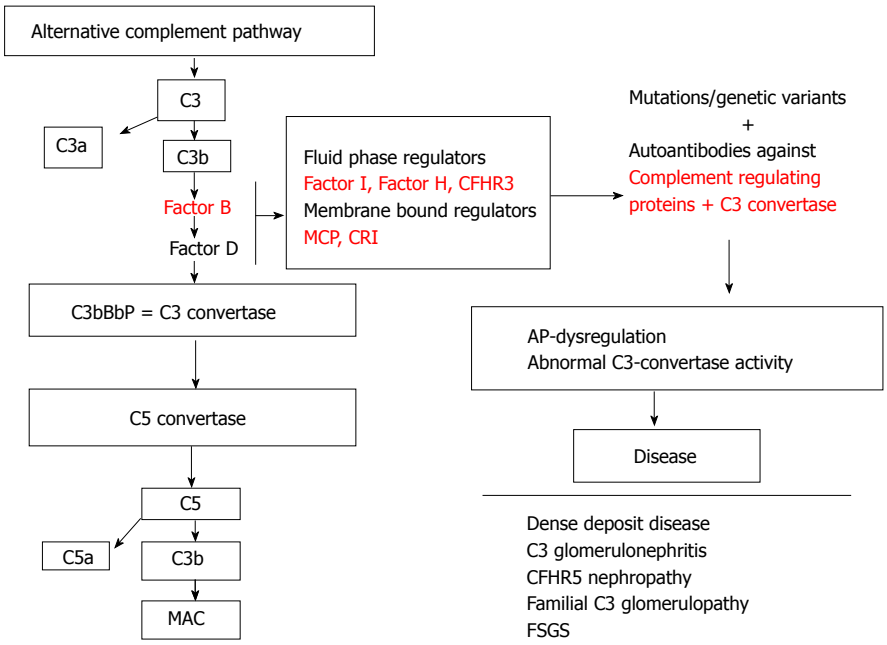

Figure 3 Dysregulation of the alternative complement cascade due to acquired or genetic factors leads to defective complement control causing a range of complement-associated glomerulopathies. CFHR3: Complement factor H-related protein 3; MCP: Membrane co-factor protein; CR1: Complement receptor 1; AP: Alternative pathway; CFHR5: Complement factor H-related protein 5; FSGS: Focal segments glomerular sclerosis; MAC: Membrane attack complex.

to regulate the alternative complement pathway. In over 60% of cases mutations have been identified in genes encoding complement regulatory proteins: CFH, CFI, MCP, thrombomodulin (THBD), and in genes encoding complement activators: CFB and C3. Complement cascade dysregulation causes a damage of endothelium leading to thrombosis and microangiopathic hemolytic anemia[35,36]. CFH mutations are observed in 25%-30% of patients with an aHUS[37]. Up to now, more than 80 mutations have been identified. Patients with aHUS and anti factor H antibodies have also been reported. These antibodies bind to short consensus repeats, thus reducing the CFH activity[38]. Reduction in MCP expression is reported in over 80% of cases with mutation in this gene[36,39]. Genetic disorders are rarely related to CFI[40]. *THBD* mutations with hyperactivity have been found in only 3%-5% of patients[41].

Recently, Noris *et al*[42] have documented that the classical HUS caused by Shiga toxin producing escherichia coli (STEC-HUS) and thrombotic thrombocytopenic purpura (TTP) are caused by inappropriate complement activation. Even if STEC-HUS, aHUS and TTP are all diseases of complement activation and recognize a common pathogenesis, we should remember that aHUS is linked to the complement dysregulation, while STEC-HUS and TTP are linked to the complement over activation and, on a pathogenetic basis, belong to the previous chapter.

In the HUS-SYNSORB Pk trial, children with STEC-HUS had increased plasma levels of Bb and C5-9 at the beginning of the study, which normalized after one month[43]. This suggests that patients with acute onset STEC-HUS have an activation of the AP in the acute phase of the disease, which normalizes within 1 mo. In the initial phases of STEC-HUS, the toxin triggers the endothelial complement deposition and interferes with the activity of the complement regulatory molecules[44]. Moreover, lack of the lectin-like domain of THBD, worsen STEC-HUS in mice[45].

Recent studies that further document the involvement of complement in STEC-HUS are those reporting the beneficial effect of Eculizumab (an anti C5 monoclonal antibody) in the outbreak of STEC-HUS induced by *E. Coli* 0104: H4 in Germany[46] and in the outbreak of STEC-HUS induced by the same strain in France[47].

Réti *et al*[48] recently reported increased levels of C3a and C5b-9 associated with decreased complement C3 levels during the acute phase of TTP. This fact indicates a complement consumption, which occurs in some TTP patients. To confirm complement involvement in TTP, in some patients refractory to treatment, eculizumab has been used with good results. These patients had severe TTP and a deficiency of disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13 (ADAMTS13) due to high titers of anti ADAMTS13 antibodies[49].

C3 glomerulopathies

Isolated C3 deposition within the glomerulus is the defining histological criterion for C3 glomerulopathy. C3 glomerulopathy is a recently introduced pathological entity defined by a glomerular pathology characterized by C3 accumulation with absent or scanty immunoglobulin deposition. In August 2012, an invited group of experts met to discuss the C3 glomerulopathy in the first C3

glomerulopathy meeting[50]. According the conclusions of the meeting and the recent paper from Barbour *et al*[51] on the basis of histological and clinical features, C3 glomerulopathies may be distinguished into: (1) DDD; and (2) C3 glomerulonephritis (C3GN). A familial form of C3GN has been recently described: the CFHR5 nephropathy.

From the patho-physiological point of view, three different mechanisms may be operating in the different conditions:

Auto antibodies: C3 nephritic factor (C3NeF) is an autoantibody that binds to a neoepitope on the AP C3 convertase. C3NeF stabilizes convertase against the CFH-mediated decay resulting in an uncontrolled C3 activation[52]. C3NeF is common in DDD, less in C3GN and absent in CFHR5 nephropathy.

Other auto-antibodies have also been described. Two patients with DDD and auto antibodies targeting both CFB and C3b have been recently identified[53]. Auto antibodies anti CFH also occur in DDD and in C3GN[54,55].

Genetic sequence variations: On genetic basis, heterozygous mutations in the *CFH, CFI* and *MCP* genes have been documented in C3GN[56] and also in MPGN type I and DDD[57]. Some of these heterozygous mutations have been also observed in patients with aHUS[58] showing similarities between the two diseases.

Genetic structure variation: As aforementioned, CFHR5 nephropathy has been described as a familial form of C3GN[59]. A mutation in complement factor H-related protein 5 in patients with glomerulonephritis has been identified *via* a genome-wide linkage study. The mutant CFHR5 protein present in patient serum had reduced affinity for surface-bound complement. Genetic abnormalities in the *CFHR3* and *CFHR1* loci were also recently reported. Such patients develop an autosomal dominant complement-mediated GN similar to CFHR5 nephropathy[60].

As aforementioned, the complement cascade is involved also in other renal conditions as the progression of renal disease and renal transplantation.

COMPLEMENT AND PROGRESSION OF RENAL DISEASE

The progression of renal disease may be mediated by tubule interstitial inflammation. Several studies have confirmed this datum and the involvement of complement activation[61]. Complement activation by tubular cells is mediated by the properdin binding. This fact is principally relevant in the case of proteinuric renal disorders. Studies *in vitro* have documented that the complement system is activated by the AP[62,63].

Complement activation may occur also in non proteinuric renal diseases as documented by Bao *et al*[64]. In this condition the C3a receptor is involved in causing renal inflammation and fibrosis.

Another important factor is the "*in situ*" produced chemokines. Genomic studies performed by Bao *et al*[65] documented that in some murine models several pro inflammatory and pro fibrotic chemokine genes are up-regulated. This activation occurs upon complement activation.

According to the aforementioned studies, targeting complement might be a useful therapeutical approach for chronic kidney disease in the future. Further studies are necessary for a better understanding of the role of complement in mediating tubule interstitial damage and consequent fibrosis.

COMPLEMENT IN RENAL TRANSPLANTATION

Transplanted kidneys principally suffer from injuries such as ischemia reperfusion (I/R) injury and rejection. Complement may mediate all these conditions.

Ischemia-reperfusion injury

The short-term consequences injuries as I/R injury and hyper acute rejection are principally related to innate immunity, while later injuries such as the antibody mediated rejection (ABMR) and the cell mediated rejection (CMR) are related either to the innate and the adaptive immune system.

I/R causes in the transplant both a vascular and parenchyma cell injury. In I/R complement is principally activated through the AP as a consequence of *in situ* generation of C3[66]. Other studies suggest the activation of MBL[67]. The majority of transplanted kidneys are retrieved from cadaveric donors. In such kidneys C3 may be present in the organ before retrieval because of donor suffering. Damman *et al*[68] found higher gene expression of C3 and increased deposition of C3d in kidney biopsies obtained from deceased grafts. Now a large scale study in the United Kingdom is analyzing in renal from deceased donors, the soluble form of C3-1 as a protecting agent for IRI and to improve graft outcomes[69]. Going in molecular details, Simone *et al*[70] documented that in renal I/R injury complement activates nicotinamide adenine dinucleotide phosphate-oxidase (NAPDH oxidase) enzymes. During renal IRI an endothelial-to-mesenchymal transition (EndMT) may occur, mediated by complement activation. EndMT may have a critical role in generating renal fibrosis[71]. Curci *et al*[72] documented that, during I/R injury, an activation of the CP and LP of the complement system occurs primarily at the endothelial cell level. In a recent study, the same authors[73] analyzed in large mammals the role of complement in the induction of EndMT by using recombinant C1 inhibitor *in vivo*.

Their data documented that the activation of the serine/threonine-specific protein kinase (Akt) pathway was essential to induced EndMT *in vitro*. In accordance,

inhibition of complement *in vivo* abrogated the Akt signaling, with inhibition of EndMT and of tissue fibrosis.

Pratt *et al*[74] documented that C3 produced by a graft and by recruited immune cells is a two phases trigger that in the early period produces a post-perfusion injury, later may contribute to late rejection associated-allograft injury. Indeed a recent study[75] documented that I/R injury can affect the systemic immune response to antigens requiring a functional alternative pathway of complement. C3 split products, C3b and C3d, deposited on antigen presenting cells (APCs), can increase allo-antigens uptake and their presentation to the T cells. So doing, C3 positive APCs potentiate the T cell response *in vitro*[74]. The role of C3 in activating T cells is confirmed by studies documenting that the macrophages deficient for the C3 have impaired capability to stimulate the T cells[76,77]. The enhancement of the effector T cell expansion by complement should be ascribed to the limited antigen induced apoptosis[78]. In addition, other studies have documented the role of complement on the iTreg. Indeed, the iTreg-mediated tolerance to alloantigen in humans[79] might be mediated by the signaling through C5a receptor and C3a receptor.

As already mentioned, complement is involved both in the CMR and in the ABMR.

Cell-mediated rejection

The complement activation through any pathway generates C3a and C5a. These anaphylatoxins bind to both APCs and T cells to stimulate and activate T cells[80]. Li *et al*[81] demonstrated that the deficiency of the C5a receptor limited the adaptive response of recipient T cells to alloantigen. C1q appears to have a regulatory role in the threshold for the T cell activation by dendritic cells[82]. Moreover, in human kidney transplants with acute rejection, C5aR expression was increased in the renal tissue and in the cells infiltrating the tubular interstitium[83]. The same authors documented that in mice treated with a C5aR antagonist the infiltration of monocyte-macrophage was significantly attenuated, perhaps as a result of reduced levels of monocyte chemo attractant protein 1 and the intercellular adhesion molecule 1. However a murine model of kidney transplantation with C4 deficiency demonstrated that a CMR can occur in the absence of the CP or of the LP activation[84]. This suggests that the AP may play the key role in CMR.

Antibody-mediated rejection

The antibody-mediated renal allograft rejection often involves either donor specific antibodies (DSAs) and the CP of complement system activation. After binding to DSAs, complement is activated. C4d is a degradation product of C4 and, because it binds and remains covalently attached at the site of complement activation, represents a useful diagnostic tool[85]. Haidar *et al*[86] discovered that the deposition of C4d on erythrocytes was even more related to histological rejection signs, thus representing a possible not invasive diagnostic tool.

C3a and C5a likely act as potent chemo tactic factors promoting the infiltration of proinflammatory cells. In addition, the MAC might directly damage the allograft[85]. Expression of the membrane-bound regulator, CD55, is inversely related to C4d staining in biopsy specimens. Indeed CD 55 (also known as DAF), which accelerates the decay of C3 and C5 convertases, might modify the severity of the rejection. An increased CD55 expression is associated with an improved long-term kidney transplant outcomes in recipients without antibody-mediated rejection, suggesting a possible role for CD55 in the kidney protection[87].

In addition the kidney, after transplantation, may be involved in clinical conditions as recurrence of some renal diseases. Recently complement involvement has been documented also in chronic antibody mediated rejection.

Atypical hemolytic uremic syndrome is associated with a high rate of recurrence and poor outcomes after kidney transplantation. Acquired or inherited dysregulation of the alternative complement pathway, thought to be the driving force of the disease, is identified in most aHUS patients[88]. Recurrent thrombotic microangiopathy is very rare in patients who had developed end stage renal failure following HUS caused by Shiga-toxin producing STEC, whereas disease recurrence is common in patients with aHUS[89]. The recurrence rate[90] of C3 glomerulopathy on renal transplantation could be approximately estimated on about 60% as derived from two small case series of Servais *et al*[57] and Little *et al*[91] and confirmed in the recent paper of Zand *et al*[92]. In such conditions anticomplement therapy could be useful.

Moreover recent data document the complement involvement also in antibody mediated chronic rejection where the "bad" activity of antibodies can also be involved in previously considered "chronic" lesions (*i.e.,* transplant glomerulopathy[93,94].

TARGETING COMPLEMENT: THERAPEUTIC STRATEGIES

Complement is clearly involved in many kidney diseases as well as in kidney transplantation. Hence targeting complement cascade at different levels may represent a new therapeutic strategy directed against the pathogenetic mechanisms.

Since the end of 2000's several review papers reported the efficacy *in vitro* or in preclinical studies of anti complement molecules[95-97]. Unfortunately in the setting of renal diseases only an anti C5 monoclonal antibody is on the market. All others molecules are either on the market for diseases not involving the kidney or are still in preclinical phases (Table 2) or failed their efficacy.

Table 2 Some of the molecules aimed to target complement, phase of the trial and renal diseases related

Compound name	Complement target	Compound class	Phase/indication
C1 inhibitor (Berinert)	C1r, C1s, MASP1, MASP2	Regulator	P 1/2 transplant
Cp40, AMY 101			PC transplant , aHUS, DDD
sCR1, CDX-1135	C3 conv, C4b, C3b	Regulator	P 1 DDD
Mirococept, APT070	C3 conv, C4b, C3b	Regulator	P 1/2 transplant
Eculizumab	C5	Ab	P 4 aHUS, P 2/3 STEC-HUS, P 2 ANCA vasculitis, P 1 transplant
Mubodina	C5	Ab	PC aHUS, DDD
Ergidina	C5	Ab	PC transplant
CCX168	C5aR	Small molecule	P 2 ANCA vasculitis
ADC-1004	C5aR	Protein	PC transplant

MASP1: Mannan-binding lectin-associated serine protease; P: Phase; PC: Preclinical; aHUS: Atypical hemolytic uremic syndrome; DDD: Dense deposit disease; sCR1: Soluble complement receptor 1; Ab: Antibodies; STEC-HUS: Shiga toxin producing *Escherichia Coli*-hemolytic uremic syndrome.

TARGETING C5

The first available anti-complement therapy is eculizumab, a fully humanized monoclonal antibody that binds with high affinity to C5 and prevents the generation of MAC[98]. Eculizumab was first approved for the treatment of paroxysmal nocturnal hemoglobinuria (PNH)[99] and more recently, for the treatment of aHUS and other kidney diseases.

To date[100] 11 trials are ongoing for aHUS, 2 trials for STEC-HUS, 2 trials for MPGN and 1 trial for ANCA related diseases. Trials with eculizumab are also ongoing in the field of kidney transplantation, in particular 8 trials for the prevention and/or the treatment of acute or chronic ABMR, 3 trials for the prevention of delayed graft function (DGF), 1 trial for the prevention of I/R injury and 1 trial for the prevention of glomerular disease recurrence after transplantation.

After initial reports of the possible beneficial effects of eculizumab in treating patients with aHUS[101,102], more recently the beneficial effect of treating aHUS by eculizumab has been documented by two studies. One study[103] reported the data from 27 patients treated in off label studies. The other study[104] reported the data of 37 patients enrolled in 2 phase Ⅱ trials. The second of these phase Ⅱ trials was notable because 80% of subjects achieved thrombotic microangiopathy event-free status. These studies were the object of debate for the issues concerning the duration and the optimal dosing of therapy[105-108]. Indeed, although eculizumab revolutioned the treatment of aHUS, several unresolved issues remain, among which whether eculizumab should be always the first line therapy for aHUS and whether the drug should be considered as a life-long therapy also taking in account the treatment high cost. In addition, in PNH, but not by now in aHUS, patients have been described with unexplained eculizumab resistance. A recent study[109,110] documented that such resistance was due to C5 variants with mutations at Arg885.

As aforementioned also STEC-HUS and TTP are caused by inappropriate complement activation[42]. The eculizumab treatment has proved effective in these conditions. After the report of 3 cases[111], the eculizumab effectiveness has been documented in two STEC-HUS outbreaks occurring in Germany and in France[46,47]. The efficacy of eculizumab in treating the TTP has also been reported[49,112], even if others advocate rituximab as the best option in treating TTP[113].

The pathogenetic similarities between aHUS and some C3 glomerulopathies might imply that eculizumab treatment could fit well in treating all these diseases[103]. The new classification of C3 glomerulopathies (previously MPGN) have already been described[51]. Only DDD and C3GN have some similarities with aHUS and eculizumab could be beneficial for such patients. To date, in literature the eculizumab use for C3 glomerulopathies is limited to 6 case reports[114-119] and the results from a 1-year, open-label study of eculizumab therapy in 6 subjects[120,121]. The treatment results differ and indicate that eculizumab may be a non adequate treatment for a subgroup of patients with DDD and C3GN. Although some investigators suggested that aHUS should be considered part of a spectrum that includes DDD and C3GN, the underlying defect is not always the same. In aHUS the endothelial damage is often due to complement dysregulation at the level of cell membrane in the solid phase[36]. The solid phase dysregulation in aHUS translates to C5 convertase dysregulation being at least equal and often greater than C3 convertase dysregulation. Hence the blockade of C5 in such conditions is expected to yield improvement. In contrast, in some cases of C3 glomerulopathies a dysregulation of the fluid phase occurs and, as a consequence, C3 convertase dysregulation is greater than C5 dysregulation. These cases, because of a feed-back effect on the C3 convertase activity, could potentially be aggravated by C5 blockade[122]. Therefore, one of the major challenges in treating patients with C3 glomerulopathy with the anti complement therapy is how to distinguish the patients with primarily C3 convertase dysregulation from the patients with primarily C5 convertase dysregulation. Blockade at the level of C3 may be an alternative to eculizumab therapy, primarily in patients with C3 glomerulopathies

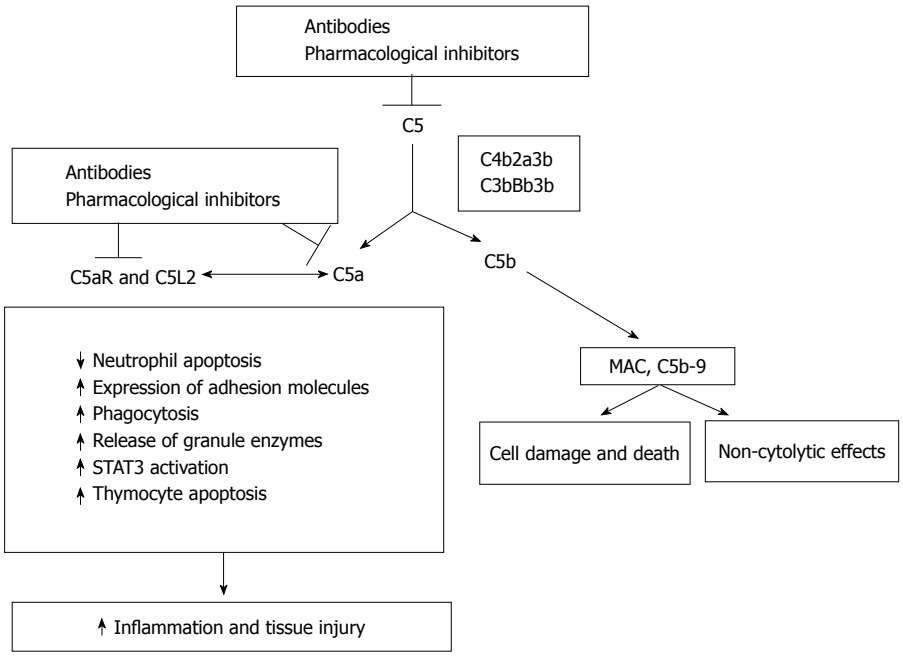

Figure 4 Significance of inhibiting the C5-C5a receptor axis. C5 convertases formed during activation of complement cascade cleave C5 into its active products. Inhibition is feasible pharmacologically or with neutralizing antibodies. MAC: Membrane attack complex; STAT3: Signal transducers and activators of transcription 3.

associated with a C3 convertase dysregulation greater than the C5 convertase dysregulation.

As mice deficient in C5 have demonstrated resistance against anti-MPO-mediated glomelulonephritis[123], an open label phase Ⅱ trial (NCT01275287), in which the patients with ANCA-associated glomerulonephritis were randomized to standard of care treatment *vs* standard of care plus eculizumab, was started. Unfortunately the trial was withdrawn because of lack of enrollment.

In the case of the recurrence after transplantation of a kidney disease susceptible to anti C5 therapy, eculizumab treatment is effective.

Zuber *et al*[124] successfully treated 22 renal transplant recipients with recurrence of aHUS.

McCaughan *et al*[117] reported a patient with DDD recurrence after kidney transplantation successfully treated by eculizumab. More recently Lonze *et al*[125] reported the cases of antiphospholipid antibody syndrome, two of them with the catastrophic variant, which were successfully treated at the time of transplantation with continuous systemic anticoagulation together with eculizumab prior to and following the live donor renal transplantation.

As aforementioned renal damage due to complement activation occurs in two phases after transplantation: during reperfusion after that the donor kidney has undergone a significant period of ischemia and during the acute rejection once the innate and adaptive immune system has recognized the donor antigens. In both conditions the complement may play a relevant role. Four clinical trials are now active aiming to control the ischemia-reperfusion injury and the consequent DGF. All these trials hypothesized that C5 cleavage is a key step in the pathogenesis of I/R injury following transplantation and its block could be an effective prophylactic tool to prevent acute kidney injury (NCT01919346, NCT01403389, NCT02145182, NCT01756508).

Eculizumab has also been successfully used in reducing antibodies in highly sensitized patients with positive cross-matches prior to transplantation[126-128]. In a larger case-control study the patients with DSAs were treated with eculizumab after transplantation and compared to the historical controls[129]. Eculizumab treatment was able in significantly lowering ABMR and in decreasing the 1-year transplant glomerulopathy incidence rate.

Table 3 summarizes all the trials ongoing with eculizumab in treating either glomerular disease and renal transplantation.

TARGETING C5a AND C5aR

C5a is a powerful anaphylatoxin that stimulates the cytokine production, enhances the T-cell activation and augments the leukocyte adhesion and the vascular permeability (Figure 4). There is an increased expression of the C5aR in transplanted kidneys with IRI or acute rejection[83,130]. Recently Cravedi *et al*[131] documented that pharmacological C5aR blockade in mice reduces the graft versus host disease, prolongs the survival and inhibits the T-cell responses. This provides the basis for future studies aimed to target C5aR. Several studies have documented that the activation of the C5-C5a receptor axis is involved in several human diseases[132]. In addition to eculizumab that to date is the only specific complement inhibitor approved for clinical use, several therapeutics targeting the C5a-C5aR axis are in different stages of clinical development ranging from preclinical studies to phase Ⅱ studies. These agents may target the axis at different levels, ranging from conversion of C5 to C5a and C5b, to inactivation of C5a, or to the inhibition of the two C5a receptors C5aR (CD88) and C5L2[132,133].

Table 3 Trials ongoing with eculizumab in renal diseases and in renal transplantation

Rank	Identifier	Status	Study name
1	NCT01221181	Active	Eculizumab therapy for dense deposit disease and C3 nephropathy
2	NCT02093533	Recruiting	Eculizumab in primary MPGN
3	NCT01275287	Active	Targeting complement activation in ANCA-vasculitis
4	NCT00844545	Completed	Open label controlled trial of eculizumab in adult patients with plasma therapy-resistant aHUS
5	NCT00844844	Completed	Open label controlled trial of eculizumab in adolescent patients with plasma therapy-resistant aHUS
6	NCT00844428	Unknown	Open label controlled trial of eculizumab in adolescent patients with plasma therapy-sensitive aHUS
7	NCT00838513	Unknown	Open label controlled trial of eculizumab in adult patients with plasma therapy-sensitive aHUS
8	NCT01194973	Unknown	An open-label, multi center clinical trial of eculizumab in adult patients with aHUS
9	NCT01193348	Unknown	An open label, Multi center clinical trial of eculizumab in pediatric patients with aHUS
10	NCT01755429	Unknown	The safety and efficacy of eculizumab in Japanese patients with aHUS
11	NCT01522170	Enrolling	aHUS observational long term follow up
12	NCT01522183	Recruiting	aHUS registry
13	NCT01770951	Completed	A retrospective, observational, non-interventional trial to assess eculizumab treatment effect in patients with aHUS
14	NCT02205541	Not yet recruiting	Eculizumab in shiga-toxin related hemolytic and uremic syndrome pediatric patients
15	NCT01410916	Completed	Safety and efficacy study of eculizumab in shiga-toxin producing *Escherichia coli* (STEC-HUS)
16	NCT01406288	Completed	Completed outbreak of HUS linked to *Escherichia coli* of serotype 0104:H4
17	NCT01756508	Recruiting	Eculizumab for prevention and treatment of kidney graft reperfusion injury
18	NCT01919346	Recruiting	Eculizumab for prevention of DGF in kidney transplantation
19	NCT01403389	Active	A study of the activity of eculizumab for prevention of DGF in deceased donor transplant
20	NCT02142182	Recruiting	A trial for prevention of DGF after kidney transplantation
21	NCT01567085	Active	Safety and efficacy of eculizumab in the prevention of AMR in sensitized recipients of a kidney transplant from a deceased donor
22	NCT02113891	Not yet recruiting	Eculizumab therapy for subclinical antibody-mediated rejection in kidney transplantation
23	NCT01095887	Active	Eculizumab added to conventional treatment in the prevention of antibody-mediated rejection in blood group incompatible living donor kidney transplantation
24	NCT01106027	Active	Dosing regimen of eculizumab added to conventional treatment in positive crossmatch deceased kidney yransplant
25	NCT01895127	Recruiting	Efficacy and safety of eculizumab for treatment of antibody-mediated rejection following renal transplantation
26	NCT00670774	Active	Dosing regimen of eculizumab added to conventional treatment in positive crossmatch living kidney transplant
27	NCT01399593	Active	Safety and efficacy of eculizumab to prevent AMR in living donor kidney transplant recipients receiving desensitization
28	NCT01327573	Active	Eculizumab therapy for chronic complement-mediated injury in kidney transplantation
29	NCT01029587	Recruiting	Eculizumab to enable renal transplantation in patients with history of catastrophic antiphospholipid antibody syndrome

MPGN: Membrano-proliferative glomerulonephritis; ANCA: Anti neutrophil cytoplasmic antibody; aHUS: Atypical hemolytic uremic syndrome; STEC-HUS: Shiga-toxin producing escherichia; DEGF: Delayed graft function; AMR: Antibody mediated rejection.

As aforementioned, after the findings that C5aR blockade protects against MPO-ANCA GN in mice[21] a clinical trial (NCT01363388) was started with a planned enrollment of 60 subjects affected by ANCA associated glomerulonephritis.

An important consideration, and a possible drawback, in blocking the C5a-C5aR axis is that the block itself might adversely affect the host defense and might counteract some of the useful recently identified functions of complement. Indeed, C5a may protect against neuron apoptosis[134], might act as an inhibitor of angiogenesis[135], and is essential for liver regeneration[136].

C1 INHIBITION

The beneficial effect of C1 inhibition on IRI has been widely studied by Castellano *et al*[71] and Curci *et al*[72]. These studies have been recently commented by Carney[73]. Purified or recombinant C1-INH is a host serine protease inhibitor that is able to block complement cascade acting either at level of classical and lectin pathway[137]. The first clinical indication of C1-INH has been hereditary angioedema. To date C1-INH has shown effects in several disease as myocardial ischemia and reperfusion injury[138], renal transplantation[139] and sepsis[140].

To date three clinical trials are ongoing in the field of kidney transplantation. The 2 first clinical trials (NCT01147302 and NCT01134510) have been made for the prevention of acute ABMR adding C1-INH to post-transplant treatment. The investigators observed that no patients developed ABMR during treatment with C1-INH and, in addition, noted a reduction in DGF due to I/R injury. As a consequence, recently a third trial with C1-INH was started (NCT02134314) to prevent DGF in patients receiving deceased donor kidney transplant.

TARGETING C3

In theory, the blockade at the level of C3 may be more effective than the anti C5 therapy, in particular for the C3 glomerulopathies when the C3 convertase

activation is prevalent over the C5 convertase. Soluble CR1 (sCR1) is among the proteins that regulate the C3 convertase. CR1 is a cell-surface glycoprotein expressed on several cells among which monocytes, APCs, T and B cells and podocytes. As a consequence sCR1 may modulate the complement cascade on all cells expressing on their surface CR1, follicular dendritic cells and a small T cells population[141-143]. In addition, CR1 is the only co-factor of factor I able to promote cleavage of inactive C3b and inactive C4b into their inactive protein fragments[144]. In normal condition only small quantities of sCR1 are in circulation. Lazar *et al*[145] and Li *et al*[146] administered high sCR1 in patients undergoing cardiac surgeries or cardiopulmonary by-pass to inhibit complement activity. These studies documented that sCR1 is effective and safe. sCR1 has been recently used in renal diseases and in renal transplantation.

Recently, Zhang *et al*[147] from Iowa University reported the results using sCR1 in mice deficient in factor H. sCR1 increases C3 serum levels and decreases C3 deposition. In the same report Zhang *et al*[147] reported the beneficial effect of treating by sCR1 a young patient affected by ESRD due to DDD. This group is currently enrolling patients for a small phase I trial of sCR1 (also called CDX-1135) in patients with DDD (NCT01791686).

C3 and C5 convertases decay is influenced by CR1. Treatment with sCR1 improved kidney transplant survival after a period of cold storage and when kidneys were transplanted across a complete major histocompatibility complex mismatch[148,149].

The effects of Mirococept (APT070) (sCR1) has been widely described by Sacks *et al*[69] and is currently the subject of a large scale study in kidney transplantation to test the superiority of Mirococept in the prevention of IRI in cadaveric renal allografts[150].

CONCLUSION

Emerging evidence has recently documented that the complement cascade as a common pathogenetic mechanism in many kidney diseases, in the chronic progression of the kidney diseases and in the kidney transplantation.

This finding led us to an improved understanding of the molecular mechanisms that are at the basis of the kidney diseases and, as a consequence allowed us to formulate a new classification of some renal diseases as the different kinds of hemolytic uremic syndromes and the membranoproliferative GN.

Among the new drugs aimed to target the complement in the renal diseases only the C5 monoclonal antibody, eculizumab, is to date on the market. Others are on clinical trials for the C3 glomerulopathies and the ANCA-mediated GN.

The use of eculizumab in treating the patients affected by aHUS has some limitations and is an example for the need of other drugs targeting the complement cascade. Indeed if eculizumab should be considered for all the patients with aHUS, because the dysregulation at C5 level is largely prevalent in this disease, the C3 glomerulopathies have variable degrees of the C5 convertase dysregulation.

In the cases were the C3 glomerulopathies are associated with a greater C3 convertase dysregulation, the blockade at the level of C3 should be the alternative to the eculizumab treatment.

The complement system is now recognized a pervasive, multifaceted mediator of transplant injury in animal models and in human transplant recipients[151]. The development of pharmacologic agents that block human complement components and receptors in the field of renal transplantation[152,153] now represents the basis of the concept that targeting complement in kidney transplant recipients will improve graft and patient survival rate.

REFERENCES

1 **Walport MJ**. Complement. First of two parts. *N Engl J Med* 2001; **344**: 1058-1066 [PMID: 11287977 DOI: 10.1056/NEJM200104053441406]

2 **Walport MJ**. Complement. Second of two parts. *N Engl J Med* 2001; **344**: 1140-1144 [PMID: 11297706 DOI: 10.1056/NEJM200104123441506]

3 **Holst B**, Raby AC, Hall JE, Labéta MO. Complement takes its Toll: an inflammatory crosstalk between Toll-like receptors and the receptors for the complement anaphylatoxin C5a. *Anaesthesia* 2012; **67**: 60-64 [PMID: 22150483 DOI: 10.1111/j.1365-2044.2011.07011.x]

4 **Zipfel PF**, Skerka C. Complement regulators and inhibitory proteins. *Nat Rev Immunol* 2009; **9**: 729-740 [PMID: 19730437 DOI: 10.1038/nri2620]

5 **Heeringa SF**, Cohen CD. Kidney diseases caused by complement dysregulation: acquired, inherited, and still more to come. *Clin Dev Immunol* 2012; **2012**: 695131 [PMID: 23227086 DOI: 10.1155/2012/695131]

6 **Kościelska-Kasprzak K**, Bartoszek D, Myszka M, Zabińska M, Klinger M. The complement cascade and renal disease. *Arch Immunol Ther Exp* (Warsz) 2014; **62**: 47-57 [PMID: 24030732 DOI: 10.1007/s00005-013-0254-x]

7 **Noris M**, Remuzzi G. Overview of complement activation and regulation. *Semin Nephrol* 2013; **33**: 479-492 [PMID: 24161035 DOI: 10.1016/j.semnephrol.2013.08.001]

8 **McCaughan JA**, O'Rourke DM, Courtney AE. The complement cascade in kidney disease: from sideline to center stage. *Am J Kidney Dis* 2013; **62**: 604-614 [PMID: 23489674 DOI: 10.1053/j.ajkd.2012.12.033]

9 **Wada T**, Nangaku M. Novel roles of complement in renal diseases and their therapeutic consequences. *Kidney Int* 2013; **84**: 441-450 [PMID: 23615508 DOI: 10.1038/ki.2013.134]

10 **Pickering MC**, Walport MJ. Links between complement abnormalities and systemic lupus erythematosus. *Rheumatology* (Oxford) 2000; **39**: 133-141 [PMID: 10725062 DOI: 10.1093/rheumatology/39.2.133]

11 **Nauta AJ**, Trouw LA, Daha MR, Tijsma O, Nieuwland R, Schwaeble WJ, Gingras AR, Mantovani A, Hack EC, Roos A. Direct binding of C1q to apoptotic cells and cell blebs induces complement activation. *Eur J Immunol* 2002; **32**: 1726-1736 [PMID: 12115656]

12 **Seelen MA**, Trouw LA, Daha MR. Diagnostic and prognostic significance of anti-C1q antibodies in systemic lupus erythematosus. *Curr Opin Nephrol Hypertens* 2003; **12**: 619-624 [PMID: 14564199]

13 **Wang Y**, Hu Q, Madri JA, Rollins SA, Chodera A, Matis LA. Amelioration of lupus-like autoimmune disease in NZB/WF1 mice after treatment with a blocking monoclonal antibody specific for complement component C5. *Proc Natl Acad Sci USA* 1996; **93**: 8563-8568 [PMID: 8710910]

14 **Turnberg D**, Cook HT. Complement and glomerulonephritis: new insights. *Curr Opin Nephrol Hypertens* 2005; **14**: 223-228 [PMID: 15821414]

15 **Ma R**, Cui Z, Liao YH, Zhao MH. Complement activation contributes to the injury and outcome of kidney in human anti-glomerular basement membrane disease. *J Clin Immunol* 2013; **33**: 172-178 [PMID: 22941511 DOI: 10.1007/s10875-012-9772-2]

16 **Chen M**, Xing GQ, Yu F, Liu G, Zhao MH. Complement deposition in renal histopathology of patients with ANCA-associated pauci-immune glomerulonephritis. *Nephrol Dial Transplant* 2009; **24**: 1247-1252 [PMID: 18940884 DOI: 10.1093/ndt/gfn586]

17 **Xing GQ**, Chen M, Liu G, Heeringa P, Zhang JJ, Zheng X, E J, Kallenberg CG, Zhao MH. Complement activation is involved in renal damage in human antineutrophil cytoplasmic autoantibody associated pauci-immune vasculitis. *J Clin Immunol* 2009; **29**: 282-291 [PMID: 19067130 DOI: 10.1007/s10875-008-9268-2]

18 **Gou SJ**, Yuan J, Chen M, Yu F, Zhao MH. Circulating complement activation in patients with anti-neutrophil cytoplasmic antibody-associated vasculitis. *Kidney Int* 2013; **83**: 129-137 [PMID: 22913983 DOI: 10.1038/ki.2012.313]

19 **Schreiber A**, Xiao H, Jennette JC, Schneider W, Luft FC, Kettritz R. C5a receptor mediates neutrophil activation and ANCA-induced glomerulonephritis. *J Am Soc Nephrol* 2009; **20**: 289-298 [PMID: 19073822 DOI: 10.1681/ASN.2008050497]

20 **Kallenberg CG**, Heeringa P. Complement is crucial in the pathogenesis of ANCA-associated vasculitis. *Kidney Int* 2013; **83**: 16-18 [PMID: 23271485 DOI: 10.1038/ki.2012.371]

21 **Xiao H**, Dairaghi DJ, Powers JP, Ertl LS, Baumgart T, Wang Y, Seitz LC, Penfold ME, Gan L, Hu P, Lu B, Gerard NP, Gerard C, Schall TJ, Jaen JC, Falk RJ, Jennette JC. C5a receptor (CD88) blockade protects against MPO-ANCA GN. *J Am Soc Nephrol* 2014; **25**: 225-231 [PMID: 24179165 DOI: 10.1681/ASN.2013020143]

22 **Beck LH**, Bonegio RG, Lambeau G, Beck DM, Powell DW, Cummins TD, Klein JB, Salant DJ. M-type phospholipase A2 receptor as target antigen in idiopathic membranous nephropathy. *N Engl J Med* 2009; **361**: 11-21 [PMID: 19571279 DOI: 10.1056/NEJMoa0810457]

23 **Beck LH**, Salant DJ. Membranous nephropathy: recent travels and new roads ahead. *Kidney Int* 2010; **77**: 765-770 [PMID: 20182413 DOI: 10.1038/ki.2010.34]

24 **Lhotta K**, Würzner R, König P. Glomerular deposition of mannose-binding lectin in human glomerulonephritis. *Nephrol Dial Transplant* 1999; **14**: 881-886 [PMID: 10328463 DOI: 10.1093/ndt/14.4.881]

25 **Val-Bernal JF**, Garijo MF, Val D, Rodrigo E, Arias M. C4d immunohistochemical staining is a sensitive method to confirm immunoreactant deposition in formalin-fixed paraffin-embedded tissue in membranous glomerulonephritis. *Histol Histopathol* 2011; **26**: 1391-1397 [PMID: 21938676]

26 **Ma H**, Sandor DG, Beck LH. The role of complement in membranous nephropathy. *Semin Nephrol* 2013; **33**: 531-542 [PMID: 24161038 DOI: 10.1016/j.semnephrol.2013.08.004]

27 **Takano T**, Elimam H, Cybulsky AV. Complement-mediated cellular injury. *Semin Nephrol* 2013; **33**: 586-601 [PMID: 24161043 DOI: 10.1016/j.semnephrol.2013.08.009]

28 **Mii A**, Shimizu A, Masuda Y, Fujita E, Aki K, Ishizaki M, Sato S, Griesemer A, Fukuda Y. Current status and issues of C1q nephropathy. *Clin Exp Nephrol* 2009; **13**: 263-274 [PMID: 19373520 DOI: 10.1007/s10157-009-0159-5]

29 **Suzuki H**, Kiryluk K, Novak J, Moldoveanu Z, Herr AB, Renfrow MB, Wyatt RJ, Scolari F, Mestecky J, Gharavi AG, Julian BA. The pathophysiology of IgA nephropathy. *J Am Soc Nephrol* 2011; **22**: 1795-1803 [PMID: 21949093 DOI: 10.1681/ASN.2011050464]

30 **Lai KN**. Pathogenesis of IgA nephropathy. *Nat Rev Nephrol* 2012; **8**: 275-283 [PMID: 22430056 DOI: 10.1038/nrneph.2012.58]

31 **Roos A**, Bouwman LH, van Gijlswijk-Janssen DJ, Faber-Krol MC, Stahl GL, Daha MR. Human IgA activates the complement system via the mannan-binding lectin pathway. *J Immunol* 2001; **167**: 2861-2868 [PMID: 11509633]

32 **Bomback AS**, Appel GB. Pathogenesis of the C3 glomerulopathies and reclassification of MPGN. *Nat Rev Nephrol* 2012; **8**: 634-642 [PMID: 23026947 DOI: 10.1038/nrneph.2012.213]

33 **Sethi S**, Nester CM, Smith RJ. Membranoproliferative glomerulonephritis and C3 glomerulopathy: resolving the confusion. *Kidney Int* 2012; **81**: 434-441 [PMID: 22157657 DOI: 10.1038/ki.2011.399]

34 **Alchi B**, Jayne D. Membranoproliferative glomerulonephritis. *Pediatr Nephrol* 2010; **25**: 1409-1418 [PMID: 19908070 DOI: 10.1007/s00467-009-1322-7]

35 **Loirat C**, Frémeaux-Bacchi V. Atypical hemolytic uremic syndrome. *Orphanet J Rare Dis* 2011; **6**: 60 [PMID: 21902819 DOI: 10.1186/1750-1172-6-60]

36 **Roumenina LT**, Loirat C, Dragon-Durey MA, Halbwachs-Mecarelli L, Sautes-Fridman C, Fremeaux-Bacchi V. Alternative complement pathway assessment in patients with atypical HUS. *J Immunol Methods* 2011; **365**: 8-26 [PMID: 21215749 DOI: 10.1016/j.jim.2010.12.020]

37 **Kavanagh D**, Goodship TH. Atypical hemolytic uremic syndrome, genetic basis, and clinical manifestations. *Hematology Am Soc Hematol Educ Program* 2011; **2011**: 15-20 [PMID: 22160007 DOI: 10.1182/asheducation-2011.1.15]

38 **Skerka C**, Józsi M, Zipfel PF, Dragon-Durey MA, Fremeaux-Bacchi V. Autoantibodies in haemolytic uraemic syndrome (HUS). *Thromb Haemost* 2009; **101**: 227-232 [PMID: 19190803 DOI: 10.1160/TH08-05-0322]

39 **Provaznikova D**, Rittich S, Malina M, Seeman T, Marinov I, Riedl M, Hrachovinova I. Manifestation of atypical hemolytic uremic syndrome caused by novel mutations in MCP. *Pediatr Nephrol* 2012; **27**: 73-81 [PMID: 21706448 DOI: 10.1007/s00467-011-1943-5]

40 **Bienaime F**, Dragon-Durey MA, Regnier CH, Nilsson SC, Kwan WH, Blouin J, Jablonski M, Renault N, Rameix-Welti MA, Loirat C, Sautés-Fridman C, Villoutreix BO, Blom AM, Fremeaux-Bacchi V. Mutations in components of complement influence the outcome of Factor I-associated atypical hemolytic uremic syndrome. *Kidney Int* 2010; **77**: 339-349 [PMID: 20016463 DOI: 10.1038/ki.2009.472]

41 **Delvaeye M**, Noris M, De Vriese A, Esmon CT, Esmon NL, Ferrell G, Del-Favero J, Plaisance S, Claes B, Lambrechts D, Zoja C, Remuzzi G, Conway EM. Thrombomodulin mutations in atypical hemolytic-uremic syndrome. *N Engl J Med* 2009; **361**: 345-357 [PMID: 19625716 DOI: 10.1056/NEJMoa0810739]

42 **Noris M**, Mescia F, Remuzzi G. STEC-HUS, atypical HUS and TTP are all diseases of complement activation. *Nat Rev Nephrol* 2012; **8**: 622-633 [PMID: 22986360 DOI: 10.1038/nrneph.2012.195]

43 **Thurman JM**, Marians R, Emlen W, Wood S, Smith C, Akana H, Holers VM, Lesser M, Kline M, Hoffman C, Christen E, Trachtman H. Alternative pathway of complement in children with diarrhea-associated hemolytic uremic syndrome. *Clin J Am Soc Nephrol* 2009; **4**: 1920-1924 [PMID: 19820137 DOI: 10.2215/CJN.02730409]

44 **Morigi M**, Galbusera M, Gastoldi S, Locatelli M, Buelli S, Pezzotta A, Pagani C, Noris M, Gobbi M, Stravalaci M, Rottoli D, Tedesco F, Remuzzi G, Zoja C. Alternative pathway activation of complement by Shiga toxin promotes exuberant C3a formation that triggers microvascular thrombosis. *J Immunol* 2011; **187**: 172-180 [PMID: 21642543 DOI: 10.4049/jimmunol.1100491]

45 **Zoja C**, Locatelli M, Pagani C, Corna D, Zanchi C, Isermann B, Remuzzi G, Conway EM, Noris M. Lack of the lectin-like domain of thrombomodulin worsens Shiga toxin-associated hemolytic uremic syndrome in mice. *J Immunol* 2012; **189**: 3661-3668 [PMID: 22942429]

46 **Kielstein JT**, Beutel G, Fleig S, Steinhoff J, Meyer TN, Hafer C, Kuhlmann U, Bramstedt J, Panzer U, Vischedyk M, Busch V, Ries W, Mitzner S, Mees S, Stracke S, Nürnberger J, Gerke P, Wiesner M, Sucke B, Abu-Tair M, Kribben A, Klause N, Schindler R, Merkel F, Schnatter S, Dorresteijn EM, Samuelsson O, Brunkhorst R. Best supportive care and therapeutic plasma exchange with or without eculizumab in Shiga-toxin-producing E. coli O104: H4 induced haemolytic-uraemic syndrome: an analysis of the German STEC-HUS registry. *Nephrol Dial Transplant* 2012; **27**: 3807-3815 [PMID: 23114903 DOI: 10.1093/ndt/gfs394]

47 **Delmas Y**, Vendrely B, Clouzeau B, Bachir H, Bui HN, Lacraz

A, Hélou S, Bordes C, Reffet A, Llanas B, Skopinski S, Rolland P, Gruson D, Combe C. Outbreak of Escherichia coli O104: H4 haemolytic uraemic syndrome in France: outcome with eculizumab. *Nephrol Dial Transplant* 2014; **29**: 565-572 [PMID: 24293658 DOI: 10.1093/ndt/gft470]

48 **Réti M**, Farkas P, Csuka D, Rázsó K, Schlammadinger Á, Udvardy ML, Madách K, Domján G, Bereczki C, Reusz GS, Szabó AJ, Prohászka Z. Complement activation in thrombotic thrombocytopenic purpura. *J Thromb Haemost* 2012; **10**: 791-798 [PMID: 22372946 DOI: 10.1111/j.1538-7836.2012.04674.x]

49 **Chapin J**, Weksler B, Magro C, Laurence J. Eculizumab in the treatment of refractory idiopathic thrombotic thrombocytopenic purpura. *Br J Haematol* 2012; **157**: 772-774 [PMID: 22409250 DOI: 10.1111/j.1365-2141.2012.09084.x]

50 **Pickering MC**, D'Agati VD, Nester CM, Smith RJ, Haas M, Appel GB, Alpers CE, Bajema IM, Bedrosian C, Braun M, Doyle M, Fakhouri F, Fervenza FC, Fogo AB, Frémeaux-Bacchi V, Gale DP, Goicoechea de Jorge E, Griffin G, Harris CL, Holers VM, Johnson S, Lavin PJ, Medjeral-Thomas N, Paul Morgan B, Nast CC, Noel LH, Peters DK, Rodríguez de Córdoba S, Servais A, Sethi S, Song WC, Tamburini P, Thurman JM, Zavros M, Cook HT. C3 glomerulopathy: consensus report. *Kidney Int* 2013; **84**: 1079-1089 [PMID: 24172683 DOI: 10.1038/ki.2013.377]

51 **Barbour TD**, Pickering MC, Cook HT. Recent insights into C3 glomerulopathy. *Nephrol Dial Transplant* 2013; **28**: 1685-1693 [PMID: 23479095 DOI: 10.1093/ndt/gfs430]

52 **Daha MR**, Fearon DT, Austen KF. C3 nephritic factor (C3NeF): stabilization of fluid phase and cell-bound alternative pathway convertase. *J Immunol* 1976; **116**: 1-7 [PMID: 1245733]

53 **Chen Q**, Müller D, Rudolph B, Hartmann A, Kuwertz-Bröking E, Wu K, Kirschfink M, Skerka C, Zipfel PF. Combined C3b and factor B autoantibodies and MPGN type II. *N Engl J Med* 2011; **365**: 2340-2342 [PMID: 22168663 DOI: 10.1056/NEJMc1107484]

54 **Jokiranta TS**, Solomon A, Pangburn MK, Zipfel PF, Meri S. Nephritogenic lambda light chain dimer: a unique human miniautoantibody against complement factor H. *J Immunol* 1999; **163**: 4590-4596 [PMID: 10510403]

55 **Sethi S**, Fervenza FC, Zhang Y, Nasr SH, Leung N, Vrana J, Cramer C, Nester CM, Smith RJ. Proliferative glomerulonephritis secondary to dysfunction of the alternative pathway of complement. *Clin J Am Soc Nephrol* 2011; **6**: 1009-1017 [PMID: 21415311 DOI: 10.2215/CJN.07110810]

56 **Servais A**, Frémeaux-Bacchi V, Lequintrec M, Salomon R, Blouin J, Knebelmann B, Grünfeld JP, Lesavre P, Noël LH, Fakhouri F. Primary glomerulonephritis with isolated C3 deposits: a new entity which shares common genetic risk factors with haemolytic uraemic syndrome. *J Med Genet* 2007; **44**: 193-199 [PMID: 17018561 DOI: 10.1136/jmg.2006.045328]

57 **Servais A**, Noël LH, Roumenina LT, Le Quintrec M, Ngo S, Dragon-Durey MA, Macher MA, Zuber J, Karras A, Provot F, Moulin B, Grünfeld JP, Niaudet P, Lesavre P, Frémeaux-Bacchi V. Acquired and genetic complement abnormalities play a critical role in dense deposit disease and other C3 glomerulopathies. *Kidney Int* 2012; **82**: 454-464 [PMID: 22456601 DOI: 10.1038/ki.2012.63]

58 **Barbour T**, Johnson S, Cohney S, Hughes P. Thrombotic microangiopathy and associated renal disorders. *Nephrol Dial Transplant* 2012; **27**: 2673-2685 [PMID: 22802583 DOI: 10.1093/ndt/gfs279]

59 **Gale DP**, de Jorge EG, Cook HT, Martinez-Barricarte R, Hadjisavvas A, McLean AG, Pusey CD, Pierides A, Kyriacou K, Athanasiou Y, Voskarides K, Deltas C, Palmer A, Frémeaux-Bacchi V, de Cordoba SR, Maxwell PH, Pickering MC. Identification of a mutation in complement factor H-related protein 5 in patients of Cypriot origin with glomerulonephritis. *Lancet* 2010; **376**: 794-801 [PMID: 20800271 DOI: 10.1016/S0140-6736(10)60670-8]

60 **Malik TH**, Lavin PJ, Goicoechea de Jorge E, Vernon KA, Rose KL, Patel MP, de Leeuw M, Neary JJ, Conlon PJ, Winn MP, Pickering MC. A hybrid CFHR3-1 gene causes familial C3 glomerulopathy. *J Am Soc Nephrol* 2012; **23**: 1155-1160 [PMID: 22626820 DOI: 10.1681/ASN.2012020166]

61 **Rodríguez-Iturbe B**, García García G. The role of tubulointerstitial inflammation in the progression of chronic renal failure. *Nephron Clin Pract* 2010; **116**: c81-c88 [PMID: 20502043 DOI: 10.1159/000314656]

62 **Gaarkeuken H**, Siezenga MA, Zuidwijk K, van Kooten C, Rabelink TJ, Daha MR, Berger SP. Complement activation by tubular cells is mediated by properdin binding. *Am J Physiol Renal Physiol* 2008; **295**: F1397-F1403 [PMID: 18753294 DOI: 10.1152/ajprenal.90313.2008]

63 **Siezenga MA**, van der Geest RN, Mallat MJ, Rabelink TJ, Daha MR, Berger SP. Urinary properdin excretion is associated with intrarenal complement activation and poor renal function. *Nephrol Dial Transplant* 2010; **25**: 1157-1161 [PMID: 19934084 DOI: 10.1093/ndt/gfp630]

64 **Bao L**, Wang Y, Haas M, Quigg RJ. Distinct roles for C3a and C5a in complement-induced tubulointerstitial injury. *Kidney Int* 2011; **80**: 524-534 [PMID: 21677637 DOI: 10.1038/ki.2011.158]

65 **Bao L**, Wang Y, Chang A, Minto AW, Zhou J, Kang H, Haas M, Quigg RJ. Unrestricted C3 activation occurs in Crry-deficient kidneys and rapidly leads to chronic renal failure. *J Am Soc Nephrol* 2007; **18**: 811-822 [PMID: 17229915 DOI: 10.1681/ASN.2006101176]

66 **Pratt JR**, Abe K, Miyazaki M, Zhou W, Sacks SH. In situ localization of C3 synthesis in experimental acute renal allograft rejection. *Am J Pathol* 2000; **157**: 825-831 [PMID: 10980122 DOI: 10.1016/S0002-9440(10)64596-8]

67 **Møller-Kristensen M**, Wang W, Ruseva M, Thiel S, Nielsen S, Takahashi K, Shi L, Ezekowitz A, Jensenius JC, Gadjeva M. Mannan-binding lectin recognizes structures on ischaemic reperfused mouse kidneys and is implicated in tissue injury. *Scand J Immunol* 2005; **61**: 426-434 [PMID: 15882434 DOI: 10.1111/j.1365-3083.2005.01591.x]

68 **Damman J**, Daha MR, van Son WJ, Leuvenink HG, Ploeg RJ, Seelen MA. Crosstalk between complement and Toll-like receptor activation in relation to donor brain death and renal ischemia-reperfusion injury. *Am J Transplant* 2011; **11**: 660-669 [PMID: 21446970 DOI: 10.1111/j.1600-6143.2011.03475.x]

69 **Sacks S**, Karegli J, Farrar CA, Asgari E, Schwaeble W, Zhou W, Smith RA. Targeting complement at the time of transplantation. *Adv Exp Med Biol* 2013; **735**: 247-255 [PMID: 23402032]

70 **Simone S**, Rascio F, Castellano G, Divella C, Chieti A, Ditonno P, Battaglia M, Crovace A, Staffieri F, Oortwijn B, Stallone G, Gesualdo L, Pertosa G, Grandaliano G. Complement-dependent NADPH oxidase enzyme activation in renal ischemia/reperfusion injury. *Free Radic Biol Med* 2014; **74**: 263-273 [PMID: 25017967]

71 **Castellano G**, Melchiorre R, Loverre A, Ditonno P, Montinaro V, Rossini M, Divella C, Battaglia M, Lucarelli G, Annunziata G, Palazzo S, Selvaggi FP, Staffieri F, Crovace A, Daha MR, Mannesse M, van Wetering S, Paolo Schena F, Grandaliano G. Therapeutic targeting of classical and lectin pathways of complement protects from ischemia-reperfusion-induced renal damage. *Am J Pathol* 2010; **176**: 1648-1659 [PMID: 20150432 DOI: 10.2353/ajpath.2010.090276]

72 **Curci C**, Castellano G, Stasi A, Divella C, Loverre A, Gigante M, Simone S, Cariello M, Montinaro V, Lucarelli G, Ditonno P, Battaglia M, Crovace A, Staffieri F, Oortwijn B, van Amersfoort E, Gesualdo L, Grandaliano G. Endothelial-to-mesenchymal transition and renal fibrosis in ischaemia/reperfusion injury are mediated by complement anaphylatoxins and Akt pathway. *Nephrol Dial Transplant* 2014; **29**: 799-808 [PMID: 24463188 DOI: 10.1093/ndt/gft516]

73 **Carney EF**. Acute kidney injury: critical role of complement in EndMT. *Nat Rev Nephrol* 2014; **10**: 183 [PMID: 24514752 DOI: 10.1038/nrneph.2014.19]

74 **Pratt JR**, Basheer SA, Sacks SH. Local synthesis of complement component C3 regulates acute renal transplant rejection. *Nat Med* 2002; **8**: 582-587 [PMID: 12042808]

75 **Fuquay R**, Renner B, Kulik L, McCullough JW, Amura C, Strassheim D, Pelanda R, Torres R, Thurman JM. Renal ischemia-reperfusion injury amplifies the humoral immune response. *J Am Soc Nephrol* 2013; **24**: 1063-1072 [PMID: 23641055 DOI: 10.1681/ASN.2012060560]

76 **Li K**, Patel H, Farrar CA, Hargreaves RE, Sacks SH, Zhou W. Complement activation regulates the capacity of proximal tubular epithelial cell to stimulate alloreactive T cell response. *J Am Soc Nephrol* 2004; **15**: 2414-2422 [PMID: 15339990 DOI: 10.1097/01.ASN.0000135974.06478.7B]

77 **Peng Q**, Li K, Patel H, Sacks SH, Zhou W. Dendritic cell synthesis of C3 is required for full T cell activation and development of a Th1 phenotype. *J Immunol* 2006; **176**: 3330-3341 [PMID: 16517700]

78 **Lalli PN**, Strainic MG, Yang M, Lin F, Medof ME, Heeger PS. Locally produced C5a binds to T cell-expressed C5aR to enhance effector T-cell expansion by limiting antigen-induced apoptosis. *Blood* 2008; **112**: 1759-1766 [PMID: 18567839 DOI: 10.1182/blood-2008-04-151068]

79 **van der Touw W**, Cravedi P, Kwan WH, Paz-Artal E, Merad M, Heeger PS. Cutting edge: Receptors for C3a and C5a modulate stability of alloantigen-reactive induced regulatory T cells. *J Immunol* 2013; **190**: 5921-5925 [PMID: 23690475 DOI: 10.4049/jimmunol.1300847]

80 **Strainic MG**, Liu J, Huang D, An F, Lalli PN, Muqim N, Shapiro VS, Dubyak GR, Heeger PS, Medof ME. Locally produced complement fragments C5a and C3a provide both costimulatory and survival signals to naive CD4+ T cells. *Immunity* 2008; **28**: 425-435 [PMID: 18328742 DOI: 10.1016/j.immuni.2008.02.001]

81 **Li Q**, Peng Q, Xing G, Li K, Wang N, Farrar CA, Meader L, Sacks SH, Zhou W. Deficiency of C5aR prolongs renal allograft survival. *J Am Soc Nephrol* 2010; **21**: 1344-1353 [PMID: 20651167 DOI: 10.1681/ASN.2009090977]

82 **Castellano G**, Woltman AM, Schlagwein N, Xu W, Schena FP, Daha MR, van Kooten C. Immune modulation of human dendritic cells by complement. *Eur J Immunol* 2007; **37**: 2803-2811 [PMID: 17899552 DOI: 10.1002/eji.200636845]

83 **Gueler F**, Rong S, Gwinner W, Mengel M, Bröcker V, Schön S, Greten TF, Hawlisch H, Polakowski T, Schnatbaum K, Menne J, Haller H, Shushakova N. Complement 5a receptor inhibition improves renal allograft survival. *J Am Soc Nephrol* 2008; **19**: 2302-2312 [PMID: 18753257 DOI: 10.1681/ASN.2007111267]

84 **Lin T**, Zhou W, Farrar CA, Hargreaves RE, Sheerin NS, Sacks SH. Deficiency of C4 from donor or recipient mouse fails to prevent renal allograft rejection. *Am J Pathol* 2006; **168**: 1241-1248 [PMID: 16565498 DOI: 10.2353/ajpath.2006.050360]

85 **Stegall MD**, Chedid MF, Cornell LD. The role of complement in antibody-mediated rejection in kidney transplantation. *Nat Rev Nephrol* 2012; **8**: 670-678 [PMID: 23026942 DOI: 10.1038/nrneph.2012.212]

86 **Haidar F**, Kisserli A, Tabary T, McGregor B, Noel LH, Réveil B, Toupance O, Rieu P, Thervet E, Legendre C, Morelon E, Issa N, Cohen JH. Comparison of C4d detection on erythrocytes and PTC-C4d to histological signs of antibody-mediated rejection in kidney transplantation. *Am J Transplant* 2012; **12**: 1564-1575 [PMID: 22420823 DOI: 10.1111/j.1600-6143.2012.04003.x]

87 **Brodsky SV**, Nadasdy GM, Pelletier R, Satoskar A, Birmingham DJ, Hadley GA, Obeidat K, Nadasdy T. Expression of the decay-accelerating factor (CD55) in renal transplants--a possible prediction marker of allograft survival. *Transplantation* 2009; **88**: 457-464 [PMID: 19696627 DOI: 10.1097/TP.0b013e3181b0517d]

88 **Zuber J**, Le Quintrec M, Morris H, Frémeaux-Bacchi V, Loirat C, Legendre C. Targeted strategies in the prevention and management of atypical HUS recurrence after kidney transplantation. *Transplant Rev* (Orlando) 2013; **27**: 117-125 [PMID: 23937869 DOI: 10.1016/j.trre.2013.07.003]

89 **Noris M**, Remuzzi G. Thrombotic microangiopathy after kidney transplantation. *Am J Transplant* 2010; **10**: 1517-1523 [PMID: 20642678 DOI: 10.1111/j.1600-6143.2010.03156.x]

90 **Mella A**, Messina M, Lavacca A, Biancone L. Complement cascade and kidney transplantation: The rediscovery of an ancient enemy. *World J Transplant* 2014; **4**: 168-175 [PMID: 25346889 DOI: 10.5500/wjt.v4.i3.168]

91 **Little MA**, Dupont P, Campbell E, Dorman A, Walshe JJ. Severity of primary MPGN, rather than MPGN type, determines renal survival and post-transplantation recurrence risk. *Kidney Int* 2006; **69**: 504-511 [PMID: 16395262 DOI: 10.1038/sj.ki.5000084]

92 **Zand L**, Lorenz EC, Cosio FG, Fervenza FC, Nasr SH, Gandhi MJ, Smith RJ, Sethi S. Clinical findings, pathology, and outcomes of C3GN after kidney transplantation. *J Am Soc Nephrol* 2014; **25**: 1110-1117 [PMID: 24357668 DOI: 10.1681/ASN.2013070715]

93 **Einecke G**, Sis B, Reeve J, Mengel M, Campbell PM, Hidalgo LG, Kaplan B, Halloran PF. Antibody-mediated microcirculation injury is the major cause of late kidney transplant failure. *Am J Transplant* 2009; **9**: 2520-2531 [PMID: 19843030 DOI: 10.1111/j.1600-6143.2009.02799.x]

94 **Sis B**, Mengel M, Haas M, Colvin RB, Halloran PF, Racusen LC, Solez K, Baldwin WM, Bracamonte ER, Broecker V, Cosio F, Demetris AJ, Drachenberg C, Einecke G, Gloor J, Glotz D, Kraus E, Legendre C, Liapis H, Mannon RB, Nankivell BJ, Nickeleit V, Papadimitriou JC, Randhawa P, Regele H, Renaudin K, Rodriguez ER, Seron D, Seshan S, Suthanthiran M, Wasowska BA, Zachary A, Zeevi A. Banff '09 meeting report: antibody mediated graft deterioration and implementation of Banff working groups. *Am J Transplant* 2010; **10**: 464-471 [PMID: 20121738 DOI: 10.1111/j.1600-6143.2009.02987.x]

95 **Kulkarni PA**, Afshar-Kharghan V. Anticomplement therapy. *Biologics* 2008; **2**: 671-685 [PMID: 19707448]

96 **Makrides SC**. Therapeutic inhibition of the complement system. *Pharmacol Rev* 1998; **50**: 59-87 [PMID: 9549758]

97 **Mollnes TE**, Kirschfink M. Strategies of therapeutic complement inhibition. *Mol Immunol* 2006; **43**: 107-121 [PMID: 16011851 DOI: 10.1016/j.molimm.2005.06.014]

98 **Bomback AS**. Anti-complement therapy for glomerular diseases. *Adv Chronic Kidney Dis* 2014; **21**: 152-158 [PMID: 24602464 DOI: 10.1053/j.ackd.2013.12.001]

99 **Hillmen P**, Young NS, Schubert J, Brodsky RA, Socié G, Muus P, Röth A, Szer J, Elebute MO, Nakamura R, Browne P, Risitano AM, Hill A, Schrezenmeier H, Fu CL, Maciejewski J, Rollins SA, Mojcik CF, Rother RP, Luzzatto L. The complement inhibitor eculizumab in paroxysmal nocturnal hemoglobinuria. *N Engl J Med* 2006; **355**: 1233-1243 [PMID: 16990386 DOI: 10.1056/NEJMoa061648]

100 ClinicalTrials.gov. Available from: URL: https: //www.clinicaltrials.gov

101 **Westra D**, Wetzels JF, Volokhina EB, van den Heuvel LP, van de Kar NC. A new era in the diagnosis and treatment of atypical haemolytic uraemic syndrome. *Neth J Med* 2012; **70**: 121-129 [PMID: 22516576]

102 **Ariceta G**, Arrizabalaga B, Aguirre M, Morteruel E, Lopez-Trascasa M. Eculizumab in the treatment of atypical hemolytic uremic syndrome in infants. *Am J Kidney Dis* 2012; **59**: 707-710 [PMID: 22196848 DOI: 10.1053/j.ajkd.2011.11.027]

103 **Zuber J**, Fakhouri F, Roumenina LT, Loirat C, Frémeaux-Bacchi V. Use of eculizumab for atypical haemolytic uraemic syndrome and C3 glomerulopathies. *Nat Rev Nephrol* 2012; **8**: 643-657 [PMID: 23026949 DOI: 10.1038/nrneph.2012.214]

104 **Legendre CM**, Licht C, Muus P, Greenbaum LA, Babu S, Bedrosian C, Bingham C, Cohen DJ, Delmas Y, Douglas K, Eitner F, Feldkamp T, Fouque D, Furman RR, Gaber O, Herthelius M, Hourmant M, Karpman D, Lebranchu Y, Mariat C, Menne J, Moulin B, Nürnberger J, Ogawa M, Remuzzi G, Richard T, Sberro-Soussan R, Severino B, Sheerin NS, Trivelli A, Zimmerhackl LB, Goodship T, Loirat C. Terminal complement inhibitor eculizumab in atypical hemolytic-uremic syndrome. *N Engl J Med* 2013; **368**: 2169-2181 [PMID: 23738544 DOI: 10.1056/NEJMoa1208981]

105 **Ring T**. Eculizumab in atypical hemolytic-uremic syndrome. *N Engl J Med* 2013; **369**: 1377-1378 [PMID: 24088106 DOI: 10.1056/NEJMc1308826]

106 **Amadio A**, Tejani AM. Eculizumab in atypical hemolytic-uremic syndrome. *N Engl J Med* 2013; **369**: 1378 [PMID: 24088107]

107 **Kistler AD**. Eculizumab in atypical hemolytic-uremic syndrome. *N Engl J Med* 2013; **369**: 1378 [PMID: 24088108]

108 **Tanimoto T**, Oshima Y, Kami M. Eculizumab in atypical hemolytic-uremic syndrome. *N Engl J Med* 2013; **369**: 1378-1379 [PMID: 24088109]

109 **Razzak M**. Anaemia: mutations in C5 explain eculizumab resistance. *Nat Rev Nephrol* 2014; **10**: 182 [PMID: 24566955 DOI: 10.1038/nrneph.2014.30]

110 **Nishimura J**, Yamamoto M, Hayashi S, Ohyashiki K, Ando K, Brodsky AL, Noji H, Kitamura K, Eto T, Takahashi T, Masuko M, Matsumoto T, Wano Y, Shichishima T, Shibayama H, Hase M, Li L, Johnson K, Lazarowski A, Tamburini P, Inazawa J, Kinoshita T, Kanakura Y. Genetic variants in C5 and poor response to eculizumab. *N Engl J Med* 2014; **370**: 632-639 [PMID: 24521109 DOI: 10.1056/NEJMoa1311084]

111 **Lapeyraque AL**, Malina M, Fremeaux-Bacchi V, Boppel T, Kirschfink M, Oualha M, Proulx F, Clermont MJ, Le Deist F, Niaudet P, Schaefer F. Eculizumab in severe Shiga-toxin-associated HUS. *N Engl J Med* 2011; **364**: 2561-2563 [PMID: 21612462 DOI: 10.1056/NEJMc1100859]

112 **Tsai E**, Chapin J, Laurence JC, Tsai HM. Use of eculizumab in the treatment of a case of refractory, ADAMTS13-deficient thrombotic thrombocytopenic purpura: additional data and clinical follow-up. *Br J Haematol* 2013; **162**: 558-559 [PMID: 23692124 DOI: 10.1111/bjh.12387]

113 **Scully M**, Goodship T. How I treat thrombotic thrombocytopenic purpura and atypical haemolytic uraemic syndrome. *Br J Haematol* 2014; **164**: 759-766 [PMID: 24387053 DOI: 10.1111/bjh.12718]

114 **Radhakrishnan S**, Lunn A, Kirschfink M, Thorner P, Hebert D, Langlois V, Pluthero F, Licht C. Eculizumab and refractory membranoproliferative glomerulonephritis. *N Engl J Med* 2012; **366**: 1165-1166 [PMID: 22435384 DOI: 10.1056/NEJMc1106619]

115 **Vivarelli M**, Pasini A, Emma F. Eculizumab for the treatment of dense-deposit disease. *N Engl J Med* 2012; **366**: 1163-1165 [PMID: 22435383 DOI: 10.1056/NEJMc1111953]

116 **Daina E**, Noris M, Remuzzi G. Eculizumab in a patient with dense-deposit disease. *N Engl J Med* 2012; **366**: 1161-1163 [PMID: 22435382 DOI: 10.1056/NEJMc1112273]

117 **McCaughan JA**, O'Rourke DM, Courtney AE. Recurrent dense deposit disease after renal transplantation: an emerging role for complementary therapies. *Am J Transplant* 2012; **12**: 1046-1051 [PMID: 22233157 DOI: 10.1111/j.1600-6143.2011.03923.x]

118 **Kerns E**, Rozansky D, Troxell ML. Evolution of immunoglobulin deposition in C3-dominant membranoproliferative glomerulopathy. *Pediatr Nephrol* 2013; **28**: 2227-2231 [PMID: 23892798 DOI: 10.1007/s00467-013-2565-x]

119 **Gurkan S**, Fyfe B, Weiss L, Xiao X, Zhang Y, Smith RJ. Eculizumab and recurrent C3 glomerulonephritis. *Pediatr Nephrol* 2013; **28**: 1975-1981 [PMID: 23689905 DOI: 10.1007/s00467-013-2503-y]

120 **Bomback AS**, Smith RJ, Barile GR, Zhang Y, Heher EC, Herlitz L, Stokes MB, Markowitz GS, D'Agati VD, Canetta PA, Radhakrishnan J, Appel GB. Eculizumab for dense deposit disease and C3 glomerulonephritis. *Clin J Am Soc Nephrol* 2012; **7**: 748-756 [PMID: 22403278 DOI: 10.2215/CJN.12901211]

121 **Herlitz LC**, Bomback AS, Markowitz GS, Stokes MB, Smith RN, Colvin RB, Appel GB, D'Agati VD. Pathology after eculizumab in dense deposit disease and C3 GN. *J Am Soc Nephrol* 2012; **23**: 1229-1237 [PMID: 22677550 DOI: 10.1681/ASN.2011121186]

122 **Nester CM**, Smith RJ. Treatment options for C3 glomerulopathy. *Curr Opin Nephrol Hypertens* 2013; **22**: 231-237 [PMID: 23318699 DOI: 10.1097/MNH.0b013e32835da24c]

123 **Xiao H**, Schreiber A, Heeringa P, Falk RJ, Jennette JC. Alternative complement pathway in the pathogenesis of disease mediated by anti-neutrophil cytoplasmic autoantibodies. *Am J Pathol* 2007; **170**: 52-64 [PMID: 17200182 DOI: 10.2353/ajpath.2007.060573]

124 **Zuber J**, Le Quintrec M, Krid S, Bertoye C, Gueutin V, Lahoche A, Heyne N, Ardissino G, Chatelet V, Noël LH, Hourmant M, Niaudet P, Frémeaux-Bacchi V, Rondeau E, Legendre C, Loirat C. Eculizumab for atypical hemolytic uremic syndrome recurrence in renal transplantation. *Am J Transplant* 2012; **12**: 3337-3354 [PMID: 22958221 DOI: 10.1111/j.1600-6143.2012.04252.x]

125 **Lonze BE**, Zachary AA, Magro CM, Desai NM, Orandi BJ, Dagher NN, Singer AL, Carter-Monroe N, Nazarian SM, Segev DL, Streiff MB, Montgomery RA. Eculizumab prevents recurrent antiphospholipid antibody syndrome and enables successful renal transplantation. *Am J Transplant* 2014; **14**: 459-465 [PMID: 24400968 DOI: 10.1111/ajt.12540]

126 **Lonze BE**, Dagher NN, Simpkins CE, Locke JE, Singer AL, Segev DL, Zachary AA, Montgomery RA. Eculizumab, bortezomib and kidney paired donation facilitate transplantation of a highly sensitized patient without vascular access. *Am J Transplant* 2010; **10**: 2154-2160 [PMID: 20636451 DOI: 10.1111/j.1600-6143.2010.03191.x]

127 **Cohney SJ**, Hughes P, Rosemary M, Walker RG, Cantwell L, Vanhardeveld E, Blecker K, Finlay M, Landgren A, Murugasu A. C5 inhibition with eculizumab to prevent antibody mediated rejection (AbMR) in patients with donor specific anti-HLA antibody (DSAb) and a positive cross match. *Am J Transplant* 2011; **11**: 483 [DOI: 10.1111/j.1600-6143.2011.03534.x]

128 **Hardinger KL**, Brennan DC. Novel immunosuppressive agents in kidney transplantation. *World J Transplant* 2013; **3**: 68-77 [PMID: 24392311 DOI: 10.5500/wjt.v3.i4.68]

129 **Stegall MD**, Diwan T, Raghavaiah S, Cornell LD, Burns J, Dean PG, Cosio FG, Gandhi MJ, Kremers W, Gloor JM. Terminal complement inhibition decreases antibody-mediated rejection in sensitized renal transplant recipients. *Am J Transplant* 2011; **11**: 2405-2413 [PMID: 21942930 DOI: 10.1111/j.1600-6143.2011.03757.x]

130 **Lewis AG**, Köhl G, Ma Q, Devarajan P, Köhl J. Pharmacological targeting of C5a receptors during organ preservation improves kidney graft survival. *Clin Exp Immunol* 2008; **153**: 117-126 [PMID: 18505432 DOI: 10.1111/j.1365-2249.2008.03678.x]

131 **Cravedi P**, Leventhal J, Lakhani P, Ward SC, Donovan MJ, Heeger PS. Immune cell-derived C3a and C5a costimulate human T cell alloimmunity. *Am J Transplant* 2013; **13**: 2530-2539 [PMID: 24033923 DOI: 10.1111/ajt.12405]

132 **Woodruff TM**, Nandakumar KS, Tedesco F. Inhibiting the C5-C5a receptor axis. *Mol Immunol* 2011; **48**: 1631-1642 [PMID: 21549429 DOI: 10.1016/j.molimm.2011.04.014]

133 **Li R**, Coulthard LG, Wu MC, Taylor SM, Woodruff TM. C5L2: a controversial receptor of complement anaphylatoxin, C5a. *FASEB J* 2013; **27**: 855-864 [PMID: 23239822 DOI: 10.1096/fj.12-220509]

134 **Mukherjee P**, Pasinetti GM. Complement anaphylatoxin C5a neuroprotects through mitogen-activated protein kinase-dependent inhibition of caspase 3. *J Neurochem* 2001; **77**: 43-49 [PMID: 11279260]

135 **Langer HF**, Chung KJ, Orlova VV, Choi EY, Kaul S, Kruhlak MJ, Alatsatianos M, DeAngelis RA, Roche PA, Magotti P, Li X, Economopoulou M, Rafail S, Lambris JD, Chavakis T. Complement-mediated inhibition of neovascularization reveals a point of convergence between innate immunity and angiogenesis. *Blood* 2010; **116**: 4395-4403 [PMID: 20625009 DOI: 10.1182/blood-2010-01-261503]

136 **Strey CW**, Markiewski M, Mastellos D, Tudoran R, Spruce LA, Greenbaum LE, Lambris JD. The proinflammatory mediators C3a and C5a are essential for liver regeneration. *J Exp Med* 2003; **198**: 913-923 [PMID: 12975457 DOI: 10.1084/jem.20030374]

137 **Ricklin D**, Lambris JD. Complement in immune and inflammatory disorders: therapeutic interventions. *J Immunol* 2013; **190**: 3839-3847 [PMID: 23564578 DOI: 10.4049/jimmunol.1203200]

138 **Lu F**, Fernandes SM, Davis AE. The effect of C1 inhibitor on myocardial ischemia and reperfusion injury. *Cardiovasc Pathol* 2013; **22**: 75-80 [PMID: 22705194 DOI: 10.1016/j.carpath.2012.05.003]

139 **Tillou X**, Poirier N, Le Bas-Bernardet S, Hervouet J, Minault D, Renaudin K, Vistoli F, Karam G, Daha M, Soulillou JP, Blancho G. Recombinant human C1-inhibitor prevents acute antibody-mediated rejection in alloimmunized baboons. *Kidney Int* 2010; **78**: 152-159 [PMID: 20336054 DOI: 10.1038/ki.2010.75]

140 **Igonin AA**, Protsenko DN, Galstyan GM, Vlasenko AV, Khachatryan NN, Nekhaev IV, Shlyapnikov SA, Lazareva NB, Herscu P. C1-esterase inhibitor infusion increases survival rates for patients with sepsis. *Crit Care Med* 2012; **40**: 770-777 [PMID: 22080632]

141 **Fang Y**, Xu C, Fu YX, Holers VM, Molina H. Expression of complement receptors 1 and 2 on follicular dendritic cells is neces-

sary for the generation of a strong antigen-specific IgG response. *J Immunol* 1998; **160**: 5273-5279 [PMID: 9605124]

142 **Rødgaard A**, Christensen LD, Thomsen BS, Wiik A, Bendixen G. Complement receptor type 1 (CR1, CD35) expression on peripheral T lymphocytes: both CD4- and CD8-positive cells express CR1. *Complement Inflamm* 1991; **8**: 303-309 [PMID: 1839374]

143 **Weiss L**, Fischer E, Haeffner-Cavaillon N, Jouvin MH, Appay MD, Bariety J, Kazatchkine M. The human C3b receptor (CR1). *Adv Nephrol Necker Hosp* 1989; **18**: 249-269 [PMID: 2522267]

144 **Morgan HP**, Schmidt CQ, Guariento M, Blaum BS, Gillespie D, Herbert AP, Kavanagh D, Mertens HD, Svergun DI, Johansson CM, Uhrín D, Barlow PN, Hannan JP. Structural basis for engagement by complement factor H of C3b on a self surface. *Nat Struct Mol Biol* 2011; **18**: 463-470 [PMID: 21317894 DOI: 10.1038/nsmb.2018]

145 **Lazar HL**, Keilani T, Fitzgerald CA, Shapira OM, Hunter CT, Shemin RJ, Marsh HC, Ryan US. Beneficial effects of complement inhibition with soluble complement receptor 1 (TP10) during cardiac surgery: is there a gender difference? *Circulation* 2007; **116**: I83-I88 [PMID: 17846331 DOI: 10.1161/]

146 **Li JS**, Sanders SP, Perry AE, Stinnett SS, Jaggers J, Bokesch P, Reynolds L, Nassar R, Anderson PA. Pharmacokinetics and safety of TP10, soluble complement receptor 1, in infants undergoing cardiopulmonary bypass. *Am Heart J* 2004; **147**: 173-180 [PMID: 14691437]

147 **Zhang Y**, Nester CM, Holanda DG, Marsh HC, Hammond RA, Thomas LJ, Meyer NC, Hunsicker LG, Sethi S, Smith RJ. Soluble CR1 therapy improves complement regulation in C3 glomerulopathy. *J Am Soc Nephrol* 2013; **24**: 1820-1829 [PMID: 23907509 DOI: 10.1681/ASN.2013010045]

148 **Patel H**, Smith RA, Sacks SH, Zhou W. Therapeutic strategy with a membrane-localizing complement regulator to increase the number of usable donor organs after prolonged cold storage. *J Am Soc Nephrol* 2006; **17**: 1102-1111 [PMID: 16510763 DOI: 10.1681/ASN.2005101116]

149 **Pratt JR**, Hibbs MJ, Laver AJ, Smith RA, Sacks SH. Allograft immune response with sCR1 intervention. *Transpl Immunol* 1996; **4**: 72-75 [PMID: 8762017]

150 An investigation into the treatment of the donor kidney to see if this improves the recovery of the kidney after transplantation. Available from: URL: http: //www.controlled-trials.com/ISRCTN49958194 [DOI 10.1186/ISRCTN49958194]

151 **Cravedi P**, Heeger PS. Complement as a multifaceted modulator of kidney transplant injury. *J Clin Invest* 2014; **124**: 2348-2354 [PMID: 24892709 DOI: 10.1172/JCI72273]

152 **Banz Y**, Rieben R. Role of complement and perspectives for intervention in ischemia-reperfusion damage. *Ann Med* 2012; **44**: 205-217 [PMID: 21254897 DOI: 10.3109/07853890.2010.535556]

153 **Chen G**, Chen S, Chen X. Role of complement and perspectives for intervention in transplantation. *Immunobiology* 2013; **218**: 817-827 [PMID: 23182708 DOI: 10.1016/j.imbio.2012.09.002]

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REVIEW

Pathogenesis of glomerular haematuria

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Abstract

Haematuria was known as a benign hallmark of some glomerular diseases, but over the last decade, new evidences pointed its negative implications on kidney disease progression. Cytotoxic effects of oxidative stress induced by hemoglobin, heme, or iron released from red blood cells may account for the tubular injury observed in human biopsy specimens. However, the precise mechanisms responsible for haematuria remain unclear. The presence of red blood cells (RBCs) with irregular contours and shape in the urine indicates RBCs egression from the glomerular capillary into the urinary space. Therefore glomerular haematuria may be a marker of glomerular filtration barrier dysfunction or damage. In this review we describe some key issues regarding epidemiology and pathogenesis of haematuric diseases as well as their renal morphological findings.

Key words: Haematuria; Pathogenesis; Glomerular filtration barrier; Dysmorphic red blood cells; Chronic kidney disease; Microscopic haematuria

Core tip: Recent advances suggest that glomerular haematuria may be a negative prognostic factor for renal function outcome. A more fragile and easily ruptured glomerular filtration barrier (GFB) may be responsible for glomerular bleeding. Several factors have been associated to this pathogenic process, including: (1) genetic alteration of GFB components, leading to a more fragile and easily ruptured GFB structure; (2) aberrant deposition of toxic molecules in the GFB; and (3) enhanced inflammatory response, as reported in autoimmune diseases, infections, or primary glomerulonephritis. In this review we fully describe these pathological mechanisms, with special interest in haematuric diseases and their renal morphological findings.

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INTRODUCTION

Haematuria is a common presenting feature of renal and urological diseases. It is described as the presence of more than 2 red blood cells (RBCs) per high-power field in the urine sediment. When the presence of RBCs in the urine is massive, the urine colour is red and is called macroscopic haematuria. Microscopic haematuria (MH) is detected by microscopical examination or dipstick, so its real incidence is unknown[1,2]. According with its origin haematuria can be glomerular or non-glomerular, however in this review we will focus exclusively on glomerular haematuria due to its implications in renal prognosis. The precise pathogenic mechanisms responsible of glomerular haematuria remain unclear. However, the identification of the specific molecular defect responsible of different genetic disorders commonly associated with haematuria has highlighted possible mechanisms. These genetic diseases originate glomerular filtration barrier (GFB) damage, leading to a more fragile and easily ruptured structure. Sometimes by directly alteration of the glomerular basement membrane (GBM) [as reported in Alport syndrome (AS), thin basement membrane nephropathy (TBMN) or hereditary angiopathy, nephropathy, aneurysms, and muscle cramps (HANAC) syndrome] or podocyte structure [Myosin heavy chain 9 (MYH9)-associated kidney disease], and others by aberrant deposition of toxic compounds like in storage disorders (Fibronectin glomerulopathy, Immunotactoid and Fibrillary glomerulonephritis). Inherit genetic mutations can also lead in an abnormal regulation of the complement alternative pathway and therefore C3 glomerular deposition [C3 glomerulopathies as complement factor H-related protein 5 (CFHR5) nephropathy or dense deposit disease], inducing a potent inflammatory response that results in phagocyte chemotaxis, with opsonization and lysis of cells which can easily explain haematuria. Haematuria can also be produced by inflammatory status as reported in autoimmune diseases [anti-neutrophil cytoplasmic antibodies (ANCA), Vasculitis, GBM disease, systemic lupus erithematosus or Cryoglobulinemia], infections (Endocapillary glomerulonephritis), or primary glomerulonephritis [IgA Nephropathy (IgAN), Membranoproliferative, Crescentic] (Table 1). In this review we will describe the molecular mechanisms responsible for histopathological findings in these diseases in order to explain the pathogenesis of haematuria and their relation with renal outcome.

PROGNOSTIC VALUE OF HAEMATURIA

Haematuria has traditionally been considered as a hallmark of some glomerular diseases, without repercussion on short and long-term kidney function[3]. However, over the last decade new evidences reported negative prognostic implications for both microscopic[4] and macroscopic haematuria[5] on the progression of renal disease. Thus, Vivante *et al*[4] reported that persistent asymptomatic isolated microscopic haematuria in 1 million young Israeli adults was significantly associated with increased risk of end stage renal disease (ESRD) after 22 years of follow up. Moreover, persistent glomerular hematuria in kidney donors has been associated with an increased risk of proteinuria and kidney disease progression at 2.3 years after donation[6].

Acute kidney injury (AKI) is a common complication of severe macroscopic haematuria, with an incidence of around 30% in IgAN patients with gross macrohaematuria bouts[7,8] and around 20% in warfarin-related nephropathy (WRN)[9]. Gutiérrez *et al*[5] reported that around 25% of IgAN patients did not recover baseline serum creatinine after cessation of macroscopic haematuria-associated AKI. In this study, duration of macroscopic bout was the more important prognostic factor determining incomplete recovery of renal function. Similarly in CFHR5-nephropathy almost all male patients who reached ESRD had episodes of macroscopic haematuria episodes after upper respiratory tract infections in childhood and adolescence[10].

The most important renal guidelines, Kidney Disease Outcomes Quality Initiative and Kidney Disease: Improving Global Outcome give a contradictory advice about haematuria management. These guidelines recommend to assess every chronic kidney disease (CKD) patient with dipstick[11,12], but haematuria is not recognized as a risk factor of CKD progression, and not recommend further monitoring or treatment in glomerulonephritis patients with isolated microscopic haematuria[13]. However they recognize that IgAN with haematuria and minimal proteinuria is a progressive disease[14], indicating that although clinical outcome for many haematuric patients is good, the lifetime risk for CKD patients may be elevated depending on the specific underlying disease.

CONSEQUENCES OF GLOMERULAR HAEMATURIA

Clinical data and basic research evidences suggest that haematuria induces renal damage. Acute tubular necrosis and intraluminal obstructive RBC casts are the most characteristic histologic findings in AKI during macroscopic hematuria. The principal mechanism of damage is the direct tubular toxicity of hemoglobin (Hb), heme, iron, or other molecules released from RBCs. It has been proposed that RBC passage throughout the GFB induces distortion of erythrocyte cytoskeleton, which is unable to maintain the cellular integrity, leading to RBC rupture. As consequence, the toxic molecules normally lock inside RBC's cytoplasm, such as Hb, heme, or iron, are released into the urinary space.

Hb is internalized into the epithelial tubular cell by the megalin/cubilin complex. Hb under the epithelial cell oxidant conditions dissociates into heme and globin. Heme oxigenase-1 (HO-1) catalyzes the conversion

Table 1 Classification of haematuric diseases by histopathological findings

Glomerular endothelial cell layer	GBM disorders	Mesangial deposits	Podocytary slit diaphragm disorders	Subendothelial/subepithelial deposit	Others
ANCA Endocapillary	Primary Alport TBMD HANAC ? LPHS Secondary Anti-GBM disease C3 glomerulopathy CFHR5 nephropathy	IgAN HSP	MYH9 disease Fabry disease	Primary GN MBP Endocapillary Crescentic Secondary GN SLE Cryoglobulinemia Fibrillar deposit Fibronectin Fibrillary Immunotactoid	WRN SCD

GBM: Glomerular basement membrane; ANCA: Antineutrophil cytoplasmic antibodies; CFHR5: Complement factor H-related 5 nephropathy; GN: Glomerulonephritis; HANAC: Hereditary angiopathy, nephropathy, aneurysms, and muscle cramps syndrome; IgAN: IgA nephropathy; LPHS: Loin back pain syndrome; MBP: Membranoproliferative; SCD: Sickle cell disease; HSP: Henoch-Schönlein purpura; SLE: Systemic lupus erythematous; TBMD: Thin basement membrane disease; WRN: Warfarin related nephropathy.

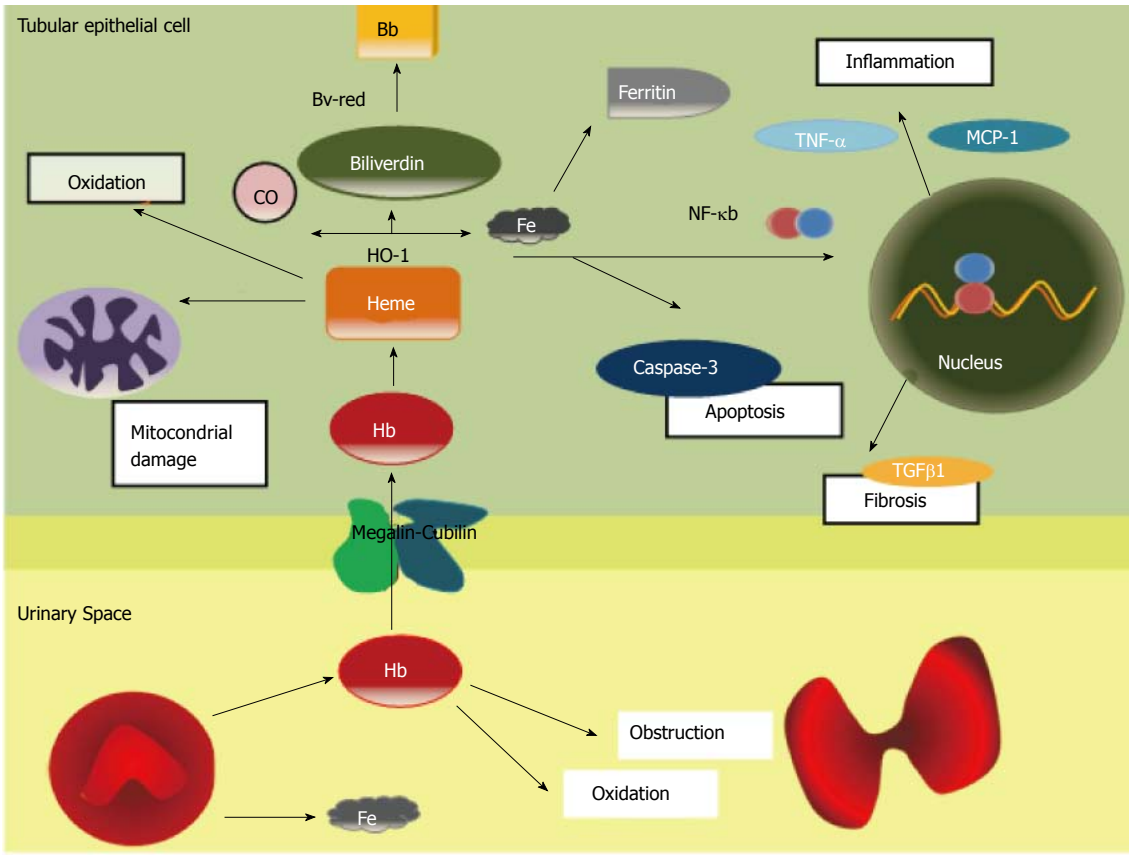

Figure 1 Haematuria-induced kidney injury in tubular cells. Hb: Haemoglobin; Bb: Bilirubin; Bv-red: Biliverdin reductase; CO: Carbon monoxide; Fe: Iron; HO-1: Heme oxygenase 1; MCP: Monocyte chemoattractant protein; NF-κb: Nuclear factor kappa b; TGF-β: Transforming growth factor beta; TNF-α: Tumour necrosis factor alpha.

of heme to biliverdin, iron and carbon monoxide[15]. At that time, the bilirubin reductase converts biliverdin in bilirubin and the iron is stored as Ferritin (Figure 1). HO-1 is now recognized as a protective molecule with anti-oxidant and anti-inflammatory properties against diverse insults in different tissues[16].

Free heme is also extremely toxic. In plasma and intracellular membranes, heme can oxidize lipids, denature proteins and perturb the cellular integrity[17]. In large amounts, heme may be a source of iron that drives oxidant injury after hypoxic and nephrotoxic insults[18]. Indirectly heme can also induce renal injury by

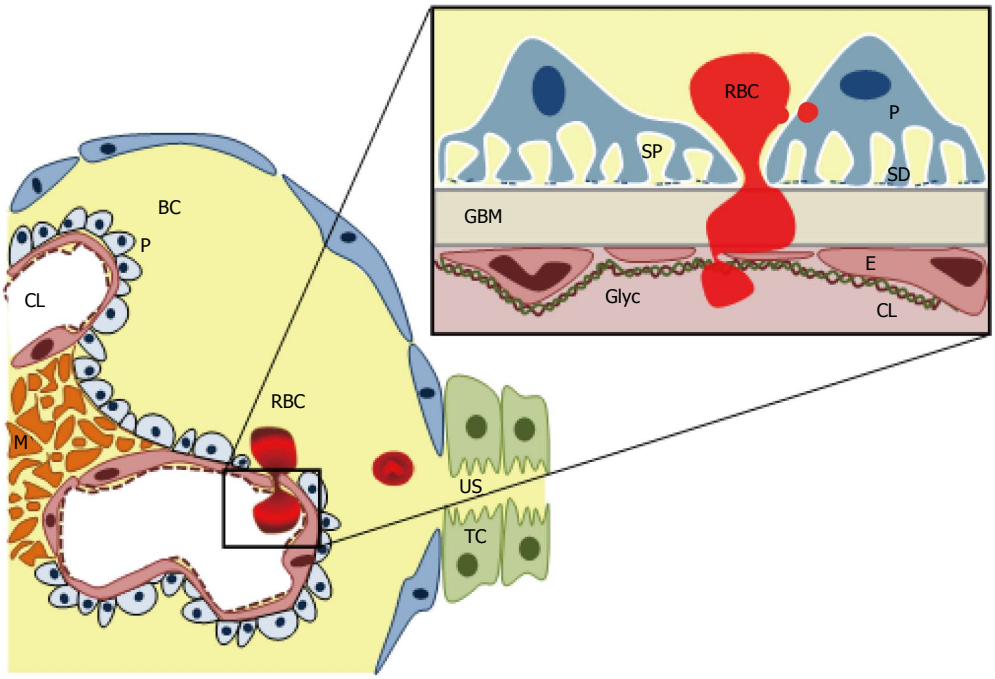

Figure 2 Glomerular filtration barrier structure and red blood cell egression leading to haematuria. CL: Capillary lumen; BC: Bowman's capsule; E: Endothelial cell; GBM: Glomerular basement membrane; Gly: Glycosaminoglicans; M: Mesangium; P: Podocyte; RBC: Red blood cell; SD: Slit diaphragm; SP: Subpodocyte space; TC: Tubular cell; US: Urinary space.

its proinflammatory effects, as inducing the chemokines such as monocyte chemoattractant protein-1 throughout the redox-sensitive transcription factor NF-KB[15]. The heme group of haemoglobin may also decrease nitric oxide availability, promoting intrarenal vasoconstriction and ischemia[19]. Finally, another possible mechanism involved in haematuria damage may be associated to a delayed dysmorphic RBC's elimination, which may explain the prolonged recovery period in patient with macrohaematuria induced AKI.

PATHOGENESIS OF HAEMATURIA

The presence of dysmorphic RBCs with irregular contours and shape in the urine is almost pathognomonic of glomerular haematuria[20] and indicates RBCs egression from the glomerular capillary into the urinary space. Therefore glomerular haematuria is a marker of the GFB dysfunction or damage[21].

GFB is an extremely complex and specialized structure[22,23], with different constituents and cell types, which allows a free permeability to water, small and midsized plasma solutes, but keeps a highly specialized selectivity for proteins and larger molecules according with size and molecular weight[24]. GFB has five major components: (1) from the vascular side, the endothelial surface layer, a complex glycosaminoglycan net which cover the endothelial layer as well as the fenestrations; (2) the endothelial cell; (3) the GBM; (4) podocytes with its interdigitating foot processes and specialized intercellular junctions, the "slit diaphragms"; and (5) finally on the urinary side, the subpodocyte space, an area delimited between the podocyte cell body and the foot processes (Figure 2). Furthermore, mesangial cell also indirectly contributes to GFB structure regulating and supporting the blood flow and the glomerular capillary structure, as well as controlling the mesangial matrix turnover (Figure 2). The GFB integrity is maintained by a complex interplay of signaling interactions between the three constituent cell types[24-26].

It has been thought that under physiological conditions, the endothelium with its fenestrations (50-100 nm) acts as molecular size sieve, self-sufficient to maintain the RBCs (6.2-8.2 μm) away from the GBM. However, haematuria in some diseases such as TBMN, Fibrillar deposit diseases or MYH9-associated kidney disease with typically intact endothelium, highlighted the key integrative role of GFB complex as a RBCs sieve. Therefore, how the RBCs, 100-fold bigger than the glomerular endothelium's pore, cross the GFB remains unclear. It is possible that a damaged GFB layer may release inflammatory or chemotactic signals promoting RBC passage throughout this layer, however the specific mechanisms have not yet found out.

According to its primary and histopathologic localization the haematuric disorders may be classified into: (1) Glomerular endothelial cell and surface layer injuries; (2) primary and secondary GBM disorders; (3) Diseases with mesangial deposition; (4) Diseases with subendothelial and subepithelial deposition; (5) Podocyte-associated disorders; and (6) Miscellaneous (Table 2).

Glomerular endothelial cell and surface layer injuries

In spite of the relatively big size of glomerular endothelial

Table 2 Possible pathogenic mechanisms of haematuria

Disease	Molecular defect	Prevalence	Main glomerular defect	Clinical expression		
				Haematuria	Proteinuria	CKD progression
Genetic disorder						
GFB structural damage						
Structural GBM damage						
ALPORT	X-linked: COL4A5 AR: COL4A3/COL4A4	1/50000	GBM	MH	Variable	100% approximately 20-30 yr
TBMD	COL4A3/COL4A4	1%	GBM	MH	Usually absent	20% CKD
HANAC	COL4A1	3 families	GBM	MH or gross	Not described	Variable
Structural podocyte damage						
MYH9	Non muscle myosin IIA heavy chain	1:100000	None	MH	Variable	ESRD by young adulthood
Storage disorders						
Fibronectine GN	Fibronectin	44 cases	Mesangial/ subendoth	60% MH	93% variable degree	ESRD at 20-60 yr
Fibrillary	10-30 nm fibrils	Rare	Mesangial /GBM	MH 47%-73% Gross 5%	Present 41%-55% nephrotic	50% ESRD in few years
Immunotactoid	> 30 nm fibrils	10-fold rarer than FGN	Mesangial/ subepith/subendoth	MH 80%	100%	17% ESRD in 3 yr
Fabry's disease	Lysosomal storage	1:3100- 1:1600	All the cells	MH	Usually nephrotic	ESRD after age 50 yr
Complement mediated						
C3 glomerulopathy	Alternative pathway	1-2 × 10^6	Mesangial/GBM	MH 87%	38%	Variable
Inflammatory disorders						
Autoimmune						
ANCA	Ab *vs* endothelium	10-20 × 10^6	Endothelium	MH	Variable	Variable
Anti GBM	Ab *vs* COL4	0.5-1 × 10^6/yr	GBM	MH	Variable	Variable
Infections (endocapillary)						
Primary GN (IgAN, membranoproliferative, crescentic)						
IgAN	Galactose-deficient IgA1	10%-16%	Mesangial	MH always 75% gross	Rare nephrotic Usual proteinuria	20% ESRD 20 yr after diagnosis
Miscellaneous						
WRN	Unknown	16.5% non-CKD 33% CKD	None	Usually MH	None	Accelerated CKD progression
LPHS	Unknown	Unknown	GBM (?)	MH or gross	Absent or minimal	GFR > 60

ANCA: Antineutrophil cytoplasmic antibodies; AR: Autosomal recesive; CKD: Chronic kidney disease; COL4A1: Alpha 1 chain of type Ⅳ collagen; COL4A3: Alpha 3 chain of type Ⅳ collagen; COL4A4: Alpha 4 chain of type Ⅳ collagen; COL4A1: Alpha 1 chain of type Ⅳ collagen; ESRD: End stage renal disease; GFB: Glomerular filtration barrier; GFR: Glomerular filtration rate; HANAC: Hereditary angiopathy, nephropathy, aneurysms, and muscle cramps syndrome; IgAN: IgA nephropathy; LPHS: Loin back pain haematuria syndrome; MH: Microscopic haematuria; TBMD: Thin basement membrane disease; WRN: Warfarin related nephropathy.

fenestrations, they play an important role in GFB perm selectivity due to its coating glycocalyx layer, composed principally by proteoglycans[27]. Glomerular endothelial cell glycocalyx and its associated surface layer retain more than 95% of the circulating proteins.

The glomerular endothelial layer is the main target of ANCAs, which attack small-vessel causing vasculitis, leading to necrotizing and crescentic glomerulonephritis. ANCA-vasculitis present an overall annual incidence of approximately 10-20 cases/million people, with an onset age peak of 65-74 years[28]. ANCA can induce the production and release of reactive oxygen species and lytic enzymes by infiltrated neutrophils[29], complement system *via* the alternative pathway[30] as well as endothelial cell as an amplification disease loop, resulting in endothelium lysis[31]. On early stages of ANCA-vasculitis the endothelial lesion could explain the onset of haematuria, although in advanced stages it could be explained by a severe GFB impairment usually involving all its layers. Although haematuria has been classically considered a marker of glomerular injury activity in ANCA, a recent report showed not repercussion of persistent haematuria (determined by dipstick) in GFR at 1 year[32]. However, in this study, persistent haematuria was associated to low baseline GFR and ANCA status.

Endothelial cell damage has been also reported in endocapillary glomerulonephritis (GN) and infection-associated GN. With a decreasing incidence over the last decades in developed countries[33], endocapillary GN is now more frequent in fragile patients, such as elder, alcoholics and intravenous drug users[34]. The typical presentation is nephritic syndrome or acute renal failure 15 d after an infection[35], in which haematuria is almost always present. Although the prognosis is excellent for children, in the 20%-74% of adults renal impairment persist[34-37]. The immune complexes produced *in situ* or deposited from circulation induce a severe inflammatory

response resulting in neutrophils chemotaxis and endocapillary hypercellularity, leading to haematuria. Endocapillary GN has been recently proposed as a C3 glomerulopathy, because the nephrogenic antigen triggers the activation of the complement alternative pathway.

GBM disorders

As previously reported, GBM has a key role on the glomerular filtration barrier permeability. GBM is composed of a dense gel-like meshwork of type Ⅳ collagen (COL4) and laminin, along with sulfated proteoglycans. The causes of GBM injury can be categorized on primary GBM disorders, as the collagen nephropathies, and secondary GBM diseases, including diseases with GBM as a target.

Primary GBM disorders: Collagen nephropathies are the main primary GBM disorders. Type Ⅳ collagen is the main component of GBM, so its mutations produce abnormal tights winding of the collagen triple helix. Type Ⅳ collagen-associated diseases are the most common hereditary disorders presenting with isolated microscopic haematuria resulting from mutations in genes for type Ⅳ collagen[38], especially on its alpha 3 (COL4A3) and 4 chains (COL4A4)[39].

AS was the first characterized GBM collagen-disorder. AS has a prevalence of 1 case/50000 live births[10]. The 85% of AS cases are due to X-linked mutations in α5 collagen chain (COL4A5), whereas the remaining 15% are due to autosomal recessive mutations in COL4A3 or COL4A4, although a minority of cases have been described as autosomal dominant sporadic mutations. X-linked AS is characterized by sensorineural hearing loss, ocular abnormalities and progressing nephropathy. These alterations are more severe in males. Autosomal reccesive Alport has the same clinical features than X-linked AS, with more aggressive and early CKD impairment (mean age at ESRD is 21 years[40]) without gender preference, and with typically asymptomatic parents genetically related. The electron microscopy show GBM thickening and thinning plus splitting and lamellation of lamina densa[41]. These alterations render in a persistent expression of foetal COL4A1 and COL4A2, being fragile and sensitive to proteases, allowing RBCs egression in the urinary space, and therefore persistent microhematuria. Persistent microhematuria is more frequent in children, often with macroscopic haematuria bouts, which suggest an exacerbating trigger factor over this chronically damaged GBM, although this promoter agent has not been yet identified. Importantly, renal function decreases progressively to ESRD before the fourth decade in 90% of patients[42,43].

Heterozygous mutations in the *COL4A3*, *COL4A4* or *COL4A5* genes produce TBMN. TBMN has an incidence of 1% and is characterized by a GBM < 150 nm. The slightly more compact GMB, due to a lack of non-collagenous molecules, is more fragile, which could explain the persistent isolated haematuria. TBMN's typical presentation include microhematuria and minimal or no proteinuria, with normal glomerular filtration rate and blood pressure. However, recent evidences showed a worse prognosis than it has been though[44], where microhematuria progress to proteinuria, to renal cysts[45] and to CKD in 26.6% of all patients, and in 48% of all patients > 50 years old[10,46].

HANAC syndrome is an extraordinary infrequent systemic basement-membrane disease, due to heterozygous mutation in *COL4A1*[47]. HANAC syndrome could present either with micro- or macroscopic bouts, related or not with impaired glomerular filtration rate and/or renal cysts. ESRD has not been yet described probably due to the low number of patients reported to date. Electron microscopy showed thickening and splitting on the basement membranes (including tubules, capillaries and GBM)[48]. The micro- and macroscopic haematuria bouts may be the result of the abnormal remodeling of the extracellular matrix and altered composition of all basement membranes[47].

Although loin pain haematuria syndrome (LPHS) is not a collagen nephropathy, we include it here due to its similarities with TBMN and AS histolopathologic features. More frequent in females (70%), LPHS presents with recurrent haematuria by the third decade of life. The electron microscopy showed abnormally thin or thick GBM[49]. It has been proposed that abnormalities on GBM allow RBCs leak into the urinary space causing intratubular obstruction and clots. The intratubular obstruction could induce interstitial edema and intraglomerular hypertension which originates further glomerular haemorrhage.

Secondary GBM disorders: Some disorders attack GBM, such as Anti-GBM disease and C3 glomerulopathy. Anti-GBM disease is characterized by autoantibodies against the alpha3 chain non-collagen 1 domain of type Ⅳ collagen. Anti-GBM disease shows an incidence of 0.5-1 case/million people per year[50]. It has been proposed that anti-GBM disease could be triggered over genetically predisposed patients (HLA-DRB1*1501 allele and genes of the FCGR and KLK families[51]) by environmental or cellular/humoral immunity factors. These auto-antibodies attack GBM disturbing its intrinsic structure, explaining the almost always present haematuria, with nephritic syndrome and crescentic glomerulonephritis.

On the other hand, the recently introduced C3 glomerulopathy, as a glomerular pathology with C3 accumulation with none significant immunoglobulin deposition[52]. C3 glomerulopathy clinically has been associated to haematuria, proteinuria and different degrees of renal dysfunction[53]. C3 glomerulopahty is secondary to an aberrant regulation of complement alternative pathway, either genetic or acquired. C3 glomerulopathies include dense deposit disease (DDD), C3 glomerulonephritis and complement factor H-related

(*CFHR*) genes mutations[54], such as hybrid *CFHR3-1* gene and an internal duplication within the *CFHR5* gene[55]. C3 glomerulopathy's incidence has been estimated in 1-2 cases/million people, independently of gender, although it has been reported an increased severity in males. DDD is characterized by linear, hyperosmiophilic, intramembranous dense deposit in lamina densa, restricted to both tubular and Bowman' s capsular basement membranes. Haematuria is observed in the 87% of the cases, mainly microscopic hematuria (68%)[53] and that persists during the follow up. Haematuria can be explained by the GBM impairment, although mesangial, subendothelial and subepithelial deposits has been also described[53]. Two evidences suggested the role of an infection triggering C3 glomerulopathies, firstly the concurrence of macroscopic bouts of haematuria with upper respiratory tract infections in CFHR5 nephropathy[54], and secondly the elevated antistreptolysin-O (a substance produced by group A Streptococcus bacteria) titters in C3 glomerulopathy[53]. Although proteinuria has pointed as the most important prognostic factor, in the Athanasiou cohort[56] all patients that reached ESRD presented macroscopic haematuria bouts associated with fever upper respiratory tract infections in the childhood and adolescence.

Mesangial deposit disorders

IgAN is the most common cause of glomerular haematuria. IgAN has an uncertain prevalence (10%-16%)[57] and is characterized by the presence of persistent isolated microscopic haematuria, with occasional macroscopical bouts associated to upper respiratory or gastrointestinal infections. Haematuria may be accompanied by proteinuria, sometimes in the nephrotic range. Although it has been consider benign, nearly 20% of patients develop ESRD within 20 years of diagnosis[58-61]. Mesangial hypercellulary is the usual histological finding, being the degree of interstitial fibrosis and tubular atrophy the strongest predictors of renal outcome[62]. However haematuria's role over IgAN outcome has not been properly addressed. Macroscopic haematuria bouts has negative implications on long-term prognosis[5] and although the prognosis of IgAN patients with isolated MH have been reported as good, almost 50% of the largest cohort presented spontaneous remission of MH during the follow up[63].

Even though mechanism of haematuria is unknown, during the episodes of macroscopic haematuria it has been detected an increase in circulating immune complexes composed of galactose-deficient IgA1 complexed with antiglycan antibodies[64]. These circulating immune complexes are deposited in the mesangium, inducing cell proliferation and secretion of several inflammatory mediators (including cytokines, growth factors and aldosterone/angiotensin) which can be released to the urinary space and induce both, podocytes and proximal tubular epithelial cells damage[65]. Therefore, these mediators could compromise GBM filtration-barrier function, allowing RBCs egression. The same pathological mechanism has been observed in Henoch-Schönlein purpura (HSP), a systemic disorder characterized by the coincidence of IgAN and leukocytoclastic vasculitis. In HSP patients, the plasma concentration of galactose-deficient IgA1 complexes are also increased and the subendothelial deposits, crescents as well as glomerular-tuft necrosis are even more frequent than in IgAN[65].

Subendothelial and subepithelial deposits diseases

Many nephropathies are characterized by the presence of deposits in subendothelial and subepithelial spaces, inducing a significant impairment in the GFB integrity and therefore haematuria.

Primary glomerulonephritis: Membranoproliferative, Endocapillary and Crescentic GN are the main primary GN associated with subendothelial and subepithelial deposits. It has been proposed that leucocytes and immune-complex can produce a severe inflammatory response activating glomerular cells and interfering with GBM structure, leading haematuria.

Fibril deposit disease: Fibronectin glomerulopathy (GFND) is a rare autosomal-dominant nephropathy due to a mutation in Fibronectin 1 (*FN1*) gene expressed[66]. FN1 is a dimeric glycoprotein constituent of the extracellular matrix. Its mutations altered the protein-dimers assembly into fibrils in the extracellular matrix and produce a disbalance between soluble and insoluble fibronectin, leading to its pathognomonic deposition in mesangium and subendothelial area[66,67]. In addition to fibronectine deposition, it has been reported IgA, C1q and fibrinogen deposits[68]. GFND may present at different ages, although mostly in adolescence or early adulthood. GFND is characterized by microhaematuria, proteinuria and hypertension. GFND patients progress to ESRD from second to sixth decade of life[66]. In these patients ESRD can recur after renal transplantation[69].

Fibrillary and Immunotactoid GN presented fibrils or microtubules deposition in mesangium, GBM or both. Immunotactoid GN can be differentiated from Fibrillary due to its typically wider fibrils with focal parallel alignment. The pathogenesis is unclear, however its response to immunosuppression pointed an underlying autoimmune condition[70]. Fibrillary GN presents deposits infiltrating both mesangium and lamina densa[71], which implied a severe impairment in the GFB allowing RBCs egression, explaining the pathogenesis of haematuria in these diseases.

Podocyte associated disorders

The podocytes are highly specialized epithelial cells, with interdigitating foot processes and specialized intercellular junctions term the "slit diaphragms", playing a key role in GFB integrity. Mutations in proteins involved

in the slit diaphragm and foot processes have been mainly associated with familial nephrotic syndrome. A previously kind of familial benign haematuria, MYH9-associated kidney disease, has been recently described as a genetic variation on *MYH9* gene. *MYH9* encode non-muscle myosin IIA heavy chain, a major protein of the actin-myosin's podocyte contractile apparatus, necessary to keep the capillary wall integrity[72]. Other autosomal-dominant syndromes as May-Hegglin anomaly and the Flechtner and Epstein syndromes also include abnormalities in *MYH9* gene, with a total incidence < 1:100000[38]. *MYH9* gene mutations presented variable degrees of sensorineural deafness and glomerulopathy[73], usually in African people. Haematuria and/or proteinuria are typically present since childhood, with a progression to ESRD by young adulthood[74]. Electron microscopy showed occasional focal thickening and splitting of the GBM[74,75]. *MYH9* mutations produce a more fragile podocyte and capillary wall which allow RBCs egression, explaining the presence of haematuria[75].

Fabry's disease also present haematuria. Fabry's disease is a lysosomal storage X-linked disorder, much more common than it has been though (1:3100[76]-1:1600[77]) and more frequent and aggressive in males. This lysosomal impairment leads to intracellular accumulations of globotriaosylceramide in almost all the human cells[78]. The globotriaosylceramide accumulation induce autophagy in podocytes and endothelial cells damage, resulting in focal and segmental sclerosis, as well as a significant impairment in the GFB, therefore leading proteinuria and haematuria[79].

Miscellaneous

There are several diseases associated to haematuria without any obvious histopathological finding to justify it. Warfarin coagulopathy (international normalized ratio > 3.0) may induce AKI, the so called WRN[80]. AKI could be caused by intratubular obstruction of RBC casts during glomerular haemorrhage, although atheroembolism[81], interstitial nephritis[82], and direct effects of warfarin on the glomerulus[83] have been also pointed. The real WRN incidence could be 16% in non-CKD and 37% in CKD patients[84]. There has been described several risk factors for WRN, including: (1) aspirin therapy; (2) drugs that increase glomerular hydrostatic pressure, such as dihydropyridine calcium channel blockers; (3) low serum albumin levels; and (4) concurrent congestive heart failure[85]. The correction of the warfarin coagulopathy with vitamin K prevents WRN, and could promote the recovery on animal models[86]. In WRN, 66% of the patients with macroscopic haematuria bouts show impairment of renal function. WRN is associated with an accelerated CKD progression and mortality rate, although this was related with the patienti's comorbidities[9]. It has been speculated that this warfarin iatrogenic coagulopathy may be observed in patients with permeable and previous "fragile" GFB (like subclinical GN or TBMD), allowing RBC egression.

Sickle cell disease (SCD) is a multisystemic disorder with homozygous or heterozygous inheritance of β-globin mutated gene, leading in the production of hemoglobin S (HbS), with a global incidence of 30/million people. HbS produce abnormally dense and rigid RBCs with tendency to sickle. The 3%-4% of SCD patients presented haematuria, although it is more frequent on heterozygotes with the sickle cell trait. The tortuous sickle RBCs can easily extravasate the glomerulus capillary wall, raising blood viscosity, and promoting microthrombi formation and ischemic necrosis in the vasa recta, and therefore inducing structural changes and haematuria[87]. Haematuria is mainly recurrent and macroscopic, and could be asymptomatic or painful due to passage of clots through the ureter. Furthermore, haemoglobinuria is also frequent is this patients due to its recurrent haemolytic anaemia crisis.

CONCLUSION

Recent findings suggest a pathogenic role for glomerular hematuria in kidney disease. Thus, the occurrence of macroscopic hematuria-associated AKI in IgAN nephropathy is associated with subsequent persistent impairment of renal function in a significant proportion of patients. An excessive anticoagulation, as a result of warfarin therapy, may also result in macroscopic hematuria-associated AKI and may compromise long-term renal function. Finally, persistent isolated microhematuria may also induce ESRD. The intrinsic pathogenical mechanism of glomerular haematuria remains unclear. Dysmorphic urinary RBCs pointed GFB dysfunction or damage as a possible alteration associated to this pathological process. Three possible pathological mechanisms may be implicated in GFB dysfunction and subsequent haematuria onset, including genetic alteration of GFB components, aberrant deposition of toxic molecules in the GFB, and an enhanced inflammatory response. However, although it has been identified some of the mechanisms involved in haematuria-associated renal damage, it is necessary to characterize new pathogenic effects of hematuria to identify new potential therapeutic targets. Future studies, in this line, will be of great interest.

REFERENCES

1 **Kelly JD**, Fawcett DP, Goldberg LC. Assessment and management of non-visible haematuria in primary care. *BMJ* 2009; **338**: a3021 [PMID: 19151065 DOI: 10.1136/bmj.a302]
2 **Cohen RA**, Brown RS. Clinical practice. Microscopic hematuria. *N Engl J Med* 2003; **348**: 2330-2338 [PMID: 12788998 DOI: 10.1056/NEJMcp012694]
3 **Clarkson AR**, Seymour AE, Thompson AJ, Haynes WD, Chan YL, Jackson B. IgA nephropathy: a syndrome of uniform morphology, diverse clinical features and uncertain prognosis. *Clin Nephrol* 1977; **8**: 459-471 [PMID: 589877]
4 **Vivante A**, Afek A, Frenkel-Nir Y, Tzur D, Farfel A, Golan E,

Chaiter Y, Shohat T, Skorecki K, Calderon-Margalit R. Persistent asymptomatic isolated microscopic hematuria in Israeli adolescents and young adults and risk for end-stage renal disease. *JAMA* 2011; **306**: 729-736 [PMID: 21846854 DOI: 10.1001/jama.2011.1141]

5 **Gutiérrez E**, González E, Hernández E, Morales E, Martínez MA, Usera G, Praga M. Factors that determine an incomplete recovery of renal function in macrohematuria-induced acute renal failure of IgA nephropathy. *Clin J Am Soc Nephrol* 2007; **2**: 51-57 [PMID: 17699387 DOI: 10.2215/CJN.02670706]

6 **Kido R**, Shibagaki Y, Iwadoh K, Nakajima I, Fuchinoue S, Fujita T, Teraoka S. Persistent glomerular hematuria in living kidney donors confers a risk of progressive kidney disease in donors after heminephrectomy. *Am J Transplant* 2010; **10**: 1597-1604 [PMID: 20353466 DOI: 10.1111/j.1600-6143.2010.03077.x]

7 **Kincaid-Smith P**, Bennett WM, Dowling JP, Ryan GB. Acute renal failure and tubular necrosis associated with hematuria due to glomerulonephritis. *Clin Nephrol* 1983; **19**: 206-210 [PMID: 6851258]

8 **Praga M**, Gutierrez-Millet V, Navas JJ, Ruilope LM, Morales JM, Alcazar JM, Bello I, Rodicio JL. Acute worsening of renal function during episodes of macroscopic hematuria in IgA nephropathy. *Kidney Int* 1985; **28**: 69-74 [PMID: 4046327]

9 **Brodsky SV**, Nadasdy T, Rovin BH, Satoskar AA, Nadasdy GM, Wu HM, Bhatt UY, Hebert LA. Warfarin-related nephropathy occurs in patients with and without chronic kidney disease and is associated with an increased mortality rate. *Kidney Int* 2011; **80**: 181-189 [PMID: 21389969 DOI: 10.1038/ki.2011.44]

10 **Deltas C**, Pierides A, Voskarides K. The role of molecular genetics in diagnosing familial hematuria(s). *Pediatr Nephrol* 2012; **27**: 1221-1231 [PMID: 21688191 DOI: 10.1007/s00467-011-1935-5]

11 **National Kidney Foundation.** K/DOQI clinical practice guidelines for chronic kidney disease: evaluation, classification, and stratification. *Am J Kidney Dis* 2002; **39**: S1-266 [PMID: 11904577]

12 KDIGO 2012 clinical practice guideline for the evaluation and management of chronic kidney disease. *Kidney Int Suppl* 2013; **3**: 1-150

13 KDIGO clinical practice guideline for glomerulonephritis. *Kidney Int Suppl* 2012; **2**: 139-274

14 **Szeto CC**, Lai FM, To KF, Wong TY, Chow KM, Choi PC, Lui SF, Li PK. The natural history of immunoglobulin a nephropathy among patients with hematuria and minimal proteinuria. *Am J Med* 2001; **110**: 434-437 [PMID: 11331053]

15 **Nath KA**. Heme oxygenase-1 and acute kidney injury. *Curr Opin Nephrol Hypertens* 2014; **23**: 17-24 [PMID: 24275768 DOI: 10.1097/01.mnh.0000437613.88158.d3]

16 **Nath KA**, Vercellotti GM, Grande JP, Miyoshi H, Paya CV, Manivel JC, Haggard JJ, Croatt AJ, Payne WD, Alam J. Heme protein-induced chronic renal inflammation: suppressive effect of induced heme oxygenase-1. *Kidney Int* 2001; **59**: 106-117 [PMID: 11135063 DOI: 10.1046/j.1523-1755.2001.00471.x]

17 **Tracz MJ**, Alam J, Nath KA. Physiology and pathophysiology of heme: implications for kidney disease. *J Am Soc Nephrol* 2007; **18**: 414-420 [PMID: 17229906 DOI: 10.1681/ASN.2006080894]

18 **Paller MS**, Jacob HS. Cytochrome P-450 mediates tissue-damaging hydroxyl radical formation during reoxygenation of the kidney. *Proc Natl Acad Sci USA* 1994; **91**: 7002-7006 [PMID: 8041736 DOI: 10.1073/pnas.91.15.7002]

19 **Heyman SN**, Brezis M. Acute renal failure in glomerular bleeding: a puzzling phenomenon. *Nephrol Dial Transplant* 1995; **10**: 591-593 [PMID: 7566565]

20 **Fairley KF**, Birch DF. Hematuria: a simple method for identifying glomerular bleeding. *Kidney Int* 1982; **21**: 105-108 [PMID: 7077941 DOI: 10.1038/ki.1982.16]

21 **Moreno JA**, Martín-Cleary C, Gutiérrez E, Rubio-Navarro A, Ortiz A, Praga M, Egido J. Haematuria: the forgotten CKD factor? *Nephrol Dial Transplant* 2012; **27**: 28-34 [PMID: 22287699 DOI: 10.1093/ndt/gfr749]

22 **Jarad G**, Miner JH. Update on the glomerular filtration barrier. *Curr Opin Nephrol Hypertens* 2009; **18**: 226-232 [PMID: 19374010 DOI: 10.1097/MNH.0b013e3283296044]

23 **Salmon AH**, Neal CR, Harper SJ. New aspects of glomerular filtration barrier structure and function: five layers (at least) not three. *Curr Opin Nephrol Hypertens* 2009; **18**: 197-205 [PMID: 19365184 DOI: 10.1097/MNH.0b013e328329f837]

24 **Menon MC**, Chuang PY, He CJ. The glomerular filtration barrier: components and crosstalk. *Int J Nephrol* 2012; **2012**: 749010 [PMID: 22934182 DOI: 10.1155/2012/749010]

25 **Eremina V**, Sood M, Haigh J, Nagy A, Lajoie G, Ferrara N, Gerber HP, Kikkawa Y, Miner JH, Quaggin SE. Glomerular-specific alterations of VEGF-A expression lead to distinct congenital and acquired renal diseases. *J Clin Invest* 2003; **111**: 707-716 [PMID: 12618525]

26 **Haraldsson BS**. The endothelium as part of the integrative glomerular barrier complex. *Kidney Int* 2014; **85**: 8-11 [PMID: 24380900 DOI: 10.1038/ki.2013.317]

27 **Fridén V**, Oveland E, Tenstad O, Ebefors K, Nyström J, Nilsson UA, Haraldsson B. The glomerular endothelial cell coat is essential for glomerular filtration. *Kidney Int* 2011; **79**: 1322-1330 [PMID: 21412215 DOI: 10.1038/ki.2011.58]

28 **Watts RA**, Scott DG, Jayne DR, Ito-Ihara T, Muso E, Fujimoto S, Harabuchi Y, Kobayashi S, Suzuki K, Hashimoto H. Renal vasculitis in Japan and the UK--are there differences in epidemiology and clinical phenotype? *Nephrol Dial Transplant* 2008; **23**: 3928-3931 [PMID: 18565978 DOI: 10.1093/ndt/gfn354]

29 **Falk RJ**, Terrell RS, Charles LA, Jennette JC. Anti-neutrophil cytoplasmic autoantibodies induce neutrophils to degranulate and produce oxygen radicals in vitro. *Proc Natl Acad Sci USA* 1990; **87**: 4115-4119 [PMID: 2161532]

30 **Schreiber A**, Xiao H, Jennette JC, Schneider W, Luft FC, Kettritz R. C5a receptor mediates neutrophil activation and ANCA-induced glomerulonephritis. *J Am Soc Nephrol* 2009; **20**: 289-298 [PMID: 19073822 DOI: 10.1681/ASN.2008050497]

31 **Kallenberg CG**. Pathophysiology of ANCA-associated small vessel vasculitis. *Curr Rheumatol Rep* 2010; **12**: 399-405 [PMID: 20878509 DOI: 10.1007/s11926-010-0138-6]

32 **Chen TK**, Murakami C, Manno RL, Geetha D. Hematuria duration does not predict kidney function at 1 year in ANCA-associated glomerulonephritis. *Semin Arthritis Rheum* 2014; **44**: 198-201 [PMID: 24775913 DOI: 10.1016/j.semarthrit.2014.03.008]

33 **Rodriguez-Iturbe B**, Musser JM. The current state of poststreptococcal glomerulonephritis. *J Am Soc Nephrol* 2008; **19**: 1855-1864 [PMID: 18667731 DOI: 10.1681/ASN.2008010092]

34 **Montseny JJ**, Meyrier A, Kleinknecht D, Callard P. The current spectrum of infectious glomerulonephritis. Experience with 76 patients and review of the literature. *Medicine* (Baltimore) 1995; **74**: 63-73 [PMID: 7891544]

35 **Sethi S**, Fervenza FC, Zhang Y, Zand L, Meyer NC, Borsa N, Nasr SH, Smith RJ. Atypical postinfectious glomerulonephritis is associated with abnormalities in the alternative pathway of complement. *Kidney Int* 2013; **83**: 293-299 [PMID: 23235567 DOI: 10.1038/ki.2012.384]

36 **Lien JW**, Mathew TH, Meadows R. Acute post-streptococcal glomerulonephritis in adults: a long-term study. *Q J Med* 1979; **48**: 99-111 [PMID: 482595]

37 **Moroni G**, Pozzi C, Quaglini S, Segagni S, Banfi G, Baroli A, Picardi L, Colzani S, Simonini P, Mihatsch MJ, Ponticelli C. Long-term prognosis of diffuse proliferative glomerulonephritis associated with infection in adults. *Nephrol Dial Transplant* 2002; **17**: 1204-1211 [PMID: 12105242]

38 **Gale DP**. How benign is hematuria? Using genetics to predict prognosis. *Pediatr Nephrol* 2013; **28**: 1183-1193 [PMID: 23325022 DOI: 10.1007/s00467-012-2399-y]

39 **Miner JH**. Developmental biology of glomerular basement membrane components. *Curr Opin Nephrol Hypertens* 1998; **7**: 13-19 [PMID: 9442357 DOI: 10.1097/00041552-199801000-00003]

40 **Oka M**, Nozu K, Kaito H, Fu XJ, Nakanishi K, Hashimura Y, Morisada N, Yan K, Matsuo M, Yoshikawa N, Vorechovsky I, Iijima K. Natural history of genetically proven autosomal recessive Alport syndrome. *Pediatr Nephrol* 2014; **29**: 1535-1544 [PMID: 24633401 DOI: 10.1007/s00467-014-2797-4]

41 **Barsotti P**, Muda AO, Mazzucco G, Massella L, Basolo B, De Marchi M, Rizzoni G, Monga G, Faraggiana T. Distribution of alpha-chains of type IV collagen in glomerular basement membranes

with ultrastructural alterations suggestive of Alport syndrome. *Nephrol Dial Transplant* 2001; **16**: 945-952 [PMID: 11328899 DOI: 10.1093/ndt/16.5.945]

42 **Longo I**, Scala E, Mari F, Caselli R, Pescucci C, Mencarelli MA, Speciale C, Giani M, Bresin E, Caringella DA, Borochowitz ZU, Siriwardena K, Winship I, Renieri A, Meloni I. Autosomal recessive Alport syndrome: an in-depth clinical and molecular analysis of five families. *Nephrol Dial Transplant* 2006; **21**: 665-671 [PMID: 16338941 DOI: 10.1093/ndt/gfi312]

43 **Jais JP**, Knebelmann B, Giatras I, De Marchi M, Rizzoni G, Renieri A, Weber M, Gross O, Netzer KO, Flinter F, Pirson Y, Verellen C, Wieslander J, Persson U, Tryggvason K, Martin P, Hertz JM, Schröder C, Sanak M, Krejcova S, Carvalho MF, Saus J, Antignac C, Smeets H, Gubler MC. X-linked Alport syndrome: natural history in 195 families and genotype- phenotype correlations in males. *J Am Soc Nephrol* 2000; **11**: 649-657 [PMID: 10752524]

44 **Carasi C**, Van't Hoff WG, Rees L, Risdon RA, Trompeter RS, Dillon MJ. Childhood thin GBM disease: review of 22 children with family studies and long-term follow-up. *Pediatr Nephrol* 2005; **20**: 1098-1105 [PMID: 15940548 DOI: 10.1007/s00467-005-1879-8]

45 **Sevillano A**, Gutierrez E, Morales E, Hernandez E, Molina M, Gonzalez E, Praga M. Multiple kidney cysts in thin basement membrane disease with proteinuria and kidney function impairment. *Clin Kidney J* 2014; **7**: 251-256 [DOI: 10.1093/ckj/sfu033]

46 **Temme J**, Peters F, Lange K, Pirson Y, Heidet L, Torra R, Grunfeld JP, Weber M, Licht C, Müller GA, Gross O. Incidence of renal failure and nephroprotection by RAAS inhibition in heterozygous carriers of X-chromosomal and autosomal recessive Alport mutations. *Kidney Int* 2012; **81**: 779-783 [PMID: 22237748 DOI: 10.1038/ki.2011.452]

47 **Plaisier E**, Chen Z, Gekeler F, Benhassine S, Dahan K, Marro B, Alamowitch S, Paques M, Ronco P. Novel COL4A1 mutations associated with HANAC syndrome: a role for the triple helical CB3[IV] domain. *Am J Med Genet A* 2010; **152A**: 2550-2555 [PMID: 20818663 DOI: 10.1002/ajmg.a.33659]

48 **Van Agtmael T**, Schlötzer-Schrehardt U, McKie L, Brownstein DG, Lee AW, Cross SH, Sado Y, Mullins JJ, Pöschl E, Jackson IJ. Dominant mutations of Col4a1 result in basement membrane defects which lead to anterior segment dysgenesis and glomerulopathy. *Hum Mol Genet* 2005; **14**: 3161-3168 [PMID: 16159887]

49 **Spetie DN**, Nadasdy T, Nadasdy G, Agarwal G, Mauer M, Agarwal AK, Khabiri H, Nagaraja HN, Nahman NS, Hartman JA, Hebert LA. Proposed pathogenesis of idiopathic loin pain-hematuria syndrome. *Am J Kidney Dis* 2006; **47**: 419-427 [PMID: 16490620]

50 **Chen M**, Cui Z, Zhao MH. ANCA-associated vasculitis and anti-GBM disease: the experience in China. *Nephrol Dial Transplant* 2010; **25**: 2062-2065 [PMID: 20228070 DOI: 10.1093/ndt/gfq134]

51 **Zhou XJ**, Lv JC, Zhao MH, Zhang H. Advances in the genetics of anti-glomerular basement membrane disease. *Am J Nephrol* 2010; **32**: 482-490 [PMID: 20962523 DOI: 10.1159/000321324]

52 **Fakhouri F**, Frémeaux-Bacchi V, Noël LH, Cook HT, Pickering MC. C3 glomerulopathy: a new classification. *Nat Rev Nephrol* 2010; **6**: 494-499 [PMID: 20606628 DOI: 10.1038/nrneph.2010.85]

53 **Medjeral-Thomas NR**, O'Shaughnessy MM, O'Regan JA, Traynor C, Flanagan M, Wong L, Teoh CW, Awan A, Waldron M, Cairns T, O'Kelly P, Dorman AM, Pickering MC, Conlon PJ, Cook HT. C3 glomerulopathy: clinicopathologic features and predictors of outcome. *Clin J Am Soc Nephrol* 2014; **9**: 46-53 [PMID: 24178974 DOI: 10.2215/CJN.04700513]

54 **Pickering MC**, D'Agati VD, Nester CM, Smith RJ, Haas M, Appel GB, Alpers CE, Bajema IM, Bedrosian C, Braun M, Doyle M, Fakhouri F, Fervenza FC, Fogo AB, Frémeaux-Bacchi V, Gale DP, Goicoechea de Jorge E, Griffin G, Harris CL, Holers VM, Johnson S, Lavin PJ, Medjeral-Thomas N, Paul Morgan B, Nast CC, Noel LH, Peters DK, Rodríguez de Córdoba S, Servais A, Sethi S, Song WC, Tamburini P, Thurman JM, Zavros M, Cook HT. C3 glomerulopathy: consensus report. *Kidney Int* 2013; **84**: 1079-1089 [PMID: 24172683 DOI: 10.1038/ki.2013.377]

55 **Medjeral-Thomas N**, Malik TH, Patel MP, Toth T, Cook HT, Tomson C, Pickering MC. A novel CFHR5 fusion protein causes C3 glomerulopathy in a family without Cypriot ancestry. *Kidney Int* 2014; **85**: 933-937 [PMID: 24067434 DOI: 10.1038/ki.2013.348]

56 **Athanasiou Y**, Voskarides K, Gale DP, Damianou L, Patsias C, Zavros M, Maxwell PH, Cook HT, Demosthenous P, Hadjisavvas A, Kyriacou K, Zouvani I, Pierides A, Deltas C. Familial C3 glomerulopathy associated with CFHR5 mutations: clinical characteristics of 91 patients in 16 pedigrees. *Clin J Am Soc Nephrol* 2011; **6**: 1436-1446 [PMID: 21566112 DOI: 10.2215/CJN.09541010]

57 **Suzuki K**, Honda K, Tanabe K, Toma H, Nihei H, Yamaguchi Y. Incidence of latent mesangial IgA deposition in renal allograft donors in Japan. *Kidney Int* 2003; **63**: 2286-2294 [PMID: 12753320 DOI: 10.1046/j.1523-1755.63.6s.2.x]

58 **Hastings MC**, Delos Santos NM, Wyatt RJ. Renal survival in pediatric patients with IgA nephropathy. *Pediatr Nephrol* 2007; **22**: 317-318 [PMID: 17024389 DOI: 10.1007/s00467-006-0303-3]

59 **Ronkainen J**, Ala-Houhala M, Autio-Harmainen H, Jahnukainen T, Koskimies O, Merenmies J, Mustonen J, Ormälä T, Turtinen J, Nuutinen M. Long-term outcome 19 years after childhood IgA nephritis: a retrospective cohort study. *Pediatr Nephrol* 2006; **21**: 1266-1273 [PMID: 16838184 DOI: 10.1007/s00467-006-0163-x]

60 **Kusumoto Y**, Takebayashi S, Taguchi T, Harada T, Naito S. Long-term prognosis and prognostic indices of IgA nephropathy in juvenile and in adult Japanese. *Clin Nephrol* 1987; **28**: 118-124 [PMID: 3665204]

61 **Syrjänen J**, Mustonen J, Pasternack A. Hypertriglyceridaemia and hyperuricaemia are risk factors for progression of IgA nephropathy. *Nephrol Dial Transplant* 2000; **15**: 34-42 [PMID: 10607765 DOI: 10.1093/ndt/15.1.34]

62 **Roberts IS**. Pathology of IgA nephropathy. *Nat Rev Nephrol* 2014; **10**: 445-454 [PMID: 24861083 DOI: 10.1038/nrneph.2014.92]

63 **Gutiérrez E**, Zamora I, Ballarín JA, Arce Y, Jiménez S, Quereda C, Olea T, Martínez-Ara J, Segarra A, Bernis C, García A, Goicoechea M, García de Vinuesa S, Rojas-Rivera J, Praga M. Long-term outcomes of IgA nephropathy presenting with minimal or no proteinuria. *J Am Soc Nephrol* 2012; **23**: 1753-1760 [PMID: 22956820]

64 **Novak J**, Tomana M, Matousovic K, Brown R, Hall S, Novak L, Julian BA, Wyatt RJ, Mestecky J. IgA1-containing immune complexes in IgA nephropathy differentially affect proliferation of mesangial cells. *Kidney Int* 2005; **67**: 504-513 [PMID: 15673298]

65 **Wyatt RJ**, Julian BA. IgA nephropathy. *N Engl J Med* 2013; **368**: 2402-2414 [PMID: 23782179 DOI: 10.1056/NEJMra1206793]

66 **Deltas C**, Pierides A, Voskarides K. Molecular genetics of familial hematuric diseases. *Nephrol Dial Transplant* 2013; **28**: 2946-2960 [PMID: 24046192 DOI: 10.1093/ndt/gft253]

67 **Strøm EH**, Banfi G, Krapf R, Abt AB, Mazzucco G, Monga G, Gloor F, Neuweiler J, Riess R, Stosiek P. Glomerulopathy associated with predominant fibronectin deposits: a newly recognized hereditary disease. *Kidney Int* 1995; **48**: 163-170 [PMID: 7564073 DOI: 10.1038/ki.1995.280]

68 **Yoshino M**, Miura N, Ohnishi T, Suzuki K, Kitagawa W, Nishikawa K, Imai H. Clinicopathological analysis of glomerulopathy with fibronectin deposits (GFND): a case of sporadic, elderly-onset GFND with codeposition of IgA, C1q, and fibrinogen. *Intern Med* 2013; **52**: 1715-1720 [PMID: 23903505 DOI: 10.2169/internalmedicine.52.0046]

69 **Gemperle O**, Neuweiler J, Reutter FW, Hildebrandt F, Krapf R. Familial glomerulopathy with giant fibrillar (fibronectin-positive) deposits: 15-year follow-up in a large kindred. *Am J Kidney Dis* 1996; **28**: 668-675 [PMID: 9158203]

70 **Javaugue V**, Karras A, Glowacki F, McGregor B, Lacombe C, Goujon JM, Ragot S, Aucouturier P, Touchard G, Bridoux F. Long-term kidney disease outcomes in fibrillary glomerulonephritis: a case series of 27 patients. *Am J Kidney Dis* 2013; **62**: 679-690 [PMID: 23759297 DOI: 10.1053/j.ajkd.2013.03.031]

71 **Nasr SH**, Valeri AM, Cornell LD, Fidler ME, Sethi S, Leung N, Fervenza FC. Fibrillary glomerulonephritis: a report of 66 cases from a single institution. *Clin J Am Soc Nephrol* 2011; **6**: 775-784 [PMID: 21441134 DOI: 10.2215/CJN.08300910]

72 **Arrondel C**, Vodovar N, Knebelmann B, Grünfeld JP, Gubler MC, Antignac C, Heidet L. Expression of the nonmuscle myosin heavy

chain IIA in the human kidney and screening for MYH9 mutations in Epstein and Fechtner syndromes. *J Am Soc Nephrol* 2002; **13**: 65-74 [PMID: 11752022]

73 **Kopp JB**, Winkler CA, Nelson GW. MYH9 genetic variants associated with glomerular disease: what is the role for genetic testing? *Semin Nephrol* 2010; **30**: 409-417 [PMID: 20807613 DOI: 10.1016/j.semnephrol.2010.06.007]

74 **Han KH**, Lee H, Kang HG, Moon KC, Lee JH, Park YS, Ha IS, Ahn HS, Choi Y, Cheong HI. Renal manifestations of patients with MYH9-related disorders. *Pediatr Nephrol* 2011; **26**: 549-555 [PMID: 21210153 DOI: 10.1007/s00467-010-1735-3]

75 **Kopp JB**. Glomerular pathology in autosomal dominant MYH9 spectrum disorders: what are the clues telling us about disease mechanism? *Kidney Int* 2010; **78**: 130-133 [PMID: 20588287 DOI: 10.1038/ki.2010.82]

76 **Spada M**, Pagliardini S, Yasuda M, Tukel T, Thiagarajan G, Sakuraba H, Ponzone A, Desnick RJ. High incidence of later-onset fabry disease revealed by newborn screening. *Am J Hum Genet* 2006; **79**: 31-40 [PMID: 16773563]

77 **Lin HY**, Chong KW, Hsu JH, Yu HC, Shih CC, Huang CH, Lin SJ, Chen CH, Chiang CC, Ho HJ, Lee PC, Kao CH, Cheng KH, Hsueh C, Niu DM. High incidence of the cardiac variant of Fabry disease revealed by newborn screening in the Taiwan Chinese population. *Circ Cardiovasc Genet* 2009; **2**: 450-456 [PMID: 20031620 DOI: 10.1161/CIRCGENETICS.109.862920]

78 **Prabakaran T**, Birn H, Bibby BM, Regeniter A, Sørensen SS, Feldt-Rasmussen U, Nielsen R, Christensen EI. Long-term enzyme replacement therapy is associated with reduced proteinuria and preserved proximal tubular function in women with Fabry disease. *Nephrol Dial Transplant* 2014; **29**: 619-625 [PMID: 24215016 DOI: 10.1093/ndt/gft452]

79 **Liebau MC**, Braun F, Höpker K, Weitbrecht C, Bartels V, Müller RU, Brodesser S, Saleem MA, Benzing T, Schermer B, Cybulla M, Kurschat CE. Dysregulated autophagy contributes to podocyte damage in Fabry's disease. *PLoS One* 2013; **8**: e63506 [PMID: 23691056 DOI: 10.1371/journal.pone.0063506]

80 **Brodsky SV**, Satoskar A, Chen J, Nadasdy G, Eagen JW, Hamirani M, Hebert L, Calomeni E, Nadasdy T. Acute kidney injury during warfarin therapy associated with obstructive tubular red blood cell casts: a report of 9 cases. *Am J Kidney Dis* 2009; **54**: 1121-1126 [PMID: 19577348 DOI: 10.1053/j.ajkd.2009.04.024]

81 **Moll S**, Huffman J. Cholesterol emboli associated with warfarin treatment. *Am J Hematol* 2004; **77**: 194-195 [PMID: 15389900]

82 **Kapoor KG**, Bekaii-Saab T. Warfarin-induced allergic interstitial nephritis and leucocytoclastic vasculitis. *Intern Med J* 2008; **38**: 281-283 [PMID: 18380703 DOI: 10.1111/j.1445-5994.2008.01646.x]

83 **Yanagita M**. Gas6, warfarin, and kidney diseases. *Clin Exp Nephrol* 2004; **8**: 304-309 [PMID: 15619028]

84 **Brodsky SV**, Collins M, Park E, Rovin BH, Satoskar AA, Nadasdy G, Wu H, Bhatt U, Nadasdy T, Hebert LA. Warfarin therapy that results in an International Normalization Ratio above the therapeutic range is associated with accelerated progression of chronic kidney disease. *Nephron Clin Pract* 2010; **115**: c142-c146 [PMID: 20413993 DOI: 10.1159/000312877]

85 **An JN**, Ahn SY, Yoon CH, Youn TJ, Han MK, Kim S, Chin HJ, Na KY, Chae DW. The occurrence of warfarin-related nephropathy and effects on renal and patient outcomes in korean patients. *PLoS One* 2013; **8**: e57661 [PMID: 23560034 DOI: 10.1371/journal.pone.0057661]

86 **Ozcan A**, Ware K, Calomeni E, Nadasdy T, Forbes R, Satoskar AA, Nadasdy G, Rovin BH, Hebert LA, Brodsky SV. 5/6 nephrectomy as a validated rat model mimicking human warfarin-related nephropathy. *Am J Nephrol* 2012; **35**: 356-364 [PMID: 22473220 DOI: 10.1159/000337918]

87 **Pham PT**, Pham PC, Wilkinson AH, Lew SQ. Renal abnormalities in sickle cell disease. *Kidney Int* 2000; **57**: 1-8 [PMID: 10620181 DOI: 10.1046/j.1523-1755.2000.00806.x]

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REVIEW

Renal dopaminergic system: Pathophysiological implications and clinical perspectives

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Abstract

Fluid homeostasis, blood pressure and redox balance in the kidney are regulated by an intricate interaction between local and systemic anti-natriuretic and natriuretic systems. Intrarenal dopamine plays a central role on this interactive network. By activating specific receptors, dopamine promotes sodium excretion and stimulates anti-oxidant and anti-inflammatory pathways. Different pathological scenarios where renal sodium excretion is dysregulated, as in nephrotic syndrome, hypertension and renal inflammation, can be associated with impaired action of renal dopamine including alteration in biosynthesis, dopamine receptor expression and signal transduction. Given its properties on the regulation of renal blood flow and sodium excretion, exogenous dopamine has been postulated as a potential therapeutic strategy to prevent renal failure in critically ill patients. The aim of this review is to update and discuss on the most recent findings about renal dopaminergic system and its role in several diseases involving the kidneys and the potential use of dopamine as a nephroprotective agent.

Key words: Dopamine; Hypertension; kidney; Na^+, K^+-ATPase; Sodium; Oxidative stress; D1 receptors; D2 receptors; Renal failure; Edema

Core tip: Renal dopaminergic system is a local and independent natriuretic system necessary to maintain the normal balance of sodium and water, blood pressure and renal redox steady state. Different findings from experimental and clinical studies highlight the participation

of renal dopamine in the pathophysiology of renal inflammation, hypertension, diabetic nephropathy and edema formation. Recent findings from experimental and clinical studies allow us to understand the complexity of this system as well as its possible contribution for future therapeutic strategies to prevent renal diseases.

Choi MR, Kouyoumdzian NM, Rukavina Mikusic NL, Kravetz MC, Rosón MI, Rodríguez Fermepin M, Fernández BE. Renal dopaminergic system: Pathophysiological implications and clinical perspectives. *World J Nephrol* 2015; 4(2): 196-212 Available from: URL: http://www.wjgnet.com/2220-6124/full/v4/i2/196.htm DOI: http://dx.doi.org/10.5527/wjn.v4.i2.196

INTRODUCTION

Kidney has all the bioenzymatic machinery necessary to possess a local dopaminergic system. Renal dopamine production depends on the precursor L-dihydroxyphenylalanine and dopa decarboxylase activity. Although dopa decarboxylase is present in high concentrations in the proximal tubular cells, L-dopa uptake by sodium dependent and independent transporters represents the limiting step for intrarenal dopamine synthesis[1-4]. Intrarenal dopamine can leave the cell through the apical border by a diffusional process, whereas plasma dopamine can be uptaken through the basal cell border by a saturable process[5]. The organic cation transporters (OCTs and OCTNs) have been postulated as potential carriers for dopamine through the tubular cells[5-7]. Finally, dopamine can be eliminated with urine flow or degraded by methylation (*via* catechol-O-methyl transferase or COMT) to 3-methoxytyramine, and by deamination (*via* monoamine oxidase or MAO) to 3,4-dihydroxyphenylacetic acid[8].

Several organs and systems are involved in the regulation of blood pressure. In particular, the kidney plays an essential role in the etiology of hypertension, but also represents a target organ vulnerable to hypertensive tissue damage. Alterations in renal tubule transport may be linked to the onset of hypertension in which dopamine could play an important role by affecting sodium handling on the proximal tubule[9]. Dopamine, as a major regulator of proximal tubule salt and water reabsorption, exerts its physiological actions through two families of receptors located in the tubular cell surface: D1-like receptors (D1 and D5) and D2-like receptors (D2, D3 and D4)[10-14]. Through activation of D1-like receptors, locally produced dopamine acts as an autocrine/paracrine natriuretic hormone by inhibiting the activity of both apical (*e.g.*, Na^+/H^+ exchange, Cl^-/HCO_3^- exchange and Na^+/Pi cotransport) and basolateral (*e.g.*, Na^+, K^+-ATPase and Na^+/HCO_3^- cotransport) transporters[15-17]. The D1-like receptors, coupled to the stimulatory G proteins Gαs and Golf, are characterized by their capacity to activate adenylate cyclase, while D2-like receptors, coupled to the inhibitory G proteins Gαi and Go, are characterized by their capacity to inhibit adenylate cyclase and modulate ion channels[18,19]. The classical signaling pathway for D1-like receptors leads to activation of adenylate cyclase and increases cyclic adenosine 3′,5′-monophosphate (cAMP) levels and protein kinase A (PKA) activation. PKA may either directly phosphorylate a target protein, such as a sodium transporting protein, or initiate a cascade of phosphorylation events by phosphorylation and activation of dopamine and cAMP-regulated phosphoprotein DARPP32[1]. D1 receptor can also stimulate phospholipase Cβ1 in renal tubules[20]. On the other hand, D2-like receptors can suppress Akt (protein kinase B) signaling pathway[21]. Both types of dopamine receptors are also linked to mitogen-activated protein kinase activation through different pathways and can interact with each other, resulting in new signaling pathways. In renal cortical cells the interaction between D1 and D2 receptors increases phospholipase C stimulation[22].

At glomerular level, dopamine increases cAMP in mesangial and podocyte cells *via* D1-like receptor and inhibits angiotensin Ⅱ-mediated contraction in mesangial cells[23,24]. Through this mechanism, dopamine induces depolarization of the podocyte that may lead to its relaxation[25]. These data suggest that dopamine can augment natriuresis and diuresis by increasing directly water and sodium filtration at glomerular level. Besides these effects on sodium and water homeostasis, it has been demonstrated that dopamine could exert anti-inflammatory and anti-oxidants properties by activation of D1-like and D2-like receptors[26-29].

To date, several studies reported that an intact dopaminergic system is required to maintain renal hemodynamic, fluid and electrolyte balance, redox steady state and blood pressure within a normal range and to antagonize the renin-angiotensin system[30,31]. In this way, alterations in dopamine production and its receptor number, function and/or post-translational modification are associated with different pathological scenarios like oxidative stress, genesis and progression of renal dysfunction, edema formation and genetic or essential hypertension. In clinical practice, dopamine is used as a first line vasoactive agent in patients with hemodynamic instability unresponsive to fluid therapy[32]. However, despite its diuretic and natriuretic properties, its clinical use in patients with renal failure remains controversial.

EFFECTS OF INTRARENAL DOPAMINE ON OXIDATIVE STRESS AND RENAL INFLAMMATION

The redox state of cells represents a balance between the generation of free radical/highly reactive species and the presence of antioxidant mechanisms. Acting as cellular messengers, reactive oxygen species (ROS) are

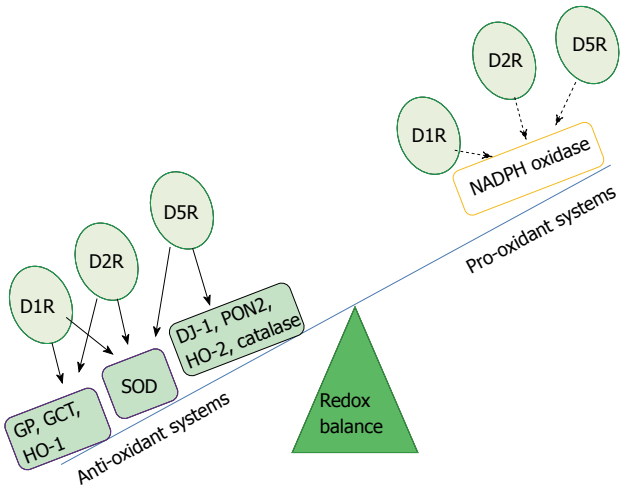

Figure 1 Dopamine receptors and regulation of redox state. Full line: Stimulation; Dotted line: Inhibition. D1R: Dopamine receptor subtype 1; D2R: Dopamine receptor subtype 2; D5R: Dopamine receptor subtype 5; NADPH: Nicotinamide adenine dinucleotide reduced form; SOD: Superoxide dismutase; HO-1: Heme oxygenase 1; HO-2: Heme oxygenase 2; PON2: Paraoxonase 2; DJ-1: Parkinson protein 7; GP: Glutathione peroxidase; GCT: Glutamyl cysteine transferase.

implicated in the destruction of invading pathogens. Pathological situations involving overproduction of free radicals (*e.g.*, atherosclerosis, hypertension, *etc.*) can lead to an increase in oxidative stress status, disrupting the normal cellular signaling mechanisms by alteration of the normal redox state of cells[33-36].

Oxidative stress and infiltration of inflammatory cells in the kidney are involved in the development of renal injury and hypertension[37]. Renal tubule cells produce both pro- and anti-inflammatory cytokines and chemokines, which are secreted across their apical and basolateral membranes, and are implicated in the development and progression of glomerular and tubular injury[38,39].

Several enzymes and receptors are involved in the regulation of the redox balance, including nicotinamide adenine dinucleotide reduced form (NADPH) oxidase and dopamine receptors (D1-like and D2-like receptors)[30]. Renal dopaminergic system represents a negative regulator of ROS. In this sense, D1, D2, and D5 receptors can exert antioxidant effects through direct and indirect inhibition of pro-oxidant enzymes, specifically, NADPH oxidase, and through stimulation of antioxidant enzymes such as superoxide dismutase (SOD) and heme-oxygenase (HO) among others, which can also indirectly inhibit NADPH oxidase activity[30,40]. Particularly, stimulation of D2 receptors in the kidney increases the expression of endogenous anti-oxidants, such as Parkinson protein 7 (PARK7 or DJ-1), paraoxonase 2, and HO-2, all of which can inhibit NADPH oxidase activity. By inhibition of phospholipase D2, the D5 receptor reduces NADPH oxidase activity. This receptor subtype also increases the expression of another antioxidant enzyme, HO-1. Finally, D1 receptor inhibits NADPH oxidase activity *via* PKA and PKC cross-talk and stimulates SOD, glutathione peroxidase, and glutamyl cysteine transferase activities (Figure 1)[30,40].

Additionally, it has been demonstrated that dopamine regulates the immune response and the inflammatory reaction by inhibiting the release of interferon γ (IFNγ), interleukin 2 (IL-2), and IL-4 and the lipopolysaccharide-stimulated production of IL-12p40 in immune cells[29,41]. Other authors showed that mice with intrarenal dopamine deficiency have increased oxidative stress and inflammatory cells infiltration; and that reduction in intrarenal dopamine synthesis is associated with increased detrimental effects of angiotensin Ⅱ on renal injury[42,43].

Experimental studies demonstrated that mice lacking D2 receptor (-/-) have increased levels of blood pressure as well as renal expression of inflammatory factors and renal injury[44,45]. To clarify if decreased D2 receptor function increases the vulnerability to renal inflammation, independently of blood pressure, Zhang *et al*[46] carried out experiments with D2 receptor (-/-) mice, and demonstrated that the treatment with apocynin (an inhibitor of NADPH oxidase) normalized blood pressure levels and decreased oxidative stress, without affecting the expression of inflammatory factors. In support of this evidence, it was reported that short-term D2 receptor silencing in one kidney (leaving the other kidney intact) in mice, induced the overexpression of inflammatory factors and markers of renal injury in the treated kidney, without increasing blood pressure levels[46]. Altogether, these studies indicate that D2 receptor impairment may cause renal inflammation as a primary effect, contributing to the subsequent development of hypertension. Polymorphisms of the human D2 receptor gene may be of clinical relevance since reduction in D2 receptor expression and function may lead to renal damage and oxidative stress[45,46].

Although these evidences indicate that alteration of the renal dopaminergic system may be associated with increased blood pressure *via* oxidative stress, this seems not to be the only mechanism by which impairment of renal dopaminergic system could lead to hypertension.

ROLE OF RENAL DOPAMINERGIC SYSTEM IN THE PATHOPHYSIOLOGY OF HYPERTENSION

Essential hypertension affects 25% of the adult population and constitutes a major risk factor for stroke, myocardial infarction, and heart and kidney failure[47-50]. The etiology of hypertension is complex and involves both genetic and environmental factors[51]. Genome-wide association studies have been able to identify 2% of genetic factors believed to influence blood pressure[52-58]. However, the studies were not designed to identify predisposing genes engaged in a complex network of gene-gene and gene-

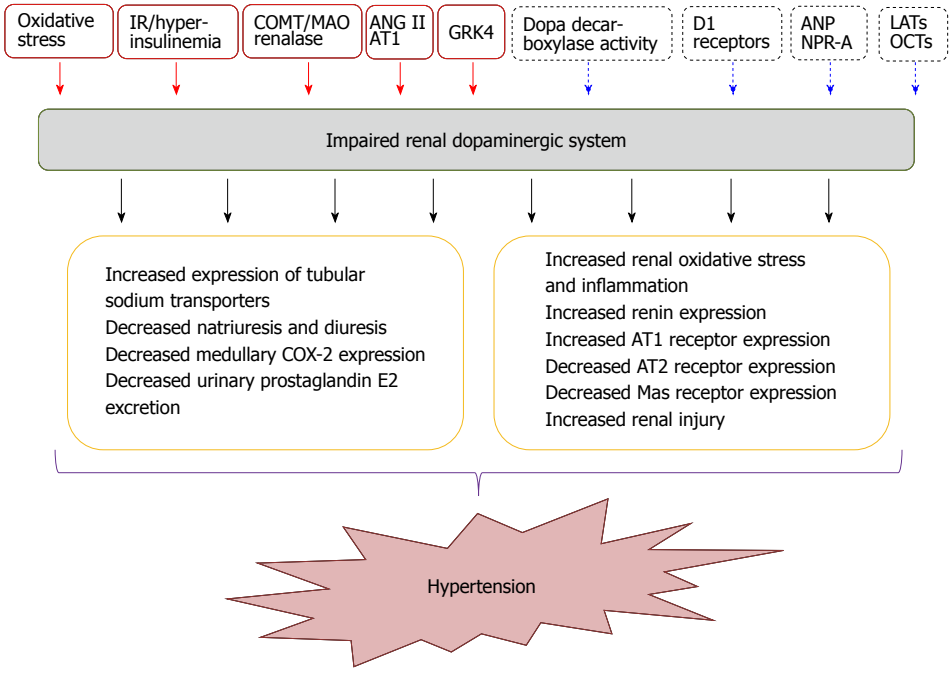

Figure 2 Impaired renal dopaminergic system and its association with hypertension. Red full squares and arrows indicate those factors that promote the impairment of renal dopamine; blue dotted squares and arrows indicate those factors that enhance renal dopaminergic system. IR: Insulin-resistance; COMT: Catechol-O-methyl-transferase; MAO: Monoamine-oxidase: AT1: Angiotensin II receptor subtype 1; AT2: Angiotensin II receptor subtype 2; COX-2: Cyclooxygenase type 2; ANP: Atrial natriuretic peptide; NPR-A: Natriuretic peptide receptor type A; LATs: L-aminoacids transporters; OCTs: Organic cationic transporters; GRK4: G-protein receptor kinase 4.

environment interactions[59]. Many genes have been proposed to cause hypertension and more than one gene is undoubtedly involved. On the other hand, the impairment of renal dopaminergic system functionality in hypertension has been extensively studied in patients as well in experimental animals, and strong evidences indicate that the alteration of this system plays a pathophysiological role in the development of different types of hypertension[30,60] (Figure 2). Several animal experimental models of hypertension exhibit one or more defects related to the renal dopaminergic system, and vice versa, different models with impairment of this system are associated with the development of hypertension (Table 1).

The interaction between renal dopamine and angiotensin Ⅱ can take place at receptors level[18]. In this way, AT2 and D1 receptors cooperatively oppose the vasoconstrictor and antinatriuretic functions elicited by angiotensin Ⅱ at AT1 receptor. It has been demonstrated that *in vivo* administration of fenoldopam (a highly selective D1-like receptor agonist) in sodium loaded Sprague Dawley rats induces the translocation of AT2 receptors from intracellular compartment to the apical plasma membranes[71]. This effect was confirmed by the fact that fenoldopam-induced natriuretic response was completely inhibited by the intrarenal co-infusion of the AT2 receptor antagonist PD123390[72]. Therefore, the alterations in D1 receptor-dependent translocation of AT2 receptor must be considered as a contributor factor for the initiation and progression of disease processes including hypertension. Blood pressure levels increase with aging, and alterations in both D1 and AT1 receptor functions are closely associated with the development of age-related hypertension[68,73-75]. Aging is a process associated with increase in oxidative stress and dysfunction of renal D1 and AT1 receptors[76-79]. Both receptors influence the activity of tubular Na^+, K^+-ATPase and contribute to maintain sodium homeostasis and blood pressure[77,79]. It has been reported in spontaneously hypertensive and obese Zucker rats an increase in oxidative stress and altered renal D1 and AT1 receptor functions[80-83].

Another possible mechanism involved in the impaired natriuretic effect in spontaneously hypertensive rats (SHRs) could be related to impaired endothelin B and D3 receptor interaction[84]. The endothelin B and dopamine receptors can interact to regulate renal function and blood pressure[85]. It has been demonstrated that activation of renal D3 receptor induces natriuresis and diuresis, but this effect is reduced in the presence of an endothelin B receptor antagonist, demonstrating that dopamine effects depends partially on endothelin B receptors. Moreover, stimulation of endothelin B receptor increases D3 receptor protein expression and vice versa in renal proximal tubule from Wistar Kyoto rats but not from SHRs[84,86]. Another study indicates that D3 receptors physically interact with proximal tubule endothelin B receptors and that the blunted natriuretic effect of dopamine in SHRs may be explained, in part, by abnormal D3/endothelin B

Table 1 Renal dopaminergic system impairment in experimental hypertension

Animal experimental model	Renal dopaminergic system impairment	Principal findings	Ref.
Spontaneously hypertensive rats	D1-like receptor function impairment caused by a defective coupling of the receptor with AC	Increased sodium reabsorption as a mechanism of hypertension	Ohbu *et al*[61]
Dahl salt-sensitive rats	D1-like receptor function impairment caused by a defective coupling of the receptor with AC	Prehypertensive Dahl salt-sensitive rats exhibit a blunted natriuretic response to dopamine compared with Dahl salt-resistant rats	Nishi *et al*[62]
DOCA salt-sensitive rats	Decreased renal dopamine production	Renal dopaminergic system is dominantly supressed in this model of hypertension	Iimura *et al*[63]
Dopamine receptor knockout mice	Defective D1-D2 like receptor/signal transduction	Impaired D1 and D2-like receptor signal pathway associated with development of hypertension	Banday *et al*[64] Zeng *et al*[65] Albrecht *et al*[66]
C57BL/6 mice	D1-like receptor function impairment associated with increased expression of GRK4 upon salt loading	Impaired ability to excrete a salt load with a resultant increase in blood pressure levels	Escano *et al*[67]
Mice with selective proximal tubule AADC deletion	Deletion of the kidney's ability to generate dopamine is associated with unbuffered response to angiotensin II that leads to hypertension and decreased longevity in mice	Increased expression of tubular sodium transporters, decreased natriuresis and diuresis in response to L-Dopa, decreased medullary COX-2 expression and urinary prostaglandin E2 excretion, increased renin and AT1 receptor expression, decreased AT2 and Mas receptor expression, and finally salt-sensitive hypertension.	Zhang *et al*[42]
Old FBN rats	Reduction of G-protein coupling in response to D1R activation associated with exaggerated AT1 receptor activity	Increase of oxidative stress	Chugh *et al*[68]
Renalase knockout mice	Alteration of urinary dopamine concentration in luminal fluid and proximal tubular transport	Impaired sodium excretion with increased blood pressure	Desir[18]
3/4 nephrectomized (3/4nx) rats	Decrease in urinary levels of dopamine and in renal AADC activity	A reduction in the natriuretic response to volume expansion with a time-dependent increase in both systolic and diastolic blood pressure	Moreira-Rodrigues *et al*[69]
Obese Zucker rats	Decrease in D1-like dopamine receptor binding sites and diminished activation of G proteins	Overproduction of ROS	Hussain *et al*[70]

GRK4: G protein receptor kinase 4; D1R: Dopamine receptor subtype 1; COX-2: Cyclooxygenase type 2; AT1: Angiotensin II receptor subtype 1; AT2: Angiotensin II receptor subtype 2; ROS: Reactive oxygen species; AC: Adenylate cyclase; FBN: Fischer 344 x Brown Norway F1.

receptor heterodimerization[85].

The interaction between prostanoids and renal dopamine on sodium and water excretion must also be considered. It has been demonstrated that the natriuretic response to dopamine was lower in Dahl salt-sensitive rats but this effect was reversed when chromosome 5 was transfered into these rats, leading to an increase of the renal expression of CYP4A protein and the production of 20-HETE[87]. Moreover, the inhibition of Na^+, K^+-ATPase activity by dopamine in the proximal tubule may be the result of the synergism between 20-HETE and the D1 signaling pathway[88]. In addition, other metabolites of arachidonic acid produced in the proximal tubule are epoxyeicosatrienoic acids and dihydroxyeicosatrienoic acids. As 20-HETE, epoxyeicosatrienoic acids can also regulate Na^+, K^+-ATPase activity and serve as second messengers for the natriuretic effects of dopamine. Since renal production of cytochrome P450 metabolites of arachidonic acid is altered in hypertension, a lower prostanoid synthesis may be involved in the impaired response to dopamine in this context[89].

Another protein that could be involved in the pathophysiology of hypertension is the novel amine oxidase, renalase[18]. Renalase is synthesized in the kidney with high expression in the proximal tubule, and then secreted into plasma and urine[19]. Renalase specifically degrades catecholamines, including dopamine. Recent findings indicate that renalase deficiency is associated with increased blood pressure and elevated circulating catecholamines[90,91]. Renalase expression depends on salt intake, and recombinant renalase exhibits a potent and prolonged hypotensive effect on blood pressure in Dahl salt-sensitive rats and rats with chronic kidney disease[92,93]. Urinary renalase metabolizes urinary catecholamines and it has been hypothesized that it might regulate dopamine concentration in the luminal fluid. However, the mechanisms of hypertension in animals with renalase deficiency and its relationship with the renal dopaminergic system are still unclear and deserve to be investigated in more detail.

Given its participation on sodium and water excretion and blood pressure regulation as well as its antioxidant properties in the kidney, alteration in renal dopaminergic system should also be considered in the pathophysiology of other diseases associated with

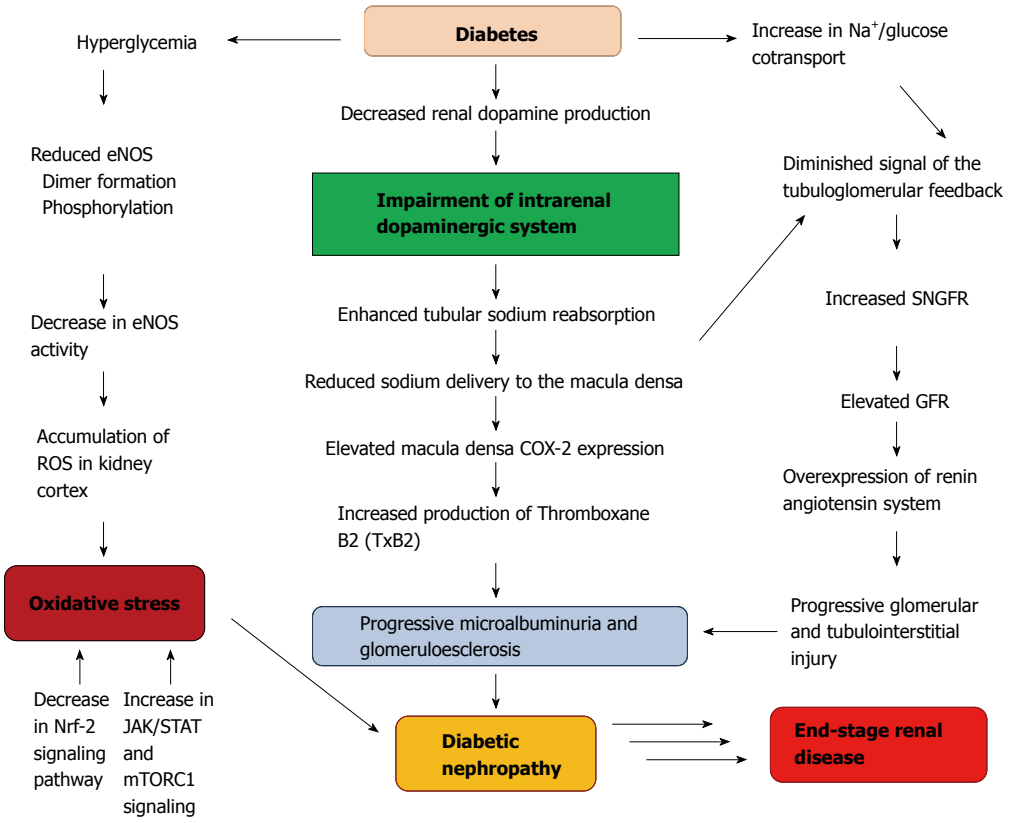

Figure 3 Association between diabetes and renal dopaminergic system in the pathophysiology of diabetic nephropathy. COX-2: Cyclooxygenase-2; GFR: Glomerular filtration rate; SNGFR: Single nephron glomerular filtration; eNOS: Endothelial nitric oxide synthase; ROS: Reactive oxygen species.

kidney damage such as diabetic nephropathy.

RENAL DOPAMINE, HYPERINSULINEMIA AND PATHOPHYSIOLOGY OF DIABETIC NEPHROPATHY

Diabetes in cursive is the most prevalent cause of end-stage kidney disease and its incidence has increased by more than 50% in the last 10 years[94]. Diabetic nephropathy is associated with elevated glomerular filtration rate, enhanced tubular sodium reabsorption, reduced sodium delivery to the macula densa and also with progressive glomerular and tubulointerstitial injuries[95,96]. Diabetic nephropathy is a major cause of mortality in both types diabetes. Adults with type 1 and 2 diabetes demonstrate insulin resistance, which is associated with diabetic nephropathy[97,98]. Experimental studies of diabetic nephropathy helped to understand the pathophysiological mechanisms underlying the disease process and allowed to identify potential molecular targets for future pharmacological treatment. In this way, it has been demonstrated a decrease in endothelial nitric oxide synthase (eNOS) activity and renal dopamine production and an increase in Nrf-2, JAK/STAT and mTORC1 signaling as contributor factors in the development of diabetic nephropathy[99]. Alteration of mechanisms involving dopamine handling and signaling by the proximal tubule cells could lead to a progressive damage in diabetic nephropathy. In addition to the renin angiotensin system, alterations of renal cyclooxygenase-2 (COX-2) function are also involved in renal hemodynamic changes and structural abnormalities observed in diabetic nephropathy[100-102]. Previous findings showed that renal dopamine inhibits COX-2 expression in the macula densa, suggesting that the impairment of intrarenal dopaminergic system observed in diabetes, may contribute to reduce the luminal offer of sodium to this area, resulting in an elevated macula densa COX-2 expression[17,103,104] (Figure 3). Another experimental study carried out in mouse models of type 1 diabetes demonstrated that enhanced proximal tubule dopamine levels by deletion of $COMT^{-/-}$ gen was associated with substantial amelioration of early hyperfiltration, decreased macula densa COX-2 expression, decreased albuminuria and glomerulopathy, and inhibition of inflammation markers, oxidative stress, and fibrosis[31,99]. Conversely, depletion of proximal tubule dopamine levels by deletion of dopa decarboxylase gen in diabetic mice developed a marked increase in albuminuria as well as increment of mesangial expansion, renal macrophage infiltration, and renal nitrotyrosine levels[31]. These findings contribute to confirm the major role played by the intrarenal dopaminergic system on the development and progression of kidney injury caused by diabetes mellitus.

In renal proximal tubule cells, insulin and dopamine

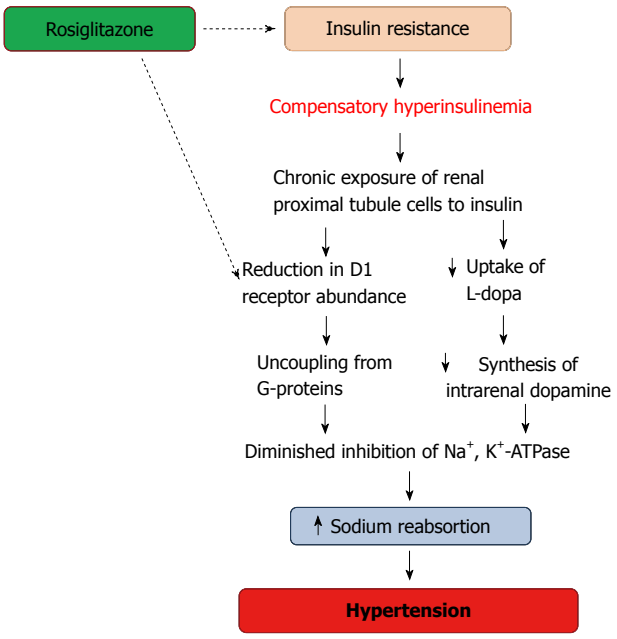

Figure 4 Association between insulin resistance and impairment of renal dopaminergic system. Full lines: Stimulation; stripped lines: Inhibition.

counter-regulate each other by opposing their effects on Na^+, K^+-ATPase activity[70]. Although insulin has been reported to enhance renal proximal tubule uptake of L-dopa in normal fed control rats, this regulatory mechanism is absent in cells isolated from animals fed with fructose, a model of insulin resistance. In addition, the chronic exposure of renal proximal tubule cells to insulin causes a reduction in D1 receptor abundance and uncoupling from G-proteins, which results in the impairment of the inhibitory effects of dopamine on Na^+, K^+-ATPase[70,105]. Hyperinsulinemic animals and patients with type 2 diabetes present a defective renal dopaminergic system[106]. In obese Zucker rats, a model of type 2 diabetes or in insulin-induced hypertension, renal D1 receptors are down-regulated and dopamine fails to produce diuresis and natriuresis[70]. Moreover, when these animals were treated with an insulin sensitizer (rosiglitazone), plasma insulin levels decreased and D1 receptor function were restored[107,108] (Figure 4). Based on these evidences, it is possible that the regulatory mechanisms of the renal dopaminergic system are impaired in diabetic nephropathy due to insulin resistance.

Previous studies have shown that in both type 1 and type 2 diabetes, expression of renal D1 receptor gene was reduced above 50% through a down-regulation mechanism that involves the extracellular cAMP-adenosine signaling pathway[70,109]. An increase in intrarenal dopamine synthesis and the subsequent stimulation of vascular D1 receptors appear to prevent early glomerular hyperfiltration in diabetic rats[110]. Conversely to the D1 like receptors, selective antagonism of D2 like receptors was demonstrated to reverse glomerular hyperfiltration induced by experimental diabetic hyperglycemia[111]. Additionally, activation of the D3 receptor in rats caused diuresis, natriuresis and increased glomerular filtration[112]. To demonstrate the renoprotective effect of a D3 receptor antagonist (A-437203), hypertensive type 2 diabetic (SHR/N-cp) rats were used to evaluate the renal effects of D3 antagonism on glomerulosclerosis damage index, glomerular volume, desmin expression as marker of podocyte damage, and urinary albumin excretion[113]. The results of this study suggest that D3 receptor antagonism has a beneficial effect on renal morphology and albuminuria, which is comparable in magnitude to that of angiotensin-converting enzyme inhibitor treatment as the gold standard[113].

Despite its role in the pathophysiology of diabetic nephropathy, the potential clinical use of dopamine in this context is still matter of basic research. Nonetheless, the therapeutic use of dopamine is restricted to its dose-dependent actions on the cardiovascular system.

DOPAMINE AS NEPHROPROTECTIVE AGENT? EXPERIMENTAL AND CLINICAL EVIDENCES IN RENAL DYSFUNCTION

Dopamine represents an essential drug in intensive care units and is still used as a first line vasopressor agent especially in hypotensive adult patients refractory to fluid resuscitation[32]. Because of its interaction with different catecholamine receptors, the pharmacological profile of dopamine is dose dependent[114]. At low doses (0.3-5 μg/kg per minute), dopamine stimulates D1 and D2 receptors inducing natriuresis, diuresis and enhances renal blood flow by renal vasodilation. At higher doses, when adrenergic stimulation prevails, dopamine increases renal blood flow through stimulation of cardiac output[32,114,115]. In healthy adult volunteers, the administration of a low dose dopamine increases renal blood flow and induces natriuresis and diuresis[116,117]. For these reasons, a low-dose dopamine represents a therapeutic option to limit or prevent renal failure in critically ill patients by increasing renal blood flow[118]. Although a low dose dopamine appears to be able to increase urinary output in critically ill adult patients at risk of renal failure, a high number of clinical studies indicate that the administration of a low dose dopamine might not be able to exert any protective effect to prevent the onset or improve the course of an established acute renal failure, but on the contrary, its use may increase its risk[32,114,119-122]. Taken all together, the nephroprotective action of a low dose dopamine in critical ill patients remains to date controversial (Table 2).

Although these evidences attempt to the clinical use of dopamine, some other findings support the clinical benefit of its use in different scenarios like cardio-renal syndrome, cardiopulmonary bypass and acute decompensated heart failure under treatment with atrial natriuretic peptide (ANP)[119,128,129]. Renal dysfunction is

Table 2 Clinical studies providing evidence against/in support of clinical use of low dose dopamine

	Study design	Results	Ref.
Against clinical use of low dose dopamine	The Australian and New Zealand Intensive Care Society (ANZICS): multicenter, randomized, double-blind, placebo-controlled	324 patients with at least two criteria for the systemic inflammatory response syndrome and clinical evidence of early renal dysfunction: continuous intravenous infusion of low-dose dopamine (2 μg/kg per minute) did not attenuate the peak serum creatinine compared with placebo. There was no statistical difference in mortality between dopamine and placebo arms	Bellomo *et al*[119]
	Meta-analysis study: 17 studies were randomized clinical trials (n = 854)	Low dose dopamine administration did not prevent mortality or the onset of acute renal failure, or the need for haemodialysis in clinically ill patients	Kellum and M Decker[120]
	Meta-analysis study: 15 randomized controlled studies	Dopamine administration did not present beneficial results in terms of serum creatinine changes and incidence of acute renal failure in clinically ill patients	Marik[121]
	Sepsis Occurrence in Acutely Ill Patients (SOAP): Cohort, multiple-center, observational study	Dopamine administration in shock patients, compared to patients who did not receive it, was associated with 20% increase in ICU and hospital mortality rates	Sakr *et al*[122]
	Renal Optimization Strategies Evaluation (ROSE) study: multicenter, double-blind, placebo-controlled randomized clinical trial	Low dose dopamine (2 μg/kg per minute) did not enhance decongestion or improved renal function when added to diuretic therapy in 360 patients with acute heart failure and renal dysfunction	Chen *et al*[123]
In support of clinical use of low dose dopamine	Dopamine in Acute Decompensated Heart Failure (DAD-HF) Trial: randomized clinical trial	The addition of low-dose dopamine (5 μg/kg per minute) to low-dose furosemide (5 mg/h) was associated with improvement in renal function profile and potassium homeostasis at 24 h and it was equally effective as high-dose furosemide (20 mg/h) alone on subjective perception of dyspnoea in 60 patients with acute decompensated heart failure	Giamouzis *et al*[124]
	Retrospective clinical study	Continuous infusion of furosemide in addition to low-dose dopamine compared to intermittent boluses of furosemide was less nephrotoxic and carried a lower readmission rate at 30 d in 116 patients with acute decompensated heart failure	Aziz *et al*[125]
	A prospective single-center randomized double-blind placebo controlled trial	The treatment with high-dose fenoldopam at 1 μg/kg per minute (short-acting D1 agonist) during cardiopulmonary bypass in 80 pediatric patients undergoing cardiac surgery for congenital heart disease significantly decreased urinary biomarkers of acute kidney injury (urinary neutrophil gelatinase-associated lipocaline and cystatin C levels) and also reduced the incidence of acute kidney injury in the postoperative period and the use of diuretics and vasodilators	Ricci *et al*[126]
	Clinical case finding	Low doses of ANP (0.0125 μg/kg per minute) with low dose dopamine (1.0 μg/kg per minute) in acute decompensated heart failure increased urine output, decreased heart rate, improved congestion with a reduced brain natriuretic peptide level, reduced serum creatinine and the levels of urinary liver-type fatty acid binding protein -a novel reno-tubular stress marker- and 8-hydroxydeoxyguanosine -an oxidative stress marker	Kamiya[127]
	Prospective randomized clinical study	Low dose dopamine infusion reduces renal tubular injury following cardiopulmonary bypass in 48 patients with normal or near normal baseline renal function	Sumeray *et al*[128]

one of the most important co-morbidities in heart failure, being a potent predictor of cardiovascular complications and mortality[130]. This relationship is commonly termed cardio-renal syndrome, where both, cardiac and renal dysfunctions, share similar pathophysiology such as activation of the renin-angiotensin-aldosterone and sympathetic nervous systems, imbalance between nitric oxide and reactive oxygen species, and inflammation[131]. Clinical guidelines recommend the treatment of heart failure or renal failure separately without consensus about how managing patients with cardio-renal syndromes[129]. Its specific treatment points out to ameliorate decreased urine output and glomerular filtration rate, increased serum creatinine, and to prevent weight loss[132]. Recent studies in this clinical setting have focused on newer therapies, including renal protective dopamine[124,125,129] (Table 2).

Acute kidney injury after cardiac operations with cardiopulmonary bypass is a life-threatening complication, with a reported incidence of up to 36%[133]. To prevent this situation diuretics have been the mainstay to promote renal function and urine flow after pediatric cardiopulmonary bypass[134,135]. Fenoldopam mesylate is a short-acting D1 agonist that

appears to improve renal function in clinical situations of reduced blood flow by enhancing renal blood flow[126,136]. Then, co-administration of both agents could be a reasonable therapeutic strategy to preserve renal function in this context (Table 2).

A primary therapeutic goal for acute heart failure is to achieve decongestion to relief symptoms without inducing renal dysfunction[137,138]. However, adult patients with acute heart failure and moderate or severe renal dysfunction are at risk for inadequate decongestion and enhanced renal dysfunction, both condition associated with worse prognosis[139]. Renal adjuvant therapies like dopamine could enhance decongestion and preserve renal function during treatment of acute heart failure. In this setting, combined therapy with ANP and dopamine might be useful to improve the management of acute decompensated heart failure without renal injury in patients who do not respond to ANP alone[127]. This beneficial interaction between both agents could be related to previous experimental findings that demonstrated that ANP stimulates dopamine uptake by tubular cells, reduces its catabolism and diminishes the turnover[140,141]. These effects may favor renal biodisponibility of dopamine in tubular cells and enhance overinhibition of renal Na^+, K^+-ATPase activity[141]. Nonetheless, future prospective studies are needed to affirm this suggestion (Table 2).

As it has been demonstrated in patients with heart failure, the interaction between two natriuretic hormones, such as ANP and dopamine, can also be present in other situations with increased extracellular fluid, such as nephrotic syndrome.

RENAL DOPAMINE IN THE PATHOGENESIS OF EDEMA FORMATION

Nephrotic syndrome exhibits increased proteinuria and enhanced sodium retention that contribute to edema and ascites formation[142]. Although reduced plasma volume and serum albumin concentration contribute to sodium retention in nephrotic syndrome, a primary intrarenal sodium handling abnormality could also be implicated in this clinical scenario[143]. In this way, experiments carried out in rats with puromycin aminonucleoside (PAN) induced nephrotic syndrome showed a blunted activity of the renal dopaminergic system evidenced by decreased urine dopamine, decreased availability of D1 receptor in renal proximal tubules and reduced dopa decarboxylase activity[144]. These findings were associated with an increase in Na^+, K^+-ATPase activity in renal proximal tubules[144]. On the other hand, renal dopamine and ANP are known to interact with each other in order to regulate sodium homeostasis[145]. The complex interaction between these two natriuretic systems is evidenced by the fact that ANP stimulates proximal tubular dopamine uptake through natriuretic peptide receptor type A (NPR-A), guanylate cyclase stimulation and protein kinase G (PKG) activation[4]. ANP also recruits silent D1 receptor from the interior of the renal tubular cells towards the plasma membrane where they become functionally active[146]. A recent work of Fernandes Cerqueira *et al*[147] demonstrated in rats with PAN-nephrotic syndrome that the increase of natriuresis and urinary cGMP excretion evoked by an acute volume expansion were blunted, despite the increased levels of circulating ANP, suggesting the unresponsiveness to ANP in this pathology. Treatment with a phosphodiesterase type 5 inhibitor (zaprinast) restored the excretion of cGMP and the natriuresis to similar levels of control rats, and increased the expression of D1 receptors in tubular cells[147]. This evidence indicates that D1 receptors are involved in ANP unresponsiveness observed in PAN-nephrotic syndrome, where the alteration of renal dopaminergic system represents a contributor factor for the edema and ascites formation (Figure 5).

Beyond its renal actions, dopamine effects on fluid homeostasis can also be exerted in other tissues. In this way, recent findings support a possible use of dopamine in edema resolution of pulmonary pathologies[148,149]. Acute lung injury and its severe form, the acute respiratory distress syndrome are prevalent causes of morbidity and mortality[150]. The outcome of patients with acute hypoxemic respiratory failure improves when lung epithelial function is restored and pulmonary edema resolves[151,152]. Pulmonary edema is cleared from the alveoli by active sodium transport, in which sodium enters into the cell *via* apical amiloride-sensitive sodium channels and pumped out from the cell *via* the basolaterally located Na^+, K^+-ATPase. Water follows the sodium gradients, resulting in alveolar fluid reabsorption[153]. Although dopamine inhibits Na^+, K^+-ATPase in the kidney and promotes natriuresis and diuresis, in alveolar cells dopamine increases, in a dose-dependent manner, lung edema clearance in rats by 40%-70% above the control clearance levels[154-156]. Experimental studies using models of lung injury have demonstrated that alveolar fluid clearance is impaired in parallel with decreased Na^+, K^+-ATPase function. In these lung injury models, dopamine (10^{-5} M) instilled into airspace increased alveolar fluid reabsorption by translocating preformed Na^+, K^+-ATPase pumps from intracellular pools (*i.e.*, late endosomal compartment) to the cell plasma membrane in alveolar epithelial-type Ⅱ cells[150,157,158]. This effect is produced through short-term and long-term fashion mechanisms. The short-term mechanism depends on the activation of D1 receptors since fenoldopam reproduces dopamine actions, meanwhile the long-term mechanism implies D2 receptors[159,160]. Accumulation of protein-rich alveolar edema fluid in acute lung injury is the result of an increased microvascular permeability[161-164]. Many experimental and human studies support the hypothesis that vascular endothelial growth factor (VEGF) plays a critical role in shaping the vascular barrier function in acute lung injury[165-169]. Although, D1

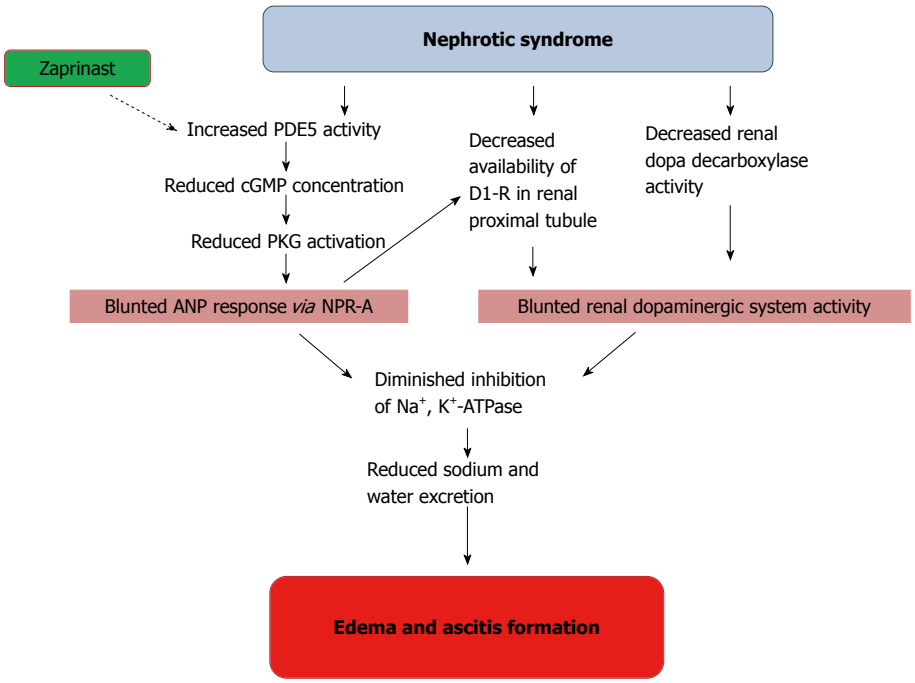

Figure 5 Impaired interactions between ANP and renal dopamine in nephrotic syndrome. Full lines: Stimulation; stripped lines: Inhibition. PDE5: Phosphodiesterase type 5.

and D2 receptors are implicated in the synthesis and trafficking of Na^+, K^+-ATPase, the D2 receptor is also implicated in the regulation of VEGF-induced vascular permeability as well as angiogenesis[159,160,170-172]. This was confirmed by the fact that a D2 receptor agonist failed to reduce pulmonary edema in D2 receptor (-/-) mice, suggesting that dopamine acts through D2 receptor to inhibit pulmonary edema-associated vascular permeability, which is mediated through VEGF-VEGFR2 signaling and conveys protective effects in an acute lung injury model[148]. Although D1 and D2 receptors subtypes seem to be beneficial to reduce edema formation, D3 receptor appears to exert an opposite effect. Several clinical findings reported that pramipexole, a potent non ergoline agent with high affinity to D3 receptors and used for Parkinson's disease, restless legs syndrome, resistant depression and bipolar depression, is associated with the development of pedal and chronic lower limb edema (with a frequency that ranges from 5% to 22.5%)[173-176]. This adverse effect disappears after the discontinuation of the drug[173-176]. Since dopamine is an important regulator of the sympathetic nervous system, aldosterone secretion, as well as adenosine triphosphate-mediated sodium/potassium channels, the peripheral effects of pramipexole at these levels could have a role[177,178]. However the relationship between D3 receptor agonism and edema formation remains unclear.

FUTURE PERSPECTIVES

An intact renal dopaminergic tonus is required for the maintenance of sodium homeostasis and normal blood pressure. By its anti-oxidative and anti-inflammatory properties, intrarenal dopamine plays a major role as a nephroprotective agent to prevent or ameliorate renal dysfunction. Oxidative stress or hyperinsulinemic states may decrease the number of functional dopaminergic receptors in the proximal tubules. In this way, it is worthwhile to test the effect of antioxidant drugs to enhance or restore the biodisponibility of these receptors. A recent observation that dopamine receptors availability in the plasma membrane may be regulated by other hormones, like ANP, could open up a possible therapeutic approach[179].

It has been emphasized the importance of endogenous dopamine and renal D1 receptor on the regulation of sodium and body fluid homeostasis. Although there is evidence that a defective renal dopaminergic system contributes to the development and maintenance of hypertension, it is still not clear what triggering factors cause the selective defects in the renal dopaminergic system. Some of these triggering factors could be an excess of sodium intake that could lead to an activation of intrarenal angiotensin Ⅱ and increase in ROS, an increase in carbohydrate intake and a high fat diet, both factors that promotes an insulin resistance state. Furthermore, the renal dopaminergic system is sensitized by a high salt intake and volume expansion, which opens the question about how intrarenal sodium sensors may influence on renal dopamine bioavailability. This approach may lead to the development of new pharmacological strategies in conditions of salt retention and hypertension. Moreover, identification of abnormalities in different steps of crucial importance for the regulation of the renal dopaminergic tonus should provide additional molecular biological tools for the early diagnosis and treatment of pre-hypertensive patients.

The fact that dopamine exerts nonselective actions upon multiple dopaminergic and adrenergic receptors must be considered, and this, can limit its therapeutic

use in renal diseases. The potential therapeutic use of exogenous dopamine and D1-like receptor agonists is limited to special conditions like critical ill patients who are at risk of kidney failure. However, given the controversial results from clinical studies the use of dopamine in this context must be examined more closely.

At last, further clinical studies must be carried out to confirm the participation of renal dopaminergic system in pathological contexts involving impaired sodium excretion as nephrotic syndrome or insulin resistance states.

CONCLUSION

Intrarenal dopamine represents a local natriuretic system with beneficial actions on blood pressure, oxidative stress and inflammation. Dopamine secreted into the tubular lumen acts *via* D1-like and D2-like receptors in an autocrine/paracrine manner to inhibit different tubular ion transporters and to regulate the production of reactive oxygen species and the inflammatory response. These renoprotective effects can be affected by situations that impair its integrity and functionality. The comprehension of the mechanisms by which renal dopaminergic system is involved in the pathogenesis and development of renal diseases may contribute to improve the diagnosis, evolution, prognosis and treatment of renal pathologies.

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REFERENCES

1 **Silva E**, Gomes P, Soares-da-Silva P. Increases in transepithelial vectorial Na+ transport facilitates Na+-dependent L-DOPA transport in renal OK cells. *Life Sci* 2006; **79**: 723-729 [PMID: 16600308 DOI: 10.1016/j.lfs.2006.02.018]

2 **Bröer A**, Klingel K, Kowalczuk S, Rasko JE, Cavanaugh J, Bröer S. Molecular cloning of mouse amino acid transport system B0, a neutral amino acid transporter related to Hartnup disorder. *J Biol Chem* 2004; **279**: 24467-24476 [PMID: 15044460 DOI: 10.1074/jbc.M400904200]

3 **Pinho MJ**, Serrão MP, Gomes P, Hopfer U, Jose PA, Soares-da-Silva P. Over-expression of renal LAT1 and LAT2 and enhanced L-DOPA uptake in SHR immortalized renal proximal tubular cells. *Kidney Int* 2004; **66**: 216-226 [PMID: 15200428 DOI: 10.1111/j.1523-1755.2004.00722.x]

4 **Correa AH**, Choi MR, Gironacci M, Valera MS, Fernández BE. Signaling pathways involved in atrial natriuretic factor and dopamine regulation of renal Na+, K+ -ATPase activity. *Regul Pept* 2007; **138**: 26-31 [PMID: 17005263 DOI: 10.1016/j.regpep.2006.08.001]

5 **Koepsell H**, Lips K, Volk C. Polyspecific organic cation transporters: structure, function, physiological roles, and biopharmaceutical implications. *Pharm Res* 2007; **24**: 1227-1251 [PMID: 17473959 DOI: 10.1007/s11095-007-9254-z]

6 **Wright SH**, Dantzler WH. Molecular and cellular physiology of renal organic cation and anion transport. *Physiol Rev* 2004; **84**: 987-1049 [PMID: 15269342 DOI: 10.1152/physrev.00040.2003]

7 **Jonker JW**, Schinkel AH. Pharmacological and physiological functions of the polyspecific organic cation transporters: OCT1, 2, and 3 (SLC22A1-3). *J Pharmacol Exp Ther* 2004; **308**: 2-9 [PMID: 14576340 DOI: 10.1124/jpet.103.053298]

8 **Armando I,** Konkalmatt P, Felder RA, Jose PA. The renal dopaminergic system: novel diagnostic and therapeutic approaches in hypertension and kidney disease. *Transl Res* 2014 [PMID: 25134060 DOI: 10.1016/j.trsl.2014.07.006]

9 **Horita S**, Seki G, Yamada H, Suzuki M, Koike K, Fujita T. Roles of renal proximal tubule transport in the pathogenesis of hypertension. *Curr Hypertens Rev* 2013; **9**: 148-155 [PMID: 23971696]

10 **Sanada H,** Xu J, Watanabe H, Jose PA, Felder RA. Differential expression and regulation of dopamine-1 (D-1) and dopamine-5 (D-5) receptor function in human kidney. *Am J Hypertens* 2000; **13:** 156

11 **Carey RM**. The intrarenal renin-angiotensin and dopaminergic systems: control of renal sodium excretion and blood pressure. *Hypertension* 2013; **61**: 673-680 [PMID: 23407646 DOI: 10.1161/HYPERTENSIONAHA.111.00241]

12 **Beaulieu JM**, Gainetdinov RR. The physiology, signaling, and pharmacology of dopamine receptors. *Pharmacol Rev* 2011; **63**: 182-217 [PMID: 21303898 DOI: 10.1124/pr.110.002642]

13 **Hussain T**, Lokhandwala MF. Renal dopamine receptors and hypertension. *Exp Biol Med* (Maywood) 2003; **228**: 134-142 [PMID: 12563019]

14 **Zeng C**, Sanada H, Watanabe H, Eisner GM, Felder RA, Jose PA. Functional genomics of the dopaminergic system in hypertension. *Physiol Genomics* 2004; **19**: 233-246 [PMID: 15548830 DOI: 101152/physiolgenomics.00127.2004]

15 **Pedrosa R**, Jose PA, Soares-da-Silva P. Defective D1-like receptor-mediated inhibition of the Cl-/HCO3- exchanger in immortalized SHR proximal tubular epithelial cells. *Am J Physiol Renal Physiol* 2004; **286**: F1120-F1126 [PMID: 14970001 DOI: 10.1152/ajprenal.00433.2003]

16 **Carey RM**. Theodore Cooper Lecture: Renal dopamine system: paracrine regulator of sodium homeostasis and blood pressure. *Hypertension* 2001; **38**: 297-302 [PMID: 11566894 DOI: 10.1161/hy0901.096422]

17 **Zhang MZ**, Yao B, Fang X, Wang S, Smith JP, Harris RC. Intrarenal dopaminergic system regulates renin expression. *Hypertension* 2009; **53**: 564-570 [PMID: 19139376 DOI: 10.1161/HYPERTENSIONAHA.108.127035]

18 **Desir GV**. Role of renalase in the regulation of blood pressure and the renal dopamine system. *Curr Opin Nephrol Hypertens* 2011; **20**: 31-36 [PMID: 21099685 DOI: 10.1097/MNH.0b013e3283412721]

19 **Li G**, Xu J, Wang P, Velazquez H, Li Y, Wu Y, Desir GV. Catecholamines regulate the activity, secretion, and synthesis of renalase. *Circulation* 2008; **117**: 1277-1282 [PMID: 18299506 DOI: 10.1161/CIRCULATIONAHA.107.732032]

20 **Yu PY**, Asico LD, Eisner GM, Jose PA. Differential regulation of renal phospholipase C isoforms by catecholamines. *J Clin Invest* 1995; **95**: 304-308 [PMID: 7814630]

21 **Souza BR**, Romano-Silva MA, Tropepe V. Dopamine D2 receptor activity modulates Akt signaling and alters GABAergic neuron development and motor behavior in zebrafish larvae. *J Neurosci* 2011; **31**: 5512-5525 [PMID: 21471388 DOI: 10.1523/JNEUROSCI.5548-10.2011]

22 **Pollack A**. Coactivation of D1 and D2 dopamine receptors: in marriage, a case of his, hers, and theirs. *Sci STKE* 2004; **2004**: pe50 [PMID: 15494560]

23 **Bek M**, Fischer KG, Greiber S, Hupfer C, Mundel P, Pavenstädt H. Dopamine depolarizes podocytes via a D1-like receptor. *Nephrol Dial Transplant* 1999; **14**: 581-587 [PMID: 10193803]

24 **Barnett R**, Singhal PC, Scharschmidt LA, Schlondorff D. Dopamine attenuates the contractile response to angiotensin II in isolated rat glomeruli and cultured mesangial cells. *Circ Res* 1986; **59**: 529-533 [PMID: 3467880]

25 **Pavenstädt H**, Kriz W, Kretzler M. Cell biology of the glomerular podocyte. *Physiol Rev* 2003; **83**: 253-307 [PMID: 12506131]

26 **Yang Z**, Asico LD, Yu P, Wang Z, Jones JE, Escano CS, Wang

X, Quinn MT, Sibley DR, Romero GG, Felder RA, Jose PA. D5 dopamine receptor regulation of reactive oxygen species production, NADPH oxidase, and blood pressure. *Am J Physiol Regul Integr Comp Physiol* 2006; **290**: R96-R104 [PMID: 16352863 DOI: 10.1152/ajpregu.00434.2005]

27 **Yu P**, Han W, Villar VA, Li H, Arnaldo FB, Concepcion GP, Felder RA, Quinn MT, Jose PA. Dopamine D1 receptor-mediated inhibition of NADPH oxidase activity in human kidney cells occurs via protein kinase A-protein kinase C cross talk. *Free Radic Biol Med* 2011; **50**: 832-840 [PMID: 21193028 DOI: 10.1016/j.freeradbiomed.2010.12.027]

28 **Nakano K**, Yamaoka K, Hanami K, Saito K, Sasaguri Y, Yanagihara N, Tanaka S, Katsuki I, Matsushita S, Tanaka Y. Dopamine induces IL-6-dependent IL-17 production via D1-like receptor on CD4 naive T cells and D1-like receptor antagonist SCH-23390 inhibits cartilage destruction in a human rheumatoid arthritis/SCID mouse chimera model. *J Immunol* 2011; **186**: 3745-3752 [PMID: 21307293 DOI: 10.4049/jimmunol.1002475]

29 **Ghosh MC**, Mondal AC, Basu S, Banerjee S, Majumder J, Bhattacharya D, Dasgupta PS. Dopamine inhibits cytokine release and expression of tyrosine kinases, Lck and Fyn in activated T cells. *Int Immunopharmacol* 2003; **3**: 1019-1026 [PMID: 12810359 DOI: 10.1016/S1567-5769(03)00100-0]

30 **Cuevas S**, Villar VA, Jose PA, Armando I. Renal dopamine receptors, oxidative stress, and hypertension. *Int J Mol Sci* 2013; **14**: 17553-17572 [PMID: 23985827 DOI: 10.3390/ijms140917553]

31 **Zhang MZ**, Yao B, Yang S, Yang H, Wang S, Fan X, Yin H, Fogo AB, Moeckel GW, Harris RC. Intrarenal dopamine inhibits progression of diabetic nephropathy. *Diabetes* 2012; **61**: 2575-2584 [PMID: 22688335 DOI: 10.2337/db12-0046]

32 **Marinosci GZ**, De Robertis E, De Benedictis G, Piazza O. Dopamine Use in Intensive Care: Are We Ready to Turn it Down? *Transl Med UniSa* 2012; **4**: 90-94 [PMID: 23905068]

33 **Yu P**, Han W, Villar VA, Yang Y, Lu Q, Lee H, Li F, Quinn MT, Gildea JJ, Felder RA, Jose PA. Unique role of NADPH oxidase 5 in oxidative stress in human renal proximal tubule cells. *Redox Biol* 2014; **2**: 570-579 [PMID: 24688893 DOI: 10.1016/j.redox.2014.01.020]

34 **Bretón-Romero R**, Lamas S. Hydrogen peroxide signaling mediator in the activation of p38 MAPK in vascular endothelial cells. *Methods Enzymol* 2013; **528**: 49-59 [PMID: 23849858 DOI: 10.1016/B978-0-12-405881-1.00003-3]

35 **Chung HS**, Wang SB, Venkatraman V, Murray CI, Van Eyk JE. Cysteine oxidative posttranslational modifications: emerging regulation in the cardiovascular system. *Circ Res* 2013; **112**: 382-392 [PMID: 23329793 DOI: 10.1161/CIRCRESAHA.112.268680]

36 **Evans JL**, Goldfine ID, Maddux BA, Grodsky GM. Are oxidative stress-activated signaling pathways mediators of insulin resistance and beta-cell dysfunction? *Diabetes* 2003; **52**: 1-8 [PMID: 12502486 DOI: 10.2337/diabetes.52.1.1]

37 **Harrison DG**, Guzik TJ, Lob HE, Madhur MS, Marvar PJ, Thabet SR, Vinh A, Weyand CM. Inflammation, immunity, and hypertension. *Hypertension* 2011; **57**: 132-140 [PMID: 21149826 DOI: 10.1161/HYPERTENSIONAHA.110.163576]

38 **Segerer S**, Schlöndorff D. Role of chemokines for the localization of leukocyte subsets in the kidney. *Semin Nephrol* 2007; **27**: 260-274 [PMID: 17533005 DOI: 10.1016/j.semnephrol.2007.02.003]

39 **Wang Y**, Tay YC, Harris DC. Proximal tubule cells stimulated by lipopolysaccharide inhibit macrophage activation. *Kidney Int* 2004; **66**: 655-662 [PMID: 15253719 DOI: 10.1111/j.1523-1755.2004.00786.x]

40 **Yang Y**, Zhang Y, Cuevas S, Villar VA, Escano C, D Asico L, Yu P, Grandy DK, Felder RA, Armando I, Jose PA. Paraoxonase 2 decreases renal reactive oxygen species production, lowers blood pressure, and mediates dopamine D2 receptor-induced inhibition of NADPH oxidase. *Free Radic Biol Med* 2012; **53**: 437-446 [PMID: 22634053 DOI: 10.1016/j.freeradbiomed.2012.05.015]

41 **Haskó G**, Szabó C, Németh ZH, Deitch EA. Dopamine suppresses IL-12 p40 production by lipopolysaccharide-stimulated macrophages via a beta-adrenoceptor-mediated mechanism. *J Neuroimmunol* 2002; **122**: 34-39 [PMID: 11777541 DOI: 10.1016/S0165-5728(01)00459-3]

42 **Zhang MZ**, Yao B, Wang S, Fan X, Wu G, Yang H, Yin H, Yang S, Harris RC. Intrarenal dopamine deficiency leads to hypertension and decreased longevity in mice. *J Clin Invest* 2011; **121**: 2845-2854 [PMID: 21701066 DOI: 10.1172/JCI57324]

43 **Yang S**, Yao B, Zhou Y, Yin H, Zhang MZ, Harris RC. Intrarenal dopamine modulates progressive angiotensin II-mediated renal injury. *Am J Physiol Renal Physiol* 2012; **302**: F742-F749 [PMID: 22169008 DOI: 10.1152/ajprenal.00583.2011]

44 **Li XX**, Bek M, Asico LD, Yang Z, Grandy DK, Goldstein DS, Rubinstein M, Eisner GM, Jose PA. Adrenergic and endothelin B receptor-dependent hypertension in dopamine receptor type-2 knockout mice. *Hypertension* 2001; **38**: 303-308 [PMID: 11566895 DOI: 10.1161/01.HYP.38.3.303]

45 **Armando I**, Wang X, Villar VA, Jones JE, Asico LD, Escano C, Jose PA. Reactive oxygen species-dependent hypertension in dopamine D2 receptor-deficient mice. *Hypertension* 2007; **49**: 672-678 [PMID: 17190875 DOI: 10.1161/01.HYP.0000254486.00883.3d]

46 **Zhang Y**, Cuevas S, Asico LD, Escano C, Yang Y, Pascua AM, Wang X, Jones JE, Grandy D, Eisner G, Jose PA, Armando I. Deficient dopamine D2 receptor function causes renal inflammation independently of high blood pressure. *PLoS One* 2012; **7**: e38745 [PMID: 22719934 DOI: 10.1371/journal.pone.0038745]

47 **Ruilope L**, Kjeldsen SE, de la Sierra A, Mancia G, Ruggenenti P, Stergiou GS, Bakris GL, Giles TD. The kidney and cardiovascular risk--implications for management: a consensus statement from the European Society of Hypertension. *Blood Press* 2007; **16**: 72-79 [PMID: 17612904 DOI: 10.1080/08037050701338985]

48 **Erdine S**, Aran SN. Current status of hypertension control around the world. *Clin Exp Hypertens* 2004; **26**: 731-738 [PMID: 15702628]

49 **Hajjar I,** Kotchen TA. Trends in prevalence, awareness, treatment, and control of hypertension in the United States, 1988–2000. *JAMA* 2003; **290**: 199-206 [PMID: 12851274]

50 **Meneton P**, Jeunemaitre X, de Wardener HE, MacGregor GA. Links between dietary salt intake, renal salt handling, blood pressure, and cardiovascular diseases. *Physiol Rev* 2005; **85**: 679-715 [PMID: 15788708 DOI: 10.1152/physrev.00056.2003]

51 **Zeng C**, Villar VA, Yu P, Zhou L, Jose PA. Reactive oxygen species and dopamine receptor function in essential hypertension. *Clin Exp Hypertens* 2009; **31**: 156-178 [PMID: 19330604 DOI: 10.1080/10641960802621283]

52 **Harrap SB**. Blood pressure genetics: time to focus. *J Am Soc Hypertens* 2009; **3**: 231-237 [PMID: 20409965 DOI: 10.1016/j.jash.2009.06.001]

53 **Adeyemo A**, Gerry N, Chen G, Herbert A, Doumatey A, Huang H, Zhou J, Lashley K, Chen Y, Christman M, Rotimi C. A genome-wide association study of hypertension and blood pressure in African Americans. *PLoS Genet* 2009; **5**: e1000564 [PMID: 19609347 DOI: 10.1371/journal.pgen.1000564]

54 **Cho YS**, Go MJ, Kim YJ, Heo JY, Oh JH, Ban HJ, Yoon D, Lee MH, Kim DJ, Park M, Cha SH, Kim JW, Han BG, Min H, Ahn Y, Park MS, Han HR, Jang HY, Cho EY, Lee JE, Cho NH, Shin C, Park T, Park JW, Lee JK, Cardon L, Clarke G, McCarthy MI, Lee JY, Lee JK, Oh B, Kim HL. A large-scale genome-wide association study of Asian populations uncovers genetic factors influencing eight quantitative traits. *Nat Genet* 2009; **41**: 527-534 [PMID: 19396169 DOI: 10.1038/ng.357]

55 **Johnson AD**, Newton-Cheh C, Chasman DI, Ehret GB, Johnson T, Rose L, Rice K, Verwoert GC, Launer LJ, Gudnason V, Larson MG, Chakravarti A, Psaty BM, Caulfield M, van Duijn CM, Ridker PM, Munroe PB, Levy D. Association of hypertension drug target genes with blood pressure and hypertension in 86,588 individuals. *Hypertension* 2011; **57**: 903-910 [PMID: 21444836 DOI: 10.1161/HYPERTENSIONAHA.110.158667]

56 **Levy D**, Ehret GB, Rice K, Verwoert GC, Launer LJ, Dehghan A, Glazer NL, Morrison AC, Johnson AD, Aspelund T, Aulchenko Y, Lumley T, Köttgen A, Vasan RS, Rivadeneira F, Eiriksdottir G, Guo X, Arking DE, Mitchell GF, Mattace-Raso FU, Smith AV, Taylor K, Scharpf RB, Hwang SJ, Sijbrands EJ, Bis J, Harris TB, Ganesh SK, O'Donnell CJ, Hofman A, Rotter JI, Coresh J, Benjamin EJ, Uitterlinden AG, Heiss G, Fox CS, Witteman JC, Boerwinkle E, Wang TJ, Gudnason V, Larson MG, Chakravarti A, Psaty BM, van Duijn CM. Genome-wide association study of blood pressure and hypertension. *Nat Genet* 2009; **41**: 677-687 [PMID: 19430479 DOI: 10.1038/ng.384]

57 **Newton-Cheh C**, Johnson T, Gateva V, Tobin MD, Bochud M, Coin L, Najjar SS, Zhao JH, Heath SC, Eyheramendy S, Papadakis K, Voight BF, Scott LJ, Zhang F, Farrall M, Tanaka T, Wallace C, Chambers JC, Khaw KT, Nilsson P, van der Harst P, Polidoro S, Grobbee DE, Onland-Moret NC, Bots ML, Wain LV, Elliott KS, Teumer A, Luan J, Lucas G, Kuusisto J, Burton PR, Hadley D, McArdle WL, Brown M, Dominiczak A, Newhouse SJ, Samani NJ, Webster J, Zeggini E, Beckmann JS, Bergmann S, Lim N, Song K, Vollenweider P, Waeber G, Waterworth DM, Yuan X, Groop L, Orho-Melander M, Allione A, Di Gregorio A, Guarrera S, Panico S, Ricceri F, Romanazzi V, Sacerdote C, Vineis P, Barroso I, Sandhu MS, Luben RN, Crawford GJ, Jousilahti P, Perola M, Boehnke M, Bonnycastle LL, Collins FS, Jackson AU, Mohlke KL, Stringham HM, Valle TT, Willer CJ, Bergman RN, Morken MA, Döring A, Gieger C, Illig T, Meitinger T, Org E, Pfeufer A, Wichmann HE, Kathiresan S, Marrugat J, O'Donnell CJ, Schwartz SM, Siscovick DS, Subirana I, Freimer NB, Hartikainen AL, McCarthy MI, O' Reilly PF, Peltonen L, Pouta A, de Jong PE, Snieder H, van Gilst WH, Clarke R, Goel A, Hamsten A, Peden JF, Seedorf U, Syvänen AC, Tognoni G, Lakatta EG, Sanna S, Scheet P, Schlessinger D, Scuteri A, Dörr M, Ernst F, Felix SB, Homuth G, Lorbeer R, Reffelmann T, Rettig R, Völker U, Galan P, Gut IG, Hercberg S, Lathrop GM, Zelenika D, Deloukas P, Soranzo N, Williams FM, Zhai G, Salomaa V, Laakso M, Elosua R, Forouhi NG, Völzke H, Uiterwaal CS, van der Schouw YT, Numans ME, Matullo G, Navis G, Berglund G, Bingham SA, Kooner JS, Connell JM, Bandinelli S, Ferrucci L, Watkins H, Spector TD, Tuomilehto J, Altshuler D, Strachan DP, Laan M, Meneton P, Wareham NJ, Uda M, Jarvelin MR, Mooser V, Melander O, Loos RJ, Elliott P, Abecasis GR, Caulfield M, Munroe PB. Genome-wide association study identifies eight loci associated with blood pressure. *Nat Genet* 2009; **41**: 666-676 [PMID: 19430483 DOI: 10.1038/ng.361]

58 **Wang Y**, O'Connell JR, McArdle PF, Wade JB, Dorff SE, Shah SJ, Shi X, Pan L, Rampersaud E, Shen H, Kim JD, Subramanya AR, Steinle NI, Parsa A, Ober CC, Welling PA, Chakravarti A, Weder AB, Cooper RS, Mitchell BD, Shuldiner AR, Chang YP. From the Cover: Whole-genome association study identifies STK39 as a hypertension susceptibility gene. *Proc Natl Acad Sci USA* 2009; **106**: 226-231 [PMID: 19114657 DOI: 10.1073/pnas.0808358106]

59 **Moore JH**, Williams SM. New strategies for identifying gene-gene interactions in hypertension. *Ann Med* 2002; **34**: 88-95 [PMID: 12108579]

60 **Wang X**, Villar VA, Armando I, Eisner GM, Felder RA, Jose PA. Dopamine, kidney, and hypertension: studies in dopamine receptor knockout mice. *Pediatr Nephrol* 2008; **23**: 2131-2146 [PMID: 18615257 DOI: 10.1007/s00467-008-0901-3]

61 **Ohbu K**, Kaskel FJ, Kinoshita S, Felder RA. Dopamine-1 receptors in the proximal convoluted tubule of Dahl rats: defective coupling to adenylate cyclase. *Am J Physiol* 1995; **268**: R231-R235 [PMID: 7840326]

62 **Nishi A**, Eklöf AC, Bertorello AM, Aperia A. Dopamine regulation of renal Na+,K(+)-ATPase activity is lacking in Dahl salt-sensitive rats. *Hypertension* 1993; **21**: 767-771 [PMID: 8099063 DOI: 10.1161/01.HYP.21.6.767]

63 **Iimura O**, Ura N, Nakagawa M. Comparative hypertensionology-renal dopaminergic activity in experimental hypertensive rats. *Clin Exp Hypertens* 1997; **19**: 117-130 [PMID: 9028640]

64 **Banday AA**, Lokhandwala MF. Dopamine receptors and hypertension. *Curr Hypertens Rep* 2008; **10**: 268-275 [PMID: 18625155]

65 **Zeng C**, Armando I, Luo Y, Eisner GM, Felder RA, Jose PA. Dysregulation of dopamine-dependent mechanisms as a determinant of hypertension: studies in dopamine receptor knockout mice. *Am J Physiol Heart Circ Physiol* 2008; **294**: H551-H569 [PMID: 18083900]

66 **Albrecht FE**, Drago J, Felder RA, Printz MP, Eisner GM, Robillard JE, Sibley DR, Westphal HJ, Jose PA. Role of the D1A dopamine receptor in the pathogenesis of genetic hypertension. *J Clin Invest* 1996; **97**: 2283-2288 [PMID: 8636408 DOI: 10.1172/JCI118670]

67 **Escano CS**, Armando I, Wang X, Asico LD, Pascua A, Yang Y, Wang Z, Lau YS, Jose PA. Renal dopaminergic defect in C57Bl/6J mice. *Am J Physiol Regul Integr Comp Physiol* 2009; **297**: R1660-R1669 [PMID: 19726707 DOI: 10.1152/ajpregu.00147.2009]

68 **Chugh G**, Lokhandwala MF, Asghar M. Altered functioning of both renal dopamine D1 and angiotensin II type 1 receptors causes hypertension in old rats. *Hypertension* 2012; **59**: 1029-1036 [PMID: 22411927 DOI: 10.1161/HYPERTENSIONAHA.112.192302]

69 **Moreira-Rodrigues M**, Sampaio-Maia B, Pestana M. Renal dopaminergic system activity in rat remnant kidney up to twenty-six weeks after surgery. *Life Sci* 2009; **84**: 409-414 [PMID: 19167406 DOI: 10.1016/j.lfs.2008.12.018]

70 **Hussain T**, Beheray SA, Lokhandwala MF. Defective dopamine receptor function in proximal tubules of obese zucker rats. *Hypertension* 1999; **34**: 1091-1096 [PMID: 10567187 DOI: 10.1161/01.HYP.34.5.1091]

71 **Padia SH**, Kemp BA, Howell NL, Keller SR, Gildea JJ, Carey RM. Mechanisms of dopamine D(1) and angiotensin type 2 receptor interaction in natriuresis. *Hypertension* 2012; **59**: 437-445 [PMID: 22203736 DOI: 10.1161/HYPERTENSIONAHA.111.184788]

72 **Salomone LJ**, Howell NL, McGrath HE, Kemp BA, Keller SR, Gildea JJ, Felder RA, Carey RM. Intrarenal dopamine D1-like receptor stimulation induces natriuresis via an angiotensin type-2 receptor mechanism. *Hypertension* 2007; **49**: 155-161 [PMID: 17116755 DOI: 10.1161/01.HYP.0000251881.89610.ee]

73 **Najjar SS**, Scuteri A, Lakatta EG. Arterial aging: is it an immutable cardiovascular risk factor? *Hypertension* 2005; **46**: 454-462 [PMID: 16103272 DOI: 10.1161/01.HYP.0000177474.06749.98]

74 **Pestana M**. Hypertension in the elderly. *Int Urol Nephrol* 2001; **33**: 563-569 [PMID: 12230296]

75 **Vasan RS**, Beiser A, Seshadri S, Larson MG, Kannel WB, D' Agostino RB, Levy D. Residual lifetime risk for developing hypertension in middle-aged women and men: The Framingham Heart Study. *JAMA* 2002; **287**: 1003-1010 [PMID: 11866648 DOI: 10.1001/jama.287.8.1003]

76 **Ames BN**, Shigenaga MK, Hagen TM. Oxidants, antioxidants, and the degenerative diseases of aging. *Proc Natl Acad Sci USA* 1993; **90**: 7915-7922 [PMID: 8367443]

77 **Beheray S**, Kansra V, Hussain T, Lokhandwala MF. Diminished natriuretic response to dopamine in old rats is due to an impaired D1-like receptor-signaling pathway. *Kidney Int* 2000; **58**: 712-720 [PMID: 10916094 DOI: 10.1046/j.1523-1755.2000.00217.x]

78 **Zemel MB**, Sowers JR. Salt sensitivity and systemic hypertension in the elderly. *Am J Cardiol* 1988; **61**: 7H-12H [PMID: 3289354]

79 **Baylis C**. Renal responses to acute angiotensin II inhibition and administered angiotensin II in the aging, conscious, chronically catheterized rat. *Am J Kidney Dis* 1993; **22**: 842-850 [PMID: 8250031]

80 **White BH**, Sidhu A. Increased oxidative stress in renal proximal tubules of the spontaneously hypertensive rat: a mechanism for defective dopamine D1A receptor/G-protein coupling. *J Hypertens* 1998; **16**: 1659-1665 [PMID: 9856367]

81 **Becker M**, Umrani D, Lokhandwala MF, Hussain T. Increased renal angiotensin II AT1 receptor function in obese Zucker rat. *Clin Exp Hypertens* 2003; **25**: 35-47 [PMID: 12597523]

82 **Banday AA**, Marwaha A, Tallam LS, Lokhandwala MF. Tempol

reduces oxidative stress, improves insulin sensitivity, decreases renal dopamine D1 receptor hyperphosphorylation, and restores D1 receptor-G-protein coupling and function in obese Zucker rats. *Diabetes* 2005; **54**: 2219-2226 [PMID: 15983225 DOI: 10.2337/diabetes.54.7.2219]

83 **Javkhedkar AA,** Lokhandwala MF, Banday AA. Higher renal AT1 receptor affinity exaggerates Ang II induced Na/K-ATPase stimulation in spontaneously hypertensive rats. *FASEB J* 2010; **24**: 707

84 **Zhang Y**, Fu C, Ren H, He D, Wang X, Asico LD, Jose PA, Zeng C. Impaired stimulatory effect of ETB receptor on D receptor in immortalized renal proximal tubule cells of spontaneously hypertensive rats. *Kidney Blood Press Res* 2011; **34**: 75-82 [PMID: 21228598 DOI: 10.1159/000323135]

85 **Zeng C**, Asico LD, Yu C, Villar VA, Shi W, Luo Y, Wang Z, He D, Liu Y, Huang L, Yang C, Wang X, Hopfer U, Eisner GM, Jose PA. Renal D3 dopamine receptor stimulation induces natriuresis by endothelin B receptor interactions. *Kidney Int* 2008; **74**: 750-759 [PMID: 18547994 DOI: 10.1038/ki.2008.247]

86 **Yu C**, Yang Z, Ren H, Zhang Y, Han Y, He D, Lu Q, Wang X, Wang X, Yang C, Asico LD, Hopfer U, Eisner GM, Jose PA, Zeng C. D3 dopamine receptor regulation of ETB receptors in renal proximal tubule cells from WKY and SHRs. *Am J Hypertens* 2009; **22**: 877-883 [PMID: 19390510 DOI: 10.1038/ajh.2009.80]

87 **Fernandez MM**, Gonzalez D, Williams JM, Roman RJ, Nowicki S. Inhibitors of 20-hydroxyeicosatetraenoic acid (20-HETE) formation attenuate the natriuretic effect of dopamine. *Eur J Pharmacol* 2012; **686**: 97-103 [PMID: 22575524 DOI: 10.1016/j.ejphar.2012.04.039]

88 **Kirchheimer C**, Mendez CF, Acquier A, Nowicki S. Role of 20-HETE in D1/D2 dopamine receptor synergism resulting in the inhibition of Na+-K+-ATPase activity in the proximal tubule. *Am J Physiol Renal Physiol* 2007; **292**: F1435-F1442 [PMID: 17264308 DOI: 10.1152/ajprenal.00176.2006]

89 **Maier KG**, Roman RJ. Cytochrome P450 metabolites of arachidonic acid in the control of renal function. *Curr Opin Nephrol Hypertens* 2001; **10**: 81-87 [PMID: 11195057 DOI: 10.1042/CS20110215]

90 **Schlaich M,** Socratous F, Eikelis N. Renalase plasma levels are associated with systolic blood pressure in patients with resistant hypertension: 9C.01. *J Hypertens* 2010; **28**: 437 [DOI: 10.1097/01.hjh.0000379519.82971.02]

91 **Desir GV,** Wu Y, Wang P. Renalase deficiency increases sympathetic tone and causes hypertension. *J Am Soc Nephrol* 2008. Available from: URL: http: //www.asn-online.org/education_and_meetings/renal_week/archives/

92 **Pestana M,** Sampaio-Maia B, Moreira-Rodrigues M. Expression of renalase in a 3/4 nephrectomy rat model. *NDT Plus* 2009; **2**: 55

93 **Desir G,** Tang L, Wang P, Li G, Velazquez H. Antihypertensive effect of recombinant renalase in Dahl salt sensitive (DSS) rats. 2010. Available from: URL: http: //www.asn-online.org/education_and_meetings/renal_week/archives/

94 **US Renal Data System.** USRDS 2008 Annual Data Report: Atlas of Chronic Kidney Disease and End-Stage Renal Disease in the United States. Bethesda, MD: National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases, 2008

95 **Vallon V**, Richter K, Blantz RC, Thomson S, Osswald H. Glomerular hyperfiltration in experimental diabetes mellitus: potential role of tubular reabsorption. *J Am Soc Nephrol* 1999; **10**: 2569-2576 [PMID: 10589696]

96 **Thomson SC**, Vallon V, Blantz RC. Kidney function in early diabetes: the tubular hypothesis of glomerular filtration. *Am J Physiol Renal Physiol* 2004; **286**: F8-15 [PMID: 14656757 DOI: 10.1152/ajprenal.00208.2003]

97 **Bjornstad P**, Maahs DM, Cherney DZ, Cree-Green M, West A, Pyle L, Nadeau KJ. Insulin sensitivity is an important determinant of renal health in adolescents with type 2 diabetes. *Diabetes Care* 2014; **37**: 3033-3039 [PMID: 25071077]

98 **Bjornstad P**, Snell-Bergeon JK, Rewers M, Jalal D, Chonchol MB, Johnson RJ, Maahs DM. Early diabetic nephropathy: a complication of reduced insulin sensitivity in type 1 diabetes. *Diabetes Care* 2013; **36**: 3678-3683 [PMID: 24026551 DOI: 10.2337/dc13-0631]

99 **Brosius FC**, Alpers CE. New targets for treatment of diabetic nephropathy: what we have learned from animal models. *Curr Opin Nephrol Hypertens* 2013; **22**: 17-25 [PMID: 23207723 DOI: 10.1097/MNH.0b013e32835b3766]

100 **Komers R**, Lindsley JN, Oyama TT, Anderson S. Cyclo-oxygenase-2 inhibition attenuates the progression of nephropathy in uninephrectomized diabetic rats. *Clin Exp Pharmacol Physiol* 2007; **34**: 36-41 [PMID: 17201733 DOI: 10.1111/j.1440-1681.2007.04534.x]

101 **Komers R**, Lindsley JN, Oyama TT, Schutzer WE, Reed JF, Mader SL, Anderson S. Immunohistochemical and functional correlations of renal cyclooxygenase-2 in experimental diabetes. *J Clin Invest* 2001; **107**: 889-898 [PMID: 11285308 DOI: 10.1172/JCI10228]

102 **Cheng HF**, Wang CJ, Moeckel GW, Zhang MZ, McKanna JA, Harris RC. Cyclooxygenase-2 inhibitor blocks expression of mediators of renal injury in a model of diabetes and hypertension. *Kidney Int* 2002; **62**: 929-939 [PMID: 12164875 DOI: 10.1046/j.1523-1755.2002.00520.x]

103 **Carranza A**, Karabatas L, Barontini M, Armando I. Decreased tubular uptake of L-3,4-dihydroxyphenylalanine in streptozotocin-induced diabetic rats. *Horm Res* 2001; **55**: 282-287 [PMID: 11805432]

104 **Marwaha A**, Banday AA, Lokhandwala MF. Reduced renal dopamine D1 receptor function in streptozotocin-induced diabetic rats. *Am J Physiol Renal Physiol* 2004; **286**: F451-F457 [PMID: 14612382 DOI: 10.1152/ajprenal.00227.2003]

105 **Carranza A**, Musolino PL, Villar M, Nowicki S. Signaling cascade of insulin-induced stimulation of L-dopa uptake in renal proximal tubule cells. *Am J Physiol Cell Physiol* 2008; **295**: C1602-C1609 [PMID: 18842830 DOI: 10.1152/ajpcell.00090.2008]

106 **Tsuchida H**, Imai G, Shima Y, Satoh T, Owada S. Mechanism of sodium load-induced hypertension in non-insulin dependent diabetes mellitus model rats: defective dopaminergic system to inhibit Na-K-ATPase activity in renal epithelial cells. *Hypertens Res* 2001; **24**: 127-135 [PMID: 11325071 DOI: 10.1291/hypres.24.127]

107 **Umrani DN**, Banday AA, Hussain T, Lokhandwala MF. Rosiglitazone treatment restores renal dopamine receptor function in obese Zucker rats. *Hypertension* 2002; **40**: 880-885 [PMID: 12468573 DOI: 10.1161/01.HYP.0000039963.01288.D3]

108 **Trivedi M**, Lokhandwala MF. Rosiglitazone restores renal D1A receptor-Gs protein coupling by reducing receptor hyperphosphorylation in obese rats. *Am J Physiol Renal Physiol* 2005; **289**: F298-F304 [PMID: 15798088 DOI: 10.1152/ajprenal.00362.2004]

109 **Kuzhikandathil EV**, Clark L, Li Y. The extracellular cAMP-adenosine pathway regulates expression of renal D1 dopamine receptors in diabetic rats. *J Biol Chem* 2011; **286**: 32454-32463 [PMID: 21803776 DOI: 10.1074/jbc.M111.268136]

110 **Barthelmebs M**, Mayer P, Thomas A, Grima M, Imbs JL. Pathophysiological role of dopamine in the kidney: effects in diabetes mellitus and after contralateral nephrectomy. *Hypertens Res* 1995; **18** Suppl 1: S131-S136 [PMID: 8529042 DOI: 10.1291/hypres.18.SupplementI_S131]

111 **Luippold G**, Beilharz M, Mühlbauer B. Reduction of glomerular hyperfiltration by dopamine D(2)-like receptor blockade in experimental diabetes mellitus. *Nephrol Dial Transplant* 2001; **16**: 1350-1356 [PMID: 11427624 DOI: 10.1093/ndt/16.7.1350]

112 **Luippold G**, Schneider S, Vallon V, Osswald H, Mühlbauer B. Postglomerular vasoconstriction induced by dopamine D(3) receptor activation in anesthetized rats. *Am J Physiol Renal Physiol* 2000; **278**: F570-F575 [PMID: 10751217]

113 **Gross ML**, Koch A, Mühlbauer B, Adamczak M, Ziebart H, Drescher K, Gross G, Berger I, Amann KU, Ritz E. Renoprotective effect of a dopamine D3 receptor antagonist in experimental type II diabetes. *Lab Invest* 2006; **86**: 262-274 [PMID: 16402031 DOI: 10.1038/labinvest.3700383]

114 **Chamorro C**, Romera MA, Martinez-Melgar JL, Pardo C, Silva JA.

Dopamine dose and renal damage. *Lancet* 2001; **357**: 1707-1708 [PMID: 11428365 DOI: 10.1016/S0140-6736(00)04838-8]
115 **Lee MR**. Dopamine and the kidney: ten years on. *Clin Sci* (Lond) 1993; **84**: 357-375 [PMID: 8482041]
116 **Mcdonald RH**, Goldberg LI, Mcnay JL, Tuttle EP. Effect of dopamine in man: augmentation of sodium excretion, glomerular filtration rate, and renal plasma flow. *J Clin Invest* 1964; **43**: 1116-1124 [PMID: 14171789]
117 **Hollenberg NK**, Adams DF, Mendell P, Abrams HL, Merrill JP. Renal vascular responses to dopamine: haemodynamic and angiographic observations in normal man. *Clin Sci Mol Med* 1973; **45**: 733-742 [PMID: 4760032]
118 **Cuthbertson BH**, Noble DW. Dopamine in oliguria. *BMJ* 1997; **314**: 690-691 [PMID: 9116537]
119 **Bellomo R**, Chapman M, Finfer S, Hickling K, Myburgh J. Low-dose dopamine in patients with early renal dysfunction: a placebo-controlled randomised trial. Australian and New Zealand Intensive Care Society (ANZICS) Clinical Trials Group. *Lancet* 2000; **356**: 2139-2143 [PMID: 11191541 DOI: 10.1016/S0140-6736(00)03495-4]
120 **Kellum JA**, M Decker J. Use of dopamine in acute renal failure: a meta-analysis. *Crit Care Med* 2001; **29**: 1526-1531 [PMID: 11505120]
121 **Marik PE**. Low-dose dopamine: a systematic review. *Intensive Care Med* 2002; **28**: 877-883 [PMID: 12122525]
122 **Sakr Y**, Reinhart K, Vincent JL, Sprung CL, Moreno R, Ranieri VM, De Backer D, Payen D. Does dopamine administration in shock influence outcome? Results of the Sepsis Occurrence in Acutely Ill Patients (SOAP) Study. *Crit Care Med* 2006; **34**: 589-597 [PMID: 16505643]
123 **Chen HH**, Anstrom KJ, Givertz MM, Stevenson LW, Semigran MJ, Goldsmith SR, Bart BA, Bull DA, Stehlik J, LeWinter MM, Konstam MA, Huggins GS, Rouleau JL, O'Meara E, Tang WH, Starling RC, Butler J, Deswal A, Felker GM, O'Connor CM, Bonita RE, Margulies KB, Cappola TP, Ofili EO, Mann DL, Dávila-Román VG, McNulty SE, Borlaug BA, Velazquez EJ, Lee KL, Shah MR, Hernandez AF, Braunwald E, Redfield MM. Low-dose dopamine or low-dose nesiritide in acute heart failure with renal dysfunction: the ROSE acute heart failure randomized trial. *JAMA* 2013; **310**: 2533-2543 [PMID: 24247300 DOI: 10.1001/jama.2013.282190]
124 **Giamouzis G**, Butler J, Starling RC, Karayannis G, Nastas J, Parisis C, Rovithis D, Economou D, Savvatis K, Kirlidis T, Tsaknakis T, Skoularigis J, Westermann D, Tschöpe C, Triposkiadis F. Impact of dopamine infusion on renal function in hospitalized heart failure patients: results of the Dopamine in Acute Decompensated Heart Failure (DAD-HF) Trial. *J Card Fail* 2010; **16**: 922-930 [PMID: 21111980 DOI: 10.1016/j.cardfail.2010.07.246]
125 **Aziz EF**, Alviar CL, Herzog E, Cordova JP, Bastawrose JH, Pamidimukala CK, Tojino A, Park TS, Musat D, Kukin M. Continuous infusion of furosemide combined with low-dose dopamine compared to intermittent boluses in acutely decompensated heart failure is less nephrotoxic and carries a lower readmission at thirty days. *Hellenic J Cardiol* 2011; **52**: 227-235 [PMID: 21642071]
126 **Ricci Z**, Luciano R, Favia I, Garisto C, Muraca M, Morelli S, Di Chiara L, Cogo P, Picardo S. High-dose fenoldopam reduces postoperative neutrophil gelatinase-associated lipocaline and cystatin C levels in pediatric cardiac surgery. *Crit Care* 2011; **15**: R160 [PMID: 21714857 DOI: 10.1186/cc10295]
127 **Kamiya M**, Sato N, Akiya M, Okazaki H, Takahashi Y, Mizuno K. A case of marked diuresis by combined dopamine and atrial natriuretic peptide administration without renal injury in acute decompensated heart failure. *Int Heart J* 2013; **54**: 243-245 [PMID: 23924940 DOI: 10.1536/ihj.54.243]
128 **Sumeray M**, Robertson C, Lapsley M, Bomanji J, Norman AG, Woolfson RG. Low dose dopamine infusion reduces renal tubular injury following cardiopulmonary bypass surgery. *J Nephrol* 2001; **14**: 397-402 [PMID: 11730274]
129 **Kim CS**. Pharmacologic Management of the Cardio-renal Syndrome. *Electrolyte Blood Press* 2013; **11**: 17-23 [PMID: 23946761 DOI: 10.5049/EBP.2013.11.1.17]
130 **Hillege HL**, Nitsch D, Pfeffer MA, Swedberg K, McMurray JJ, Yusuf S, Granger CB, Michelson EL, Ostergren J, Cornel JH, de Zeeuw D, Pocock S, van Veldhuisen DJ. Renal function as a predictor of outcome in a broad spectrum of patients with heart failure. *Circulation* 2006; **113**: 671-678 [PMID: 16461840]
131 **Bongartz LG**, Cramer MJ, Doevendans PA, Joles JA, Braam B. The severe cardiorenal syndrome: 'Guyton revisited'. *Eur Heart J* 2005; **26**: 11-17 [PMID: 15615794]
132 **Koniari K**, Parissis J, Paraskevaidis I, Anastasiou-Nana M. Treating volume overload in acutely decompensated heart failure: established and novel therapeutic approaches. *Eur Heart J Acute Cardiovasc Care* 2012; **1**: 256-268 [PMID: 24062916 DOI: 10.1177/2048872612457044]
133 **Zappitelli M**, Bernier PL, Saczkowski RS, Tchervenkov CI, Gottesman R, Dancea A, Hyder A, Alkandari O. A small post-operative rise in serum creatinine predicts acute kidney injury in children undergoing cardiac surgery. *Kidney Int* 2009; **76**: 885-892 [PMID: 19641482 DOI: 10.1038/ki.2009.270]
134 **Picca S**, Ricci Z, Picardo S. Acute kidney injury in an infant after cardiopulmonary bypass. *Semin Nephrol* 2008; **28**: 470-476 [PMID: 18790366 DOI: 10.1016/j.semnephrol.2008.05.006]
135 **van der Vorst MM**, Ruys-Dudok van Heel I, Kist-van Holthe JE, den Hartigh J, Schoemaker RC, Cohen AF, Burggraaf J. Continuous intravenous furosemide in haemodynamically unstable children after cardiac surgery. *Intensive Care Med* 2001; **27**: 711-715 [PMID: 11398698]
136 **Tumlin JA**. Impaired blood flow in acute kidney injury: pathophysiology and potential efficacy of intrarenal vasodilator therapy. *Curr Opin Crit Care* 2009; **15**: 514-519 [PMID: 19829105 DOI: 10.1097/MCC.0b013e328332f6f9]
137 **Lindenfeld J**, Albert NM, Boehmer JP, Collins SP, Ezekowitz JA, Givertz MM, Katz SD, Klapholz M, Moser DK, Rogers JG, Starling RC, Stevenson WG, Tang WH, Teerlink JR, Walsh MN. HFSA 2010 Comprehensive Heart Failure Practice Guideline. *J Card Fail* 2010; **16**: e1-194 [PMID: 20610207 DOI: 10.1016/j.cardfail.2010.04.004]
138 **Yancy CW**, Jessup M, Bozkurt B, Butler J, Casey DE, Drazner MH, Fonarow GC, Geraci SA, Horwich T, Januzzi JL, Johnson MR, Kasper EK, Levy WC, Masoudi FA, McBride PE, McMurray JJ, Mitchell JE, Peterson PN, Riegel B, Sam F, Stevenson LW, Tang WH, Tsai EJ, Wilkoff BL. 2013 ACCF/AHA guideline for the management of heart failure: a report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines. *J Am Coll Cardiol* 2013; **62**: e147-e239 [PMID: 23747642 DOI: 10.1161/CIR.0b013e31829e8776]
139 **Ronco C**, McCullough P, Anker SD, Anand I, Aspromonte N, Bagshaw SM, Bellomo R, Berl T, Bobek I, Cruz DN, Daliento L, Davenport A, Haapio M, Hillege H, House AA, Katz N, Maisel A, Mankad S, Zanco P, Mebazaa A, Palazzuoli A, Ronco F, Shaw A, Sheinfeld G, Soni S, Vescovo G, Zamperetti N, Ponikowski P. Cardio-renal syndromes: report from the consensus conference of the acute dialysis quality initiative. *Eur Heart J* 2010; **31**: 703-711 [PMID: 20037146 DOI: 10.1093/eurheartj/ehp507]
140 **Fernández BE**, Correa AH, Choi MR. Atrial natriuretic factor stimulates renal dopamine uptake mediated by natriuretic peptide-type A receptor. *Regul Pept* 2005; **124**: 137-144 [PMID: 15544851]
141 **Correa AH**, Choi MR, Gironacci M, Aprile F, Fernández BE. Atrial natriuretic factor decreases renal dopamine turnover and catabolism without modifying its release. *Regul Pept* 2008; **146**: 238-242 [PMID: 17963868]
142 **Humphreys MH**. Mechanisms and management of nephrotic edema. *Kidney Int* 1994; **45**: 266-281 [PMID: 8127018]
143 **Deschênes G**, Wittner M, Stefano A, Jounier S, Doucet A. Collecting duct is a site of sodium retention in PAN nephrosis: a rationale for amiloride therapy. *J Am Soc Nephrol* 2001; **12**: 598-601 [PMID: 11181809]

144 **Sampaio-Maia B**, Moreira-Rodrigues M, Serrão P, Pestana M. Blunted renal dopaminergic system activity in puromycin aminonucleoside-induced nephrotic syndrome. *Nephrol Dial Transplant* 2006; **21**: 314-323 [PMID: 16204272]

145 **Marin-Grez M**, Briggs JP, Schubert G, Schnermann J. Dopamine receptor antagonists inhibit the natriuretic response to atrial natriuretic factor (ANF). *Life Sci* 1985; **36**: 2171-2176 [PMID: 3158789]

146 **Trivedi M**, Narkar VA, Hussain T, Lokhandwala MF. Dopamine recruits D1A receptors to Na-K-ATPase-rich caveolar plasma membranes in rat renal proximal tubules. *Am J Physiol Renal Physiol* 2004; **287**: F921-F931 [PMID: 15265765]

147 **Fernandes-Cerqueira C**, Sampaio-Maia B, Quelhas-Santos J, Moreira-Rodrigues M, Simões-Silva L, Blazquez-Medela AM, Martinez-Salgado C, Lopez-Novoa JM, Pestana M. Concerted action of ANP and dopamine D1-receptor to regulate sodium homeostasis in nephrotic syndrome. *Biomed Res Int* 2013; **2013**: 397391 [PMID: 23956981 DOI: 10.1155/2013/397391]

148 **Vohra PK**, Hoeppner LH, Sagar G, Dutta SK, Misra S, Hubmayr RD, Mukhopadhyay D. Dopamine inhibits pulmonary edema through the VEGF-VEGFR2 axis in a murine model of acute lung injury. *Am J Physiol Lung Cell Mol Physiol* 2012; **302**: L185-L192 [PMID: 22003095 DOI: 10.1152/ajplung.00274.2010]

149 **Adir Y**, Sznajder JI. Regulation of lung edema clearance by dopamine. *Isr Med Assoc J* 2003; **5**: 47-50 [PMID: 12592959]

150 **Ware LB**, Matthay MA. The acute respiratory distress syndrome. *N Engl J Med* 2000; **342**: 1334-1349 [PMID: 10793167 DOI: 10.1056/NEJM200005043421806]

151 **Ware LB**, Matthay MA. Alveolar fluid clearance is impaired in the majority of patients with acute lung injury and the acute respiratory distress syndrome. *Am J Respir Crit Care Med* 2001; **163**: 1376-1383 [PMID: 11371404 DOI: 10.1164/ajrccm.163.6.2004035]

152 **Sznajder JI**. Strategies to increase alveolar epithelial fluid removal in the injured lung. *Am J Respir Crit Care Med* 1999; **160**: 1441-1442 [PMID: 10556102]

153 **Dematte JE**, Sznajder JI. Mechanisms of pulmonary edema clearance: from basic research to clinical implication. *Intensive Care Med* 2000; **26**: 477-480 [PMID: 10872144]

154 **Goldberg LI**. Cardiovascular and renal actions of dopamine: potential clinical applications. *Pharmacol Rev* 1972; **24**: 1-29 [PMID: 4554480]

155 **McGrath B**, Bode K, Luxford A, Howden B, Jablonski P. Effects of dopamine on renal function in the rat isolated perfused kidney. *Clin Exp Pharmacol Physiol* 1985; **12**: 343-352 [PMID: 3912084]

156 **Barnard ML**, Ridge KM, Saldias F, Friedman E, Gare M, Guerrero C, Lecuona E, Bertorello AM, Katz AI, Sznajder JI. Stimulation of the dopamine 1 receptor increases lung edema clearance. *Am J Respir Crit Care Med* 1999; **160**: 982-986 [PMID: 10471628 DOI: 10.1164/ajrccm.160.3.9812003]

157 **Ridge KM**, Dada L, Lecuona E, Bertorello AM, Katz AI, Mochly-Rosen D, Sznajder JI. Dopamine-induced exocytosis of Na,K-ATPase is dependent on activation of protein kinase C-epsilon and -delta. *Mol Biol Cell* 2002; **13**: 1381-1389 [PMID: 11950946 DOI: 10.1091/mbc.01-07-0323]

158 **Lecuona E**, Garcia A, Sznajder JI. A novel role for protein phosphatase 2A in the dopaminergic regulation of Na,K-ATPase. *FEBS Lett* 2000; **481**: 217-220 [PMID: 11007967 DOI: 10.1016/S0014-5793(00)02009-3]

159 **Bertorello AM**, Sznajder JI. The dopamine paradox in lung and kidney epithelia: sharing the same target but operating different signaling networks. *Am J Respir Cell Mol Biol* 2005; **33**: 432-437 [PMID: 16234332 DOI: 10.1165/rcmb.2005-0297TR]

160 **Guerrero C**, Lecuona E, Pesce L, Ridge KM, Sznajder JI. Dopamine regulates Na-K-ATPase in alveolar epithelial cells via MAPK-ERK-dependent mechanisms. *Am J Physiol Lung Cell Mol Physiol* 2001; **281**: L79-L85 [PMID: 11404249]

161 **Leaver SK**, Evans TW. Acute respiratory distress syndrome. *BMJ* 2007; **335**: 389-394 [PMID: 17717368 DOI: 10.1136/bmj.39293.624699.AD]

162 **Matthay MA**, Calfee CS. Therapeutic value of a lung protective ventilation strategy in acute lung injury. *Chest* 2005; **128**: 3089-3091 [PMID: 16304246 DOI: 10.1378/chest.128.5.3089]

163 **Ware LB**, Kaner RJ, Crystal RG, Schane R, Trivedi NN, McAuley D, Matthay MA. VEGF levels in the alveolar compartment do not distinguish between ARDS and hydrostatic pulmonary oedema. *Eur Respir J* 2005; **26**: 101-105 [PMID: 15994395 DOI: 0.1183/09031936.05.00106604]

164 **Wheeler AP**, Bernard GR. Acute lung injury and the acute respiratory distress syndrome: a clinical review. *Lancet* 2007; **369**: 1553-1564 [PMID: 17482987 DOI: 10.1016/S0140-6736(07)60604-7]

165 **Becker PM**, Alcasabas A, Yu AY, Semenza GL, Bunton TE. Oxygen-independent upregulation of vascular endothelial growth factor and vascular barrier dysfunction during ventilated pulmonary ischemia in isolated ferret lungs. *Am J Respir Cell Mol Biol* 2000; **22**: 272-279 [PMID: 10696063 DOI: 10.1165/ajrcmb.22.3.3814]

166 **Bhandari V**, Choo-Wing R, Lee CG, Yusuf K, Nedrelow JH, Ambalavanan N, Malkus H, Homer RJ, Elias JA. Developmental regulation of NO-mediated VEGF-induced effects in the lung. *Am J Respir Cell Mol Biol* 2008; **39**: 420-430 [PMID: 18441284 DOI: 10.1165/rcmb.2007-0024OC]

167 **Godzich M**, Hodnett M, Frank JA, Su G, Pespeni M, Angel A, Howard MB, Matthay MA, Pittet JF. Activation of the stress protein response prevents the development of pulmonary edema by inhibiting VEGF cell signaling in a model of lung ischemia-reperfusion injury in rats. *FASEB J* 2006; **20**: 1519-1521 [PMID: 16793871 DOI: 10.1096/fj.05-4708fje]

168 **Gurkan OU**, O'Donnell C, Brower R, Ruckdeschel E, Becker PM. Differential effects of mechanical ventilatory strategy on lung injury and systemic organ inflammation in mice. *Am J Physiol Lung Cell Mol Physiol* 2003; **285**: L710-L718 [PMID: 12754185 DOI: 0.1152/ajplung.00044.2003]

169 **Kaner RJ**, Crystal RG. Pathogenesis of high altitude pulmonary edema: does alveolar epithelial lining fluid vascular endothelial growth factor exacerbate capillary leak? *High Alt Med Biol* 2004; **5**: 399-409 [PMID: 15671629 DOI: 10.1089/ham.2004.5.399]

170 **Basu S**, Nagy JA, Pal S, Vasile E, Eckelhoefer IA, Bliss VS, Manseau EJ, Dasgupta PS, Dvorak HF, Mukhopadhyay D. The neurotransmitter dopamine inhibits angiogenesis induced by vascular permeability factor/vascular endothelial growth factor. *Nat Med* 2001; **7**: 569-574 [PMID: 11329058 DOI: 10.1038/87895]

171 **Chakroborty D**, Sarkar C, Mitra RB, Banerjee S, Dasgupta PS, Basu S. Depleted dopamine in gastric cancer tissues: dopamine treatment retards growth of gastric cancer by inhibiting angiogenesis. *Clin Cancer Res* 2004; **10**: 4349-4356 [PMID: 15240521 DOI: 10.1158/1078-0432.CCR-04-0059]

172 **Sarkar C**, Chakroborty D, Mitra RB, Banerjee S, Dasgupta PS, Basu S. Dopamine in vivo inhibits VEGF-induced phosphorylation of VEGFR-2, MAPK, and focal adhesion kinase in endothelial cells. *Am J Physiol Heart Circ Physiol* 2004; **287**: H1554-H1560 [PMID: 15371263 DOI: 10.1152/ajpheart.00272.2004]

173 **Goldberg JF**, Burdick KE, Endick CJ. Preliminary randomized, double-blind, placebo-controlled trial of pramipexole added to mood stabilizers for treatment-resistant bipolar depression. *Am J Psychiatry* 2004; **161**: 564-566 [PMID: 14992985 DOI: 10.1176/appi.ajp.161.3.564]

174 **Kleiner-Fisman G**, Fisman DN. Risk factors for the development of pedal edema in patients using pramipexole. *Arch Neurol* 2007; **64**: 820-824 [PMID: 17420306 DOI: 10.1001/archneur.64.6.noc60158]

175 **Tan EK**, Ondo W. Clinical characteristics of pramipexole-induced peripheral edema. *Arch Neurol* 2000; **57**: 729-732 [PMID: 10815140 DOI: 10.1001/archneur.57.5.729]

176 **Sato S**, Hori T, Tsutsumi K, Asada T. Pramipexole-induced peripheral edema in a patient with bipolar depression. *J Neuropsychiatry Clin Neurosci* 2011; **23**: E20 [PMID: 22231331 DOI: 10.1176/appi.neuropsych.23.4.e20]

177 **Biglan KM**, Holloway RG, McDermott MP, Richard IH. Risk factors for somnolence, edema, and hallucinations in early Parkinson disease. *Neurology* 2007; **69**: 187-195 [PMID: 17620552]

178 **Zavala JA**, Munhoz RP, Teive HA. Pramipexole-related chronic lower limb oedema in a patient with Parkinson's disease. *J Clin Neurosci* 2012; **19**: 1298-1299 [PMID: 22721884 DOI: 10.1016/

j.jocn.2011.12.011]
179 **Aperia AC**. Intrarenal dopamine: a key signal in the interactive regulation of sodium metabolism. *Annu Rev Physiol* 2000; **62**: 621-647 [PMID: 10845105]

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REVIEW

Oxidative stress as a potential causal factor for autoimmune hemolytic anemia and systemic lupus erythematosus

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Abstract

The kidneys and the blood system mutually exert influence in maintaining homeostasis in the body. Because the kidneys control erythropoiesis by producing erythropoietin and by supporting hematopoiesis, anemia is associated with kidney diseases. Anemia is the most prevalent genetic disorder, and it is caused by a deficiency of glucose 6-phosphate dehydrogenase (G6PD), for which sulfhydryl oxidation due to an insufficient supply of NADPH is a likely direct cause. Elevated reactive oxygen species (ROS) result in the sulfhydryl oxidation and hence are another potential cause for anemia. ROS are elevated in red blood cells (RBCs) under superoxide dismutase (SOD1) deficiency in C57BL/6 mice. SOD1 deficient mice exhibit characteristics similar to autoimmune hemolytic anemia (AIHA) and systemic lupus erythematosus (SLE) at the gerontic stage. An examination of AIHA-prone New Zealand Black (NZB) mice, which have normal *SOD1* and *G6PD* genes, indicated that ROS levels in RBCs are originally high and further elevated during aging. Transgenic overexpression of human SOD1 in erythroid cells effectively suppresses ROS elevation and ameliorates AIHA symptoms such as elevated anti-RBC antibodies and premature death in NZB mice. These results support the hypothesis that names oxidative stress as a risk factor for AIHA and other autoimmune diseases such as SLE. Herein we discuss the association between oxidative stress and SLE pathogenesis based mainly on the genetic and phenotypic characteristics of NZB and New Zealand white mice and provide insight into the mechanism of SLE pathogenesis.

Key words: Autoimmune hemolytic anemia; Systemic lupus erythematosus; Red blood cells; New Zealand black mice; New Zealand white mice

Core tip: Superoxide dismutase (SOD1) deficient C57BL/6 mice exhibit characteristics similar to autoimmune hemolytic anemia (AIHA) and systemic lupus erythematosus (SLE) at the gerontic stage. An examination of AIHA-prone New Zealand Black (NZB) mice indicated that reactive oxygen species (ROS) levels in red blood cells are originally high and

further elevated during aging. Transgenic overexpression of human SOD1 in erythroid cells effectively suppresses ROS elevation and ameliorates AIHA symptoms in NZB mice. Herein we discuss the association between oxidative stress and SLE pathogenesis based mainly on the genetic and phenotypic characteristics of NZB and New Zealand white mice.

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INTRODUCTION

The kidney has multiple functions that include maintaining the functions of homeostasis such as the excretion of waste, maintenance of the electrolyte balance of body fluids, and endocrine secretion. As an endocrine organ, the kidney plays an essential role in erythropoiesis by producing erythropoietin that supports hematopoiesis in bone marrow[1]. Chronic kidney disease causes renal anemia by reducing erythropoietin production, and, hence, exogenous erythropoietin is widely used as a potent medicine for the treatment of patients with renal anemia[2]. Defected iron metabolism due to chronic inflammation and cytokine imbalance is also involved in chronic kidney disease-induced anemia[3].

A variety of contributing factors including defected hematopoiesis and accelerated hemolysis are involved in anemic pathogenesis. Glucose 6-phosphate dehydrogenase (G6PD) deficiency, which is the most common genetic defect in the human population[4], causes an insufficient supply of NADPH in RBCs and results in anemia. Although the actual mechanism of anemia due to the G6PD deficiency is not totally understood, the involvement of sulfhydryl oxidation is suspected to be a contributing factor.

Aberrant immune responses in some autoimmune diseases also cause anemia. Autoimmune hemolytic anemia (AIHA) is the pathological condition whereby antibodies attack RBCs, and it often precedes a diagnosis of systemic lupus erythematosus (SLE)[5,6]. Both genetic and environmental factors are involved in the etiology of AIHA and SLE, but molecular mechanisms for a majority of the diseases are largely ambiguous. Reactive oxygen species (ROS) are elevated and appear to be a likely underlying mechanism for these pathological conditions[7-9]. In this review article we discuss recent advances in the research on AIHA and SLE from the viewpoint of oxidative stress using animal models.

G6PD deficiency and oxidative stress

ROS are produced under various conditions such as inflammation and hypoxia-reperfusion injury, and they are involved in a variety of diseases including anemia and renal failure[10]. While reduction-oxidation (redox) reactions play essential roles in metabolic reactions, which includes oxidative phosphorylation that consumes respired oxygen, ROS are simultaneously produced as byproducts. Meanwhile, hemoglobin (Hb), which constitutes a major protein (5 mmol/L) in RBCs and contains Fe(Ⅱ)-heme. When hemoglobin is oxygenated ($Hb\text{-}O_2$), a part of $Hb\text{-}O_2$ suffers autoxidation to methemoglobin (MetHb), which possesses Fe(Ⅲ)-heme and is unable to bind oxygen, and releases superoxide[11,12]. Calculation has shown that the rate for the autoxidation of hemoglobin is 2%-3% (in humans) and 4% (in mice) of total hemoglobin per day. Thus, RBCs are under oxidative stress constitutively, and cellular components face the risk of oxidative damage (Figure 1). NADPH is the principle electron donor for most redox systems that include antioxidation by glutathione peroxidase-glutathione reductase and peroxiredoxin (Prdx)-thioredoxin reductase axes[13] and reductive carbonyl detoxification by the aldo-keto reductase family. Under healthy conditions, the resultant methemoglobin is reduced back by methemoglobin reductase in a NADPH-dependent manner and kept at low levels.

Elimination of the resultant ROS and maintaining the redox potential within cells are prerequisites for the survival for RBCs, so that antioxidative enzymes, such as superoxide dismutase (SOD), catalase, glutathione peroxidase, and Prdx, have crucial roles in keeping RBCs healthy. Antioxidants with a small molecular weight, notably glutathione and vitamin C (ascorbic acid), also play roles in redox homeostasis. Oxidative stress induced by SOD and Prdx deficiencies participate in the pathogenesis of anemia, as described below.

Approximately 60 years have passed since the discovery of G6PD deficiency, but the actual mechanism of G6PD deficiency-triggered anemia remains undefined[4]. Because G6PD is the rate-determining enzyme in the pentose phosphate pathway and is involved in the production of NADPH, a G6PD deficiency shifts the cellular redox balance to an oxidized state[14]. Meanwhile, most redox proteins, excluding the ones possessing electron-accepting prosthetic groups, consist of reactive sulfhydryl residues, which are also highly susceptible to oxidative modification. Oxidized or aged proteins undergo proteolytic degradation, and RBCs lack the cellular organelle and protein synthesis machinery that is necessary for their renewal. Thus, oxidative stress appears to cause selective decreases in redox-sensitive proteins. The production of NADPH is elevated by activated G6PD in response to oxidative stress[15], and it supports the reductive detoxification of ROS and oxidized molecules, although its activation mechanism is still unclear.

Mouse models developing anemia, AIHA, and SLE

There are animal models that are applicable to research

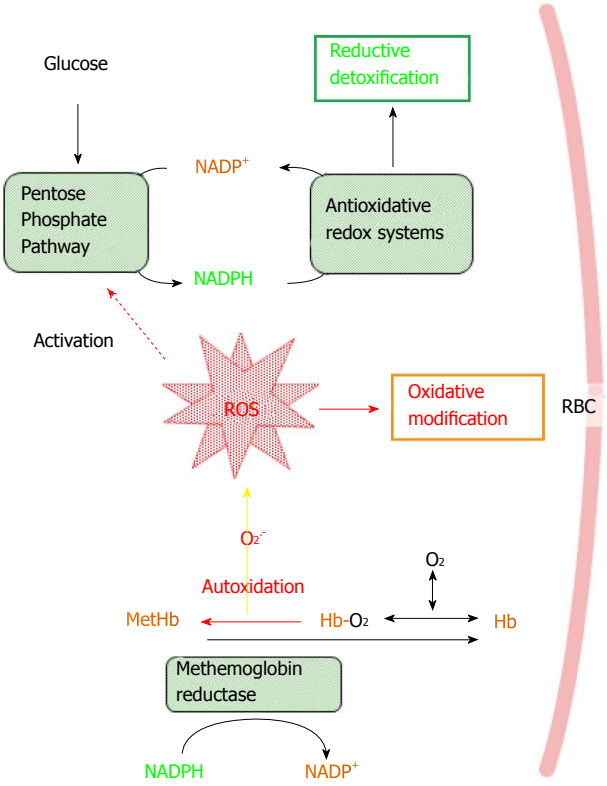

Figure 1 Oxidative stress and antioxidative/redox systems in red blood cells. A part of oxygenized hemoglobin (Hb-O_2) is autoxidized to methemoglobin (MetHb) and releases superoxide ($O_2^{\cdot-}$), which may cause oxidative damage to RBCs. MetHb can be reduced back to Hb by methemoglobin reductase in NADPH-dependent manner. G6PD, a rate-determining enzyme in the pentose phosphate pathway, is involved in the production of NADPH that supports antioxid/redox systems as well as methemoglobin reduction by donating redox potential. G6PD deficiency affects entire antioxidative/redox systems, which can consequently accelerate the destruction of RBCs and lead to anemia. RBCs: Red blood cells. NADPH: Triphosphopyridine nucleotide.

into the etiology of anemia. For example, a direct cause for iron deficiency anemia is defective hemoglobin synthesis due to insufficient heme supply. The involvement of oxidative stress has been implicated in the pathogenesis of some types of chemically induced anemia, such as that induced by pheynylhydrazine[16].

Several strains of animals that spontaneously develop anemia have been used for pathophysiological examinations. New Zealand Black (NZB) mice constitute a strain that develops AIHA during late middle age, at around 40-50 wk. IgG bound to RBCs increases from about 3 mo of age and induces anemia from about 6 mo of age onward[5]. AIHA is exacerbated by an aberrant immune system with notably impaired $CD4^+CD25^+$ regulatory T cells[17] and a Th1 and Th2 cytokine imbalance[18]. Peripheral B-1 cells appear to be a source of autoantibody-producing cells[19]. A dominant T-cell epitope in AIHA is a major glycosylated membrane protein of RBCs, which is also known as an anion transporter band 3[20]. When the *AE1* gene encoding band 3 is deleted, the congenic NZB mice still produce autoantibodies against another glycoprotein, glycophorin, and develop AIHA[21]. Thus, a defect in these glycoproteins is not a primary cause, but other latent abnormalities remain.

Defected genes have been identified in pathological model animals for SLE, MRL/*lpr* and MRL/*gld* mice[22]. Mutations in Fas and Fas ligand genes cause SLE in MRL/*lpr* and MRL/*gld* mice, respectively, *via* malfunctioning apoptotic removal of self-recognizing preB cells at an infant stage[23]. Although mutations in FAS/APO-1 and Fas ligand are found in human SLE patients[24-26], the incidence is not high. Thus, causal factors for SLE are still largely unknown in the human population. (NZB x NZW) F1 mice are another SLE model animal and show characteristics similar to human SLE[27]. While NZB mice spontaneously develop AIHA symptoms that are limited to the blood system, (NZB x NZW) F1 mice exhibit symptoms in a systemic fashion that include lupus nephritis and cardiovascular abnormalities[28-30]. Although NZW mice possess a larval defect in the immune system, they show virtually normal phenotypes and survival times. Genetic analysis of NZW mice has advanced in the past decade, and the latent factor responsible for the onset of SLE has been unveiled.

Anemia observed in antioxidative enzyme gene-modified mice

Because antioxidation plays an essential role in maintaining RBC function, a deficiency of antioxidative enzymes occasionally exerts severe damage to RBCs. Anemia is caused by a deficiency of antioxidative enzymes SOD1[31], SOD2[32], Prdx1[33], and Prdx2[34], but not by deficiencies of glutathione peroxidase 1[35] or catalase[36]. Phenotypic characteristics regarding anemia differ in genetically modified mice, as follows.

SOD1 DEFICIENCY

Among three SOD isozymes present in mammals, SOD1 is a sole superoxide-scavenging enzyme in mature RBCs, and its deficiency causes anemia[31]. Hemoglobin is a major protein in RBCs, and suffers autoxidation, which results in the production of superoxide[11,12]. Without SOD1, the radical chain reaction initiated by superoxide oxidatively damages RBCs, and ultimately accelerates their destruction. Thus, SOD1-deficient RBCs show a shortened life span that is approximately 60%-70% that of the RBCs of wild-type mice[31].

SOD1 deficiency accelerates hemolysis in the blood and phagocytotic removal of RBCs by liver Kupffer cells[37]. An elevation of ROS levels in RBCs, oxidation of RBC components, and augmented production of autoantibodies in RBCs have been observed in SOD1 deficient C57BL/6 (B6) mice[31]. Elevated production of antibodies against lipid peroxidation products, 4-hydroxynonenal and acrolein, occurs[15]. A general antioxidant, *N*-acetyl cysteine (NAC), ameliorates these phenotypes and suppresses anemia and AIHA development. Restricted expression of human *SOD1* in erythroid cells suppresses oxidative stress in RBCs,

which rescues aberrant phenotypes related to anemia and autoimmune responses in *SOD1*-deficient B6 mice. This substantial amount of evidence supports the notion that overproduced ROS due to *SOD1* deficiency can trigger anemia.

Superoxide is continuously produced from oxygenized hemoglobin[11], and hence it is regarded as one of the sources for ROS. Based on theoretical calculation[12], an approximate 200-fold elevation in superoxide results from *SOD1* deficiency. Superoxide would conversely result in the conversion of hemoglobin to methemoglobin and enhance the oxidative modification of RBCs. A marked reduction in glutathione peroxidase 1 protein and its activity is seen in SOD1 deficiency[38], which is caused by an irreversible inactivation *via* conversion of the catalytic selenocysteine to dihydroalanine by elevated ROS[39]. However, the contribution of this low glutathione peroxidase 1 activity to anemia is ambiguous because a deficiency of either glutathione peroxidase 1 or catalase does not cause hematological abnormalities in mice[35,36]. Because thioredoxin reductase is also a selenoenzyme[40], inactivation by the elevated ROS due to a SOD1 deficiency may have a role in the destruction of RBCs.

SOD2 DEFICIENCY

Mice lacking SOD2, a mitochondria-specific isoform, in the whole body show dilated cardiomyopathy, hepatic lipid accumulation and early neonatal death[41]. Hematopoietic chimeras in which all blood cells are derived from the fetal liver stem cells of *SOD2*-deficient mice are employed to examine the effect of SOD2 deficiency on hematopoiesis. The chimera mice are persistently anemic and characteristically similar to the human disorder sideroblastic anemia[32]. Enhanced protein oxidation and altered membrane deformation appear to reduce the life span of RBCs[42,43]. SOD2-deficient reticulocytes reveal up-regulated transferrin receptors[44] and mitochondrial proliferation and mitochondrial membrane thickening[45]. It is noteworthy that mature RBCs, which do not possess mitochondria, show an elevated production of ROS, abundant iron-stainable granules, and oxidatively damaged proteins. These observations imply that the life-span of the resultant RBCs is reduced due to oxidative damage that is experienced before final maturation of the erythroid cells.

PRDX DEFICIENCY

Among 6 Prdx family members, deficiency of either *Prdx1*[33] or *Prdx2*[34] causes hemolytic anemia. *Prdx1*-deficient mice show increased ROS, hemoglobin instability, Heinz body formation, and a decreased erythrocyte life span[33]. Cancers develop in some organs of *Prdx1*-deficient mice, but a causal connection to anemia is unknown. Prdx2 is a predominant form of Prdx family members in RBCs[46] and its function in RBCs has been thoroughly characterized. Prdx2 exists as either a stable dimer or a hyperoxidized form in RBCs[47]. Prdx2 functions in a dimer form with a head-to-tail arrangement. During the peroxidase reaction two pairs of disulfide bonds between the catalytic Cys at the N-terminus and the resolving Cys at the C-terminus in the two subunits are formed as an intermediate[13]. However, Prdx2 appears to function as a non-catalytic scavenger of peroxides in RBCs due to an insufficient thioredoxin-thioredoxin reductase system[48,49]. Sulfenic acid is a physiological intermediate of sulfhydryl groups in the catalytic Cys, but excessively produced hydrogen peroxide hyperoxidizes it to sulfinic acid and then sulfonic acid during the reaction cycle of Prdx, which results in a loss of peroxidase activity[13]. The slow turnover rate of Prdx2 increases the chance for hyperoxidization by hydrogen peroxide in RBCs. Sulfinic acid in Prdx can be converted back to sulfhydryl by sulfiredoxin in an ATP-dependent manner in many cells[50,51]. However, because of an insufficient amount of sulfiredoxin in RBCs, hyperoxidized Prdx2 would proceed to proteolytic removal. Although cyclic changes of the hyperoxidized Prdx has been shown in cultured RBCs[52], this phenomenon cannot be explained by virtue of sulfiredoxin but may be caused by the proteolytic removal of hyperoxidized Prdx2. Because Prdx2 is involved in maintaining hemoglobin stability[53], hemolytic anemia found in Prdx2-deficient mice may be related to the decrease in the life-span of hemoglobin.

Oxidative stress as a potential cause for anemia and autoimmune responses in NZB mice

SOD1-deficient mice produce anti-RBC autoantibody and ultimately develop lupus nephritis-like symptoms in the gerontic stage[15,31], so that we hypothesized that oxidative stress is one of the causal factors for some autoimmune diseases such as SLE and AIHA in C57BL/6 mice. However *SOD1* deficiency is far from a physiologic condition, and we have tried to validate this hypothesis based on physiological conditions using NZB mice.

NZB mice[54] and (NZB × NZW)F1 mice[55] are the established model animals that spontaneously develop AIHA and SLE, respectively, at around 40-50 wk of age. Abnormal proteolytic cleavage of the membrane proteins of RBCs has been proposed as a likely cause because the cleaved membrane proteins, such as band 3, are highly antigenic[56-58]. However, elevated proteolytic activity in the RBCs of AIHA patients or AIHA-prone mice is unknown. Despite extensive investigation on the etiology of the mice, it remains unclear what actually triggers the autoantibody production in the NZB mice[5].

We first recognized that the ROS levels are originally high at a young age (4 wk) and increase as NZB mice age compared to control mice[38]. Increases in the autoantibodies against RBCs show a correlation with the elevated levels of ROS in RBCs. Antioxidants such as NAC suppress autoantibody production in the mice, supporting the oxidative stress theory of AIHA in mice. The onset of AIHA occurs prematurely and

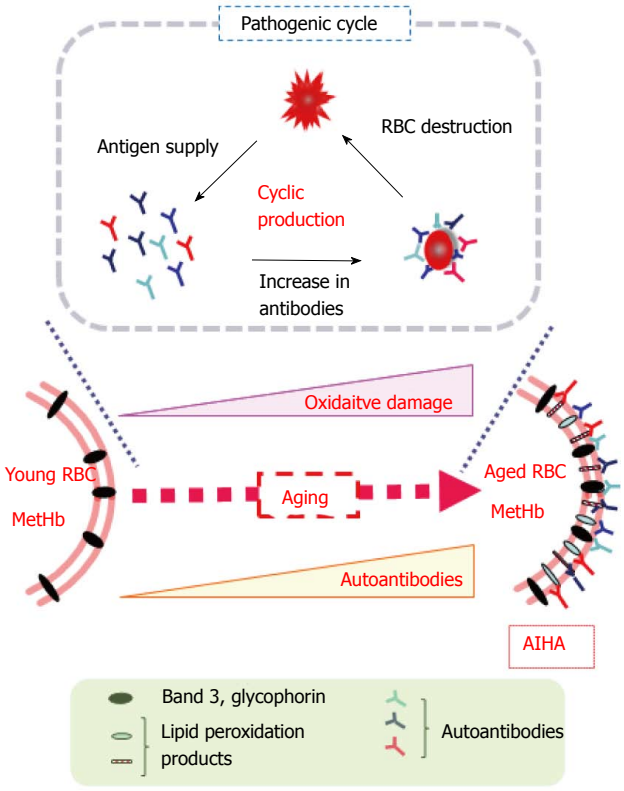

Figure 2 Hypothetical role of oxidative stress in triggering the autoimmune reaction against red blood cells in New Zealand Black mice. Elevated ROS trigger oxidative modification of RBC components and result in the production of oxidatively modified compounds such as 4-hydroxy 2-nonenal and acrolein that are highly antigenic. During aging, the oxidation of susceptible molecules and the production of antibodies recognizing them occurs repeatedly, which results in an accumulation of epitopes and autoantibodies. The elevated levels of autoantibodies ultimately trigger AIHA in aged NZB mice. RBCs: Red blood cells; AIHA: Autoimmune hemolytic anemia; MetHb: Methemoglobin; NZB: New Zealand Black; ROS: Reactive oxygen species.

mortality increases in the *SOD1*-deficient congenic NZB mice compared with control NZB mice[59]. The transgenic expression of human SOD1 in RBCs reduces oxidative stress to RBCs and oxidative modification of lipids and proteins and consequently rescues the AIHA phenotypes in NZB mice. Figure 2 provides a schematic mechanism for the onset of AIHA based on our hypothesis regarding the oxidative damage of RBCs. Because oxidative modification elevates during aging and oxidized molecules are highly antigenic, oxidative stress would elevate the autoantibodies by increasing the autoantigens, and would ultimately cause AIHA onset.

Either suppression of the antioxidative/redox system or activation of ROS generation causes the elevated ROS in RBCs. As mentioned above, the mechanism of G6PD deficiency-induced anemia is attributed to a short supply of NADPH, which triggers the oxidation of sulfhydryls in RBCs[4]. However, no report has shown abnormalities in glucose G6PD in the RBCs of NZB mice. Regarding the antioxidative enzymes catalase, SOD, glutathione peroxidase, and glutathione reductase, nonsynonymous nucleotide polymorphisms have been identified in the genes in 10 inbred mouse strains, including NZB mice[60]. Thus, the origin of oxidative stress in NZB mice is unclear as of this writing.

Oxidative stress as a potential cause for SLE

Superoxide anion diffuses across the RBC membrane *via* the anion channel band 3 protein[61], which is a potent antigenic molecule in RBCs[56-58]. ROS appear to derive from inside the RBCs because lipid peroxides are high in the RBCs but about the same in plasma among the congenic mouse groups. Based on theoretical calculation, more than 100 mol/L of superoxide is released daily from hemoglobin autoxidation[12].

Involvement of oxidative stress has been implied in the pathogenesis of human SLE. For example, lipid peroxidation product 4-hydroxy 2-nonenal may modify Ro60, which is the 60-kDa autoantigen of autoimmunity in both SLE Sjögren syndrome, and differentially participate in Sjögren syndrome or SLE[62,63]. Children with SLE carry increased levels of 4-hydroxy 2-nonenal-modified proteins in plasma[64]. Plasma concentrations of 4-hydroxy 2-nonenal as well as malondialdehyde and oxidized glutathione increase during aging in human plasma and RBCs[65]. Both mitochondrial electron transport chain activity at complex I and oxygen consumption are increased in the lymphocytes of SLE patients[66]. On the contrary, NAC suppresses oxygen consumption and hydrogen peroxide levels. Other studies have shown the beneficial effects that antioxidants such as vitamin E, all-trans-retinoic acid, fish oil, and cystamine has on (NZB X NZW)F1 mice[67-69].

Oxidative modification is caused by relatively large amounts of ROS and generally causes oxidation in a non-specific manner. While lymphocytes are defective, and aberrant immune responses occur in AIHA and SLE, it is unclear how they are stimulated to produce autoantibodies. Because oxidized cells are efficiently phagocytosed by macrophages, there is more chance for the immune system to recognize the resultant oxidized molecules as antigens[70,71]. In fact, oxidatively modified albumin is well recognized by the antibodies from SLE patients[72], and oxidatively modified lipids are identified as epitopes for innate immunity and are responsible for diseases such as atherogenesis[73-75]. Lipid peroxidation products, such as 4-hydroxy 2-nonenal and acrolein, have been identified as bona fide epitopes for autoantibodies on RBC membranes[15]. Thus, oxidative stress participates in the formation of novel epitopes by oxidizing proteins and lipids. It is also noteworthy that anti-DNA antibodies, which are typically elevated in SLE patients, also recognize 4-hydroxy 2-nonenal-bound proteins[76,77].

Hypothetical mechanism for SLE onset in (NZB x NZW)F1 mice

An early genetic study suggests that three genes, one from NZB and two from NZW mice, are involved in

the development of SLE in the (NZB x NZW) F1 mice and that the gene from NZB mice should function dominantly[78]. Recent genetic studies have indicated several candidate genes for AIHA and/or SLE in model mice[79]. Three major genomic intervals (Sle1, Sle2, and Sle3) have been identified on the New Zealand mouse strains and regarded as systemic autoimmune disease susceptibility loci in NZM2410 mice, which is an acute lupus-prone strain derived from a cross between NZB and NZW[28,30]. High titers of IgG autoantibodies against nuclear proteins and DNA are produced by B6 mice congenic for the *Sle1* locus[80]. T cells specific for histone are present[81], implicating *Sle1* in the loss of tolerance that leads to the development of antinuclear antibodies. The *Sle1b* sublocus contains the SLAM (signaling lymphocyte activating molecule) family (Slamf) genes derived from the lupus-prone NZW mice[82,83].

Several candidate genes for autoimmune diseases in humans have also been screened out by genome-wide association studies[84]. Those genes include *HLA*, *STAT4*, and *PTPN22*. Among them, an allelic variant of protein tyrosine phosphatase nonreceptor 22 (*PTPN22*) shows the most promise because it has been associated with multiple human autoimmune diseases, such as type 1 diabetes, rheumatoid arthritis, and SLE. *PTPN22* encodes lymphoid tyrosine phosphatase (Lyp) which participates in the negative regulation of T-cell receptor (TCR) proximal signaling[85,86]. Lyp is also referred to as PEST domain-enriched tyrosine phosphatase (Pep), and it suppresses the activity of the Src family protein tyrosine kinases and inhibits T-cell activity[87]. Because PEST domain, which is rich in proline (P)-glutamate (E)-serine (S)-threonine (T), undergoes rapid degradation, Lyp is vulnerable to proteolytic cleavage. Lyp reportedly negatively regulates T cell receptor signaling[88,89], and the decreased activity would conversely activate the signaling pathway.

In the past two decades, the signaling function of ROS has attracted much attention in the research field of oxidative stress. In this aspect, ROS specifically inactivates susceptible molecules, *e.g.*, phosphotyrosine phosphatase (PTP) families such as PTP1B, Cdc25, SHP1 and SHP2[90,91]. PTP has reactive cys-SH at its catalytic center, which is a preferred target of locally produced ROS. Multiple reports have indicated that PTP variants are linked to human hereditary disorders[92], which indicate that PTP activities play pivotal roles and hence oxidative inactivation affects a variety of cells including lymphocytes.

Because Lyp/Pep is a member of the PTP superfamily and easily oxidized by ROS such as hydrogen peroxide, it may play a role in the sustained activation of lymphocytes, and, hence, it would also play a role in the autoimmune response. A Pep variant (Pep-R619W; Rep with substitution of arginine-619 to Tryptophane-619) protein linked to autoimmune disease is more rapidly degraded and shows greater association with, and *in vitro* cleavage by, calpain 1 than normal allele Pep-R619[93]. Conversely, Pep overexpression in T cells attenuates autoimmune diabetes in NOD mice by preferentially modulating TCR signaling-mediated functions in diabetogenic T cells but not in regulatory T cells[94]. Lyp-R620W is also involved in the breakdown of peripheral tolerance and in the entry of autoreactive B cells into the naive B cell compartment. Moreover, lymphocytes with a variant of Pep-R619W, corresponding to human Lyp-R620W, are hyper-responsive to antigen-receptor engagement. Thus, Pep-R619W uniquely modulates T and B cell homeostasis, leading to a loss in tolerance[95].

Elevated ROS would cause inactivation of Lyp/Pep by oxidizing catalytic Cys and may accelerate its degradation *via* the PEST domain. If ROS inactivates Lyp/Pep, the incidence of autoimmune response would be elevated. This oxidative stress-triggered SLE onset is only hypothetical at this moment and hence requires direct demonstration. The crystallographic analysis of Lyp shows a unique disulfide bond that may play a role in protecting the enzyme from irreversible oxidation[96], and hydrogen peroxide actually inactivates the Lyp phosphatase to a lesser extent compared with CD45 phosphatase[97]. Based on the literature and our own observations, we can propose a hypothetical model to explain SLE onset in (NZB x NZW)F1 mice (Figure 3). Because the F1 mice inherit a SLAM variant from NZW mice and high levels of ROS from NZB mice, which may oxidatively inactivate the Lyp/Pep, lymphocytes are hyper-activated, leading to SLE onset in aged mice. Low CD45 phosphotyrosine phosphatase activities that have been reported by two groups[98,99] may support our hypothesis.

Potential roles of oxidative stress in lupus nephritis

Lupus nephritis is a serious pathological condition of SLE. The incidence of SLE in women is nine times greater than in men[100], while the sex difference is not observed for the autoantibody production in SOD1-deficient mice[31]. Immune complex formation and complement activation are major causes, but other pathogenic factor is involved in lupus nephritis[101]. Despite deficiency of the gamma chain of the Fc receptor in F1 mice, ameliorated glomerulonephritis, immune complex deposition still occurs[102]. Thus, glomerular deposition of C1q as immune complexes, complement activation, and Fc gamma receptor activation together appear to be required for the renal damage[103]. As discussed above, oxidative stress is a potent risk factor for the autoantibody production by affecting immune system and hence would be involved in the kidney damage by increasing the immune complex deposition. However, since kidney is the organ considerably susceptible to oxidative damage[104], elevated ROS may directly affect the renal function and be an independent risk factor for lupus nephritis in SLE.

Perspectives

In addition to the results of studies on the supplementary administration of antioxidative compounds,

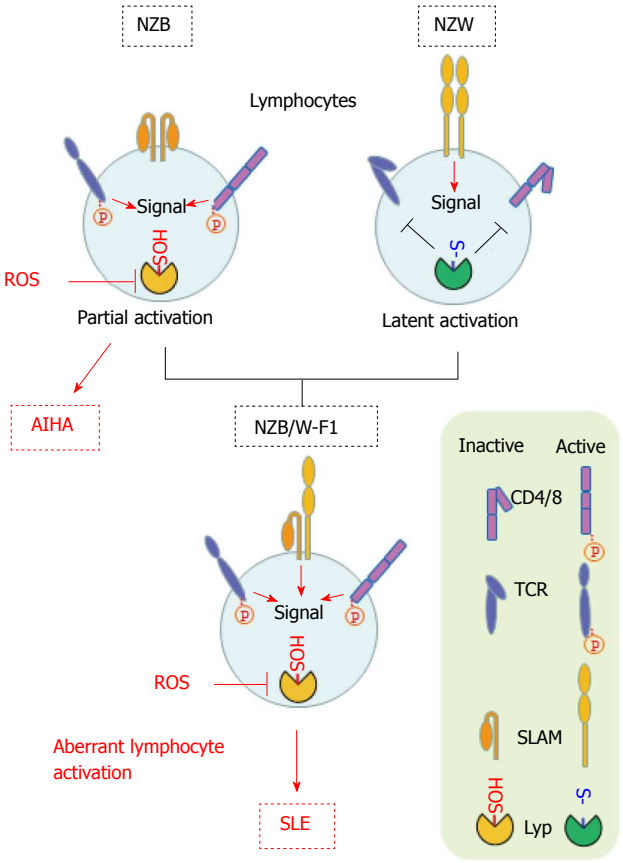

Figure 3 Hypothetical model for triggering systemic lupus erythematosus in (NZB x NZW) F1 mice. Lyp/pep suppressively regulates lymphocyte receptors such as CD4/8 and TCR. Production of ROS are originally high in the cells derived from NZB mice and oxidize the sulfhydryl group in the catalytic Cys to sulfenic acid (-SOH). So that Lyp/pep proteins in lymphocytes in the F1 mice are prone to oxidative inactivation. On the other hand, lymphocytes in NZW mice possess the variant form of SLAM that would be responsible for sustained activation of the lymphocytes. (NZB x NZW) F1 mice inherit this potentially pathogenic nature from parental strains. SLE: Systemic lupus erythematosus; ROS: Reactive oxygen species; NZB: New Zealand black; TCR: T-cell receptor; AIHA: Autoimmune hemolytic anemia.

observations from pathological models and genetically modified mice support the view that ROS are one of the underlying mechanisms for AIHA and/or SLE. ROS cause opposing responses; they trigger cell growth arrest and accelerate cellular senescence, but stimulate the cellular proliferation on the other hand[90,91]. In the latter case, transient elevation in ROS levels occurs when cells are stimulated by growth factor and is involved in sustaining the signal transduction. Antioxidant therapy appears to be effective, but may be potentially adverse because of a possible impairment of the ROS signaling during proliferation of hematopoietic cells. Elucidation of target molecules by oxidative modification and pathogenesis could lead to safer forms of preventive and therapeutic treatment.

REFERENCES

1 **Bauer C**, Kurtz A. Oxygen sensing in the kidney and its relation to erythropoietin production. *Annu Rev Physiol* 1989; **51**: 845-856 [PMID: 2653209 DOI: 10.1146/annurev.ph.51.030189.004213]

2 **Sato Y**, Yanagita M. Renal anemia: from incurable to curable. *Am J Physiol Renal Physiol* 2013; **305**: F1239-F1248 [PMID: 23884144 DOI: 10.1152/ajprenal.00233.2013]

3 **Canavesi E**, Alfieri C, Pelusi S, Valenti L. Hepcidin and HFE protein: Iron metabolism as a target for the anemia of chronic kidney disease. *World J Nephrol* 2012; **1**: 166-176 [PMID: 24175256 DOI: 10.5527/wjn.v1.i6.166]

4 **Beutler E**. Glucose-6-phosphate dehydrogenase deficiency: a historical perspective. *Blood* 2008; **111**: 16-24 [PMID: 18156501 DOI: 10.1182/blood-2007-04-077412]

5 **Izui S**. Autoimmune hemolytic anemia. *Curr Opin Immunol* 1994; **6**: 926-930 [PMID: 7710716 DOI: 10.1016/0952-7915(94)90015-9]

6 **Scatizzi JC**, Haraldsson MK, Pollard KM, Theofilopoulos AN, Kono DH. The Lbw2 locus promotes autoimmune hemolytic anemia. *J Immunol* 2012; **188**: 3307-3314 [PMID: 22371393 DOI: 10.4049/jimmunol.1103561]

7 **Gordon C**, Bradley H, Waring RH, Emery P. Abnormal sulphur oxidation in systemic lupus erythematosus. *Lancet* 1992; **339**: 25-26 [PMID: 1345954 DOI: 10.1016/0140-6736(92)90144-R]

8 **Dhaliwal G**, Cornett PA, Tierney LM. Hemolytic anemia. *Am Fam Physician* 2004; **69**: 2599-2606 [PMID: 15202694]

9 **Perl A**. Oxidative stress in the pathology and treatment of systemic lupus erythematosus. *Nat Rev Rheumatol* 2013; **9**: 674-686 [PMID: 24100461 DOI: 10.1038/nrrheum.2013.147]

10 **Halliwell B,** Gutteridge JMC. Free Radical Biology and Medicine. 4th ed. Oxford: Oxford University Press, 2007

11 **Winterbourn CC**. Oxidative reactions of hemoglobin. *Methods Enzymol* 1990; **186**: 265-272 [PMID: 2172706 DOI: 10.1016/0076-6879(90)86118-F]

12 **Johnson RM**, Goyette G, Ravindranath Y, Ho YS. Hemoglobin autoxidation and regulation of endogenous H2O2 levels in erythrocytes. *Free Radic Biol Med* 2005; **39**: 1407-1417 [PMID: 16274876 DOI: 10.1016/j.freeradbiomed.2005.07.002]

13 **Rhee SG**, Woo HA, Kil IS, Bae SH. Peroxiredoxin functions as a peroxidase and a regulator and sensor of local peroxides. *J Biol Chem* 2012; **287**: 4403-4410 [PMID: 22147704 DOI: 10.1074/jbc.R111.283432]

14 **Mason PJ**, Bautista JM, Gilsanz F. G6PD deficiency: the genotype-phenotype association. *Blood Rev* 2007; **21**: 267-283 [PMID: 17611006 DOI: 10.1016/j.blre.2007.05.002]

15 **Iuchi Y**, Okada F, Takamiya R, Kibe N, Tsunoda S, Nakajima O, Toyoda K, Nagae R, Suematsu M, Soga T, Uchida K, Fujii J. Rescue of anaemia and autoimmune responses in SOD1-deficient mice by transgenic expression of human SOD1 in erythrocytes. *Biochem J* 2009; **422**: 313-320 [PMID: 19515016 DOI: 10.1042/BJ20090176]

16 **Criswell KA**, Sulkanen AP, Hochbaum AF, Bleavins MR. Effects of phenylhydrazine or phlebotomy on peripheral blood, bone marrow and erythropoietin in Wistar rats. *J Appl Toxicol* 2000; **20**: 25-34 [PMID: 10641014 DOI: 10.1002/(SICI)1099-1263(200001/02)20:1<25: : AID-JAT624>3.0.CO; 2-7]

17 **Mqadmi A**, Zheng X, Yazdanbakhsh K. CD4+CD25+ regulatory T cells control induction of autoimmune hemolytic anemia. *Blood* 2005; **105**: 3746-3748 [PMID: 15637139 DOI: 10.1182/blood-2004-12-4692]

18 **Fagiolo E**, Toriani-Terenzi C. Mechanisms of immunological tolerance loss versus erythrocyte self-antigens and autoimmune hemolytic anemia. *Autoimmunity* 2003; **36**: 199-204 [PMID: 14563012 DOI: 10.1080/0891693031000151238]

19 **Murakami M**, Yoshioka H, Shirai T, Tsubata T, Honjo T. Prevention of autoimmune symptoms in autoimmune-prone mice by elimination of B-1 cells. *Int Immunol* 1995; **7**: 877-882 [PMID: 7547714 DOI: 10.1093/intimm/7.5.877]

20 **Fossati-Jimack L**, Azeredo da Silveira S, Moll T, Kina T, Kuypers FA, Oldenborg PA, Reininger L, Izui S. Selective increase of autoimmune epitope expression on aged erythrocytes in mice: implications in anti-erythrocyte autoimmune responses. *J Autoimmun* 2002; **18**: 17-25 [PMID: 11869043 DOI: 10.1006/jaut.2001.0563]

21 **Hall AM**, Ward FJ, Shen CR, Rowe C, Bowie L, Devine A, Urbaniak SJ, Elson CJ, Barker RN. Deletion of the dominant autoantigen in NZB mice with autoimmune hemolytic anemia: effects on

autoantibody and T-helper responses. *Blood* 2007; **110**: 4511-4517 [PMID: 17785581 DOI: 10.1182/blood-2007-06-094383]
22 **Santiago-Raber ML**, Laporte C, Reininger L, Izui S. Genetic basis of murine lupus. *Autoimmun Rev* 2004; **3**: 33-39 [PMID: 14871647 DOI: 10.1016/S1568-9972(03)00062-4]
23 **Nagata S**, Suda T. Fas and Fas ligand: lpr and gld mutations. *Immunol Today* 1995; **16**: 39-43 [PMID: 7533498 DOI: 10.1016/0167-5699(95)80069-7]
24 **Wu J**, Wilson J, He J, Xiang L, Schur PH, Mountz JD. Fas ligand mutation in a patient with systemic lupus erythematosus and lymphoproliferative disease. *J Clin Invest* 1996; **98**: 1107-1113 [PMID: 8787672 DOI: 10.1172/JCI118892]
25 **Vaishnaw AK**, Toubi E, Ohsako S, Drappa J, Buys S, Estrada J, Sitarz A, Zemel L, Chu JL, Elkon KB. The spectrum of apoptotic defects and clinical manifestations, including systemic lupus erythematosus, in humans with CD95 (Fas/APO-1) mutations. *Arthritis Rheum* 1999; **42**: 1833-1842 [PMID: 10513797 DOI: 10.1002/1529-0131(199909)42: 9<1833: : AID-ANR7>3.0.CO; 2-Q]
26 **Kojima T**, Horiuchi T, Nishizaka H, Sawabe T, Higuchi M, Harashima SI, Yoshizawa S, Tsukamoto H, Nagasawa K, Niho Y. Analysis of fas ligand gene mutation in patients with systemic lupus erythematosus. *Arthritis Rheum* 2000; **43**: 135-139 [PMID: 10643709 DOI: 10.1002/1529-0131(200001)43: 1<135: : AID-ANR17>3.0.CO; 2-Q]
27 **Dubois EL**, Horowitz RE, Demopoulos HB, Teplitz R. NZB/NZW mice as a model of systemic lupus erythematosus. *JAMA* 1966; **195**: 285-289 [PMID: 4159181 DOI: 10.1001/jama.1966.03100040091025]
28 **Morel L**, Rudofsky UH, Longmate JA, Schiffenbauer J, Wakeland EK. Polygenic control of susceptibility to murine systemic lupus erythematosus. *Immunity* 1994; **1**: 219-229 [PMID: 7889410 DOI: 10.1016/1074-7613(94)90100-7]
29 **Morel L**, Mohan C, Yu Y, Croker BP, Tian N, Deng A, Wakeland EK. Functional dissection of systemic lupus erythematosus using congenic mouse strains. *J Immunol* 1997; **158**: 6019-6028 [PMID: 9190957]
30 **Morel L**, Croker BP, Blenman KR, Mohan C, Huang G, Gilkeson G, Wakeland EK. Genetic reconstitution of systemic lupus erythematosus immunopathology with polycongenic murine strains. *Proc Natl Acad Sci USA* 2000; **97**: 6670-6675 [PMID: 10841565 DOI: 10.1073/pnas.97.12.6670]
31 **Iuchi Y**, Okada F, Onuma K, Onoda T, Asao H, Kobayashi M, Fujii J. Elevated oxidative stress in erythrocytes due to a SOD1 deficiency causes anaemia and triggers autoantibody production. *Biochem J* 2007; **402**: 219-227 [PMID: 17059387 DOI: 10.1042/BJ20061386]
32 **Martin FM**, Bydlon G, Friedman JS. SOD2-deficiency sideroblastic anemia and red blood cell oxidative stress. *Antioxid Redox Signal* 2006; **8**: 1217-1225 [PMID: 16910769 DOI: 10.1089/ars.2006.8.1217]
33 **Neumann CA**, Krause DS, Carman CV, Das S, Dubey DP, Abraham JL, Bronson RT, Fujiwara Y, Orkin SH, Van Etten RA. Essential role for the peroxiredoxin Prdx1 in erythrocyte antioxidant defence and tumour suppression. *Nature* 2003; **424**: 561-565 [PMID: 12891360 DOI: 10.1038/nature01819]
34 **Lee TH**, Kim SU, Yu SL, Kim SH, Park DS, Moon HB, Dho SH, Kwon KS, Kwon HJ, Han YH, Jeong S, Kang SW, Shin HS, Lee KK, Rhee SG, Yu DY. Peroxiredoxin II is essential for sustaining life span of erythrocytes in mice. *Blood* 2003; **101**: 5033-5038 [PMID: 12586629 DOI: 10.1182/blood-2002-08-2548]
35 **Johnson RM**, Goyette G, Ravindranath Y, Ho YS. Red cells from glutathione peroxidase-1-deficient mice have nearly normal defenses against exogenous peroxides. *Blood* 2000; **96**: 1985-1988 [PMID: 10961904]
36 **Ho YS**, Xiong Y, Ma W, Spector A, Ho DS. Mice lacking catalase develop normally but show differential sensitivity to oxidant tissue injury. *J Biol Chem* 2004; **279**: 32804-32812 [PMID: 15178682]
37 **Starzyński RR**, Canonne-Hergaux F, Willemetz A, Gralak MA, Woliński J, Styś A, Olszak J, Lipiński P. Haemolytic anaemia and alterations in hepatic iron metabolism in aged mice lacking Cu,Zn-superoxide dismutase. *Biochem J* 2009; **420**: 383-390 [PMID: 19296829 DOI: 10.1042/BJ20082137]
38 **Iuchi Y**, Kibe N, Tsunoda S, Suzuki S, Mikami T, Okada F, Uchida K, Fujii J. Implication of oxidative stress as a cause of autoimmune hemolytic anemia in NZB mice. *Free Radic Biol Med* 2010; **48**: 935-944 [PMID: 20079426 DOI: 10.1016/j.freeradbiomed.2010.01.012]
39 **Cho CS**, Lee S, Lee GT, Woo HA, Choi EJ, Rhee SG. Irreversible inactivation of glutathione peroxidase 1 and reversible inactivation of peroxiredoxin II by H2O2 in red blood cells. *Antioxid Redox Signal* 2010; **12**: 1235-1246 [PMID: 20070187 DOI: 10.1089/ars.2009.2701]
40 **Lu J**, Holmgren A. Selenoproteins. *J Biol Chem* 2009; **284**: 723-727 [PMID: 18757362 DOI: 10.1074/jbc.R800045200]
41 **Li Y**, Huang TT, Carlson EJ, Melov S, Ursell PC, Olson JL, Noble LJ, Yoshimura MP, Berger C, Chan PH, Wallace DC, Epstein CJ. Dilated cardiomyopathy and neonatal lethality in mutant mice lacking manganese superoxide dismutase. *Nat Genet* 1995; **11**: 376-381 [PMID: 7493016]
42 **Friedman JS**, Rebel VI, Derby R, Bell K, Huang TT, Kuypers FA, Epstein CJ, Burakoff SJ. Absence of mitochondrial superoxide dismutase results in a murine hemolytic anemia responsive to therapy with a catalytic antioxidant. *J Exp Med* 2001; **193**: 925-934 [PMID: 11304553]
43 **Mohanty JG**, Nagababu E, Friedman JS, Rifkind JM. SOD2 deficiency in hematopoietic cells in mice results in reduced red blood cell deformability and increased heme degradation. *Exp Hematol* 2013; **41**: 316-321 [PMID: 23142655 DOI: 10.1016/j.exphem.2012.10.017]
44 **Martin FM**, Xu X, von Löhneysen K, Gilmartin TJ, Friedman JS. SOD2 deficient erythroid cells up-regulate transferrin receptor and down-regulate mitochondrial biogenesis and metabolism. *PLoS One* 2011; **6**: e16894 [PMID: 21326867 DOI: 10.1371/journal.pone.0016894]
45 **Friedman JS**, Lopez MF, Fleming MD, Rivera A, Martin FM, Welsh ML, Boyd A, Doctrow SR, Burakoff SJ. SOD2-deficiency anemia: protein oxidation and altered protein expression reveal targets of damage, stress response, and antioxidant responsiveness. *Blood* 2004; **104**: 2565-2573 [PMID: 15205258]
46 **Johnson RM**, Ho YS, Yu DY, Kuypers FA, Ravindranath Y, Goyette GW. The effects of disruption of genes for peroxiredoxin-2, glutathione peroxidase-1, and catalase on erythrocyte oxidative metabolism. *Free Radic Biol Med* 2010; **48**: 519-525 [PMID: 19969073 DOI: 10.1016/j.freeradbiomed.2009.11.021]
47 **Cox AG**, Winterbourn CC, Hampton MB. Measuring the redox state of cellular peroxiredoxins by immunoblotting. *Methods Enzymol* 2010; **474**: 51-66 [PMID: 20609904 DOI: 10.1016/S0076-6879(10)74004-0]
48 **Low FM**, Hampton MB, Peskin AV, Winterbourn CC. Peroxiredoxin 2 functions as a noncatalytic scavenger of low-level hydrogen peroxide in the erythrocyte. *Blood* 2007; **109**: 2611-2617 [PMID: 17105810]
49 **Low FM**, Hampton MB, Winterbourn CC. Peroxiredoxin 2 and peroxide metabolism in the erythrocyte. *Antioxid Redox Signal* 2008; **10**: 1621-1630 [PMID: 18479207 DOI: 10.1089/ars.2008.2081]
50 **Biteau B**, Labarre J, Toledano MB. ATP-dependent reduction of cysteine-sulphinic acid by S. cerevisiae sulphiredoxin. *Nature* 2003; **425**: 980-984 [PMID: 14586471]
51 **Woo HA**, Jeong W, Chang TS, Park KJ, Park SJ, Yang JS, Rhee SG. Reduction of cysteine sulfinic acid by sulfiredoxin is specific to 2-cys peroxiredoxins. *J Biol Chem* 2005; **280**: 3125-3128 [PMID: 15590625 DOI: 10.1074/jbc.C400496200]
52 **O'Neill JS**, Reddy AB. Circadian clocks in human red blood cells. *Nature* 2011; **469**: 498-503 [PMID: 21270888 DOI: 10.1038/nature09702]
53 **Han YH**, Kim SU, Kwon TH, Lee DS, Ha HL, Park DS, Woo EJ, Lee SH, Kim JM, Chae HB, Lee SY, Kim BY, Yoon do Y, Rhee SG, Fibach E, Yu DY. Peroxiredoxin II is essential for preventing hemolytic anemia from oxidative stress through maintaining hemoglobin stability. *Biochem Biophys Res Commun* 2012; **426**: 427-432 [PMID: 22960070 DOI: 10.1016/j.bbrc.2012.08.113]
54 **Helyer BJ**, Howie JB. Spontaneous auto-immune disease in NZB/

BL mice. *Br J Haematol* 1963; **9**: 119-131 [PMID: 13953665 DOI: 10.1111/j.1365-2141.1963.tb05450.x]
55 **Theofilopoulos AN**, Dixon FJ. Murine models of systemic lupus erythematosus. *Adv Immunol* 1985; **37**: 269-390 [PMID: 3890479 DOI: 10.1016/S0065-2776(08)60342-9]
56 **Beppu M**, Mizukami A, Nagoya M, Kikugawa K. Binding of anti-band 3 autoantibody to oxidatively damaged erythrocytes. Formation of senescent antigen on erythrocyte surface by an oxidative mechanism. *J Biol Chem* 1990; **265**: 3226-3233 [PMID: 2303447]
57 **Poole J**. Red cell antigens on band 3 and glycophorin A. *Blood Rev* 2000; **14**: 31-43 [PMID: 10805259 DOI: 10.1054/blre.1999.0124]
58 **Shen CR**, Youssef AR, Devine A, Bowie L, Hall AM, Wraith DC, Elson CJ, Barker RN. Peptides containing a dominant T-cell epitope from red cell band 3 have in vivo immunomodulatory properties in NZB mice with autoimmune hemolytic anemia. *Blood* 2003; **102**: 3800-3806 [PMID: 12829598 DOI: 10.1182/blood-2002-07-2125]
59 **Konno T**, Otsuki N, Kurahashi T, Kibe N, Tsunoda S, Iuchi Y, Fujii J. Reactive oxygen species exacerbate autoimmune hemolytic anemia in New Zealand Black mice. *Free Radic Biol Med* 2013; **65**: 1378-1384 [PMID: 24095725 DOI: 10.1016/j.freeradbiomed.2013.09.021]
60 **Guo Z**, Higuchi K, Mori M. Spontaneous hypomorphic mutations in antioxidant enzymes of mice. *Free Radic Biol Med* 2003; **35**: 1645-1652 [PMID: 14680687 DOI: 10.1016/j.freeradbiomed.2003.09.017]
61 **Lynch RE**, Fridovich I. Permeation of the erythrocyte stroma by superoxide radical. *J Biol Chem* 1978; **253**: 4697-4699 [PMID: 207707]
62 **Scofield RH**, Kurien BT, Ganick S, McClain MT, Pye Q, James JA, Schneider RI, Broyles RH, Bachmann M, Hensley K. Modification of lupus-associated 60-kDa Ro protein with the lipid oxidation product 4-hydroxy-2-nonenal increases antigenicity and facilitates epitope spreading. *Free Radic Biol Med* 2005; **38**: 719-728 [PMID: 15721982 DOI: 10.1016/j.freeradbiomed.2004.11.001]
63 **Kurien BT**, Porter A, Dorri Y, Iqbal S, D'Souza A, Singh A, Asfa S, Cartellieri M, Mathias K, Matsumoto H, Bachmann M, Hensley K, Scofield RH. Degree of modification of Ro60 by the lipid peroxidation by-product 4-hydroxy-2-nonenal may differentially induce Sjögren syndrome or systemic lupus erythematosus in BALB/c mice. *Free Radic Biol Med* 2011; **50**: 1222-1233 [PMID: 20946951 DOI: 10.1016/j.freeradbiomed.2010.10.687]
64 **Grune T**, Michel P, Sitte N, Eggert W, Albrecht-Nebe H, Esterbauer H, Siems WG. Increased levels of 4-hydroxynonenal modified proteins in plasma of children with autoimmune diseases. *Free Radic Biol Med* 1997; **23**: 357-360 [PMID: 9214570 DOI: 10.1016/S0891-5849(96)00586-2]
65 **Gil L**, Siems W, Mazurek B, Gross J, Schroeder P, Voss P, Grune T. Age-associated analysis of oxidative stress parameters in human plasma and erythrocytes. *Free Radic Res* 2006; **40**: 495-505 [PMID: 16551576 DOI: 10.1080/10715760600592962]
66 **Doherty E**, Oaks Z, Perl A. Increased mitochondrial electron transport chain activity at complex I is regulated by N-acetylcysteine in lymphocytes of patients with systemic lupus erythematosus. *Antioxid Redox Signal* 2014; **21**: 56-65 [PMID: 24673154 DOI: 10.1089/ars.2013.5702]
67 **Kinoshita K**, Yoo BS, Nozaki Y, Sugiyama M, Ikoma S, Ohno M, Funauchi M, Kanamaru A. Retinoic acid reduces autoimmune renal injury and increases survival in NZB/W F1 mice. *J Immunol* 2003; **170**: 5793-5798 [PMID: 12759464 DOI: 10.4049/jimmunol.170.11.5793]
68 **Halade GV**, Rahman MM, Bhattacharya A, Barnes JL, Chandrasekar B, Fernandes G. Docosahexaenoic acid-enriched fish oil attenuates kidney disease and prolongs median and maximal life span of autoimmune lupus-prone mice. *J Immunol* 2010; **184**: 5280-5286 [PMID: 20368275 DOI: 10.4049/jimmunol.0903282]
69 **Hsu TC**, Chiu CC, Wang YW, Tzang BS. Effects of cystamine on antioxidant activities and regulatory T cells in lupus-prone mice. *J Cell Mol Med* 2013; **17**: 1308-1315 [PMID: 23905628 DOI: 10.1111/jcmm.12107]
70 **Greenberg ME**, Sun M, Zhang R, Febbraio M, Silverstein R, Hazen SL. Oxidized phosphatidylserine-CD36 interactions play an essential role in macrophage-dependent phagocytosis of apoptotic cells. *J Exp Med* 2006; **203**: 2613-2625 [PMID: 17101731 DOI: 10.1084/jem.20060370]
71 **López-Revuelta A**, Sánchez-Gallego JI, García-Montero AC, Hernández-Hernández A, Sánchez-Yagüe J, Llanillo M. Membrane cholesterol in the regulation of aminophospholipid asymmetry and phagocytosis in oxidized erythrocytes. *Free Radic Biol Med* 2007; **42**: 1106-1118 [PMID: 17349937 DOI: 10.1016/j.freeradbiomed.2007.01.010]
72 **Rasheed Z**, Ahmad R, Rasheed N, Ali R. Enhanced recognition of reactive oxygen species damaged human serum albumin by circulating systemic lupus erythematosus autoantibodies. *Autoimmunity* 2007; **40**: 512-520 [PMID: 17966041 DOI: 10.1080/08916930701574331]
73 **Binder CJ**, Shaw PX, Chang MK, Boullier A, Hartvigsen K, Hörkkö S, Miller YI, Woelkers DA, Corr M, Witztum JL. The role of natural antibodies in atherogenesis. *J Lipid Res* 2005; **46**: 1353-1363 [PMID: 15897601 DOI: 10.1194/jlr.R500005-JLR200]
74 **Chou MY**, Hartvigsen K, Hansen LF, Fogelstrand L, Shaw PX, Boullier A, Binder CJ, Witztum JL. Oxidation-specific epitopes are important targets of innate immunity. *J Intern Med* 2008; **263**: 479-488 [PMID: 18410591 DOI: 10.1111/j.1365-2796.2008.01968.x]
75 **Hazen SL**. Oxidized phospholipids as endogenous pattern recognition ligands in innate immunity. *J Biol Chem* 2008; **283**: 15527-15531 [PMID: 18285328 DOI: 10.1074/jbc.R700054200]
76 **Toyoda K**, Nagae R, Akagawa M, Ishino K, Shibata T, Ito S, Shibata N, Yamamoto T, Kobayashi M, Takasaki Y, Matsuda T, Uchida K. Protein-bound 4-hydroxy-2-nonenal: an endogenous triggering antigen of antI-DNA response. *J Biol Chem* 2007; **282**: 25769-25778 [PMID: 17588942 DOI: 10.1074/jbc.M703039200]
77 **Otaki N**, Chikazawa M, Nagae R, Shimozu Y, Shibata T, Ito S, Takasaki Y, Fujii J, Uchida K. Identification of a lipid peroxidation product as the source of oxidation-specific epitopes recognized by anti-DNA autoantibodies. *J Biol Chem* 2010; **285**: 33834-33842 [PMID: 20736172 DOI: 10.1074/jbc.M110.165175]
78 **Knight JG**, Adams DD. Three genes for lupus nephritis in NZB x NZW mice. *J Exp Med* 1978; **147**: 1653-1660 [PMID: 681876 DOI: 10.1084/jem.147.6.1653]
79 **Kono DH**, Theofilopoulos AN. Genetics of SLE in mice. *Springer Semin Immunopathol* 2006; **28**: 83-96 [PMID: 16972052 DOI: 10.1007/s00281-006-0030-7]
80 **Mohan C**, Alas E, Morel L, Yang P, Wakeland EK. Genetic dissection of SLE pathogenesis. Sle1 on murine chromosome 1 leads to a selective loss of tolerance to H2A/H2B/DNA subnucleosomes. *J Clin Invest* 1998; **101**: 1362-1372 [PMID: 9502778]
81 **Sobel ES**, Satoh M, Chen Y, Wakeland EK, Morel L. The major murine systemic lupus erythematosus susceptibility locus Sle1 results in abnormal functions of both B and T cells. *J Immunol* 2002; **169**: 2694-2700 [PMID: 12193743 DOI: 10.4049/jimmunol.169.5.2694]
82 **Wandstrat AE**, Nguyen C, Limaye N, Chan AY, Subramanian S, Tian XH, Yim YS, Pertsemlidis A, Garner HR, Morel L, Wakeland EK. Association of extensive polymorphisms in the SLAM/CD2 gene cluster with murine lupus. *Immunity* 2004; **21**: 769-780 [PMID: 15589166 DOI: 10.1016/j.immuni.2004.10.009]
83 **Wang A**, Batteux F, Wakeland EK. The role of SLAM/CD2 polymorphisms in systemic autoimmunity. *Curr Opin Immunol* 2010; **22**: 706-714 [PMID: 21094032 DOI: 10.1016/j.coi.2010.10.014]
84 **Delgado-Vega A**, Sánchez E, Löfgren S, Castillejo-López C, Alarcón-Riquelme ME. Recent findings on genetics of systemic autoimmune diseases. *Curr Opin Immunol* 2010; **22**: 698-705 [PMID: 20933377 DOI: 10.1016/j.coi.2010.09.002]
85 **Cloutier JF**, Veillette A. Cooperative inhibition of T-cell antigen receptor signaling by a complex between a kinase and a phosphatase. *J Exp Med* 1999; **189**: 111-121 [PMID: 9874568 DOI: 10.1084/jem.189.1.111]
86 **Cohen S**, Dadi H, Shaoul E, Sharfe N, Roifman CM. Cloning and characterization of a lymphoid-specific, inducible human protein tyrosine phosphatase, Lyp. *Blood* 1999; **93**: 2013-2024 [PMID:

10068674]
87 **Veillette A**, Rhee I, Souza CM, Davidson D. PEST family phosphatases in immunity, autoimmunity, and autoinflammatory disorders. *Immunol Rev* 2009; **228**: 312-324 [PMID: 19290936 DOI: 10.1111/j.1600-065X.2008.00747.x]
88 **Gianchecchi E**, Palombi M, Fierabracci A. The putative role of the C1858T polymorphism of protein tyrosine phosphatase PTPN22 gene in autoimmunity. *Autoimmun Rev* 2013; **12**: 717-725 [PMID: 23261816 DOI: 10.1016/j.autrev.2012.12.003]
89 **Zheng J**, Petersen F, Yu X. The role of PTPN22 in autoimmunity: learning from mice. *Autoimmun Rev* 2014; **13**: 266-271 [PMID: 24189282 DOI: 10.1016/j.autrev.2013.10.011]
90 **Savitsky PA**, Finkel T. Redox regulation of Cdc25C. *J Biol Chem* 2002; **277**: 20535-20540 [PMID: 11925443 DOI: 10.1074/jbc.M201589200]
91 **Weibrecht I**, Böhmer SA, Dagnell M, Kappert K, Ostman A, Böhmer FD. Oxidation sensitivity of the catalytic cysteine of the protein-tyrosine phosphatases SHP-1 and SHP-2. *Free Radic Biol Med* 2007; **43**: 100-110 [PMID: 17561098 DOI: 10.1016/j.freeradbiomed.2007.03.021]
92 **Hendriks WJ**, Pulido R. Protein tyrosine phosphatase variants in human hereditary disorders and disease susceptibilities. *Biochim Biophys Acta* 2013; **1832**: 1673-1696 [PMID: 23707412 DOI: 10.1016/j.bbadis.2013.05.022]
93 **Zhang J**, Zahir N, Jiang Q, Miliotis H, Heyraud S, Meng X, Dong B, Xie G, Qiu F, Hao Z, McCulloch CA, Keystone EC, Peterson AC, Siminovitch KA. The autoimmune disease-associated PTPN22 variant promotes calpain-mediated Lyp/Pep degradation associated with lymphocyte and dendritic cell hyperresponsiveness. *Nat Genet* 2011; **43**: 902-907 [PMID: 21841778 DOI: 10.1038/ng.904]
94 **Yeh LT**, Miaw SC, Lin MH, Chou FC, Shieh SJ, Chuang YP, Lin SH, Chang DM, Sytwu HK. Different modulation of Ptpn22 in effector and regulatory T cells leads to attenuation of autoimmune diabetes in transgenic nonobese diabetic mice. *J Immunol* 2013; **191**: 594-607 [PMID: 23752610 DOI: 10.4049/jimmunol.1203380]
95 **Dai X**, James RG, Habib T, Singh S, Jackson S, Khim S, Moon RT, Liggitt D, Wolf-Yadlin A, Buckner JH, Rawlings DJ. A disease-associated PTPN22 variant promotes systemic autoimmunity in murine models. *J Clin Invest* 2013; **123**: 2024-2036 [PMID: 23619366 DOI: 10.1172/JCI66963]
96 **Tsai SJ**, Sen U, Zhao L, Greenleaf WB, Dasgupta J, Fiorillo E, Orrú V, Bottini N, Chen XS. Crystal structure of the human lymphoid tyrosine phosphatase catalytic domain: insights into redox regulation. *Biochemistry* 2009; **48**: 4838-4845 [PMID: 19371084 DOI: 10.1021/bi900166y]
97 **Bayley R**, Yang P, Buckley CD, Young SP. Measuring the specific activity of the protein tyrosine phosphatase Lyp. *J Immunol Methods* 2013; **388**: 33-39 [PMID: 23219421 DOI: 10.1016/j.jim.2012.11.011]
98 **Takeuchi T**, Pang M, Amano K, Koide J, Abe T. Reduced protein tyrosine phosphatase (PTPase) activity of CD45 on peripheral blood lymphocytes in patients with systemic lupus erythematosus (SLE). *Clin Exp Immunol* 1997; **109**: 20-26 [PMID: 9218819 DOI: 10.1046/j.1365-2249.1997.4371334.x]
99 **Blasini AM**, Alonzo E, Chacón R, Riera R, Stekman IL, Rodriguez MA. Abnormal pattern of tyrosine phosphorylation in unstimulated peripheral blood T lymphocytes from patients with systemic lupus erythematosus. *Lupus* 1998; **7**: 515-523 [PMID: 9863892 DOI: 10.1191/096120398678920604]
100 **Whitacre CC**. Sex differences in autoimmune disease. *Nat Immunol* 2001; **2**: 777-780 [PMID: 11526384 DOI: 10.1038/ni0901-777]
101 **Sterner RM,** Hartono SP, Grande JP. The Pathogenesis of Lupus Nephritis. *J Clin Cell Immunol* 2014; **5**: 205 [PMID: 25133068]
102 **Clynes R**, Dumitru C, Ravetch JV. Uncoupling of immune complex formation and kidney damage in autoimmune glomerulonephritis. *Science* 1998; **279**: 1052-1054 [PMID: 9461440 DOI: 10.1126/science.279.5353.1052]
103 **Trouw LA,** Groeneveld TW, Seelen MA, Duijs JM, Bajema IM, Prins FA, Kishore U, Salant DJ, Verbeek JS, van Kooten C, Daha MR. Anti-C1q autoantibodiesdeposit in glomeruli but are only pathogenic in combination with glomerular C1q-containing immune complexes. *J Clin Invest* 2004; **114**: 679-688 [PMID: 15343386 DOI: 10.1172/JCI200421075]
104 **Kooman JP**, Kotanko P, Schols AM, Shiels PG, Stenvinkel P. Chronic kidney disease and premature ageing. *Nat Rev Nephrol* 2014; **10**: 732-742 [PMID: 25287433 DOI: 10.1038/nrneph.2014.185]

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Impact of obesity on kidney function and blood pressure in children

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Abstract

In recent years, obesity has become an increasingly important epidemic health problem in children and adolescents. The prevalence of the overweight status in children grew from 5% to 11% from 1960s to 1990s. The epidemic of obesity has been paralleled by an increase in the incidence of chronic kidney disease (CKD) and hypertension. Results of several studies have demonstrated that obesity and metabolic syndrome were independent predictors of renal injury. The pathophysiology of obesity related hypertension is complex, including activation of sympathetic nervous system, renin angiotensin aldosterone system, hyperinsulinemia and inflammation. These same mechanisms likely contribute to the development of increased blood pressure in children. This review summarizes the recent epidemiologic data linking obesity with CKD and hypertension in children, as well as the potential mechanisms.

Key words: Obesity; Chronic kidney disease; End-stage renal failure; Hypertension; Blood pressure

Core tip: Excess weight gain appears to be a major risk factor for chronic kidney disease and hypertension. The potential mechanisms involve insulin resistance, inflammation, renal renin-angiotensin-aldosterone hyperactivity, and sympathetic nervous system hyperactivity. Increased awareness is needed in children for early diagnosis and implementation of prevention and treatment measures.

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INTRODUCTION

Throughout the world, the increasing rate of childhood obesity has been steadily on the rise over the past

decades. In the first decade of this century, up to 28% of school and 12% of preschool children were determined to be overweight or obese in developed countries, and the international obesity task force addressed childhood obesity as a global "public health crisis"[1]. The impact of obesity on metabolic disease has been well demonstrated, and recently there is increasing evidence that obesity appears to be an independent risk factor for chronic kidney disease (CKD). Baseline body mass index (BMI) has been suggested as an independent predictor of CKD progression[2]. Obesity is strongly associated with the two most common causes of end-stage renal disease (ESRD), namely hypertension and diabetes. In addition, the metabolic syndrome, a major consequence of obesity, also seems to be an independent risk factor for ESRD[3]. Recent evidence also supports the hypothesis that reduced insulin sensitivity and hyperinsulinemia are among the most important factors leading to renal injury[4]. In concert with the increasing prevalence of obesity in children, hypertension has also made an epidemiological shift. Hypertension is a common feature present in a large proportion of obese and overweight individuals. It is correlated with the degree of obesity and significantly increased the risk of coronary artery, stroke and peripheral artery diseases. Moreover, the burden of hypertension attributable to obesity is very high[5]. This review focuses on the impact of obesity on the kidney and blood pressure in children as well as the mechanisms linking obesity to CKD and hypertension.

IMPACT OF OBESITY ON THE KIDNEY FUNCTION

Epidemiology of obesity

The BMI has been used to define obesity based on associated health risk factors in adult individuals. The National Institute of Health (NIH) in the United States determined an adult with a BMI of < 18.5 as underweight, 18.5-24.9 as normal, 25-29.9 as overweight and > 30 as obese. However, the criteria used to define children who are overweight or obese has varied. Most studies concerning childhood obesity or overweight in the United States are based on the Centers for Disease Control and Prevention (CDC) growth charts in 2000. The CDC defined children with > 85th percentile BMI to be overweight and BMI > 95th percentile to be obese[6]. The National Health and Nutrition Examination Survey (NHANES) data demonstrated almost doubling in prevalence of children with BMI > 85th percentile from 1999 to 2004. Recently, the NHANES showed stable prevalence of high BMI in children < 19 years old, with 10% of infants and toddlers < 2 years old with a weight-for-height $\geqslant$ 95th percentile, 17% of children aged 2-19 years old $\geqslant$ 95th percentile, and 32% $\geqslant$ 85th percentile of BMI for age[7,8].

Obesity and the risk factors for CKD

Childhood obesity is fast becoming a worldwide epidemic, and the state of being overweight/obese continues to persist into adolescence. Clustering of cardiovascular risk factors has been shown in obese children with the highest degree of insulin resistance, and these children are likely to develop obesity related kidney damage. In fact, there is a rapidly increasing prevalence of overweight and obese patients with CKD[9]. Reports in a Californian cohort of 330252 persons suggested a strong dose-response relationship between the baseline BMI and the risk of CKD. According to recent studies, obesity also appears to be an independent risk factor for CKD in children. Pediatric nephrology patients had consistently markedly higher BMI z-scores than the normal population at a tertiary center in Canada over a period of two decades. In another study of children with renal transplants, kidneys obtained from obese donors (BMI > 30 kg/m^2) had a lower glomerular filtration rate (GFR) and higher allograft dysfunction rate than kidneys obtained from lean individuals (BMI < 25 kg/m^2)[10-13]. Furthermore, Pantoja Zuzuárregui *et al*[14] demonstrated that obese children have larger kidneys than those of normal weight patients.

Role of obesity in CKD initiation

This question still remains whether obesity and obesity-related metabolic syndrome could directly induce renal injury. Though the theory needs to be confirmed by cause-and effect studies, more and more epidemiologic studies and clinical observations suggested that the obesity metabolic syndrome played a key role in the development of CKD[15]. Recently, several researches indicated that CKD is temporarily related to obesity independently of hypertension. Bonnet *et al*[16] demonstrated that excessive body weight was considered to be a new independent risk factor for clinical and pathological progression in IgA nephritis. Obesity was also shown to independently affect the process of CKD, for instance in patients with unilateral renal agenesis[17] or after unilateral nephrectomy[18]. In addition, kidneys that were obtained from obese individuals (BMI > 30 kg/m^2) were more likely to correlate with a lower GFR and a higher rate of renal allograft dysfunction than kidneys that were obtained from lean donors[12]. These results indicated that obesity could contribute to or even initiate the development of CKD. Kincaid-Smith challenged the long-held notion that hypertension accounts for > 30% of cases of ESRD in the United States and suggested that insulin resistance may be the real culprits in the development of glomerulosclerosis[19]. This notion is supported by the fact that there are no pathologic studies or large clinical studies to provide strong evidence of a relation between

hypertension and ESRD[18].

Obesity related glomerulopathy

Obesity is associated with glomerular hyperfiltration and hypertension. Obesity related glomerulopathy (ORG) is clinically characterized by moderate proteinuria, minimal edema, lower serum cholesterol and higher serum albumin[20]. ORG has been described as a secondary form of focal segmental glomerulosclerosis (FSGS) occurring in obese patients. The first research between obesity and renal injury was reported in 1974[21]. One year later, Cohen also described the presence of significant glomerular enlargement, variable widening of mesangial regions and mild hypercellularity in obese patients, and these features also were found even in children as young as 3 years old[22]. Obese children have larger kidneys and increased renal blood flow than normal weight individuals of similar age. Recently, some reports of improvement in ORG with reduction in body weight were demonstrated. In a recent clinical report, a 17-year-old girl with ORG and nephrotic-range proteinuria, one year after bariatric surgery, her renal function was normal and had no proteinuria[23]. However, the improvement in proteinuria might not correlate with histological change. The pathology of ORG may be biased by the fact that most of the kidney samples were obtained in patients with proteinuria. It suggested that ORG could not be the histopathological feature in nonproteinuric obese individuals with renal dysfunction.

Metabolic syndrome, inflammation and renal injury

The metabolic syndrome or insulin resistance syndrome represents a clustering of CKD risk factors. According to Bogalusa Heart study, metabolic syndrome was characterized as having four of the aforementioned components at or above the 75th percentile for age and gender in children[24]. The primary cause of the metabolic syndrome seems to be obesity. In the NHANES Ⅲ study, the prevalence of metabolic syndrome was 28.7% in overweight adolescents, compared with 0.1% in those with normal BMI and 6.1% in adolescents at risk of being overweight[25]. Up to 90% of overweight individuals had at least one component of the syndrome, and about 56% had two components of the syndrome. There is a plausible association between metabolic syndrome and obesity. One of the important features of metabolic syndrome is insulin resistance. Insulin resistance may lead to a proinflammatory state in obese children. Plasma concentrations of some inflammatory mediators such as tumor necrosis factor (TNF-α), C-reactive protein (CRP) and interleukin (IL)-6 were increased in patients with metabolic syndrome[26]. These results suggest that inflammation is a key risk factor for obesity and inflammation has been strongly associated with the metabolic syndrome. Recent evidence shows that inflammation is linked to obesity in CKD patients. Beddhu *et al*[27] found that in the NHANES III cohort, the metabolic syndrome was associated with greater odds for inflammation at various levels of creatinine clearance. Wu *et al*[28] showed that lipid metabolism related genes and inflammatory cytokines were increased in glomeruli of patients with ORG compared with gender and age matched glomeruli of control kidney samples. Ramkumar *et al*[29] also demonstrated a strong relationship between high BMI and inflammation characterized by a CRP level > 3 mg/dL in patients with CKD. These findings strengthen the notion that inflammatory risk factors and lipid byproducts play a key role in the progress of renal dysfunction in obese patients. Strong evidence shows that obesity, in particular central body fat distribution, has been implicated in kidney dysfunction. In fact, obesity and overweight are associated with many other risk factors, *i.e.,* hyperinsulinemia, hypertension, impaired glucose metabolism and hyperlipidemia, renin-angiotensin-aldosterone (RAAS) activity, oxidative stress and proinflammatory cytokines. Above all, reduced insulin sensitivity presents the most important relationship between obesity and other metabolic complications (Figure 1), which leads to CKD[30,31].

IMPACT OF OBESITY ON BLOOD PRESSURE

Epidemiology

Hypertension is a common feature in a large proportion of obese and overweight individuals. It is correlated with the degree of obesity and significantly exaggerated the risk of stroke, coronary and kidney disease. The association between obesity and hypertension in children has been reported in many studies. Rosner *et al*[32] collected data from 8 US epidemiological studies including over 47000 children and the results demonstrate that blood pressures differ between white and black children in relation to their body size. They found the risk of increased blood pressure was markedly higher in the upper compared with the lower decile of BMI irrespective of race, age and gender. Freedman *et al*[33] showed that overweight children were 4.5 and 2.4 times as likely to have increased systolic and diastolic blood pressure, respectively, than normal children. Sorof *et al*[34] recently demonstrated that there was a 3 times prevalence of hypertension in obese compared with non-obese adolescents in a school based hypertension and obesity screening study.

Obesity as a major cause of hypertension

More recent evidence shows that excess weight gain is one of the best predictors of the development of obesity. In addition, blood pressure is closely correlated with BMI and other biochemical and anthropometric indices of obesity, such as serum insulin, leptin and waist to hip ratio[5,35]. The strong relationship between obesity and hypertension cannot be attributed to

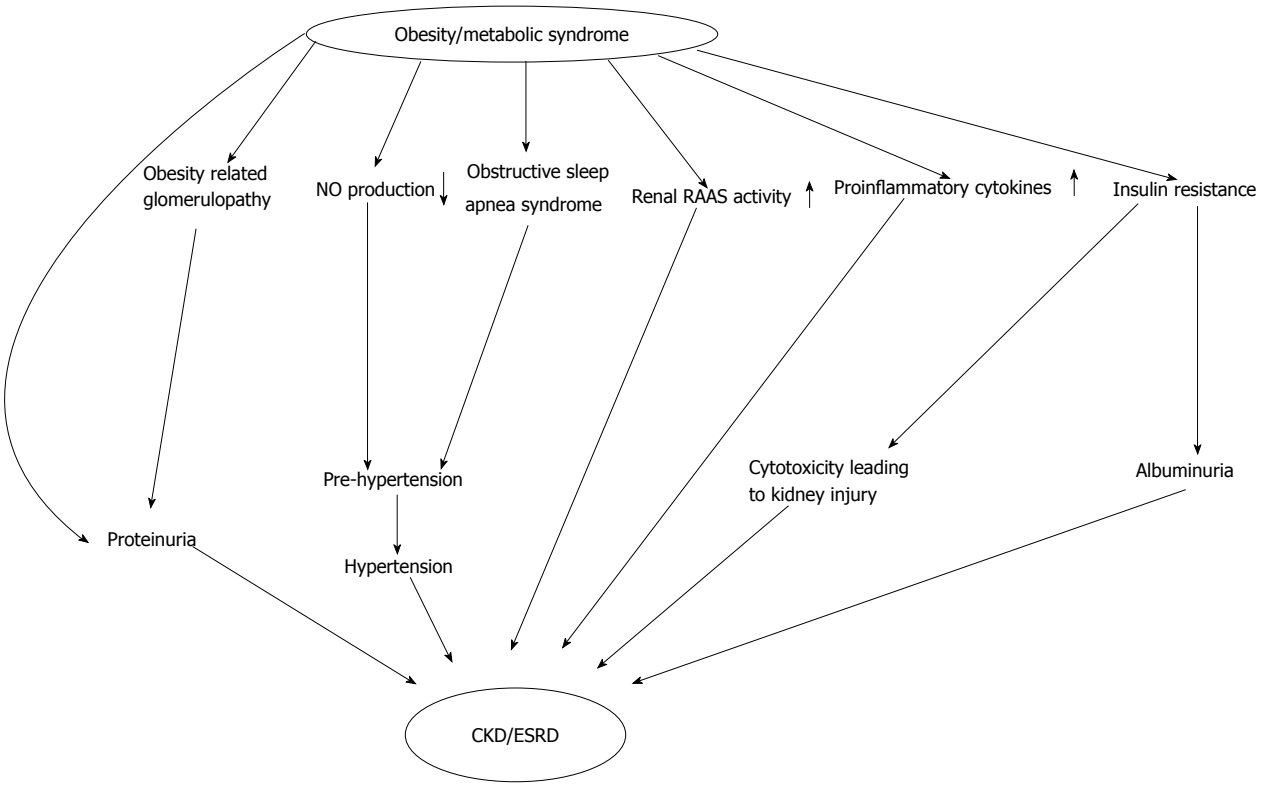

Figure 1 Obesity leads to progression of chronic kidney disease through various pathways. NO: Nitric oxide; RAAS: Renal angiotensin aldosterone system; CKD: Chronic kidney disease; ESRD: End-stage renal disease.

genetic factors, because the association between obesity and hypertension has been observed in diverse populations throughout the world. Although the precise contribution of excess weight to hypertension has not been clearly established, Garrison *et al*[5] reported that about 78% hypertension in men and 65% in women may be directly attributed to excess body mass. Moreover, this association between obesity and hypertension can be modified by factors, such as the duration of obesity and the distribution of body fat. Clinical research has also demonstrated the therapeutic role of weight loss for reducing blood pressure. Even weight loss in "normotensive" overweight individuals can decrease the blood pressure. Experimental research of dietary-induced or genetic animal models of obesity has permitted mechanistic insights into these factors that link hypertension and obesity. Dobrian *et al*[36] showed that weight gain induced by long-term high-fat diets consistently increased blood pressure in a rat model[37]. In addition, renal and metabolic changes observed in animal models of diet-induced obesity seem to mimic very closely the findings in obese humans.

Mechanisms of hypertension in obesity

Obesity-associated hypertension is a complex multi-factorial disease, including activation of RAAS, altered vascular function and increased sympathetic nervous system (SNS)[38]. The potential relationship among these mechanisms is shown in Figure 2. Insulin resistance alone, or in combination with hyperleptinemia, activates the SNS, which cause vasoconstriction and reduced renal blood flow, leading in turn to activation of RAAS and water and sodium retention[39]. The serum level of leptin has a strong association with increased blood pressure, and eventually activated SNS. In addition, recent reports show that other mechanisms may be involved in the pathogenesis of hypertension in obese children, such as proinflammatory cytokines and oxidative stress pathway. These signaling pathways likely contribute to increased arterial stiffness and endothelial dysfunction (Figure 2)[40]. Moreover, sleep apnea syndrome or poor sleep quality often increase the risk of the development of hypertension in obese children[41]. The potential mechanisms for sleep apnea or poor sleep quality may be triggered by intermittent hypoxia and increased inflammatory cytokines, and may eventually exacerbate the progression of hypertension in obese individuals. Pacifico *et al*[42] demonstrated that low serum $25(OH)_2D_3$ levels were associated with metabolic syndrome and hypertension in Caucasian children and adolescents. This suggests that low vitamin D level often observed in obese children, may have a strong association with hypertension and metabolic syndrome. The uric acid may be also involved in obesity-induced hypertension. A high fructose diet can lead to hyperuricaemia owing to increased uric acid production by adipose tissue in obese individuals[43]. Several researches have demonstrated a strong relationship between uric acid and hypertension in children and adolescents. The Moscow Children's hypertension study showed hyperuricaemia (> 8.0 mg/dL) only in 9.5% of children with normal blood

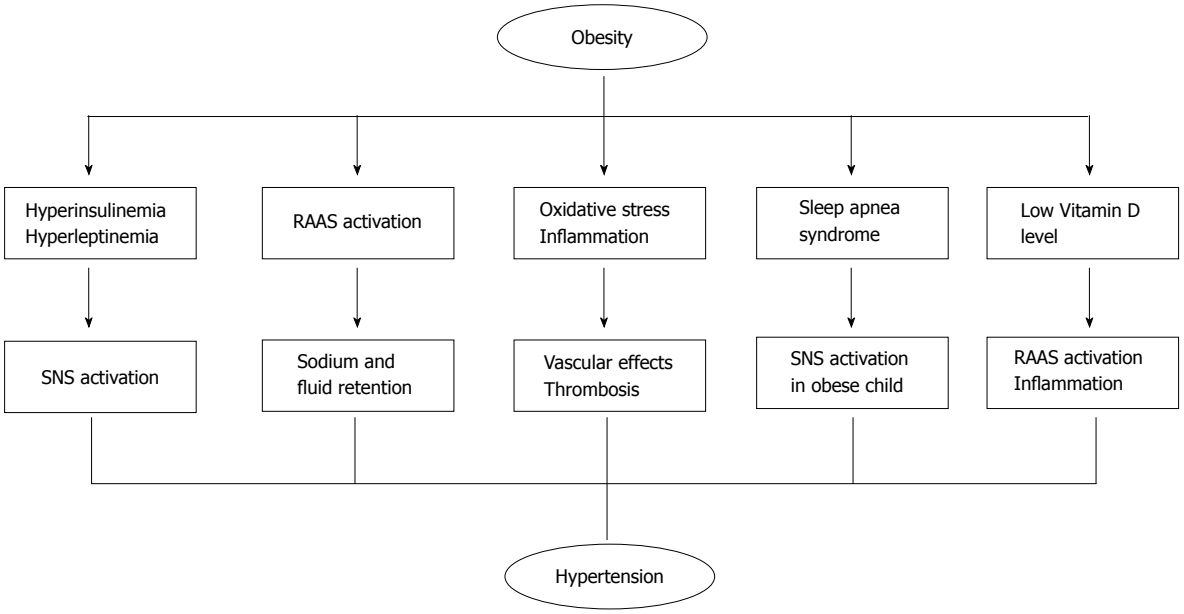

Figure 2 Mechanisms of hypertension in obesity. RAAS: Renal angiotensin aldosterone system; SNS: Sympathetic nervous system.

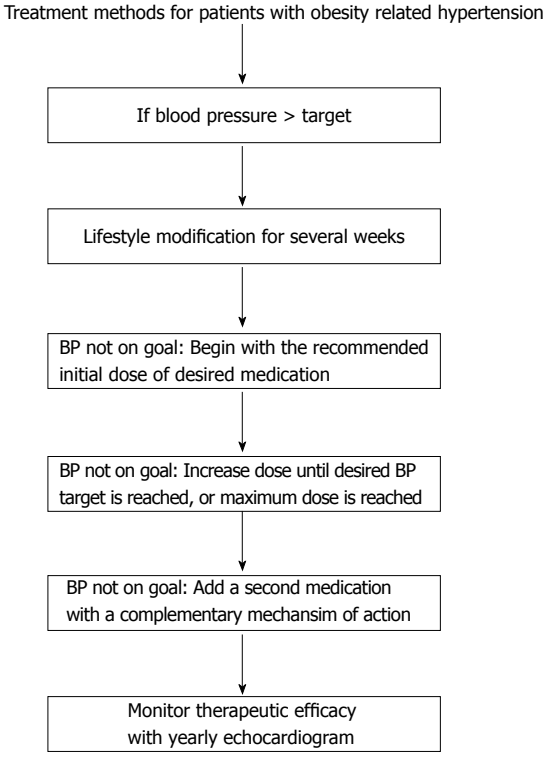

Figure 3 Stepped-care approach to antihypertensive therapy in obese children. BP: Blood pressure.

pressure, but as high as 49% in children with borderline hypertension, and up to 73% of children with moderate and severe hypertension[44]. These findings also need to be confirmed by large-scale epidemiological studies.

Therapeutic approaches for obesity related hypertension

Lifestyle interventions were recommended for all the obese children with hypertension. These include increased physical activity, low sodium diet and other healthy dietary choices for weight loss[45]. The effects of high sodium intake may have an important role of elevated blood pressure in overweight and obese adolescents compared with the general individuals. In addition, decreasing sodium intake may have a beneficial effect on blood pressure in obese individuals[46]. Pharmacological and surgical options were limited for the treatment of obese children. Calcium channel blockers and angiotensin converting enzyme (ACE) inhibitors are the most frequently prescribed drugs for primary hypertension in children and adolescents[47]. Because of the important role of RAAS and SNS activation in obesity related hypertension, ACE inhibitors are considered a very good choice for the treatment of hypertension[48]. Moreover, ACE inhibitors and angiotensin receptor blockers may have additional reno-protective role in obese patients[45]. Beta blockers may impair lipid and glucose metabolism and they are not preferably the first choice therapy in obese hypertensive individuals[49,50].

The current approach for obesity-related hypertension in children is summarized in Figure 3[51]. Lastly, obesity related hypertension should be considered a chronic medical condition and likely requires long-term treatment.

CONCLUSION

Obesity has reached epidemic proportions and continues to be a growing problem worldwide. Excess weight gain appears to be a major risk factor for CKD and hypertension. The potential mechanisms involve insulin resistance, inflammation, renal RAAS hyperactivity, SNS hyperactivity, and perhaps other unknown mechanisms. Obesity related renal injury and hypertension is already well recognized in the adult population. Increased awareness is needed in children for early diagnosis and

implementation of prevention and treatment measures.

REFERENCES

1 **Lobstein T**, Baur L, Uauy R; IASO International Obesity TaskForce. Obesity in children and young people: a crisis in public health. *Obes Rev* 2004; **5** Suppl 1: 4-104 [PMID: 15096099 DOI: 10.1111/j.1467-789X.2004.00133.x]

2 **Wang Y**, Chen X, Song Y, Caballero B, Cheskin LJ. Association between obesity and kidney disease: a systematic review and meta-analysis. *Kidney Int* 2008; **73**: 19-33 [PMID: 17928825 DOI: 10.1038/sj.ki.5002586]

3 **Wahba IM**, Mak RH. Obesity and obesity-initiated metabolic syndrome: mechanistic links to chronic kidney disease. *Clin J Am Soc Nephrol* 2007; **2**: 550-562 [PMID: 17699463 DOI: 10.2215/CJN.04071206]

4 **Sarafidis PA**, Ruilope LM. Insulin resistance, hyperinsulinemia, and renal injury: mechanisms and implications. *Am J Nephrol* 2006; **26**: 232-244 [PMID: 16733348 DOI: 10.1159/000093632]

5 **Garrison RJ**, Kannel WB, Stokes J, Castelli WP. Incidence and precursors of hypertension in young adults: the Framingham Offspring Study. *Prev Med* 1987; **16**: 235-251 [PMID: 3588564 DOI: 10.1016/0091-7435(87)90087-9]

6 **Kuczmarski RJ**, Ogden CL, Guo SS, Grummer-Strawn LM, Flegal KM, Mei Z, Wei R, Curtin LR, Roche AF, Johnson CL. 2000 CDC Growth Charts for the United States: methods and development. *Vital Health Stat 11* 2002; **(246)**: 1-190 [PMID: 12043359]

7 **Ogden CL**, Carroll MD, Curtin LR, McDowell MA, Tabak CJ, Flegal KM. Prevalence of overweight and obesity in the United States, 1999-2004. *JAMA* 2006; **295**: 1549-1555 [PMID: 16595758 DOI: 10.1001/jama.295.13.1549]

8 **Ogden CL**, Carroll MD, Curtin LR, Lamb MM, Flegal KM. Prevalence of high body mass index in US children and adolescents, 2007-2008. *JAMA* 2010; **303**: 242-249 [PMID: 20071470 DOI: 10.1001/jama.2009.2012]

9 **Flegal KM**, Carroll MD, Ogden CL, Johnson CL. Prevalence and trends in obesity among US adults, 1999-2000. *JAMA* 2002; **288**: 1723-1727 [PMID: 12365955 DOI: 10.1001/jama.288.14.1723]

10 **Hsu CY**, McCulloch CE, Iribarren C, Darbinian J, Go AS. Body mass index and risk for end-stage renal disease. *Ann Intern Med* 2006; **144**: 21-28 [PMID: 16389251 DOI: 10.7326/0003-4819-144-1-200601030-00006]

11 **Filler G**, Reimão SM, Kathiravelu A, Grimmer J, Feber J, Drukker A. Pediatric nephrology patients are overweight: 20 years' experience in a single Canadian tertiary pediatric nephrology clinic. *Int Urol Nephrol* 2007; **39**: 1235-1240 [PMID: 17899438 DOI: 10.1007/s11255-007-9258-y]

12 **Espinoza R**, Gracida C, Cancino J, Ibarra A. Effect of obese living donors on the outcome and metabolic features in recipients of kidney transplantation. *Transplant Proc* 2006; **38**: 888-889 [PMID: 16647499 DOI: 10.1016/j.transproceed.2006.02.044]

13 **Ding W**, Mak RH. Early markers of obesity-related renal injury in childhood. *Pediatr Nephrol* 2015; **30**: 1-4 [PMID: 25322907]

14 **Pantoja Zuzuárregui JR**, Mallios R, Murphy J. The effect of obesity on kidney length in a healthy pediatric population. *Pediatr Nephrol* 2009; **24**: 2023-2027 [PMID: 19475431 DOI: 10.1007/s00467-009-1202-1]

15 **Chen J**, Muntner P, Hamm LL, Jones DW, Batuman V, Fonseca V, Whelton PK, He J. The metabolic syndrome and chronic kidney disease in U.S. adults. *Ann Intern Med* 2004; **140**: 167-174 [PMID: 14757614 DOI: 10.7326/0003-4819-140-3-200402030-00007]

16 **Bonnet F**, Deprele C, Sassolas A, Moulin P, Alamartine E, Berthezène F, Berthoux F. Excessive body weight as a new independent risk factor for clinical and pathological progression in primary IgA nephritis. *Am J Kidney Dis* 2001; **37**: 720-727 [PMID: 11273871 DOI: 10.1016/S0272-6386(01)80120-7]

17 **González E**, Gutiérrez E, Morales E, Hernández E, Andres A, Bello I, Díaz-González R, Leiva O, Praga M. Factors influencing the progression of renal damage in patients with unilateral renal agenesis and remnant kidney. *Kidney Int* 2005; **68**: 263-270 [PMID: 15954916 DOI: 10.1111/j.1523-1755.2005.00401.x]

18 **Marcantoni C**, Ma LJ, Federspiel C, Fogo AB. Hypertensive nephrosclerosis in African Americans versus Caucasians. *Kidney Int* 2002; **62**: 172-180 [PMID: 12081576 DOI: 10.1046/j.1523-1755.2002.00420.x]

19 **Kincaid-Smith P.** Hypothesis: Obesity and the insulin resistance syndrome play a major role in end-stage renal failure attributed to hypertension and labed "hypertensive nephrosclerosis. *J Hypertens* 2004; **22**: 1051-1055 [PMID: 15167435 DOI: 10.1097/00004872-200406000-00001]

20 **Srivastava T**. Nondiabetic consequences of obesity on kidney. *Pediatr Nephrol* 2006; **21**: 463-470 [PMID: 16491417 DOI: 10.1007/s00467-006-0027-4]

21 **Weisinger JR**, Kempson RL, Eldridge FL, Swenson RS. The nephrotic syndrome: a complication of massive obesity. *Ann Intern Med* 1974; **81**: 440-447 [PMID: 4416380 DOI: 10.7326/0003-4819-81-4-440]

22 **Cohen AH**. Massive obesity and the kidney. A morphologic and statistical study. *Am J Pathol* 1975; **81**: 117-130 [PMID: 1180328]

23 **Fowler SM**, Kon V, Ma L, Richards WO, Fogo AB, Hunley TE. Obesity-related focal and segmental glomerulosclerosis: normalization of proteinuria in an adolescent after bariatric surgery. *Pediatr Nephrol* 2009; **24**: 851-855 [PMID: 18941798 DOI: 10.1007/s00467-008-1024-6]

24 **Chen W**, Bao W, Begum S, Elkasabany A, Srinivasan SR, Berenson GS. Age-related patterns of the clustering of cardiovascular risk variables of syndrome X from childhood to young adulthood in a population made up of black and white subjects: the Bogalusa Heart Study. *Diabetes* 2000; **49**: 1042-1048 [PMID: 10866058 DOI: 10.2337/diabetes.49.6.1042]

25 **Cook S**, Weitzman M, Auinger P, Nguyen M, Dietz WH. Prevalence of a metabolic syndrome phenotype in adolescents: findings from the third National Health and Nutrition Examination Survey, 1988-1994. *Arch Pediatr Adolesc Med* 2003; **157**: 821-827 [PMID: 12912790 DOI: 10.1001/archpedi.157.8.821]

26 **Wisse BE**. The inflammatory syndrome: the role of adipose tissue cytokines in metabolic disorders linked to obesity. *J Am Soc Nephrol* 2004; **15**: 2792-2800 [PMID: 15504932 DOI: 10.1097/01.ASN.0000141966.69934.21]

27 **Beddhu S**, Kimmel PL, Ramkumar N, Cheung AK. Associations of metabolic syndrome with inflammation in CKD: results From the Third National Health and Nutrition Examination Survey (NHANES III). *Am J Kidney Dis* 2005; **46**: 577-586 [PMID: 16183411 DOI: 10.1053/j.ajkd.2005.06.014]

28 **Wu Y**, Liu Z, Xiang Z, Zeng C, Chen Z, Ma X, Li L. Obesity-related glomerulopathy: insights from gene expression profiles of the glomeruli derived from renal biopsy samples. *Endocrinology* 2006; **147**: 44-50 [PMID: 16210374 DOI: 10.1210/en.2005-0641]

29 **Ramkumar N**, Cheung AK, Pappas LM, Roberts WL, Beddhu S. Association of obesity with inflammation in chronic kidney disease: a cross-sectional study. *J Ren Nutr* 2004; **14**: 201-207 [PMID: 15483779 DOI: 10.1016/S1051-2276(04)00133-5]

30 **Weiss R**, Kaufman FR. Metabolic complications of childhood obesity: identifying and mitigating the risk. *Diabetes Care* 2008; **31** Suppl 2: S310-S316 [PMID: 18227502 DOI: 10.2337/dc08-s273]

31 **Gunta SS**, Mak RH. Is obesity a risk factor for chronic kidney disease in children? *Pediatr Nephrol* 2013; **28**: 1949-1956 [PMID: 23150030 DOI: 10.1007/s00467-012-2353-z]

32 **Rosner B**, Prineas R, Daniels SR, Loggie J. Blood pressure differences between blacks and whites in relation to body size among US children and adolescents. *Am J Epidemiol* 2000; **151**: 1007-1019 [PMID: 10853640 DOI: 10.1093/oxfordjournals.aje.a010129]

33 **Freedman DS**, Dietz WH, Srinivasan SR, Berenson GS. The relation of overweight to cardiovascular risk factors among children and adolescents: the Bogalusa Heart Study. *Pediatrics* 1999; **103**: 1175-1182 [PMID: 10353925 DOI: 10.1542/peds.103.6.1175]

34 **Sorof JM**, Poffenbarger T, Franco K, Bernard L, Portman RJ. Isolated systolic hypertension, obesity, and hyperkinetic hemodynamic states in children. *J Pediatr* 2002; **140**: 660-666 [PMID: 12072867 DOI: 10.1067/mpd.2002.125228]

35 **He Q**, Ding ZY, Fong DY, Karlberg J. Blood pressure is associated with body mass index in both normal and obese children. *Hypertension* 2000; **36**: 165-170 [PMID: 10948072 DOI: 10.1161/01.HYP.36.2.165]

36 **Dobrian AD**, Davies MJ, Schriver SD, Lauterio TJ, Prewitt RL. Oxidative stress in a rat model of obesity-induced hypertension. *Hypertension* 2001; **37**: 554-560 [PMID: 11230334 DOI: 10.1161/01.HYP.37.2.554]

37 **Hall JE**. The kidney, hypertension, and obesity. *Hypertension* 2003; **41**: 625-633 [PMID: 12623970 DOI: 10.1161/01.HYP.0000052314.95497.78]

38 **Kotchen TA**. Obesity-related hypertension: epidemiology, pathophysiology, and clinical management. *Am J Hypertens* 2010; **23**: 1170-1178 [PMID: 20706196 DOI: 10.1038/ajh.2010.172]

39 **Flynn J**. The changing face of pediatric hypertension in the era of the childhood obesity epidemic. *Pediatr Nephrol* 2013; **28**: 1059-1066 [PMID: 23138756 DOI: 10.1007/s00467-012-2344-0]

40 **Bucher BS**, Ferrarini A, Weber N, Bullo M, Bianchetti MG, Simonetti GD. Primary hypertension in childhood. *Curr Hypertens Rep* 2013; **15**: 444-452 [PMID: 23897423 DOI: 10.1007/s11906-013-0378-8]

41 **Archbold KH**, Vasquez MM, Goodwin JL, Quan SF. Effects of sleep patterns and obesity on increases in blood pressure in a 5-year period: report from the Tucson Children's Assessment of Sleep Apnea Study. *J Pediatr* 2012; **161**: 26-30 [PMID: 22284918 DOI: 10.1016/j.jpeds.2011.12.034]

42 **Pacifico L**, Anania C, Osborn JF, Ferraro F, Bonci E, Olivero E, Chiesa C. Low 25(OH)D3 levels are associated with total adiposity, metabolic syndrome, and hypertension in Caucasian children and adolescents. *Eur J Endocrinol* 2011; **165**: 603-611 [PMID: 21753070 DOI: 10.1530/EJE-11-0545]

43 **Johnson RJ**, Nakagawa T, Sanchez-Lozada LG, Shafiu M, Sundaram S, Le M, Ishimoto T, Sautin YY, Lanaspa MA. Sugar, uric acid, and the etiology of diabetes and obesity. *Diabetes* 2013; **62**: 3307-3315 [PMID: 24065788 DOI: 10.2337/db12-1814]

44 **Yanik M**, Feig DI. Serum urate: a biomarker or treatment target in pediatric hypertension? *Curr Opin Cardiol* 2013; **28**: 433-438 [PMID: 23736817 DOI: 10.1097/HCO.0b013e32836205ff]

45 **Halbach SM**, Flynn J. Treatment of obesity-related hypertension in children and adolescents. *Curr Hypertens Rep* 2013; **15**: 224-231 [PMID: 23475645 DOI: 10.1007/s11906-013-0334-7]

46 **Yang Q**, Zhang Z, Kuklina EV, Fang J, Ayala C, Hong Y, Loustalot F, Dai S, Gunn JP, Tian N, Cogswell ME, Merritt R. Sodium intake and blood pressure among US children and adolescents. *Pediatrics* 2012; **130**: 611-619 [PMID: 22987869 DOI: 10.1542/peds.2011-3870]

47 **Yoon EY**, Cohn L, Rocchini A, Kershaw D, Freed G, Ascione F, Clark S. Antihypertensive prescribing patterns for adolescents with primary hypertension. *Pediatrics* 2012; **129**: e1-e8 [PMID: 22144698 DOI: 10.1542/peds.2011-0877]

48 **Thatcher S**, Yiannikouris F, Gupte M, Cassis L. The adipose renin-angiotensin system: role in cardiovascular disease. *Mol Cell Endocrinol* 2009; **302**: 111-117 [PMID: 19418627 DOI: 10.1016/j.mce.2009.01.019]

49 **Che Q**, Schreiber MJ, Rafey MA. Beta-blockers for hypertension: are they going out of style? *Cleve Clin J Med* 2009; **76**: 533-542 [PMID: 19726558 DOI: 10.3949/ccjm.76a.09030]

50 **Jacob S**, Rett K, Henriksen EJ. Antihypertensive therapy and insulin sensitivity: do we have to redefine the role of beta-blocking agents? *Am J Hypertens* 1998; **11**: 1258-1265 [PMID: 9799045 DOI: 10.1016/S0895-7061(98)00141-1]

51 **Ferguson MA,** Flynn JT. Rational use of antihypertensive medications in children. *Pediatr Nephrol* 2014; **29**: 979-988 [PMID: 23715784 DOI: 10.1007/s00467-013-2510-z]

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Strategies to optimize shock wave lithotripsy outcome: Patient selection and treatment parameters

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Abstract

Shock wave lithotripsy (SWL) was introduced in 1980, modernizing the treatment of upper urinary tract stones, and quickly became the most commonly utilized technique to treat kidney stones. Over the past 5-10 years, however, use of SWL has been declining because it is not as reliably effective as more modern technology. SWL success rates vary considerably and there is abundant literature predicting outcome based on patient- and stone-specific parameters. Herein we discuss the ways to optimize SWL outcomes by reviewing proper patient selection utilizing stone characteristics and patient features. Stone size, number, location, density, composition, and patient body habitus and renal anatomy are all discussed. We also review the technical parameters during SWL that can be controlled to improve results further, including type of anesthesia, coupling, shock wave rate, focal zones, pressures, and active monitoring. Following these basic principles and selection criteria will help maximize success rate.

Key words: Shock wave lithotripsy; Kidney stones; Nephrolithiasis; Treatment outcome; Optimization

Core tip: Shock wave lithotripsy is a commonly utilized technology for kidney stone treatment that has declining efficacy over the past decade. The paper outlines how to optimize outcomes with proper patient selection and control of treatment parameters.

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INTRODUCTION

Shock wave lithotripsy (SWL) was introduced in 1980, modernizing the treatment of upper urinary tract stones. Prior to the SWL era, proximal ureteral and renal calculi required major operations with a prolonged recovery time. Because SWL is a non-invasive surgical procedure with a low complication rate allowing same day discharges, it has been the most commonly utilized treatment of kidney stones

Table 1 Stone criteria for shock wave lithotripsy

Sub-optimal features suggesting alternate therapy
Stone size > 2 cm
Multiple stones
Lower pole stone
Hounsfield unit > 1000
History of cystine, calcium oxalate monohydrate, matrix stones

over the past 3 decades[1-3]. Over the past 5-10 years, however, use of SWL has been declining and just recently, a group in Canada showed ureteroscopy has surpassed it as the most common treatment of nephrolithiasis[1-4]. While ureteroscopy is more invasive than SWL, it is still minimally invasive, with a low morbidity profile, and it is more reliably definitive than SWL requiring fewer subsequent procedures to establish stone-free status[5]. As SWL technology has transformed to a more convenient and easier process, success rates have declined. SWL outcomes, however, can be optimized with careful patient selection and control of specific treatment parameters. Herein, we review how to maximize the success rate of SWL and reduce failures by defining the appropriate range of uses and outlining what technical factors can be controlled to improve efficacy.

PATIENT SELECTION

Success rate of SWL varies considerably. This variability is a direct result of well-established stone-specific and patient-specific features. While the American Urological Association guidelines for management of ureteral calculi cite SWL as a primary treatment option if intervention is needed, and the technology could theoretically be used on any urinary stone, selectivity is crucial to maximize efficacy[6] .

Stones have varying responsiveness to SWL depending on several aspects. Stone size and number, location, density, and composition all affect the stone-free rate following SWL (Table 1). The American Urological Association Guideline on the management of staghorn calculi recommends against SWL as monotherapy because of poor outcomes, with only 54% overall stone-free rate, and increased complications (pain, obstruction, infection, bleeding, loss of kidney)[7]. SWL may be appropriate as an adjunctive procedure following percutaneous nephrolithotomy for staghorn calculi if there is a small residual stone. In general, it is still recommended that nephroscopy be the final procedure performed to confirm stone clearance in this setting[7]. If SWL is used as monotherapy for staghorn calculi, then a stent or nephrostomy tube should be placed prior to intervention, though the drainage mostly helps to prevent complications, and does not necessarily improve outcome. Multiple procedures are generally required for this scenario.

While staghorn is the extreme of large stone size, any stone over 2 cm is associated with an inferior outcome when treated with SWL[8-11]. Larger stones usually require more procedures and have increased complications such as obstruction from steinstrasse or larger fragment passage. If a stone is larger than 2 cm, then an alternate treatment may be best. In addition to stone size, total stone burden should be considered when electing treatment. If there are several stones throughout the kidney or bilateral stones amenable to single stage ureteroscopy *vs* multi-stage SWL then the patient should be counseled that stone-free rate may be higher with fewer procedures with the former option.

In addition to stone burden dispersed throughout the kidney making SWL less ideal, different stone locations affect success rates of the procedure. Specifically, there is an abundance of literature showing a lower stone-free rate for kidney calculi located in the lower pole treated with SWL with highest success rates in renal pelvic, upper pole and ureteropelvic junction stones[12-15]. Lower pole 1, a prospective, multicenter, randomized controlled trial evaluating treatment outcome for lower pole kidney stones, illustrated a 37% *vs* 95% stone-free rate for SWL *vs* percutaneous nephrolithotomy[12]. Outcome worsened further for lower pole kidney stones larger than 2 cm when treated with SWL (stone free rate 14%)[12]. This inferior outcome is directly related to the infundibulopelvic angle and lack of fragment clearance, rather than actual successful fragmentation. Success rates can be further delineated with measurements of infundibular width and length. One research group evaluated these anatomical features using intravenous pyelogram measurements and better stone clearance with SWL was achieved in kidneys with a wide infundibulopelvic angle or a short length and a broad width[15].

In addition to kidney stone locations, ureteral stone location affects outcome as well. Lower stone free rates are seen with distal ureteral stones, particularly stones greater than 1 cm, and SWL is not recommended as the primary treatment option but is an acceptable secondary alternative[6]. In general, SWL of the pelvis (distal ureteral stones) is avoided in women of childbearing age due to the theoretical risk of adjacent adnexal injury[6,16].

Both how hard a stone is and its composition also affect outcome of SWL. Density alone is a great predictor of successful fragmentation. Several groups have found that Hounsfield unit (HU) measurement of the stone on computed tomography imaging is associated with stone-free rate[17-19]. One group reported treatment failure in close to 50% of patients for stones great than 1000 HU[19]. Another study found at least 3 SWL sessions were required 70% of the time if HU was more than 750, and stone-free rate was still only 65%[18]. Specific stones compositions are more dense than others, and therefore have well-established resistance to SWL. Brushite, cystine, and

Table 2 Patient criteria for shock wave lithotripsy

Sub-optimal features suggesting alternate therapy
Obesity - skin to stone distance > 10 cm
Pelvic kidney
Horseshoe kidney
Calyceal diverticulum

Table 3 Absolute contraindications to shock wave lithotripsy

Anticoagulation
Bleeding diathesis
Pregnancy
Severe skeletal malformations
Distal obstruction
Infection associated with obstruction

calcium oxalate monohydrate are well-known to have very poor responses to SWL[7,20-24]. If suspicious for these stone compositions based on prior history or crystal presence on urinalysis, SWL is best avoided and another treatment selected. Matrix stones, while not dense, are made of organic matter and do not break with SWL[25]. Ureteroscopy or percutaneous nephrolithotomy should be used to treat this rare stone type if known.

Once the checklist for SWL has been reviewed for ideal stone characteristics, patient-specific features need to be evaluated. Body habitus and renal anatomy both affect SWL outcome (Table 2). Obesity, specifically skin to stone distance (SSD) measured on axial imaging, predicts outcome, with greater than 9 or 10 cm having a poor result[26-28]. This is because the shock wave fired loses energy as it travels through excess body fat in a patient with an elevated body mass index[29]. Pelvic kidneys and horseshoe kidneys also have a lower stone-free rate with a greater number of SWL sessions needed to achieve success[30,31]. SWL is generally not recommended in patients with a calculus in a calyceal diverticulum. While some patients may have symptomatic relief with stone fragmentation, stone-free rate is only 21% because the diverticular neck does not allow for stone passage[32]. If the ostium of the diverticulum is well-visualized, the stone is small, and the diverticula fills with contrast, success rates have been shown to be improved[33]. Hydronephrosis and renal insufficiency are also associated with lower success rates but the mechanism for this is unknown[34]. Anticoagulation, bleeding disorders, pregnancy, severe skeletal malformations, distal obstruction, and infection associated with obstruction are all absolute contraindications to SWL (Table 3)[6,35].

While some patients may still choose SWL despite not satisfying all criteria, keeping these general principles in mind regarding stone-specific characteristics and patient features when electing SWL will improve the procedure success rate.

Table 4 Technical factors that optimize shock wave lithotripsy outcome

General anesthesia
Optimal coupling
Low shock wave rate (60 shocks per minute)
Wider focal zone
Active intraoperative monitoring

TREATMENT PARAMETERS

Once SWL is selected as the procedure for definitive management based on the above criteria, several technical parameters during the procedure can be controlled to also optimize outcomes (Table 4).

The first way to improve outcome begins before the procedure even starts when selecting anesthesia. With more modern lithotripters having a narrow focal zone, unforeseen movements may shift the location of the stone out of the treatment zone, thus delivering shocks to surrounding tissue instead of the desired target. One way to minimize movement is to administer general anesthesia, as the anesthesiologist can control respirations with adjustments of rate and volume as needed, thus providing more control over kidney and stone motion. Several studies have shown improved SWL outcomes with higher stone free rates using general anesthesia *vs* sedation[36,37].

The next way to improve outcome is during the preparation. The original lithotripter in 1980 immersed patients completely in a bathtub and therefore used water as the medium to couple the shock wave to the patient. This was the optimal coupler as there was no air present to dissipate any energy. With miniaturization of the technology, most lithotripter machines now have a dry treatment head and use gel or oil for coupling. This has negatively impacted the outcome as air bubbles that form within the medium dampen the energy and reduce the impact on the stone. Efficacy can be reduced by as much as 40% with the presence of as few as 2% of air pockets[38]. Avoiding patient movement or repositioning during the procedure will lessen the impact of this effect minimizing the number of air pockets created. Additionally, medium application as a large volume mound directly from the stock container has been shown to minimize air bubble creation far more than dispensing from a squirt bottle or applying with the hand[39].

Once ready to initiate SWL several settings can be adjusted as well to optimize outcome. Shock wave rate can be set prior to initiating treatment and a slow rate of 60 shocks per minute has been shown to not only reduce tissue injury but also have a superior stone free rates[40-45]. This optimal rate has been confirmed by several studies including a meta-analysis of randomized controlled trials[46]. If the lithotripter being used, allows for control of focal zone size and

pressures, a wider zone with lower pressures have been shown to have the best outcomes while reducing tissue injury[47-50]. Another setting recommendation for SWL is pre-treating the stone at a low energy for 100-200 shock waves and then pausing for several minutes prior to going to a higher energy[50,51]. While this does not necessarily improve efficacy of SWL it does improve outcome by decreasing injury to the kidney[52-54]. Once the procedure begins, active monitoring of the stone location with continuous ultrasound or spot fluoroscopy every couple of minutes or every 100-200 shocks, will confirm that the target is still appropriately positioned within the treatment zone.

Following these general guidelines for control of technical parameters during SWL will help to optimize outcome and improve stone free rates while minimizing tissue injury.

CONCLUSION

SWL is an excellent treatment modality for upper urinary tract treatment stones however success rate has decreased in the recent years secondary to changes in the machine design. Careful patient and stone selection and control of technical parameters improves stone free rates and will more likely result in a successful outcome.

REFERENCES

1 **Pearle MS**, Calhoun EA, Curhan GC. Urologic diseases in America project: urolithiasis. *J Urol* 2005; **173**: 848-857 [PMID: 15711292]
2 **Ordon M**, Urbach D, Mamdani M, Saskin R, D'A Honey RJ, Pace KT. The surgical management of kidney stone disease: a population based time series analysis. *J Urol* 2014; **192**: 1450-1456 [PMID: 24866599 DOI: 10.1016/j.juro.2014.05.095]
3 **Kerbl K**, Rehman J, Landman J, Lee D, Sundaram C, Clayman RV. Current management of urolithiasis: progress or regress? *J Endourol* 2002; **16**: 281-288 [PMID: 12184077 DOI: 10.1089/089277902760102758]
4 **Scales CD**, Krupski TL, Curtis LH, Matlaga B, Lotan Y, Pearle MS, Saigal C, Preminger GM. Practice variation in the surgical management of urinary lithiasis. *J Urol* 2011; **186**: 146-150 [PMID: 21575964 DOI: 10.1016/j.juro.2011.03.018]
5 **Scales CD**, Lai JC, Dick AW, Hanley JM, van Meijgaard J, Setodji CM, Saigal CS. Comparative effectiveness of shock wave lithotripsy and ureteroscopy for treating patients with kidney stones. *JAMA Surg* 2014; **149**: 648-653 [PMID: 24839228]
6 **Preminger GM**, Tiselius HG, Assimos DG, Alken P, Buck C, Gallucci M, Knoll T, Lingeman JE, Nakada SY, Pearle MS, Sarica K, Türk C, Wolf JS. 2007 guideline for the management of ureteral calculi. *J Urol* 2007; **178**: 2418-2434 [PMID: 17993340]
7 **Preminger GM**, Assimos DG, Lingeman JE, Nakada SY, Pearle MS, Wolf JS. Chapter 1: AUA guideline on management of staghorn calculi: diagnosis and treatment recommendations. *J Urol* 2005; **173**: 1991-2000 [PMID: 15879803 DOI: 10.1097/01.ju.0000161171.67806.2a]
8 **Lingeman JE**, Coury TA, Newman DM, Kahnoski RJ, Mertz JH, Mosbaugh PG, Steele RE, Woods JR. Comparison of results and morbidity of percutaneous nephrostolithotomy and extracorporeal shock wave lithotripsy. *J Urol* 1987; **138**: 485-490 [PMID: 3625845]
9 **Galvin DJ**, Pearle MS. The contemporary management of renal and ureteric calculi. *BJU Int* 2006; **98**: 1283-1288 [PMID: 17125486 DOI: 10.1111/j.1464-410X.2006.06514.x]
10 **Abe T**, Akakura K, Kawaguchi M, Ueda T, Ichikawa T, Ito H, Nozumi K, Suzuki K. Outcomes of shockwave lithotripsy for upper urinary-tract stones: a large-scale study at a single institution. *J Endourol* 2005; **19**: 768-773 [PMID: 16190825]
11 **Egilmez T**, Tekin MI, Gonen M, Kilinc F, Goren R, Ozkardes H. Efficacy and safety of a new-generation shockwave lithotripsy machine in the treatment of single renal or ureteral stones: Experience with 2670 patients. *J Endourol* 2007; **21**: 23-27 [PMID: 17263603]
12 **Albala DM**, Assimos DG, Clayman RV, Denstedt JD, Grasso M, Gutierrez-Aceves J, Kahn RI, Leveillee RJ, Lingeman JE, Macaluso JN, Munch LC, Nakada SY, Newman RC, Pearle MS, Preminger GM, Teichman J, Woods JR. Lower pole I: a prospective randomized trial of extracorporeal shock wave lithotripsy and percutaneous nephrostolithotomy for lower pole nephrolithiasis-initial results. *J Urol* 2001; **166**: 2072-2080 [PMID: 11696709 DOI: 10.1016/S0022-5347(05)65508-5]
13 **Weld KJ**, Montiglio C, Morris MS, Bush AC, Cespedes RD. Shock wave lithotripsy success for renal stones based on patient and stone computed tomography characteristics. *Urology* 2007; **70**: 1043-1046; discussion 1046-1047 [PMID: 18158009]
14 **Pearle MS**, Lingeman JE, Leveillee R, Kuo R, Preminger GM, Nadler RB, Macaluso J, Monga M, Kumar U, Dushinski J, Albala DM, Wolf JS, Assimos D, Fabrizio M, Munch LC, Nakada SY, Auge B, Honey J, Ogan K, Pattaras J, McDougall EM, Averch TD, Turk T, Pietrow P, Watkins S. Prospective, randomized trial comparing shock wave lithotripsy and ureteroscopy for lower pole caliceal calculi 1 cm or less. *J Urol* 2005; **173**: 2005-2009 [PMID: 15879805]
15 **Elbahnasy AM**, Clayman RV, Shalhav AL, Hoenig DM, Chandhoke P, Lingeman JE, Denstedt JD, Kahn R, Assimos DG, Nakada SY. Lower-pole caliceal stone clearance after shockwave lithotripsy, percutaneous nephrolithotomy, and flexible ureteroscopy: impact of radiographic spatial anatomy. *J Endourol* 1998; **12**: 113-119 [PMID: 9607435 DOI: 10.1016/S0022-5347(01)63699-1]
16 **Carol PR,** Shi RY. Genetic toxicity of high energy shockwaves: assessment using the induction of mutations or micronuclei in Chinese hamster ovary. *J Urol* 1986; **135**: 292
17 **Saw KC**, McAteer JA, Fineberg NS, Monga AG, Chua GT, Lingeman JE, Williams JC. Calcium stone fragility is predicted by helical CT attenuation values. *J Endourol* 2000; **14**: 471-474 [PMID: 10954300 DOI: 10.1089/end.2000.14.471]
18 **Gupta NP**, Ansari MS, Kesarvani P, Kapoor A, Mukhopadhyay S. Role of computed tomography with no contrast medium enhancement in predicting the outcome of extracorporeal shock wave lithotripsy for urinary calculi. *BJU Int* 2005; **95**: 1285-1288 [PMID: 15892818 DOI: 10.1111/j.1464-410X.2005.05520.x]
19 **Joseph P**, Mandal AK, Singh SK, Mandal P, Sankhwar SN, Sharma SK. Computerized tomography attenuation value of renal calculus: can it predict successful fragmentation of the calculus by extracorporeal shock wave lithotripsy? A preliminary study. *J Urol* 2002; **167**: 1968-1971 [PMID: 11956419 DOI: 10.1016/S0022-5347(05)65064-1]
20 **Williams JC**, Saw KC, Paterson RF, Hatt EK, McAteer JA, Lingeman JE. Variability of renal stone fragility in shock wave lithotripsy. *Urology* 2003; **61**: 1092-1096; discussion 1097 [PMID: 12809867 DOI: 10.1016/S0090-4295(03)00349-2]
21 **Chow GK**, Streem SB. Contemporary urological intervention for cystinuric patients: immediate and long-term impact and implications. *J Urol* 1998; **160**: 341-344; discussion 344-345 [PMID: 9679873 DOI: 10.1016/S0022-5347(01)62889-1]
22 **Kachel TA**, Vijan SR, Dretler SP. Endourological experience with cystine calculi and a treatment algorithm. *J Urol* 1991; **145**: 25-28 [PMID: 1984093]
23 **Hockley NM,** Lingeman JE, Hutchinson CL. Relative efficacy of extracorporeal shock wave lithotripsy and percutaneous nephrolithotomy in the management of cystine calculi. *J Endourol* 1989; **2**: 273-285 [DOI: 10.1089/end.1989.3.273]
24 **Klee LW**, Brito CG, Lingeman JE. The clinical implications of

brushite calculi. *J Urol* 1991; **145**: 715-718 [PMID: 2005685]
25 **Bani-Hani AH**, Segura JW, Leroy AJ. Urinary matrix calculi: our experience at a single institution. *J Urol* 2005; **173**: 120-123 [PMID: 15592051 DOI: 10.1097/01.ju.0000145868.18824.25]
26 **Thomas R**, Cass AS. Extracorporeal shock wave lithotripsy in morbidly obese patients. *J Urol* 1993; **150**: 30-32 [PMID: 8510269]
27 **Perks AE**, Schuler TD, Lee J, Ghiculete D, Chung DG, D'A Honey RJ, Pace KT. Stone attenuation and skin-to-stone distance on computed tomography predicts for stone fragmentation by shock wave lithotripsy. *Urology* 2008; **72**: 765-769 [PMID: 18674803 DOI: 10.1016/j.urology.2008.05.04]
28 **Pareek G**, Hedican SP, Lee FT, Nakada SY. Shock wave lithotripsy success determined by skin-to-stone distance on computed tomography. *Urology* 2005; **66**: 941-944 [PMID: 16286099 DOI: 10.1016/j.urology.2005.05.011]
29 **Pareek G**, Armenakas NA, Panagopoulos G, Bruno JJ, Fracchia JA. Extracorporeal shock wave lithotripsy success based on body mass index and Hounsfield units. *Urology* 2005; **65**: 33-36 [PMID: 15667858 DOI: 10.1016/j.urology.2004.08.004]
30 **Tunc L**, Tokgoz H, Tan MO, Kupeli B, Karaoglan U, Bozkirli I. Stones in anomalous kidneys: results of treatment by shock wave lithotripsy in 150 patients. *Int J Urol* 2004; **11**: 831-836 [PMID: 15479286 DOI: 10.1111/j.1442-2042.2004.00916.x]
31 **Viola D**, Anagnostou T, Thompson TJ, Smith G, Moussa SA, Tolley DA. Sixteen years of experience with stone management in horseshoe kidneys. *Urol Int* 2007; **78**: 214-218 [PMID: 17406129 DOI: 10.1159/000099340]
32 **Turna B**, Raza A, Moussa S, Smith G, Tolley DA. Management of calyceal diverticular stones with extracorporeal shock wave lithotripsy and percutaneous nephrolithotomy: long-term outcome. *BJU Int* 2007; **100**: 151-156 [PMID: 17552962 DOI: 10.1111/j.1464-410X.2007.06911.x]
33 **Streem SB**, Yost A. Treatment of caliceal diverticular calculi with extracorporeal shock wave lithotripsy: patient selection and extended followup. *J Urol* 1992; **148**: 1043-1046 [PMID: 1507327]
34 **Hung SF**, Chung SD, Wang SM, Yu HJ, Huang HS. Chronic kidney disease affects the stone-free rate after extracorporeal shock wave lithotripsy for proximal ureteric stones. *BJU Int* 2010; **105**: 1162-1167 [PMID: 19930180 DOI: 10.1111/j.1464-410X.2009.08974.x]
35 **D'Addessi A**, Vittori M, Racioppi M, Pinto F, Sacco E, Bassi P. Complications of extracorporeal shock wave lithotripsy for urinary stones: to know and to manage them-a review. *ScientificWorldJournal* 2012; **2012**: 619820 [PMID: 22489195 DOI: 10.1100/2012/619820]
36 **Sorensen C**, Chandhoke P, Moore M, Wolf C, Sarram A. Comparison of intravenous sedation versus general anesthesia on the efficacy of the Doli 50 lithotriptor. *J Urol* 2002; **168**: 35-37 [PMID: 12050487]
37 **Eichel L**, Batzold P, Erturk E. Operator experience and adequate anesthesia improve treatment outcome with third-generation lithotripters. *J Endourol* 2001; **15**: 671-673 [PMID: 11697394 DOI: 10.1089/08927790152596217]
38 **Pishchalnikov YA**, Neucks JS, VonDerHaar RJ, Pishchalnikova IV, Williams JC, McAteer JA. Air pockets trapped during routine coupling in dry head lithotripsy can significantly decrease the delivery of shock wave energy. *J Urol* 2006; **176**: 2706-2710 [PMID: 17085200 DOI: 10.1016/j.juro.2006.07.149]
39 **Neucks JS**, Pishchalnikov YA, Zancanaro AJ, VonDerHaar JN, Williams JC, McAteer JA. Improved acoustic coupling for shock wave lithotripsy. *Urol Res* 2008; **36**: 61-66 [PMID: 18172634 DOI: 10.1007/s00240-007-0128-y]
40 **Evan AP**, McAteer JA, Connors BA, Blomgren PM, Lingeman JE. Renal injury during shock wave lithotripsy is significantly reduced by slowing the rate of shock wave delivery. *BJU Int* 2007; **100**: 624-627; discussion 627-628 [PMID: 17550415 DOI: 10.1111/j.1464-410X.2007.07007.x]
41 **Kato Y**, Yamaguchi S, Hori J, Okuyama M, Kakizaki H. Improvement of stone comminution by slow delivery rate of shock waves in extracorporeal lithotripsy. *Int J Urol* 2006; **13**: 1461-1465 [PMID: 17118017 DOI: 10.1111/j.1442-2042.2006.01609.x]
42 **Chacko J**, Moore M, Sankey N, Chandhoke PS. Does a slower treatment rate impact the efficacy of extracorporeal shock wave lithotripsy for solitary kidney or ureteral stones? *J Urol* 2006; **175**: 1370-1373; discussion 1373-1374 [PMID: 16515999 DOI: 10.1016/S0022-5347(05)00683-X]
43 **Pace KT**, Ghiculete D, Harju M, Honey RJ. Shock wave lithotripsy at 60 or 120 shocks per minute: a randomized, double-blind trial. *J Urol* 2005; **174**: 595-599 [PMID: 16006908 DOI: 10.1097/01.ju.0000165156.90011.95]
44 **Connors BA**, Evan AP, Blomgren PM, Handa RK, Willis LR, Gao S, McAteer JA, Lingeman JE. Extracorporeal shock wave lithotripsy at 60 shock waves/min reduces renal injury in a porcine model. *BJU Int* 2009; **104**: 1004-1008 [PMID: 19338532 DOI: 10.1111/j.1464-410X.2009.08520.x]
45 **Madbouly K**, El-Tiraifi AM, Seida M, El-Faqih SR, Atassi R, Talic RF. Slow versus fast shock wave lithotripsy rate for urolithiasis: a prospective randomized study. *J Urol* 2005; **173**: 127-130 [PMID: 15592053]
46 **Semins MJ**, Trock BJ, Matlaga BR. The effect of shock wave rate on the outcome of shock wave lithotripsy: a meta-analysis. *J Urol* 2008; **179**: 194-197; discussion 197 [PMID: 18001796 DOI: 10.1016/j.juro.2007.08.173]
47 **Sapozhnikov OA**, Maxwell AD, MacConaghy B, Bailey MR. A mechanistic analysis of stone fracture in lithotripsy. *J Acoust Soc Am* 2007; **121**: 1190-1202 [PMID: 17348540 DOI: 10.1121/1.2404894]
48 **Cleveland RO**, Sapozhnikov OA. Modeling elastic wave propagation in kidney stones with application to shock wave lithotripsy. *J Acoust Soc Am* 2005; **118**: 2667-2676 [PMID: 16266186 DOI: 10.1121/1.2032187]
49 **Eisenmenger W**, Du XX, Tang C, Zhao S, Wang Y, Rong F, Dai D, Guan M, Qi A. The first clinical results of "wide-focus and low-pressure" ESWL. *Ultrasound Med Biol* 2002; **28**: 769-774 [PMID: 12113789 DOI: 10.1016/S0301-5629(02)00507-0]
50 **McAteer JA**, Bailey MR, Williams Jr JC, Cleveland RO, Evan AP. Strategies for improved shock wave lithotripsy. *Minerva Urol Nefrol* 2005; **57**: 271-287 [PMID: 16247349]
51 **Zhou Y**, Cocks FH, Preminger GM, Zhong P. The effect of treatment strategy on stone comminution efficiency in shock wave lithotripsy. *J Urol* 2004; **172**: 349-354 [PMID: 15201809]
52 **McAteer JA**, Evan AP, Williams JC, Lingeman JE. Treatment protocols to reduce renal injury during shock wave lithotripsy. *Curr Opin Urol* 2009; **19**: 192-195 [PMID: 19195131 DOI: 10.1097/MOU.0b013e32831e16e3]
53 **Connors BA**, Evan AP, Blomgren PM, Handa RK, Willis LR, Gao S. Effect of initial shock wave voltage on shock wave lithotripsy-induced lesion size during step-wise voltage ramping. *BJU Int* 2009; **103**: 104-107 [PMID: 18680494 DOI: 10.1111/j.1464-410X.2008.07922.x]
54 **Willis LR**, Evan AP, Connors BA, Handa RK, Blomgren PM, Lingeman JE. Prevention of lithotripsy-induced renal injury by pretreating kidneys with low-energy shock waves. *J Am Soc Nephrol* 2006; **17**: 663-673 [PMID: 16452495 DOI: 10.1681/ASN.2005060634]

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Primary and secondary hyperoxaluria: Understanding the enigma

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Abstract

Hyperoxaluria is characterized by an increased urinary excretion of oxalate. Primary and secondary hyperoxaluria are two distinct clinical expressions of hyperoxaluria. Primary hyperoxaluria is an inherited error of metabolism due to defective enzyme activity. In contrast, secondary hyperoxaluria is caused by increased dietary ingestion of oxalate, precursors of oxalate or alteration in intestinal microflora. The disease spectrum extends from recurrent kidney stones, nephrocalcinosis and urinary tract infections to chronic kidney disease and end stage renal disease. When calcium oxalate burden exceeds the renal excretory ability, calcium oxalate starts to deposit in various organ systems in a process called systemic oxalosis. Increased urinary oxalate levels help to make the diagnosis while plasma oxalate levels are likely to be more accurate when patients develop chronic kidney disease. Definitive diagnosis of primary hyperoxaluria is achieved by genetic studies and if genetic studies prove inconclusive, liver biopsy is undertaken to establish diagnosis. Diagnostic clues pointing towards secondary hyperoxaluria are a supportive dietary history and tests to detect increased intestinal absorption of oxalate. Conservative treatment for both types of hyperoxaluria includes vigorous hydration and crystallization inhibitors to decrease calcium oxalate precipitation. Pyridoxine is also found to be helpful in approximately 30% patients with primary hyperoxaluria type 1. Liver-kidney and isolated kidney transplantation are the treatment of choice in primary hyperoxaluria type 1 and type 2 respectively. Data is scarce on role of transplantation in primary hyperoxaluria type 3 where there are no reports of end stage renal disease so far. There are ongoing investigations into newer modalities of diagnosis and treatment of hyperoxaluria. Clinical differentiation between primary and secondary hyperoxaluria and further between the types of primary hyperoxaluria is very important because of implications in treatment and diagnosis. Hyperoxaluria continues to be a challenging disease and a high index of clinical suspicion is often the first step on the path to accurate diagnosis and management.

Key words: Primary hyperoxaluria; Transplantation; Renal stones; Secondary hyperoxaluria; Renal failure

Core tip: Hyperoxaluria is a disorder characterized by increased urinary oxalate excretion. Primary hyperoxaluria is an inherited defect of oxalate metabolism while secondary hyperoxaluria is seen in states of increased ingestion of oxalate, its precursors or altered gut flora. These disorders can lead to recurrent renal stones, nephrocalcinosis and eventually end stage renal disease. Despite these common features, the sub types of hyperoxaluria differ in their pathogenesis, severity of clinical presentation and treatment plan. Prompt clinical recognition and distinction between these disorders is essential not only for timely intervention but also impacts prognosis in patients with hyperoxaluria.

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INTRODUCTION

Oxalate is the ionic form of oxalic acid and is derived from various animal and plant sources. Oxalate is excreted mainly through the kidneys. Hyperoxaluria is a state of disordered metabolism characterized by an increased urinary excretion of oxalate. The normal daily oxalate excretion in healthy individuals ranges between 10-40 mg per 24 h. Concentrations exceeding 40-45 mg per 24 h are considered as clinical hyperoxaluria[1-3]. This may result from increased endogenous production of oxalate in primary hyperoxaluria (PH) or from increased intestinal absorption or increased intake of oxalate precursors in secondary hyperoxaluria (SH).

Hyperoxaluria has the potential to cause devastating consequences which can present as early as infancy or in the sixth decade of life and if not addressed appropriately, can cause significant morbidity and mortality including End Stage Renal Disease (ESRD)[4]. Elevated plasma oxalate levels lead to oxalate deposition in various organ systems. Systemic oxalosis should be prevented but the diagnosis is often delayed in more than 40% of patients. In a survey by Hoppe *et al*[5], 30% of the patients were diagnosed only when they had already reached ESRD. In some cases, the diagnosis may first be made when the disease recurs following renal transplant[6]. Hyperoxaluria continues to be a challenging disease and appropriate treatment requires a high index of suspicion and a timely diagnosis.

This review highlights the mechanisms underlying both primary and secondary hyperoxaluria, clinical manifestations, important elements in screening and diagnosis, and our current knowledge of modalities of treatment.

SOURCES OF OXALATE

Oxalate is obtained from exogenous sources as well as endogenous synthesis. Oxalate is abundantly found in plant and animal sources. Dietary sources richest in oxalate include nuts, plums, chocolate, beetroot, strawberries, rhubarb, tofu and spinach[1,7]. Juicing is a recent popular trend where a diet based mainly on fruits and vegetable juices is consumed and may supply a very high amount of daily oxalate[8,9]. Studies have demonstrated that as the dietary intake of oxalate increases, so does the urinary concentration of oxalate[10]. Endogenous synthesis of oxalate occurs in the liver[11] through a pathway that generates glyoxalate as an intermediate molecule[12]. Glyoxalate is synthesized from oxidation of glycolate through enzymatic action of glycolate oxidase or from metabolism of hydroxyproline which is found in collagen or dietary sources. Increased glyoxalate is converted to oxalate by action of lactate dehydrogenase in the absence of enzymatic activity as is seen in the various types of PH[12,13]. This pathway is depicted in Figure 1.

RENAL HANDLING OF OXALATE

Renal oxalate handling comprises glomerular filtration, tubular secretion and tubular reabsorption[14,15]. Glomerular filtration depends on the plasma oxalate levels while tubular transport is mediated by SLC26 family of transport proteins. SLC26A1 mediates oxalate uptake into the cell across the basolateral membrane in exchange for sulfate[16,17]. On the apical side of the tubular cells, SLC26A6 is the dominant chloride-oxalate exchanger which promotes chloride reabsorption in exchange for oxalate secretion and has been implicated in the development of renal stones. This exchanger also mediates intestinal secretion of oxalate and loss of this exchanger has been shown to promote increased intestinal absorption of oxalate in the small intestine[18,19]. In rat kidney, tubular reabsorption has been demonstrated in the S1 and S2 segments of the proximal tubule[14] which may help decrease the tendency for calcium oxalate supersaturation in the earlier parts of the nephron[3].

Overall, the contribution of tubular secretion in addition to glomerular filtration is critical in regulating plasma oxalate levels as a strong correlation has been demonstrated between high plasma oxalate levels and oxalate secretion[20]. It has also been noted that tubular oxalate secretion is increased in PH patients possibly in an attempt to mitigate the life threatening consequences of systemic oxalosis[21]. Increased tubular secretion has also been noted in patients with hyperoxaluria following intestinal bypass[22].

GENETIC AND BIOCHEMICAL BASIS OF DISEASE

Primary hyperoxaluria

Primary hyperoxaluria type 1 (PH1) is the most common and severe form of PH. It accounts for approximately 80% of the cases of PH and is caused by defect in the

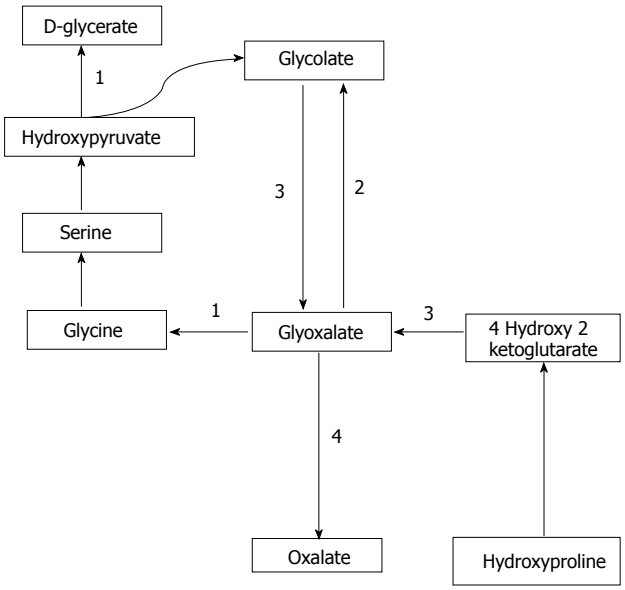

Figure 1 Pathway of oxalate synthesis and enzymatic defects in PH. A: PH1, alanine glyoxalate aminotransferase; B: PH 2, glycolate reductase hydroxy pyruvate reductase; C: PH 3, 4-hydroxy 2-ketoglutarate aldolase; D: Lactate dehydrogenase.

Vitamin B6 dependent hepatic peroxisomal enzyme, Alanine Glyoxalate Aminotransferase (AGT). This enzyme catalyzes the transamination of L-alanine and glyoxalate to pyruvate and glycine. The enzyme defect has been attributed to a mutation in the *AGXT* gene located on chromosome 2[23,24].

Primary hyperoxaluria type 2 (PH2) represents about 10% of the patients with PH. Dysfunction of the enzyme glyoxalate/hydroxypyruvate reductase (GRHPR) occurs secondary to a mutation in the *GRHPR* gene located on chromosome 10[25-27]. Consequently, there is increased urinary excretion of L-glyceric acid and oxalate.

Primary hyperoxaluria type 3 (PH 3) is a recently described entity and it occurs in 10% PH cases. The genetic defect in PH3 has been localized to the *HOGA1* gene located on chromosome 9 which codes for the mitochondrial 4-hydroxy 2-oxoglutarate aldolase[28]. This enzyme breaks down 4-hydroxy 2-oxoglutarate into pyruvate and glyoxalate which in turn is converted into oxalate.

SECONDARY HYPEROXALURIA

The causes of SH are increase in dietary and intestinal absorption (enteric hyperoxaluria), excessive intake of oxalate precursors and alteration in intestinal microflora.

Increased dietary intake of oxalate

Oxalate rich dietary sources include rhubarb and spinach and daily intake may be in excess of 1000 mg/d[29]. Increased dietary absorption may occur in "juicing" which is being propagated as a health fad for clearing toxins from the body and also for weight loss. Previously dietary oxalate was thought to make only a minimal (10%-20%) contribution to the amount of oxalate excreted in urine but studies have shown that this is not correct. In a study by Holmes *et al*[10], dietary intake contributed to about 50% of the oxalate secretion proving that dietary ingestion is an important determinant in total oxalate excretion. Bioavailability of oxalate from food and, thus, urinary oxalate, is also influenced by the forms of oxalate in the food, techniques of food processing and cooking and other constituents in the meal[30]. Dietary ingestion of oxalate is reduced by concurrent ingestion of calcium or magnesium which complex with oxalate and form insoluble salts[10,31].

Hyperoxaluria associated with fat malabsorption

Fat malabsorption increases the intestinal absorption of oxalate due to increased intestinal permeability to oxalate and formation of calcium and fatty acid complexes leading to increased amounts of soluble oxalate. An intact colon is required for increased oxalate absorption *via* this mechanism[32]. This form of hyperoxaluria is seen in partial gastrectomy, bariatric surgery, jejunoileal bypass, and inflammatory bowel disease[7,33].

Role of oxalobacter formigenes

Oxalobacter formigenes (*O. formigenes*) is an aerobic gram negative bacterium that uses oxalate as its energy source and decreases intestinal absorption of oxalate and thus reduces urinary oxalate excretion[34,35]. This has been well documented in both human and animal experiments[36,37]. Loss of this bacterium occurs after the use of antibiotics[38] and its restoration may have a role in treatment of hyperoxaluria.

Excess intake of oxalate precursors

Ascorbic acid (Vitamin C) is a precursor of oxalate and intake of excessive quantities of vitamin C may result in precipitation of calcium oxalate[39,40]. Oxalate is a product of ethylene glycol causing calcium oxalate deposition and renal failure[41,42]. Hyperoxaluria has also been reported following renal transplantation due to mobilization of oxalate and deposition within the renal allograft[43]. Increased intestinal absorption of oxalate and tubular secretion has also been reported in patients with cystic fibrosis leading to hyperoxaluria[3,44,45].

"Juicing" deserves a special mention as it supplies a high amount of daily oxalate. The increased amount of fluid intake in the juices increases the paracellular absorption of oxalate in the intestines. This may overwhelm the ability of the kidney to excrete the increased dietary load especially in patients with chronic kidney disease. Oxalate is ingested in the fruits and vegetables used to make the juices such as kiwi, spinach and beetroot. Low calcium intake and ingestion of excess of vitamin C is also noted which

together with the oxalate intake heighten the risk of acute kidney injury[8,9].

CLINICAL PRESENTATION

The prevalence of PH1 is approximately 1-3 cases per million population[46,47]. At least 1% of the ESRD seen in the pediatric population is attributable to PH1 in European and Japanese studies[48,49]. It is more frequently seen in Kuwaiti and Tunisian populations where consanguineous marriages are practiced[50,51]. PH1 is the most severe type of PH although there is significant variability in its clinical presentation. Patients may present early in life during infancy with life threatening oxalosis and failure to thrive or in adulthood after passing an occasional stone. Overall, the disease is characterized by recurrent nephrolithiasis and progressive nephrocalcinosis leading to renal damage and as a result, the majority of the patients reach ESRD during 3rd-5th decade of life[52,53].

PH2 is a less aggressive form of PH with better preservation of renal function and lower incidence of end stage renal disease and less severe nephrocalcinosis compared to PH1. The differences are accounted for by the higher oxalate excretion in PH1 and altered urine composition with reduced urinary levels of citrate and magnesium in PH1 compared to PH2[54].

PH3 generally presents with recurrent nephrolithiasis in the early decades of life. It is also characterized by the increase in urinary calcium levels and genetic defects in the *HOGA1* gene have also been implicated in cases of idiopathic calcium oxalate urolithiasis[55]. The disease course is more benign compared to other forms and although limited clinical data is available, no cases of ESRD have been reported to date with PH3[56,57].

Patients with secondary hyperoxaluria have a predisposition to developing recurrent calcium oxalate stones due to the underlying disorder. This leads to worsening renal damage and progression to ESRD. Systemic oxalosis is less common in secondary hyperoxaluria but reported in some severe cases of Crohn's disease[58].

SYSTEMIC OXALOSIS

Calcium oxalate salts are poorly soluble in body fluids. Calcium oxalate deposits within renal tissue as nephrocalcinosis and also forms renal stones (nephrolithiasis). This leads to progressive renal injury and inflammation and tubular obstruction leading to interstitial fibrosis, declining renal function and eventually ESRD[52,59].

When glomerular filtration rate (GFR) drops below 30-40 mL/min per 1.73 m^2, renal capacity to excrete calcium oxalate is significantly impaired. At this stage, calcium oxalate starts to deposit in extra renal tissues in a process called systemic oxalosis. Calcium oxalate deposits have been reported in the myocardium, cardiac conduction system, kidneys, bones and bone marrow. This leads to cardiomyopathy, heart block and other cardiac conduction defects, vascular disease, retinopathy, synovitis, oxalate osteopathy and anemia that is noted to be resistant to treatment[52,60,61].

SCREENING FOR HYPEROXALURIA

Screening for hyperoxaluria must be undertaken in every child with the first episode of renal stone and all adults who present with recurrent calcium oxalate stones. Screening should also be done at first presentation of nephrocalcinosis or family history of stone disease at any age. Furthermore, screening must be offered to relatives of an index case. PH1 should be strongly considered in the differential in any patient with renal failure of unknown etiology, particularly when there is nephrocalcinosis with reduced renal function or a high occurrence of renal stones. Presence of monohydrate calcium oxalate crystals in biological fluids or tissues is also a strong pointer towards primary hyperoxaluria and should be followed up with additional testing[62].

DIAGNOSIS

Diagnosis of hyperoxaluria is established using a combination of clinical, radiological, biochemical, histopathological and genetic studies in primary hyperoxaluria. Precise diagnosis is of paramount importance for prognostic and treatment implications and also for prenatal screening in appropriate cases where PH is suspected.

In patients with a clinical suspicion for hyperoxaluria, the diagnostic workup should begin with ultrasound or other radiological imaging of the kidneys and the rest of the urinary tract to confirm the presence of nephrocalcinosis and urolithiasis[2,53]. Stone analysis should be done and may yield the initial diagnostic clues for PH. Stones in PH are composed of monohydrate calcium oxalate (whewellite) which assume a dumbbell shaped form[63].

The initial biochemical tests include urinary oxalate excretion preferably measured in 24 h urine collection and adjustment of the oxalate excretion per 1.73 m^2 of the body surface area is recommended[2]. Urinary oxalate: urinary creatinine ratios can be used but age specific normal values must be known. These values however should be interpreted with caution as the ratios decline in early life and are also subject to variability based on nutritional intake. Oxaluria must be confirmed using two urine samples. PH is characterized by urinary oxalate excretion > 1.0 mmol/1.73 m^2 per 24 h in majority and in some cases may exceed 2.0 mmol/1.73 m^2/ 24 h in contrast to the normal urinary excretion which is typically < 0.45 mmol/1.73 m^2 per 24 h. In patients with hyperoxaluria > 0.8 mmol/1.73 m^2 per 24 h, urinary glycolate and glycerate levels should be measured. About two thirds of PH1 patients have elevated urinary glycolate levels but it is important to remember that normal glycolate levels do not exclude

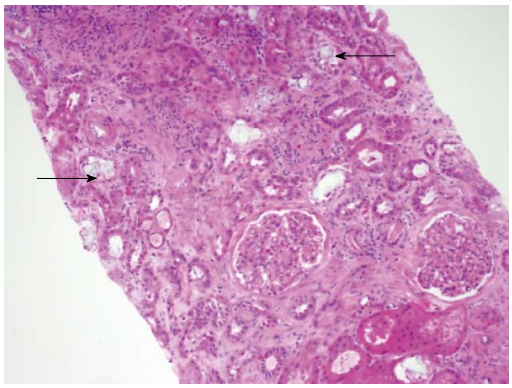

Figure 2 Calcium oxalate deposition in the renal tubules (black arrows).

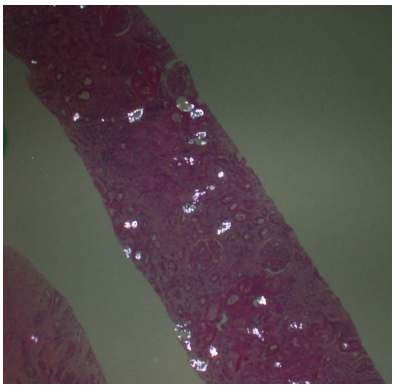

Figure 3 Examination of renal biopsy specimen under polarized light. Calcium oxalate crystals depict a characteristic birefringence.

the diagnosis. Urinary glycerate levels are noted to be high in PH2 patients[2,53].

As GFR declines, urinary excretion of oxalate decreases and the urinary oxalate estimation may no longer be accurate. Plasma oxalate should be measured in these circumstances. In PH patients with ESRD, plasma oxalate levels are typically higher than 80 μmol/L while in non PH hyperoxaluric patients, the plasma oxalate level may range between 30-80 μmol/L[64-66]. This is in contrast to plasma oxalate levels of 1-5 μmol/L in normal subjects[1].

Non-invasive, definitive diagnosis of PH is provided by testing of *AGXT*, *GRHPR* and *HOGA1* genes. There are 150 known mutations for *AGXT*[67], 16 for *GRHP*[26] and 15 for *HOGA1*[28,55-57,68]. Williams *et al*[69] showed that targeted analysis of the three most common mutations in *AGXT* (c.33_34insC, c.508G>A, and c.731T>C) provides the diagnosis in 34.5% PH1 patients while exon sequencing of exon 1, 4 and 7 increases the yield and allows diagnosis in 50% PH1 patients. Prenatal diagnosis can be done by testing chorionic villi. In patients with one or no known mutation, intragenic and extragenic linkage analysis is recommended for diagnosis[70,71] .When DNA screening is non diagnostic but clinical suspicion is high, liver biopsy is undertaken for establishing the diagnosis. However, this is an invasive method and carries a high risk of complications like bleeding[53].

In SH, stones are usually mixed (whewellite and weddellite) in contrast to PH. The excretion of urinary oxalate is increased in SH and may be > 0.7 mmol/1.73 m^2 per 24 h but in some cases may exceed 1.0 mmol/1.73 m^2 per 24 h[2,72,73]. Other available diagnostic tests include use of PCR in stool samples to identify *oxalobacter formigenes*[74,75]. Also, Increased intestinal oxalate absorption can be assessed by an absorption test using (^{13}C2) oxalate[76]. This test can help identify hyperabsorbers who would benefit from dietary interventions focusing on lowering oxalate and increasing calcium in the diet. This diagnostic test also helps to differentiate between primary and secondary forms of hyperoxaluria[33].

Radiological imaging may aid in diagnosis of multisystem involvement. Renal involvement, apart from urolithiasis, may show two distinct patterns: medullary nephrocalcinosis which is evaluated well on ultrasound while CT scan is a better modality for diagnosis of cortical nephrocalcinosis. CT may also be helpful in detection of calcium oxalate deposition in various other organ systems like bowel wall, muscle and arteries. The effects on the heart can be evaluated by electrocardiography and echocardiography. Skin biopsy may be necessary for skin lesions secondary to calcium oxalate deposition which can resemble the lesions of calciphylaxis[62]. On histopathological examination, calcium oxalate crystals demonstrate a characteristic birefringence when examined under polarized light. Figures 2 and 3 demonstrate calcium oxalate deposition in renal tissue.

TREATMENT

Conservative measures

Conservative measures are recommended soon after the diagnosis is made. High fluid intake is vital in preventing stone formation[77]. Patients with hyperoxaluria should be advised to increase their fluid intake to 3-4 L/d[53,60]. In infants and children, a gastrostomy tube may have to be placed to achieve this and special attention should be given to fluid intake in states of fluids losses like vomiting and diarrhea[57,62].

Dietary interventions do not play a major role in the management of primary hyperoxaluria as absorption of oxalate from the intestine is very small. In a study by Sikora *et al*[78], intestinal absorption of oxalate in patients with PH was noted to be less than 7%. This was attributed to less absorption and translocation of the SLC26A6 transporters favoring oxalate secretion over absorption. On the other hand, diet modification is a very important element in the treatment of secondary hyperoxaluria where efforts should be made to reduce oxalate intake in the diet. Calcium intake should not be restricted as it complexes with oxalate and prevents its absorption[10]. However, excessive intake of Vitamin C should be avoided.

Role of pyridoxine

Pyridoxine supplementation has been shown to be

beneficial in patients with PH1. Pyridoxine functions as a cofactor for the enzyme AGT which is defective in PH1. Administration of supraphysiological doses of pyridoxine may stabilize this enzyme and also enhance its enzymatic activity[57]. The recommended initial dose of pyridoxine is 5 mg/kg with a maximum dose of 20 mg/kg[79]. Pyridoxine has been demonstrated to be effective in only 30% of the patients[80,81] and therapeutic success is noted by a approximately 30% reduction in urine oxalate excretion after 3 mo of pyridoxine supplementation at the maximal dose[53,60]. Certain genotypes (508G>A (Gly170Arg) and 454T>A (Phe153Ile) are known to be more responsive to pyridoxine treatment than others[82,83] although pyridoxine therapy should be tested in all patients with PH1. Early initiation of pyridoxine treatment and compliance with the treatment regimen in pyridoxine responsive patients may help to prevent renal failure in PH1[57].

Urinary alkalinization

Alkalinization of the urine is well known to prevent stone formation as citrate complexes with calcium and thus decreases the amount of calcium oxalate available for precipitation. This same principle can be used in patients with hyperoxaluria. Potassium citrate can be used at a dose of 0.1-0.15 g/kg body weight[84]. Urinary pH must be maintained between 6.2 and 6.8[7]. In patients with renal failure, potassium salt can be replaced by sodium citrate[85]. Other inhibitors of crystallization are orthophosphate[86] and magnesium[7] though there is no conclusive evidence that magnesium therapy alone inhibits stone formation.

Probiotics (O. formigenes)

Despite our knowledge of *O. formigenes* and its use of oxalate as an energy source, the use of probiotics to reduce urinary oxalate excretion has not been demonstrated in human studies[57,87]. The results in animal studies however have been encouraging[88,89].

Management of renal stones

For management of renal stones, endoscopy is currently the procedure of choice as it allows direct visualization of the stones. Extracorporeal shock wave lithotripsy (ESWL) has been the standard of treatment for many years. However, with use of this technique, the shock waves may be mistakenly used on areas of nephrocalcinosis instead of stones due to lack of direct visual assessment which is achieved with endoscopy[90]. Further, gravel in the urinary tract following the ESWL procedure may form a nidus for calcium oxalate deposition and recurrent stone formation in patients with hyperoxaluria. In contrast, endoscopy allows complete retrieval of stones and their fragments and yields excellent results[62].

Renal replacement therapy

Patients reaching ESRD need optimization of renal replacement therapy to ensure adequate oxalate removal. Oxalate deposition occurs when the oxalate levels reach the threshold for supersaturation which is estimated to be 30-45 μmol/L. Hemodialysis (HD) removes oxalate more efficiently than peritoneal dialysis (PD)[66]. However, there is significant oxalate rebound following hemodialysis and levels can reach 80% of the pre-hemodialysis levels[91]. The weekly removal of oxalate by hemodialysis or peritoneal dialysis has been calculated to be 6-10 mmol/1.73 m^2[92,93] which leaves patients in a positive oxalate balance and at high risk for systemic deposition. Illies *et al*[94] studied 6 patients with PH1 who were on dialysis and awaiting liver transplant. Based on their observations, they made recommendations for improvement of the dialysis prescription. Dialysis should be initiated early (around GFR of 20-30 mL/min per 1.73 m^2) before ESRD is reached. Dialysis should be done with high flux dialyzers and maximum possible blood flow rate. To improve efficiency of oxalate removal by HD, additional sessions per week are preferable as compared to more time per session. Combination of HD and PD may be used to further enhance oxalate elimination. The timing of HD and PD should be coordinated as PD may be more efficient in removing oxalate in the later phases of the interdialytic period when rebound is much higher than in the earlier interdialytic phase. Efforts should be made to keep the oxalate level below 50 μmol/L[94]. The intensification of dialysis may pose a burden on the patient and family and it is important to keep this in mind while designing an individualized dialysis plan.

Transplantation

Transplantation must be planned when GFR falls between 15-30 mL/min per 1.73 m^2. As the defective enzyme is liver specific in PH1, these patients require preemptive liver, sequential liver- kidney, or combined liver-kidney transplantation. Transplantation strategy is decided based on individual presentation and clinical course as disease expression may vary among patients with PH1. Preemptive liver transplantation can be considered in patients who have progressive renal disease and approach a GFR of 50 mL/min per 1.73 m^2. Sequential liver-kidney transplantation can be performed in children who are small for a combined liver-kidney transplant[95]. In contrast, combined liver-kidney transplant is best suited for patients who are on chronic renal replacement therapy and not responsive to pyridoxine[57]. Isolated kidney transplantation may be the procedure of choice for adult patients who are sensitive to pyridoxine[96]. However, in isolated renal transplant, allograft survival rates have been reported to be inferior in patients with primary hyperoxaluria compared to patients who received renal transplant for a non PH1 cause of ESRD[48]. Thus, caution should be exercised while advocating this approach.

For patients with PH2, isolated kidney transplantation

Table 1 Comparison between primary and secondary hyperoxaluria

Clinical feature	Primary hyperoxaluria	Secondary hyperoxaluria
Etiology	Inborn error of metabolism with specific enzymatic defects PH 1: Alanine glyoxalate aminotransferase PH 2: Glyoxalate/hydroxypyruvate reductase PH 3: 4-hydroxy 2-oxoglutarate aldolase	Increased dietary intake of oxalate or precursors Increased intestinal absorption Altered intestinal microflora
Clinical presentation	PH 1: Recurrent stones, nephrocalcinosis, ESRD common Clinical heterogeneity in presentation, varies from an infantile to an adult onset form PH 2: Recurrent stones, nephrocalcinosis less common, ESRD has been reported (approximately 20% cases) PH 3: Hypercalciuria with hyperoxaluria is reported, no reports to date of ESRD	Recurrent renal stones, nephrocalcinosis, CKD and ESRD
Systemic oxalosis	Frequent part of the presentation	Less common but may occur in severe cases of inflammatory bowel disease or short bowel syndrome
Diagnosis:		
History	Family history is often suggestive with other affected relatives	Dietary history may be an important pointer towards the diagnosis
Urinary excretion	> 1.0 mmol/1.73 m^2 BSA	Usually < 1.0 mmol/1.73 m^2 BSA but in some cases of enteric hyperoxaluria may extend into the primary range
Composition of renal stones	95% calcium oxalate monohydrate (whewellite)	Mixed stones (whewellite and weddellite)
Other diagnostic points	Plasma oxalate levels in ESRD are > 60-80 mmol/L as compared from non-PH causes of ESRD	^{14}C test can be used to assess for increased intestinal absorption
Treatment:		
General measures:	Daily fluid intake > 3.0 L/d Pyridoxine in PH1 Urinary alkalinization Thiazides for PH3 Renal replacement therapy when ESRD occurs	Hydration and urinary alkalinization Renal replacement therapy when ESRD occurs
Specific measures: Dietary management	No role as dietary absorption is < 5%	Important role as dietary absorption is > 40%
O. formigenes	No role in management	No role demonstrated in human studies
Transplantation	PH1: Liver kidney transplant (combined or sequential) Isolated kidney transplant in pyridoxine sensitive adult patients PH2: Isolated kidney transplant PH3: No role of kidney transplant	Limited data available regarding transplants for treatment of SH

PH: Primary hyperoxaluria; SH: Secondary hyperoxaluria; CKD: Chronic kidney disease; ESRD: End stage renal disease; BSA: Body surface area.

Table 2 Additional resources for information on hyperoxaluria

Resource	Web address
Oxalosis and Hyperoxaluria Foundation	http://www.ohf.org/
Rare Disease Initiative of the Renal Association	http://rarerenal.org/
Rare Diseases Clinical Research Network (links to the Rare Kidney Stone Consortium)	http://www.rarediseasesnetwork.org/
Children Living with Inherited Metabolic Diseases	http://www.climb.org.uk/
Genetics Home Reference	http://ghr.nlm.nih.gov/
Office of Rare Diseases Research	http://rarediseases.info.nih.gov/
National Organization for Rare Disorders	http://www.rarediseases.org/

is the preferred treatment of choice[53,57] as the defective enzyme is found in various body tissues[97]. For patients with PH3, there are no reports of ESRD to date and as a result, no recommendations for renal transplantation have been made in this subset of PH patients[57].

In secondary hyperoxaluria, there is a paucity of data regarding renal transplantation in those who develop ESRD. There is an increased risk for allograft dysfunction by the rapid release of oxalate from systemic deposits leading to recurrent nephrocalcinosis. Ceulemans *et al*[98] performed combined intestinal and kidney transplants in a patient with hyperoxaluria due to short bowel syndrome which may be a promising approach in patients with enteric hyperoxaluria but this needs to be evaluated in larger studies. The differences between primary and secondary hyperoxaluria are depicted in Table 1.

Future directions

Gene therapy, chaperone treatment, liver cell transplantation and proteomic analysis of urine for diagnosis are amongst the new approaches being evaluated for

management of patients with primary hyperoxaluria[7,57,62].

Additional resources

There are numerous online resources for physicians and patients to obtain more information about hyperoxaluria. The resources and their web address are outlined in Table 2.

REFERENCES

1 **Asplin JR**. Hyperoxaluric calcium nephrolithiasis. *Endocrinol Metab Clin North Am* 2002; **31**: 927-949 [PMID: 12474639 DOI: 10.1016/S0889-8529(02)00030-0]

2 **Milliner DS**. The primary hyperoxalurias: an algorithm for diagnosis. *Am J Nephrol* 2005; **25**: 154-160 [PMID: 15855742 DOI: 10.1159/000085407]

3 **Robijn S**, Hoppe B, Vervaet BA, D'Haese PC, Verhulst A. Hyperoxaluria: a gut-kidney axis? *Kidney Int* 2011; **80**: 1146-1158 [PMID: 21866092 DOI: 10.1038/ki.2011.287]

4 **Arena R**, Cahalin LP. Evaluation of cardiorespiratory fitness and respiratory muscle function in the obese population. *Prog Cardiovasc Dis* 2014; **56**: 457-464 [PMID: 24438738 DOI: 10.1016/j.pcad.2013.08.001]

5 **Hoppe B**, Langman CB. A United States survey on diagnosis, treatment, and outcome of primary hyperoxaluria. *Pediatr Nephrol* 2003; **18**: 986-991 [PMID: 12920626 DOI: 10.1007/s00467-003-1234-x]

6 **Spasovski G**, Beck BB, Blau N, Hoppe B, Tasic V. Late diagnosis of primary hyperoxaluria after failed kidney transplantation. *Int Urol Nephrol* 2010; **42**: 825-829 [PMID: 20020206 DOI: 10.1007/s11255-009-9690-2]

7 **Lorenzo V**, Torres A, Salido E. Primary hyperoxaluria. *Nefrologia* 2014; **34**: 398-412 [PMID: 24798559 DOI: 10.3265/Nefrologia.pre2014.Jan.12335]

8 **Lien YH**. Juicing is not all juicy. *Am J Med* 2013; **126**: 755-756 [PMID: 23968899 DOI: 10.1016/j.amjmed.2013.04.007]

9 **Getting JE**, Gregoire JR, Phul A, Kasten MJ. Oxalate nephropathy due to 'juicing': case report and review. *Am J Med* 2013; **126**: 768-772 [PMID: 23830537 DOI: 10.1016/j.amjmed.2013.03.019]

10 **Holmes RP**, Goodman HO, Assimos DG. Contribution of dietary oxalate to urinary oxalate excretion. *Kidney Int* 2001; **59**: 270-276 [PMID: 11135080 DOI: 10.1046/j.1523-1755.2001.00488.x]

11 **Farinelli MP**, Richardson KE. Oxalate synthesis from [14C1]glycollate and [14C1]glyoxylate in the hepatectomized rat. *Biochim Biophys Acta* 1983; **757**: 8-14 [PMID: 6838909 DOI: 10.1016/0304-4165(83)90146-0]

12 **Holmes RP**, Assimos DG. Glyoxylate synthesis, and its modulation and influence on oxalate synthesis. *J Urol* 1998; **160**: 1617-1624 [PMID: 9783918 DOI: 10.1016/S0022-5347(01)62363-2]

13 **Marengo SR**, Romani AM. Oxalate in renal stone disease: the terminal metabolite that just won't go away. *Nat Clin Pract Nephrol* 2008; **4**: 368-377 [PMID: 18523430 DOI: 10.1038/ncpneph0845]

14 **Knight TF**, Sansom SC, Senekjian HO, Weinman EJ. Oxalate secretion in the rat proximal tubule. *Am J Physiol* 1981; **240**: F295-F298 [PMID: 7223887]

15 **Osswald H**, Hautmann R. Renal elimination kinetics and plasma half-life of oxalate in man. *Urol Int* 1979; **34**: 440-450 [PMID: 494445 DOI: 10.1159/000280294]

16 **Mount DB**, Romero MF. The SLC26 gene family of multifunctional anion exchangers. *Pflugers Arch* 2004; **447**: 710-721 [PMID: 12759755 DOI: 10.1007/s00424-003-1090-3]

17 **Sindić A**, Chang MH, Mount DB, Romero MF. Renal physiology of SLC26 anion exchangers. *Curr Opin Nephrol Hypertens* 2007; **16**: 484-490 [PMID: 17693766 DOI: 10.1097/MNH.0b013e3282e7d7d0]

18 **Jiang Z**, Asplin JR, Evan AP, Rajendran VM, Velazquez H, Nottoli TP, Binder HJ, Aronson PS. Calcium oxalate urolithiasis in mice lacking anion transporter Slc26a6. *Nat Genet* 2006; **38**: 474-478 [PMID: 16532010 DOI: 10.1038/ng1762]

19 **Freel RW**, Hatch M, Green M, Soleimani M. Ileal oxalate absorption and urinary oxalate excretion are enhanced in Slc26a6 null mice. *Am J Physiol Gastrointest Liver Physiol* 2006; **290**: G719-G728 [PMID: 16373425 DOI: 10.1152/ajpgi.00481.2005]

20 **Bergsland KJ**, Zisman AL, Asplin JR, Worcester EM, Coe FL. Evidence for net renal tubule oxalate secretion in patients with calcium kidney stones. *Am J Physiol Renal Physiol* 2011; **300**: F311-F318 [PMID: 21123489 DOI: 10.1152/ajprenal.00411.2010]

21 **Kasidas GP**, Nemat S, Rose GA. Plasma oxalate and creatinine and oxalate/creatinine clearance ratios in normal subjects and in primary hyperoxaluria. Evidence for renal hyperoxaluria. *Clin Chim Acta* 1990; **191**: 67-77 [PMID: 2073734]

22 **Lindsjö M**, Fellström B, Danielson BG, Kasidas GP, Rose GA, Ljunghall S. Hyperoxaluria or hypercalciuria in nephrolithiasis: the importance of renal tubular functions. *Eur J Clin Invest* 1990; **20**: 546-554 [PMID: 2124987 DOI: 10.1111/j.1365-2362.1990.tb01900.x]

23 **Danpure CJ**, Jennings PR. Peroxisomal alanine: glyoxylate aminotransferase deficiency in primary hyperoxaluria type I. *FEBS Lett* 1986; **201**: 20-24 [PMID: 3709805 DOI: 10.1016/0014-5793(86)80563-4]

24 **Purdue PE**, Takada Y, Danpure CJ. Identification of mutations associated with peroxisome-to-mitochondrion mistargeting of alanine/glyoxylate aminotransferase in primary hyperoxaluria type 1. *J Cell Biol* 1990; **111**: 2341-2351 [PMID: 1703535 DOI: 10.1083/jcb.111.6.2341]

25 **Cramer SD**, Ferree PM, Lin K, Milliner DS, Holmes RP. The gene encoding hydroxypyruvate reductase (GRHPR) is mutated in patients with primary hyperoxaluria type II. *Hum Mol Genet* 1999; **8**: 2063-2069 [PMID: 10484776 DOI: 10.1093/hmg/8.11.2063]

26 **Cregeen DP**, Williams EL, Hulton S, Rumsby G. Molecular analysis of the glyoxylate reductase (GRHPR) gene and description of mutations underlying primary hyperoxaluria type 2. *Hum Mutat* 2003; **22**: 497 [PMID: 14635115 DOI: 10.1002/humu.9200]

27 **Williams HE**, Smith LH. Hyperoxaluria in L-glyceric aciduria: possible pathogenic mechanism. *Science* 1971; **171**: 390-391 [PMID: 4321474 DOI: 10.1126/science.171.3969.390]

28 **Belostotsky R**, Seboun E, Idelson GH, Milliner DS, Becker-Cohen R, Rinat C, Monico CG, Feinstein S, Ben-Shalom E, Magen D, Weissman I, Charon C, Frishberg Y. Mutations in DHDPSL are responsible for primary hyperoxaluria type III. *Am J Hum Genet* 2010; **87**: 392-399 [PMID: 20797690 DOI: 10.1016/j.ajhg.2010.07.023]

29 **Holmes RP**, Kennedy M. Estimation of the oxalate content of foods and daily oxalate intake. *Kidney Int* 2000; **57**: 1662-1667 [PMID: 10760101 DOI: 10.1046/j.1523-1755.2000.00010.x]

30 **Massey LK**. Food oxalate: factors affecting measurement, biological variation, and bioavailability. *J Am Diet Assoc* 2007; **107**: 1191-1194; quiz 1195-1196 [PMID: 17604750 DOI: 10.1016/j.jada.2007.04.007]

31 **Lemann J**, Pleuss JA, Worcester EM, Hornick L, Schrab D, Hoffmann RG. Urinary oxalate excretion increases with body size and decreases with increasing dietary calcium intake among healthy adults. *Kidney Int* 1996; **49**: 200-208 [PMID: 8770968 DOI: 10.1038/ki.1996.27]

32 **Worcester EM**. Stones from bowel disease. *Endocrinol Metab Clin North Am* 2002; **31**: 979-999 [PMID: 12474641 DOI: 10.1016/S0889-8529(02)00035-X]

33 **Karaolanis G**, Lionaki S, Moris D, Palla VV, Vernadakis S. Secondary hyperoxaluria: a risk factor for kidney stone formation and renal failure in native kidneys and renal grafts. *Transplant Rev* (Orlando) 2014; **28**: 182-187 [PMID: 24999029 DOI: 10.1016/j.trre.2014.05.004]

34 **Mittal RD**, Kumar R. Gut-inhabiting bacterium Oxalobacter formigenes: role in calcium oxalate urolithiasis. *J Endourol* 2004; **18**: 418-424 [PMID: 15253809 DOI: 10.1089/0892779041271706]

35 **Hatch M**, Cornelius J, Allison M, Sidhu H, Peck A, Freel RW. Oxalobacter sp. reduces urinary oxalate excretion by promoting enteric oxalate secretion. *Kidney Int* 2006; **69**: 691-698 [PMID: 16518326 DOI: 10.1038/sj.ki.5000162]

36 **Hoppe B**, Beck B, Gatter N, von Unruh G, Tischer A, Hesse A, Laube N, Kaul P, Sidhu H. Oxalobacter formigenes: a potential tool

for the treatment of primary hyperoxaluria type 1. *Kidney Int* 2006; **70**: 1305-1311 [PMID: 16850020 DOI: 10.1038/sj.ki.5001707]
37 **Sidhu H**, Schmidt ME, Cornelius JG, Thamilselvan S, Khan SR, Hesse A, Peck AB. Direct correlation between hyperoxaluria/oxalate stone disease and the absence of the gastrointestinal tract-dwelling bacterium Oxalobacter formigenes: possible prevention by gut recolonization or enzyme replacement therapy. *J Am Soc Nephrol* 1999; **10** Suppl 14: S334-S340 [PMID: 10541258]
38 **Stewart CS**, Duncan SH, Cave DR. Oxalobacter formigenes and its role in oxalate metabolism in the human gut. *FEMS Microbiol Lett* 2004; **230**: 1-7 [PMID: 14734158 DOI: 10.1016/S0378-1097(03)00864-4]
39 **Canavese C**, Petrarulo M, Massarenti P, Berutti S, Fenoglio R, Pauletto D, Lanfranco G, Bergamo D, Sandri L, Marangella M. Long-term, low-dose, intravenous vitamin C leads to plasma calcium oxalate supersaturation in hemodialysis patients. *Am J Kidney Dis* 2005; **45**: 540-549 [PMID: 15754276 DOI: 10.1053/j.ajkd.2004.10.025]
40 **Nasr SH**, Kashtanova Y, Levchuk V, Markowitz GS. Secondary oxalosis due to excess vitamin C intake. *Kidney Int* 2006; **70**: 1672 [PMID: 17080154 DOI: 10.1038/sj.ki.5001724]
41 **Stapenhorst L**, Hesse A, Hoppe B. Hyperoxaluria after ethylene glycol poisoning. *Pediatr Nephrol* 2008; **23**: 2277-2279 [PMID: 18696123 DOI: 10.1007/s00467-008-0917-8]
42 **Kraut JA**, Kurtz I. Toxic alcohol ingestions: clinical features, diagnosis, and management. *Clin J Am Soc Nephrol* 2008; **3**: 208-225 [PMID: 18045860 DOI: 10.2215/CJN.03220807]
43 **Worcester EM**, Fellner SK, Nakagawa Y, Coe FL. Effect of renal transplantation on serum oxalate and urinary oxalate excretion. *Nephron* 1994; **67**: 414-418 [PMID: 7969673 DOI: 10.1159/000188014]
44 **Terribile M**, Capuano M, Cangiano G, Carnovale V, Ferrara P, Petrarulo M, Marangella M. Factors increasing the risk for stone formation in adult patients with cystic fibrosis. *Nephrol Dial Transplant* 2006; **21**: 1870-1875 [PMID: 16595587 DOI: 10.1093/ndt/gfl067]
45 **Hoppe B**, von Unruh GE, Blank G, Rietschel E, Sidhu H, Laube N, Hesse A. Absorptive hyperoxaluria leads to an increased risk for urolithiasis or nephrocalcinosis in cystic fibrosis. *Am J Kidney Dis* 2005; **46**: 440-445 [PMID: 16129205 DOI: 10.1053/j.ajkd.2005.06.003]
46 **Cochat P**, Deloraine A, Rotily M, Olive F, Liponski I, Deries N. Epidemiology of primary hyperoxaluria type 1. Société de Néphrologie and the Société de Néphrologie Pédiatrique. *Nephrol Dial Transplant* 1995; **10** Suppl 8: 3-7 [PMID: 8592622]
47 **van Woerden CS**, Groothoff JW, Wanders RJ, Davin JC, Wijburg FA. Primary hyperoxaluria type 1 in The Netherlands: prevalence and outcome. *Nephrol Dial Transplant* 2003; **18**: 273-279 [PMID: 12543880]
48 **Harambat J**, van Stralen KJ, Espinosa L, Groothoff JW, Hulton SA, Cerkauskiene R, Schaefer F, Verrina E, Jager KJ, Cochat P. Characteristics and outcomes of children with primary oxalosis requiring renal replacement therapy. *Clin J Am Soc Nephrol* 2012; **7**: 458-465 [PMID: 22223608 DOI: 10.2215/CJN.07430711]
49 **Hattori S**, Yosioka K, Honda M, Ito H. The 1998 report of the Japanese National Registry data on pediatric end-stage renal disease patients. *Pediatr Nephrol* 2002; **17**: 456-461 [PMID: 12107812 DOI: 10.1007/s00467-002-0848-8]
50 **Al-Eisa AA**, Samhan M, Naseef M. End-stage renal disease in Kuwaiti children: an 8-year experience. *Transplant Proc* 2004; **36**: 1788-1791 [PMID: 15350478 DOI: 10.1016/j.transproceed.2004.07.024]
51 **Kamoun A**, Lakhoua R. End-stage renal disease of the Tunisian child: epidemiology, etiologies, and outcome. *Pediatr Nephrol* 1996; **10**: 479-482 [PMID: 8865247 DOI: 10.1007/s004670050143]
52 **Cochat P**, Liutkus A, Fargue S, Basmaison O, Ranchin B, Rolland MO. Primary hyperoxaluria type 1: still challenging! *Pediatr Nephrol* 2006; **21**: 1075-1081 [PMID: 16810517 DOI: 10.1007/s00467-006-0124-4]
53 **Hoppe B**, Beck BB, Milliner DS. The primary hyperoxalurias. *Kidney Int* 2009; **75**: 1264-1271 [PMID: 19225556 DOI: 10.1038/ki.2009.32]
54 **Milliner DS**, Wilson DM, Smith LH. Phenotypic expression of primary hyperoxaluria: comparative features of types I and II. *Kidney Int* 2001; **59**: 31-36 [PMID: 11135054 DOI: 10.1046/j.1523-1755.2001.00462.x]
55 **Monico CG**, Rossetti S, Belostotsky R, Cogal AG, Herges RM, Seide BM, Olson JB, Bergstrahl EJ, Williams HJ, Haley WE, Frishberg Y, Milliner DS. Primary hyperoxaluria type III gene HOGA1 (formerly DHDPSL) as a possible risk factor for idiopathic calcium oxalate urolithiasis. *Clin J Am Soc Nephrol* 2011; **6**: 2289-2295 [PMID: 21896830 DOI: 10.2215/CJN.02760311]
56 **Williams EL**, Bockenhauer D, van't Hoff WG, Johri N, Laing C, Sinha MD, Unwin R, Viljoen A, Rumsby G. The enzyme 4-hydroxy-2-oxoglutarate aldolase is deficient in primary hyperoxaluria type 3. *Nephrol Dial Transplant* 2012; **27**: 3191-3195 [PMID: 22391140 DOI: 10.1093/ndt/gfs039]
57 **Hoppe B**. An update on primary hyperoxaluria. *Nat Rev Nephrol* 2012; **8**: 467-475 [PMID: 22688746 DOI: 10.1038/nrneph.2012.113]
58 **Hueppelshaeuser R**, von Unruh GE, Habbig S, Beck BB, Buderus S, Hesse A, Hoppe B. Enteric hyperoxaluria, recurrent urolithiasis, and systemic oxalosis in patients with Crohn's disease. *Pediatr Nephrol* 2012; **27**: 1103-1109 [PMID: 22366809 DOI: 10.1007/s00467-012-2126-8]
59 **Rumsby G**, Cochat P. Primary hyperoxaluria. *N Engl J Med* 2013; **369**: 2163 [PMID: 24283239 DOI: 10.1056/NEJMc1311606]
60 **Leumann E**, Hoppe B. The primary hyperoxalurias. *J Am Soc Nephrol* 2001; **12**: 1986-1993 [PMID: 11518794]
61 **Bobrowski AE**, Langman CB. The primary hyperoxalurias. *Semin Nephrol* 2008; **28**: 152-162 [PMID: 18359396 DOI: 10.1016/j.semnephrol.2008.01.008]
62 **Cochat P**, Hulton SA, Acquaviva C, Danpure CJ, Daudon M, De Marchi M, Fargue S, Groothoff J, Harambat J, Hoppe B, Jamieson NV, Kemper MJ, Mandrile G, Marangella M, Picca S, Rumsby G, Salido E, Straub M, van Woerden CS. Primary hyperoxaluria Type 1: indications for screening and guidance for diagnosis and treatment. *Nephrol Dial Transplant* 2012; **27**: 1729-1736 [PMID: 22547750 DOI: 10.1093/ndt/gfs078]
63 **Daudon M**, Jungers P, Bazin D. Peculiar morphology of stones in primary hyperoxaluria. *N Engl J Med* 2008; **359**: 100-102 [PMID: 18596285 DOI: 10.1056/NEJMc0800990]
64 **Marangella M**, Petrarulo M, Mandolfo S, Vitale C, Cosseddu D, Linari F. Plasma profiles and dialysis kinetics of oxalate in patients receiving hemodialysis. *Nephron* 1992; **60**: 74-80 [PMID: 1738418 DOI: 10.1159/000186708]
65 **Marangella M**, Petrarulo M, Vitale C, Cosseddu D, Linari F. Plasma and urine glycolate assays for differentiating the hyperoxaluria syndromes. *J Urol* 1992; **148**: 986-989 [PMID: 1507356]
66 **Hoppe B**, Kemper MJ, Bökenkamp A, Portale AA, Cohn RA, Langman CB. Plasma calcium oxalate supersaturation in children with primary hyperoxaluria and end-stage renal failure. *Kidney Int* 1999; **56**: 268-274 [PMID: 10411702 DOI: 10.1046/j.1523-1755.1999.00546.x]
67 **Williams EL**, Acquaviva C, Amoroso A, Chevalier F, Coulter-Mackie M, Monico CG, Giachino D, Owen T, Robbiano A, Salido E, Waterham H, Rumsby G. Primary hyperoxaluria type 1: update and additional mutation analysis of the AGXT gene. *Hum Mutat* 2009; **30**: 910-917 [PMID: 19479957 DOI: 10.1002/humu.21021]
68 **Riedel TJ**, Knight J, Murray MS, Milliner DS, Holmes RP, Lowther WT. 4-Hydroxy-2-oxoglutarate aldolase inactivity in primary hyperoxaluria type 3 and glyoxylate reductase inhibition. *Biochim Biophys Acta* 2012; **1822**: 1544-1552 [PMID: 22771891 DOI: 10.1016/j.bbadis.2012.06.014]
69 **Williams E**, Rumsby G. Selected exonic sequencing of the AGXT gene provides a genetic diagnosis in 50% of patients with primary hyperoxaluria type 1. *Clin Chem* 2007; **53**: 1216-1221 [PMID: 17495019 DOI: 10.1373/clinchem.2006.084434]
70 **Rumsby G**. Experience in prenatal diagnosis of primary hyperoxaluria type 1. *J Nephrol* 1998; **11** Suppl 1: 13-14 [PMID: 9604802]
71 **Rumsby G**, Uttley WS, Kirk JM. First trimester diagnosis of primary hyperoxaluria type I. *Lancet* 1994; **344**: 1018 [PMID: 7934395

DOI: 10.1016/S0140-6736(94)91675-6]
72 **Rifkin SI**. Transplantation for renal failure secondary to enteric hyperoxaluria: a case report. *J Med Case Rep* 2007; **1**: 31 [PMID: 17592638 DOI: 10.1186/1752-1947-1-31]
73 **Hoppe B**, Hesse A, Brömme S, Rietschel E, Michalk D. Urinary excretion substances in patients with cystic fibrosis: risk of urolithiasis? *Pediatr Nephrol* 1998; **12**: 275-279 [PMID: 9655356 DOI: 10.1007/s004670050452]
74 **Kwak C**, Jeong BC, Lee JH, Kim HK, Kim EC, Kim HH. Molecular identification of Oxalobacter formigenes with the polymerase chain reaction in fresh or frozen fecal samples. *BJU Int* 2001; **88**: 627-632 [PMID: 11678762 DOI: 10.1046/j.1464-4096.2001.02395.x]
75 **Kodama T**, Akakura K, Mikami K, Ito H. Detection and identification of oxalate-degrading bacteria in human feces. *Int J Urol* 2002; **9**: 392-397 [PMID: 12165021]
76 **Hesse A**, Schneeberger W, Engfeld S, Von Unruh GE, Sauerbruch T. Intestinal hyperabsorption of oxalate in calcium oxalate stone formers: application of a new test with [13C2]oxalate. *J Am Soc Nephrol* 1999; **10** Suppl 14: S329-S333 [PMID: 10541257]
77 **Borghi L**, Meschi T, Amato F, Briganti A, Novarini A, Giannini A. Urinary volume, water and recurrences in idiopathic calcium nephrolithiasis: a 5-year randomized prospective study. *J Urol* 1996; **155**: 839-843 [PMID: 8583588 DOI: 10.1016/S0022-5347(01)66321-3]
78 **Sikora P**, von Unruh GE, Beck B, Feldkötter M, Zajaczkowska M, Hesse A, Hoppe B. [13C2]oxalate absorption in children with idiopathic calcium oxalate urolithiasis or primary hyperoxaluria. *Kidney Int* 2008; **73**: 1181-1186 [PMID: 18337715 DOI: 10.1038/ki.2008.63]
79 **Hoppe B**, Latta K, von Schnakenburg C, Kemper MJ. Primary hyperoxaluria--the German experience. *Am J Nephrol* 2005; **25**: 276-281 [PMID: 15961947 DOI: 10.1159/000086358]
80 **Gibbs DA**, Watts RW. The action of pyridoxine in primary hyperoxaluria. *Clin Sci* 1970; **38**: 277-286 [PMID: 5416155]
81 **Watts RW**, Veall N, Purkiss P, Mansell MA, Haywood EF. The effect of pyridoxine on oxalate dynamics in three cases of primary hyperoxaluria (with glycollic aciduria). *Clin Sci* (Lond) 1985; **69**: 87-90 [PMID: 4064559]
82 **van Woerden CS**, Groothoff JW, Wijburg FA, Annink C, Wanders RJ, Waterham HR. Clinical implications of mutation analysis in primary hyperoxaluria type 1. *Kidney Int* 2004; **66**: 746-752 [PMID: 15253729 DOI: 10.1111/j.1523-1755.2004.00796.x]
83 **Monico CG**, Rossetti S, Olson JB, Milliner DS. Pyridoxine effect in type I primary hyperoxaluria is associated with the most common mutant allele. *Kidney Int* 2005; **67**: 1704-1709 [PMID: 15840016 DOI: 10.1111/j.1523-1755.2005.00267.x]
84 **Leumann E**, Hoppe B, Neuhaus T. Management of primary hyperoxaluria: efficacy of oral citrate administration. *Pediatr Nephrol* 1993; **7**: 207-211 [PMID: 8476722 DOI: 10.1007/BF00864405]
85 **Marangella M**, Bagnis C, Bruno M, Vitale C, Petrarulo M, Ramello A. Crystallization inhibitors in the pathophysiology and treatment of nephrolithiasis. *Urol Int* 2004; **72** Suppl 1: 6-10 [PMID: 15133325 DOI: 10.1159/000076583]
86 **Milliner DS**, Eickholt JT, Bergstralh EJ, Wilson DM, Smith LH. Results of long-term treatment with orthophosphate and pyridoxine in patients with primary hyperoxaluria. *N Engl J Med* 1994; **331**: 1553-1558 [PMID: 7969325 DOI: 10.1056/NEJM199412083312304]
87 **Hoppe B**, Groothoff JW, Hulton SA, Cochat P, Niaudet P, Kemper MJ, Deschênes G, Unwin R, Milliner D. Efficacy and safety of Oxalobacter formigenes to reduce urinary oxalate in primary hyperoxaluria. *Nephrol Dial Transplant* 2011; **26**: 3609-3615 [PMID: 21460356 DOI: 10.1093/ndt/gfr107]
88 **Grujic D**, Salido EC, Shenoy BC, Langman CB, McGrath ME, Patel RJ, Rashid A, Mandapati S, Jung CW, Margolin AL. Hyperoxaluria is reduced and nephrocalcinosis prevented with an oxalate-degrading enzyme in mice with hyperoxaluria. *Am J Nephrol* 2009; **29**: 86-93 [PMID: 18698135 DOI: 10.1159/000151395]
89 **Hatch M**, Gjymishka A, Salido EC, Allison MJ, Freel RW. Enteric oxalate elimination is induced and oxalate is normalized in a mouse model of primary hyperoxaluria following intestinal colonization with Oxalobacter. *Am J Physiol Gastrointest Liver Physiol* 2011; **300**: G461-G469 [PMID: 21163900 DOI: 10.1152/ajpgi.00434.2010]
90 **Kerbl K**, Clayman RV. Endourologic treatment of nephrocalcinosis. *Urology* 2000; **56**: 508 [PMID: 10962330 DOI: 10.1016/S0090-4295(00)00675-0]
91 **Yamauchi T**, Quillard M, Takahashi S, Nguyen-Khoa M. Oxalate removal by daily dialysis in a patient with primary hyperoxaluria type 1. *Nephrol Dial Transplant* 2001; **16**: 2407-2411 [PMID: 11733634]
92 **Hoppe B**, Graf D, Offner G, Latta K, Byrd DJ, Michalk D, Brodehl J. Oxalate elimination via hemodialysis or peritoneal dialysis in children with chronic renal failure. *Pediatr Nephrol* 1996; **10**: 488-492 [PMID: 8865249 DOI: 10.1007/s004670050145]
93 **Watts RW**, Veall N, Purkiss P. Oxalate dynamics and removal rates during haemodialysis and peritoneal dialysis in patients with primary hyperoxaluria and severe renal failure. *Clin Sci* (Lond) 1984; **66**: 591-597 [PMID: 6368103]
94 **Illies F**, Bonzel KE, Wingen AM, Latta K, Hoyer PF. Clearance and removal of oxalate in children on intensified dialysis for primary hyperoxaluria type 1. *Kidney Int* 2006; **70**: 1642-1648 [PMID: 16955107 DOI: 10.1038/sj.ki.5001806]
95 **Brinkert F**, Ganschow R, Helmke K, Harps E, Fischer L, Nashan B, Hoppe B, Kulke S, Müller-Wiefel DE, Kemper MJ. Transplantation procedures in children with primary hyperoxaluria type 1: outcome and longitudinal growth. *Transplantation* 2009; **87**: 1415-1421 [PMID: 19424045 DOI: 10.1097/TP.0b013e3181a27939]
96 **Saborio P**, Scheinman JI. Transplantation for primary hyperoxaluria in the United States. *Kidney Int* 1999; **56**: 1094-1100 [PMID: 10469379 DOI: 10.1046/j.1523-1755.1999.00619.x]
97 **Giafi CF**, Rumsby G. Primary hyperoxaluria type 2: enzymology. *J Nephrol* 1998; **11** Suppl 1: 29-31 [PMID: 9604806]
98 **Ceulemans LJ**, Nijs Y, Nuytens F, De Hertogh G, Claes K, Bammens B, Naesens M, Evenepoel P, Kuypers D, Vanrenterghem Y, Monbaliu D, Pirenne J. Combined kidney and intestinal transplantation in patients with enteric hyperoxaluria secondary to short bowel syndrome. *Am J Transplant* 2013; **13**: 1910-1914 [PMID: 23730777 DOI: 10.1111/ajt.12305]

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MINIREVIEWS

Treatment of hypogonadotropic male hypogonadism: Case-based scenarios

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Abstract

The aim of this study is to review four case-based scenarios regarding the treatment of symptomatic hypogonadism in men. The article is designed as a review of published literature. We conducted a PubMed literature search for the time period of 1989-2014, concentrating on 26 studies investigating the efficacy of various therapeutic options on semen analysis, pregnancy outcomes, time to recovery of spermatogenesis, as well as serum and intratesticular testosterone levels. Our results demonstrated that exogenous testosterone suppresses intratesticular testosterone production, which is an absolute prerequisite for normal spermatogenesis. Cessation of exogenous testosterone should be recommended for men desiring to maintain their fertility. Therapies that protect the testis involve human chorionic gonadotropin (hCG) therapy or selective estrogen receptor modulators (SERMs), but may also include low dose hCG with exogenous testosterone. Off-label use of SERMs, such as clomiphene citrate, are effective for maintaining testosterone production long-term and offer the convenience of representing a safe, oral therapy. At present, routine use of aromatase inhibitors is not recommended based on a lack of long-term data. We concluded that exogenous testosterone supplementation decreases sperm production. It was determined that clomiphene citrate is a safe and effective therapy for men who desire to maintain fertility. Although less frequently used in the general population, hCG therapy with or without testosterone supplementation represents an alternative treatment.

Key words: Hypogonadism; Selective estrogen receptor modulator; Male fertility; Clomiphene; Human chorionic gonadotropin

Core tip: Symptomatic hypogonadism is both a common and growing health issue. Our four case-based scenarios assess different treatment options for hypogonadotropic male hypogonadism such as clomiphene citrate, human chorionic gonadotropin, and anastrozole. Furthermore, we provide clinical recommendations that can help physicians when confronted with situations such as the ones presented in this article.

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INTRODUCTION

According to a recent study by Mulligan *et al*[1], symptomatic hypogonadism affects approximately 40% of men aged 45 years or older. With the maturation of the Baby Boomer population, it is anticipated that there may be a significant increase in men desiring children at an older age. Testosterone therapies have been increasingly used in aging men, as well as men of reproductive age. A study by Samplaski *et al*[2] showed that 88.4% of men were azoospermic while on exogenous testosterone. In addition, this study demonstrated that cessation of therapy led to recovery of spermatogenesis in most infertile males. More startlingly, an estimated 6.5 million men in the United States will have hypogonadism by 2025[3]. Out of concern for this growing epidemic, this article will review four case-based scenarios concerning the treatment of hypogonadism in men. These case studies will include an assessment of the efficacy of potential treatment options as well as provide clinical recommendations for physicians.

CASE STUDIES

Case 1: The infertile male who presents while on testosterone therapy

In our first case-based scenario, a 38-year-old hypogonadal male patient has severely abnormal semen analyses as a result of use of testosterone therapy. His baseline Testosterone (T) level is 260 ng/dL. This male's baseline semen analysis prior to T therapy is 50 million/mL at 60% motility and now presents with 8 million sperm/mL with motility of 40%. This patient has been on a topical T therapy for 1 year and his luteinizing hormone (LH) is 4 mIU/L and follicle-stimulating hormone (FSH) level is 4 mIU/mL. This male commonly presents with infertility after being treated with testosterone therapy for symptomatic hypogonadism. Severe oligozoospermia and azoospermia be seen. Often times, the prescribing physician has not asked the patient about interest in fertility or has expressed unawareness of the detrimental effects of testosterone therapy on spermatogenesis.

Commentary

Exogenous testosterone is detrimental for spermatogenesis: Exogenous testosterone's mechanism creates a negative feedback on the hypothalamic-pituitary axis. This effectively decreases the production of gonadotropin and gonadotropin-releasing hormone. Consequently, the secretion of FSH and LH are also inhibited. These impairments on hormones result in overall decreases in intratesticular testosterone levels (ITT) as well as testosterone production. Typically, ITT concentrations are roughly fifty to one hundred times serum levels. Exogenous testosterone treatment can suppress ITT production to such an extent that spermatogenesis can be dramatically compromised at ITT concentrations to less than 20 ng/mL[4]. ITT is an absolute requirement for normal spermatogenesis. Without ITT, one can develop azoospermia[5,6]. However, the rates of success in recovering spermatogenesis after use of exogenous T are generally quite favorable.

Contraceptive studies using testosterone demonstrate that spermatogenesis may return after cessation of testosterone therapy: In one investigation, Gu *et al*[7] from China administered 500 mg of testosterone undecanoate monthly for 30 mo. The study used a primary outcome of pregnancy rate. In more than 1500 person-years of exposure in the 24-mo efficacy phase, only nine pregnancies were reported (855 men) resulting in a failure rate of 1.1 per 100 men. Forty-three men (4.8%) did not attain azoospermia or severe oligozoospermia ($< 1 \times 10^6$ sperm/mL). One hundred and eight days was the median time to the onset of azoospermia or severe oligozoospermia. Only two participants did not return to a normal fertility range of spermatogenesis. Spermatogenesis recovered at a median time of 196 d which was calculated from the beginning of the recovery phase. Recovery of sperm concentrations to baseline values was 182 d and to normal sperm output ($> 20 \times 10^6$/mL) was 230 d. Most notably, all but 17 participants who completed the 12-mo recovery period returned to normal levels of spermatogenesis. Furthermore, 15 of the 17 patients who did not recover returned to normal reference levels after an additional 3-mo follow-up. Although this study had a follow-up period of 2.5 years, it is important to note that longer-term data are not available in the published literature.

Recovery of spermatogenesis may be prolonged for some men, and may vary based on ethnicity: In a separate investigation, Liu *et al*[8] performed an integrated, multivariate analysis of 30 studies published between 1990-2005, in which semen analyses were recorded each month until recovery to a threshold of 20 million/mL. One thousand five hundred and forty-nine healthy eugonadal men aged between 18 and 51 years were treated with either androgens or androgens plus progestagens. The strength of this large meta-analysis was > 1200 man-years of treatment and > 700 man-years of post-treatment recovery. It required median times of 3.4, 3.0 and 2.5 mo for sperm to recover to thresholds of 20, 10, and 3 million per mL, respectively. Shorter treatment duration, shorter-acting testosterone preparations, older age, higher sperm concentrations at baseline, Asian origin, faster suppression of spermatogenesis, and lower LH levels at baseline identified with higher rates of recovery. As this

contraceptive trial was performed in men of Chinese ethnicity, similar trials with men of other ethnicities might not be reliable.

It should be advised that recovery of spermatogenesis might be prolonged for a small number of men, which may be of a larger concern with advanced maternal age. This study concluded that hormonal male contraceptive regimens demonstrate complete reversibility within an anticipated time course. However, the absence of pregnancy outcome data is a noteworthy limitation of the published literature. In addition, it is critical to stress that none of the literature measures time to fecundity and pregnancy outcomes do not correlate with semen analysis data.

Low-dose human chorionic gonadotropin maintains ITT in normal men with testosterone-induced hypogonadism: Low dose human chorionic gonadotropin (hCG) with intramuscular testosterone enanthate (200 mg/wk) can also maintain ITT and serum testosterone levels[9]. Some men are reluctant to stop testosterone therapy due to the symptomatic benefit, despite understanding the fertility risk. Use of hCG with testosterone may be a viable alternative for this select group of men. The use of hCG with intramuscular testosterone was initially studied for the development of a male contraceptive agent. Coviello *et al*[9] administered low doses of hCG (0, 125, 250, or 500 IU every other day) to normal men during this 3 wk study and measured serum and ITT levels. While the administration of testosterone alone resulted in profound decreases in ITT concentrations (94% from baseline in the TE and placebo hCG group), the addition of low dose hCG resulted in maintenance of the ITT levels. Although serum T increased from baseline in all groups, ITT remained significantly higher than serum T in all four groups after treatment. Despite supraphysiologic doses of exogenous testosterone, high levels of ITT can be maintained with the low-dose hCG.

Prevention of azoospermia and maintenance of fertility in hypogonadal men on TRT with low dose hCG: Hsieh *et al*[10] also studied the effect of hCG administration with testosterone replacement therapy on spermatogenesis. In this small series, ten men received short-acting testosterone preparations in addition to low doses of hCG. The key finding of this study was that spermatogenesis was maintained. Although there was a relatively small decrease in sperm density, no men became azoospermic.

Clinical recommendation

Discontinuing testosterone therapy alone may be adequate to return spermatogenesis to baseline levels as suggested by the hormonal contraception trials. However, men studied in these contraceptive trials most commonly had normal baseline T levels and semen analyses, and may not reflect men presenting with low T or infertility. In our experience symptomatic hypogonadal men may often be reluctant as their symptoms return. These men would especially benefit from medical therapy.

Case 2: The subfertile male who has been prescribed testosterone therapy to improve sperm production and fertility

The second case-based scenario is one of a 33-year-old subfertile male who has been treated with testosterone therapy to improve fertility potential. He had a sperm concentration of 12 million sperm/mL and motility of 45% prior to T therapy. His baseline T level was 400 ng/dL. His baseline serum T level was 270 ng/dL (normal is 300-800 ng/dL). The LH level was low normal at 3 mIU/L and the serum FSH was 5 mIU/mL. indicative of mid-normal range. Upon presentation, this male has seen his physician for infertility and has been found to have impaired semen quality. His physician prescribed an intramuscular T preparation 6 mo ago, not recognized the potential for a detrimental effect on spermatogenesis. At present, his sperm density is 2 million sperm/mL and the motility is 40%. The serum T level on therapy is 600 ng/dL. Upon physical examination, the testes are normal in size and there is no sign of varicocele. Otherwise, this patient is healthy and does not use any illicit drugs. His prolactin and estradiol was normal. This gentleman has an abnormal semen analysis and low T with an unspecified cause.

Commentary

Testosterone therapy does not improve spermatogenesis and should not used by men of reproductive age: This practice is not unusual. Ko *et al*[11] conducted a recent survey of United States urologists. The survey observed that up to 25% of these urologists have used testosterone therapy in an effort to improve spermatogenesis. However, this thought process is incorrect. As discussed before, testosterone therapy results in a mechanism that impairs spermatogenesis. Furthermore, in a recent study, testosterone use has increased greatly from 2000 to 2011 in the United States, specifically since 2008. This can be attributed to novel types of testosterone therapy, a greater awareness of symptoms associated with below normal testosterone levels, and consumer marketing by pharmaceutical companies. This investigation revealed that 12.4% of men in the United States who began testosterone therapy were between 18 and 39 years of age with 74% being between 40 and 64 years of age[12]. This is of upmost concern since it suggests that testosterone is being given to men that could be in their reproductive years.

Clomiphene citrate may improve serum testosterone levels: Clomiphene citrate (CC) can be a fairly effective treatment option in increasing serum testosterone levels[13,14]. Clomiphene is a selective

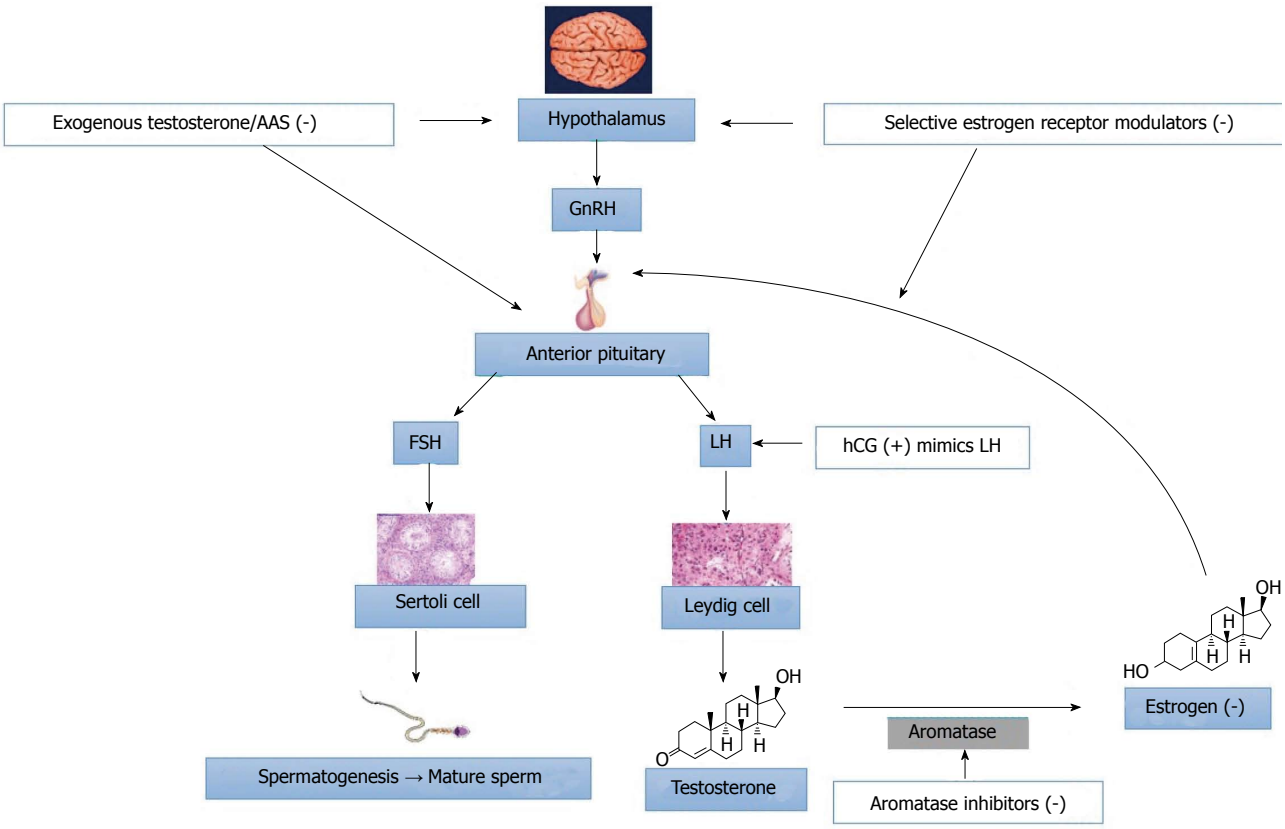

Figure 1 Hypogonadism therapeutic hormones and mechanism of action. Exogenous T and anabolic steroids negatively affect the HPG axis. SERMs such as CC stop the negative feedback of estrogen on the HPG axis. hCG stimulates Leydig cells. Aromatase inhibitors inhibit the conversion of T to E. LH: Luteinizing hormone; FSH: Follicle-stimulating hormone; AAS: Anabolic-Androgenic steroid; hCG: Human chorionic gonadotropin; GnRH: Gonadotropin-releasing hormone.

estrogen receptor modulator (SERM)[15]. This class of medications competitively binds to estrogen receptors on the hypothalamus and the pituitary gland (Figure 1). Accordingly, the pituitary gland recognizes less estrogen and creates more LH, which escalates overall testosterone production by the testes. CC is administrated orally with a common dosing that starts at 25 mg every other day with an upward titration to 50 mg daily, as needed. When LH and FSH levels are already high, it is not as effective in raising serum testosterone levels. Currently, use of hormonal dynamic testings, such as clomiphene or hCG stimulation testing, are not well-defined or commonly used. Potential side effects include gynecomastia, hypertension, cataracts, weight gain, and acne.

Clomiphene citrate as a treatment option for patients with hypogonadism: In a recent study by Da Ros *et al*[16], clomiphene citrate was tested for effectiveness in restoring endogenous testosterone production. In these trials, 125 men with an average age of 62 years were given clomiphene citrate (25 mg daily). Before treatment, all men had either below normal or low normal testosterone levels. Moreover, all patients complained about decreases in libido. The average follow up was 6 mo. Post-treatment testosterone levels increased by an average of 115%. The study concluded that clomiphene citrate should be considered as a therapy for male patients with hypogonadism.

Clomiphene citrate is an effective and less expensive treatment option: Taylor *et al*[17] conducted a study in which CC gave rise to significant increases in testosterone levels from baseline values. This was similar to increases made in testosterone gel replacement therapy (TGRT). One hundred and four men began CC (50 mg every other day) or TGRT (5 gm of 1% gel). The average follow up was 23 mo for CC or 46 mo for TGRT. Average post-treatment testosterone levels were 573 ng/dL (average baseline 277 ng/dL) in the CC group and 553 ng/dL (average baseline 221 ng/dL) in the TGRT group. The authors observed that the cost per month of CC was about $190 less than the cost of Testim® 1% (5 gm daily) at $270 and Androgel® 1% (5 gm daily) at $265. Compared with TGRT, CC demonstrates a less expensive option for men with hypogonadism, representing efficacy with minor side effects.

Long-term use of Clomiphene citrate is a safe way to improve serum testosterone levels: A similar study on clomiphene was performed by Moskovic *et al*[18] where forty-six hypogonadal males with an average age of 44 years were treated with clomiphene citrate for more than 12 mo. The main outcome measures were long-term results of clomiphene treatment on

hypogonadal males and predictors of response. Average baseline serum T levels were 228 ng/dL. Post-treatment serum T levels were 612 ng/dL, 562 ng/dL, and 582 ng/dL after 1, 2, and 3 years, respectively. Patients were also given the Androgen Deficiency in Aging Males (ADAM) questionnaire. The average pre-treatment ADAM score was 7 as compared to an ADAM score of 3 after 1 year of treatment. This investigation concluded that CC is effective as a long-term therapy for men with symptomatic hypogonadism. In addition, CC can improve many of the ADAM symptoms.

In an earlier study from the same institution, Katz *et al*[3] concluded that long-term use of CC improved serum testosterone levels to normal in a safe and effective manner. In this analysis, eighty-six men between 22 and 37 years old with hypogonadism (T levels < 300 ng/dL) were assessed and treated for an average of 19 mo. The participating men started with 25 mg of CC every other day. They were then titrated to 50 mg every other day. 550 ng/dL was the goal testosterone level. Once preferred testosterone levels were reached, testosterone/onadotropin levels were measured biannually. With regards to questions on the Androgen Deficiency in Aging Males (ADAM) questionnaire, advances were noted in each area excluding loss of height. Five of the ten variables saw significant improvement including feeling sad/grumpy, lack of energy, decreased life enjoyment, decreased libido, and decreased sports performance. This study demonstrates that CC is both an effective and safe testosterone therapy substitute in hypogonadal men.

A randomized, prospective trial of CC for men with hypogonadism with normal semen parameters is vital to confirm the recommendation for the use of SERMs for fertility preservation. It is essential that this study show that semen profiles are not negatively affected. A purified androgenic isomer of generic clomiphene is presently completing phase Ⅲ clinical trials in the United States[19]. The anticipated patient is the overweight, hypogonadal male interested in maintaining fertility potential.

Oral enclomiphene citrate initiates production of serum testosterone and sperm in men with low testosterone: Androxal®, or enclomiphene citrate, is the trans-isomer of clomiphene citrate[20]. Enclomiphene citrate is currently completing phase Ⅲ clinical trials in the United States and may in the future be another alternative treatment to testosterone therapies. In a randomized study by Kaminetsky *et al*[21], the investigators compared levels of testosterone, FSH, and LH after hypogonadal males used either oral enclomiphene citrate or testosterone gel. Twelve male subjects were assigned to either of the two treatments. At baseline, the average testosterone level for all patients was 165 ± 66 pg/dL. Treatment with both enclomiphene and testosterone gel raised serum testosterone levels back to the normal range. Both groups had about the same serum T levels after 3 mo and 6 mo. After 6 mo, serum T levels were 525 ± 256 pg/dL for enclomiphene and 545 ± 268 pg/dL for testosterone gel. The distinguishing factors between these two treatments are their FSH and LH levels as well as their sperm counts. Only enclomiphene citrate was associated with rises in FSH and LH as well as sperm counts. All of the enclomiphene citrate subjects had sperm counts above 75 million/mL, with an average sperm count of 176 million/mL. In contrast, the testosterone gel subjects did not surpass sperm counts of more than 12 million/mL. These findings were also evident throughout the follow-up period. This study suggests that enclomiphene citrate may prove to be a superior treatment as it is effective in increasing testosterone as well as sperm counts. The rise in FSH and LH levels could also point towards a shift back to normal endogenous testosterone production.

Wiehle *et al*[22] carried out another study for enclomiphene citrate. This randomized study also compared the effects of enclomiphene (Androxal®) *vs* AndroGel®, a transdermal testosterone. Enclomiphene citrate was given in three different doses: 6.25 mg, 12.5 mg and 25 mg Androxal®. Forty-four men with testosterone levels less than 350 ng/dL at baseline were included in the study. Their average age was 53 years. After six weeks of treatment, patients who took 25 mg enclomiphene had an average testosterone level of 604 ± 160 ng/dL while patients on the transdermal testosterone had an average testosterone level of 500 ± 278 ng/dL. While these results were almost equivalent, AndroGel® patients saw a decrease in FSH and LH levels whereas enclomiphene patients saw an increase. These outcomes correlate with the results of the aforementioned study. This study concluded that enclomiphene citrate was capable of increasing serum T and LH levels.

Repros Therapeutics Inc[19] observed the effect of 12 d of use of clomiphene citrate, enclomiphene, and zuclomiphene in baboons. All of the animal subjects were administered 1.5 mg of one treatment per day. Zuclomiphene did have much of a significant effect on increasing testosterone levels from baseline levels of 170 ng/dL. Enclomiphene had a much greater effect (8-fold increase to 1144 ng/dL) than clomiphene citrate (5-fold increase to 559 ng/dL). However, neither clomiphene nor enclomiphene demonstrated any effect on FSH or LH levels. This could be due to a flaw in the study.

Clinical recommendation

Similar to the first case study, testosterone (T) therapy should be stopped, and treatment with clomiphene should begin. Cessation of T therapy should be the first treatment concern for nearly all men who are interested in preserving their fertility. Longer durations of T therapy are likely to have more significant effect on the return of testosterone but undoubtedly the amount of T would be expected to have an effect on return of spermatogenesis. Clomiphene would only be expected

to benefit men with secondary hypogonadism based on its mechanism of action. It is important to assess serum LH levels prior to therapy to determine that these levels are low or normal.

Case 3: The symptomatic hypogonadal male desiring to preserve his fertility

In the third case-based scenario, a 42-year-old male patient with symptomatic hypogonadism has a desire to father children at an unspecified future time. Upon presentation, this male has symptomatic hypogonadism without a specific underlying cause. While he knows he wants to have children in the future, he does not have a clear idea regarding timeframe. He is not married and does not have any children. This male's baseline T is 220 ng/dL. His LH is 4 mIU/L and FSH level is 4 mIU/mL. Semen analysis is 26 million sperm/mL with motility of 70%. He is healthy, has a normal physical exam and is currently not on any therapy.

Commentary

Clomiphene citrate results in similar satisfaction and efficacy to testosterone therapy: There has been concern that clomiphene citrate may not result in as much symptomatic improvement compared to testosterone therapies. There are no prospective, controlled trials to confirm or refute this concern. In a recent retrospective, age-matched comparison, Ramasamy *et al*[23] assessed their results using the ADAM questionnaire and serum T levels in 31 men on topical testosterone, 31 men on injectable testosterone and 31 men on clomiphene. Clomiphene-treated men had similar total testosterone levels to topical testosterone-treated males. Men on injectable testosterone had the highest serum T levels. Similar ADAM questionnaire satisfaction was noted between treatment groups. The authors concluded that testosterone supplementation regimens and clomiphene citrate are efficacious for improving serum total testosterone. No difference in overall hypogonadal symptoms was noted among men on any testosterone supplementation therapy. Despite lower serum total testosterone, men on clomiphene citrate and testosterone gels reported satisfaction similar to that of men treated with testosterone injections.

Exogenous hCG increases serum testosterone levels thus increasing ITT concentrations: Although most men taking testosterone for contraceptive use trials recover their baseline spermatogenesis, this recovery could take up to 18 mo and may not always happen. Use of clomiphene is generally effective, but off-label. High quality safety studies of greater than 18 to 24 mo of use are lacking. Another treatment option could be hCG.

hCG is an LH analog that stimulates Leydig cell to produce more testosterone. hCG stimulates testosterone production by the Leydig cells by functioning as an LH analogue. hCG can be extracted from urine as well as other recombinant sources. Exogenous hCG increases serum testosterone levels and ITT concentrations. hCG alone can only maintain spermatogenesis for a short period of time. In a small case series directed by Depenbusch *et al*[24], thirteen azoospermic men with hypogonadotropic hypogonadism were initially administered hCG and human menopausal gonadotropin (hMG) to induce spermatogenesis. hCG was then administered 500-2500 IU hCG subcutaneously biweekly alone for up to two years (range 3-24 mo). After 12 mo of treatment, sperm counts decreased gradually but remained present in all patients, except for one who became azoospermic. The declining sperm counts demonstrate that FSH is crucial for the continuation of normal spermatogenesis.

Low levels of hCG increase ITT concentrations and serum testosterone levels: Treating patients with a high dose of hCG is not necessary with regards to the upkeep of spermatogenesis. In a study conducted by Roth *et al*[25], 37 normal patients became experimentally gonadotropin deficient through the use of GnRH antagonists. The patients were then randomized and treated with 0, 15, 60, or 125 IU SC hCG every other day or 7.5 g testosterone gel daily for a duration of 10 d. Steroid measurements at baseline and endpoint were taken after obtaining testicular fluid by percutaneous aspiration. ITT concentrations increased proportionally to the dose from 77 nmol/L to 923 nmol/L in the 0- and 125-IU treatment groups, respectively ($P < 0.001$). Furthermore, significant correlation ($P < 0.01$) existed between serum hCG and both ITT and serum T levels. The study established that low dose hCG treatment dose-dependently increases ITT concentrations in normal men brought about with gonadotropin deficiency. Even though hCG can be advantageous in increasing serum T levels, this treatment can be very costly. In addition, the fact that this medication is applied through an injection can deter potential users.

Aromatase inhibitors as a treatment option: Another therapeutic target is the aromatase enzyme, which converts testosterone to estradiol. It is mainly located in the testes, brain, adipose tissue, and liver. Estradiol inhibits the secretion of gonadotropins, which may affect the production of ITT. The purpose of aromatase inhibitors is to inhibit the process of converting androgens to estrogen, effectively increasing T, LH, and FSH levels and having effects similar to those of anti-estrogens. This type of treatment has been employed to stimulate spermatogenesis and bring about improvements to male fertility. Aromatase inhibitors may be more beneficial than antiestrogens in male patients with low serum T to estradiol ratios (< 10) and who are obese.

Typically, aromatase inhibitors have been classified into two types: steroidal or non-steroidal. Anastrozole

Table 1 Treatment options for hypogonadism in men desiring to preserve their fertility

Name of medication	Dosing and administration	Side effects	Anticipated results
Clomiphene	Oral: 25 mg every other day with max of 50 mg daily	Gynecomastia, weight gain, hypertension, cataracts, and acne	Recovery of spermatogenetic function, increases in testosterone levels
hCG	Intramuscular injection; 125-500 IU every other day	Headache, restlessness, tiredness, swelling of the ankles/feet, mental/mood changes, pain/swelling of the breast	Recovery of spermatogenetic function, increases in testosterone levels
hMG	Intramuscular injection; 75 IU three times a week	Gynecomastia, dizziness, fainting, headache, loss of appetite, irregular heartbeat	Recovery of spermatogenetic function
Anastrozole	Oral; either 0.5 or 1 mg	Blood pressure increase, anorexia, malaise, rash, peripheral edema, aches glossitis, paresthesias vomiting/nausea	Improvements in T/E2 ratio, increases in sperm concentration

IU: International units; hMG: Human menopausal gonadotropin; T/E2 ratio: Testosterone/estradiol ratio; hCG: Human chorionic gonadotropin.

and letrozole are examples of non-steroidal aromatase inhibitors. Typically, adrenal steroid supplementation is not required. Although aromatase inhibition by these two medications is almost 100%, their administration does not completely suppress estradiol levels in men and actually decreases the plasma T to estradiol ratio by 77%. This incomplete suppression may be linked to the high levels of circulating testosterone in men and may provide an advantage by limiting the adverse side effect profile.

Men with conditions including idiopathic male infertility, primarily men with lower serum testosterone to estradiol ratios (< 10), and men with hypogonadism, often related to obesity have been treated with aromatase inhibitors. They have also been used in men with Klinefelter's syndrome in order to stabilize serum T levels prior to testicular sperm extraction.

Testolactone or anastrozole may increase sperm quality and concentrations: Raman *et al*[26] conducted a study in order to detect a difference between treatment with testolactone or anastrozole. The male patients in this study had T/E2 ratios that were less than 10. The patients were treated with either testolactone or anastrozole. Both treatments resulted in significant improvements in sperm concentrations, morphology, motility, and T/E2 ratios. With regards to the small group of 25 infertile, oligospermic men treated with anastrozole, sperm concentration increased substantially from 5.5 to 15.6 million per mL, and the total motile sperm concentration per ejaculate increased from 833 to 2931 million ($P < 0.005$). The study reported no changes in the azoospermic group treated with anastrozole. In addition, pregnancy rates were not reported for any of the patients regardless of improvements in the semen parameter.

Clinical recommendation

Options for therapy include long-term CC with or without drug holidays, testosterone with low dose hCG, testosterone with or without drug holidays, or alternating combinations of the prior options (Table 1). Katz *et al*[3] conducted a study demonstrating the effectiveness of treating 86 hypogonadal men with low-dose clomiphene over the course of 19 mo. No major side effects were reported and improvements were made in more than three items on the ADAM questionnaire for 60% of the patients. Long term studies with anastrozole are lacking.

Case 4: The anabolic steroid abuser presents with symptomatic hypogonadism

Lastly, the fourth case-based scenario is centered on a 30-year-old male with symptomatic hypogonadism due to chronic anabolic steroid abuse. He completed 3 cycles of nandrolone over the last two years. These men are typically using anabolic steroids to improve muscle mass and body image. Long-term use in cycles are commonly observed. Sophisticated hormonal regimens that are self-prescribed have evolved. Severe impairments in spermatogenesis may be seen and may be permanent depending on the duration and potency of agents used. He has been off anabolic steroids for 3 mo. At present time his T level is 170 ng/dL and his LH and FSH are low at 1.5 (UNITS). His semen analysis is 1 million sperm/mL with 10% motility. Upon physical examination, the testes have mild atrophy and his overall physique is still muscular.

Commentary

Chronic anabolic steroid use is detrimental to spermatogenesis: Anabolic-androgenic steroids use is currently widespread as they are now readily available in over-the-counter medicines. According to recent evidence, illegal use of anabolic steroids may be the most prevalent cause of symptomatic hypogonadism in young men[27]. Other side effects of anabolic steroid use include hepatotoxicity, cholestasis, renal failure, gynecomastia, and infertility[28]. Furthermore, a meta-analysis study comprised of 197 studies was done on the epidemiology of anabolic steroids use[29]. The study resulted in a global lifetime prevalence rate of 3.3%. This data demonstrates the prevalence of this problem, which is connected to symptomatic hypogonadism.

Men using anabolic steroids may be deficient in ITT concentrations required to sustain spermatogenesis even though they may have serum androgen concentrations that are between normal and high. Many males who use anabolic steroids acquire hypogonadotropic hypogonadism along with an ensuing atrophy of the testes.

The user of anabolic steroids often has azoospermia or oligozoospermia as well as irregularities of sperm morphology and motility[30,31]. For men with hypogonadotropic hypogonadism from anabolic steroid abuse, administration of intramuscular injections of hCG at doses of 2000 to 3000 units 2 to 3 times per week for 4 mo can initiate spermatogenesis[32,33].

Cessation of anabolic steroid abuse may reverse suppression of spermatogenesis: Mills *et al*[34] tested the recovery of spermatogenesis after exogenous testosterone administration in 26 men with a recent history of anabolic steroid use. In this relatively small study, all men discontinued exogenous testosterone usage and began treatment with human chorionic gonadotropin (hCG) 3000 units IM every other day. The treatment lasted for at least 3 mo. In regards to the two men who remained azoospermic, one had insufficient follow-up and the other was suspected of continued anabolic steroid use. Men who were using intramuscular testosterone (hCG) at the time of presentation recovered spermatogenesis in an average of 3.1 mo. However, men receiving transdermal testosterone supplementation at the time of presentation took an average of 7.4 mo to recover. Mills *et al*[34] concluded that impairment of fertility following testosterone replacement therapy suppression is reversible and that the rate of sperm may be related to the delivery system.

Clinical recommendation

hCG with or without human menopausal gonadotropin has been used most commonly to restore fertility. hCG may be considered, but it requires frequent injections and can produce side effects (Table 1). Occasionally, hCG can exacerbate depression and irritability in hypogonadal men. Cessation of the anabolic steroids with use of clomiphene may be the most beneficial. Non-responders to hCG will require the addition of human menopausal gonadotropin with a daily injection of 75 IU[34].

CONCLUSION

Men wishing for future fertility should refrain from utilizing exogenous testosterone due to the potential for long-term detrimental effects on spermatogenesis. In a minority of cases, spermatogenesis is not recovered, although it is difficult to say whether this is due to testosterone treatment or to the natural evolution of the condition. Clomiphene citrate, an oral selective estrogen receptor modulator, is an off-label yet innocuous and potent therapy for men who wish to retain future potential fertility. hCG therapy, although less used, with or without testosterone supplementation represents an alternative treatment. Currently, it is not recommended to repeatedly use aromatase inhibitors due to a paucity of long-standing data.

REFERENCES

1 **Mulligan T**, Frick MF, Zuraw QC, Stemhagen A, McWhirter C. Prevalence of hypogonadism in males aged at least 45 years: the HIM study. *Int J Clin Pract* 2006; **60**: 762-769 [PMID: 16846397 DOI: 10.1111/j.1742-1241.2006.00992.x]

2 **Samplaski MK**, Loai Y, Wong K, Lo KC, Grober ED, Jarvi KA. Testosterone use in the male infertility population: prescribing patterns and effects on semen and hormonal parameters. *Fertil Steril* 2014; **101**: 64-69 [PMID: 24094422 DOI: 10.1016/j.fertnstert.2013.09.003]

3 **Katz DJ**, Nabulsi O, Tal R, Mulhall JP. Outcomes of clomiphene citrate treatment in young hypogonadal men. *BJU Int* 2012; **110**: 573-578 [PMID: 22044663 DOI: 10.1111/j.1464-410X.2011.10702.x]

4 **Zirkin BR**, Santulli R, Awoniyi CA, Ewing LL. Maintenance of advanced spermatogenic cells in the adult rat testis: quantitative relationship to testosterone concentration within the testis. *Endocrinology* 1989; **124**: 3043-3049 [PMID: 2498065 DOI: 10.1210/endo-124-6-3043]

5 **McLachlan RI**, O'Donnell L, Meachem SJ, Stanton PG, de K, Pratis K, Robertson DM. Hormonal regulation of spermatogenesis in primates and man: insights for development of the male hormonal contraceptive. *J Androl* 2002; **23**: 149-162 [PMID: 11868805 DOI: 10.1002/j.1939-4640.2002.tb02607.x]

6 **Weinbauer GF**, Nieschlag E. Gonadotrophin-releasing hormone analogue-induced manipulation of testicular function in the monkey. *Hum Reprod* 1993; **8** Suppl 2: 45-50 [PMID: 8276968 DOI: 10.1093/humrep/8.suppl_2.45]

7 **Gu Y**, Liang X, Wu W, Liu M, Song S, Cheng L, Bo L, Xiong C, Wang X, Liu X, Peng L, Yao K. Multicenter contraceptive efficacy trial of injectable testosterone undecanoate in Chinese men. *J Clin Endocrinol Metab* 2009; **94**: 1910-1915 [PMID: 19293262 DOI: 10.1210/jc.2008-1846]

8 **Liu PY**, Swerdloff RS, Christenson PD, Handelsman DJ, Wang C. Rate, extent, and modifiers of spermatogenic recovery after hormonal male contraception: an integrated analysis. *Lancet* 2006; **367**: 1412-1420 [PMID: 16650651 DOI: 10.1016/S0140-6736(06)68614-5]

9 **Coviello AD**, Matsumoto AM, Bremner WJ, Herbst KL, Amory JK, Anawalt BD, Sutton PR, Wright WW, Brown TR, Yan X, Zirkin BR, Jarow JP. Low-dose human chorionic gonadotropin maintains intratesticular testosterone in normal men with testosterone-induced gonadotropin suppression. *J Clin Endocrinol Metab* 2005; **90**: 2595-2602 [PMID: 15713727 DOI: 10.1210/jc.2004-0802]

10 **Hsieh TC**, Pastuszak AW, Hwang K, Lipshultz LI. Concomitant intramuscular human chorionic gonadotropin preserves spermatogenesis in men undergoing testosterone replacement therapy. *J Urol* 2013; **189**: 647-650 [PMID: 23260550 DOI: 10.1016/j.juro.2012.09.043]

11 **Ko EY**, Siddiqi K, Brannigan RE, Sabanegh ES. Empirical medical therapy for idiopathic male infertility: a survey of the American Urological Association. *J Urol* 2012; **187**: 973-978 [PMID: 22264467 DOI: 10.1016/j.juro.2011.10.137]

12 **Layton JB**, Li D, Meier CR, Sharpless JL, Stürmer T, Jick SS, Brookhart MA. Testosterone lab testing and initiation in the United Kingdom and the United States, 2000 to 2011. *J Clin Endocrinol Metab* 2014; **99**: 835-842 [PMID: 24423353 DOI: 10.1210/jc.2013-3570]

13 **Tenover JS**, Bremner WJ. The effects of normal aging on the response of the pituitary-gonadal axis to chronic clomiphene administration in men. *J Androl* 1991; **12**: 258-263 [PMID: 1917692 DOI: 10.1002/j.1939-4640.1991.tb00265.x]

14 **Guay AT**, Bansal S, Heatley GJ. Effect of raising endogenous testosterone levels in impotent men with secondary hypogonadism: double blind placebo-controlled trial with clomiphene citrate. *J Clin Endocrinol Metab* 1995; **80**: 3546-3552 [PMID: 8530597 DOI: 10.1210/jcem.80.12.8530597]

15 **Shelly W**, Draper MW, Krishnan V, Wong M, Jaffe RB. Selective estrogen receptor modulators: an update on recent clinical findings.

Obstet Gynecol Surv 2008; **63**: 163-181 [PMID: 18279543 DOI: 10.1097/OGX.0b013e31816400d7]

16 **Da Ros CT**, Averbeck MA. Twenty-five milligrams of clomiphene citrate presents positive effect on treatment of male testosterone deficiency - a prospective study. *Int Braz J Urol* 2012; **38**: 512-518 [PMID: 22951175 DOI: 10.1590/S1677-55382012000400011]

17 **Taylor F**, Levine L. Clomiphene citrate and testosterone gel replacement therapy for male hypogonadism: efficacy and treatment cost. *J Sex Med* 2010; **7**: 269-276 [PMID: 19694928 DOI: 10.1111/j.1743-6109.2009.01454.x]

18 **Moskovic DJ**, Katz DJ, Akhavan A, Park K, Mulhall JP. Clomiphene citrate is safe and effective for long-term management of hypogonadism. *BJU Int* 2012; **110**: 1524-1528 [PMID: 22458540 DOI: 10.1111/j.1464-410X.2012.10968.x]

19 **Repros Therapeutics Inc.** A Randomized, Double Blind, Placebo-Controlled, Multi-Center Phase III Study in Men With Acquired Hypogonadotropic Hypogonadism to Compare Changes in Testosterone and Sperm Concentration Following Treatment With 12.5 mg or 25 mg Androxal or AndroGel 1.62%. Bethesda (MD): National Library of Medicine (US). Available from: URL: http: //clinicaltrials.gov/show/NCT01993225 NLM Identifier: NCT01993225

20 **Kaminetsky J**, Hemani ML. Clomiphene citrate and enclomiphene for the treatment of hypogonadal androgen deficiency. *Expert Opin Investig Drugs* 2009; **18**: 1947-1955 [PMID: 19938905 DOI: 10.1517/13543780903405608]

21 **Kaminetsky J**, Werner M, Fontenot G, Wiehle RD. Oral enclomiphene citrate stimulates the endogenous production of testosterone and sperm counts in men with low testosterone: comparison with testosterone gel. *J Sex Med* 2013; **10**: 1628-1635 [PMID: 23530575 DOI: 10.1111/jsm.12116]

22 **Wiehle R,** Cunningham GR, Pitteloud N, Wike J, Hsu K, Fontenot GK, Rosner M, Dwyer A, Podolski J. Testosterone Restoration by Enclomiphene Citrate in Men with Secondary Hypogonadism: Pharmacodynamics and Pharmacokinetics. *BJU Int* 2013 Jul 12; Epub ahead of print [PMID: 23875626 DOI: 10.1111/bju.12363]

23 **Ramasamy R**, Scovell JM, Kovac JR, Lipshultz LI. Testosterone supplementation versus clomiphene citrate for hypogonadism: an age matched comparison of satisfaction and efficacy. *J Urol* 2014; **192**: 875-879 [PMID: 24657837 DOI: 10.1016/j.juro.2014.03.089]

24 **Depenbusch M**, von Eckardstein S, Simoni M, Nieschlag E. Maintenance of spermatogenesis in hypogonadotropic hypogonadal men with human chorionic gonadotropin alone. *Eur J Endocrinol* 2002; **147**: 617-624 [PMID: 12444893 DOI: 10.1530/eje.0.1470617]

25 **Roth MY**, Page ST, Lin K, Anawalt BD, Matsumoto AM, Snyder CN, Marck BT, Bremner WJ, Amory JK. Dose-dependent increase in intratesticular testosterone by very low-dose human chorionic gonadotropin in normal men with experimental gonadotropin deficiency. *J Clin Endocrinol Metab* 2010; **95**: 3806-3813 [PMID: 20484472 DOI: 10.1210/jc.2010-0360]

26 **Raman JD**, Schlegel PN. Aromatase inhibitors for male infertility. *J Urol* 2002; **167**: 624-629 [PMID: 11792932 DOI: 10.1016/S0022-5347(01)69099-2]

27 **Coward RM**, Rajanahally S, Kovac JR, Smith RP, Pastuszak AW, Lipshultz LI. Anabolic steroid induced hypogonadism in young men. *J Urol* 2013; **190**: 2200-2205 [PMID: 23764075 DOI: 10.1016/j.juro.2013.06.010]

28 **Rahnema CD**, Lipshultz LI, Crosnoe LE, Kovac JR, Kim ED. Anabolic steroid-induced hypogonadism: diagnosis and treatment. *Fertil Steril* 2014; **101**: 1271-1279 [PMID: 24636400 DOI: 10.1016/j.fertnstert.2014.02.002]

29 **Sagoe D**, Molde H, Andreassen CS, Torsheim T, Pallesen S. The global epidemiology of anabolic-androgenic steroid use: a meta-analysis and meta-regression analysis. *Ann Epidemiol* 2014; **24**: 383-398 [PMID: 24582699 DOI: 10.1016/j.annepidem.2014.01.009]

30 **Basaria S**. Androgen abuse in athletes: detection and consequences. *J Clin Endocrinol Metab* 2010; **95**: 1533-1543 [PMID: 20139230 DOI: 10.1210/jc.2009-1579]

31 **Fronczak CM**, Kim ED, Barqawi AB. The insults of illicit drug use on male fertility. *J Androl* 2012; **33**: 515-528 [PMID: 21799144 DOI: 10.2164/jandrol.110.011874]

32 **Turek PJ**, Williams RH, Gilbaugh JH, Lipshultz LI. The reversibility of anabolic steroid-induced azoospermia. *J Urol* 1995; **153**: 1628-1630 [PMID: 7714991 DOI: 10.1016/S0022-5347(01)67482-2]

33 **Menon DK**. Successful treatment of anabolic steroid-induced azoospermia with human chorionic gonadotropin and human menopausal gonadotropin. *Fertil Steril* 2003; **79** Suppl 3: 1659-1661 [PMID: 12801577 DOI: 10.1016/S0015-0282(03)00365-0]

34 **Mills JN,** Grober ED, Khera M, Fenig DM, Sathymoorthy K, Lipshultz LI. Recovery of Spermatogenesis After Exogenous Testosterone Administration. *J Urol* 2008; **179**: 656-657 [DOI: 10.1016/S0022-5347(08)61918-7]

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Is there a role for systemic targeted therapy after surgical treatment for metastases of renal cell carcinoma?

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Abstract

Metastatic renal cell carcinoma (mRCC) is a challenging disease. Despite the new targeted therapies, complete remissions occur only in 1%-3% of the cases, and the most effective first-line treatment drugs have reached a ceiling in overall survival (ranging from 9 to 49 mo). Metastasectomy remains to be the only curative option in most patients with mRCC. Prognostic nomograms have been recently published, so we have tools to classify patients in risk groups, allowing us to detect the cases with the higher risk of recurrence after metastasectomy. Although sparse, there is some evidence of effectiveness of neoadjuvant targeted therapy before metastasectomy; but with an increase in surgical complications due to the effects of these new drugs in tissue healing. We have aimed to answer the question: Is there a role for systemic targeted therapy after surgical treatment for metastases of renal cell carcinoma? We have made a search in Pubmed database. As far as we know, evidence is low and it's based in case reports and small series of patients treated with adjuvant drugs after neoadjuvant therapy plus metastasectomy in cases of partial response to initial systemic treatment. Despite the limitations and high risk of bias, promising results and cases with long-term survival with this approach have been described. Two ongoing clinical trials may answer the question that concerns us.

Key words: Metastatic renal cell carcinoma; Targeted therapy; Metastasectomy; Surgery; Adjuvant treatment

Core tip: We have made a search in Pubmed database looking for evidence to support adjuvant systemic therapy after metastasectomy in metastatic renal cell carcinoma. As far as we know, evidence is low and it's based in case reports and small series of patients. Despite the limitations and high risk of bias, promising results and cases with long-term survival with this approach have been described. Two ongoing clinical trials may answer the question that concerns us.

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INTRODUCTION

Renal cell carcinoma (RCC) represents 2%-3% of all cancers[1]. We know that the last two decades there has been 2% increase per year in its incidence worldwide[2].

According to largest published series, approximately 20%-30% of patients with renal cell carcinoma present metastasis at time of diagnosis. Besides, another 20%-40% of patients with localized disease who have had a surgical treatment, either partial or radical nephrectomy, will have progression during follow-up[3].

The more frequently affected organs are lungs, lymph nodes, liver and bone[4]. Nowadays, there are six targeted therapies approved for mRCC treatment. These new agents have completely changed the treatment and prognostic of patients with mRCC, but the cure is rare with medical treatment alone. Metastasectomy when feasible remains a curative option in some patients[5].

These are some of the reasons why metastatic renal cell carcinoma (mRCC) is a challenging disease. The present review aims to clarify if there is an evidence to support combination of metastasectomy and adjuvant systemic targeted therapy in mRCC.

LITERATURE STUDY

We have made a search in Pubmed database, using the key words: "renal cell carcinoma", "metastatic renal cell carcinoma", "renal cell carcinoma metastasis", "metastasectomy", "neoadjuvant treatment", "adjuvant treatment", "local treatment", "surgery"; in all languages and no date restrictions.

We included in the review all the studies that underwent the inclusion criteria: surgical treatment of metastatic renal cell carcinoma (yes/no), with emphasis on those that focused on neoadjuvant/adjuvant systemic therapy and mestastasectomy.

RESULTS

Epidemiology of mRCC treated with metastasectomy

As mentioned above, around 20%-30% of patients with RCC have metastases when diagnosed, and 20%-40% of those with localized advanced disease will progress to metastatic disease.

The most commonly affected organ in mRCC is the lungs. Lymph nodes, liver, bone, adrenal glands and brain are other typical sites; but there are reported metastases in rare organs, like pancreas, skin, bladder, *etc.*

In a recent publication, the distribution according to different organs was: 45.2% in lungs, 29.5% in bone, 21.8% in lymph nodes and 20.3% in liver. It was observed that in patients with multiple metastatic sites, 16% and 49%, brain and bone were affected, respectively[6].

Without treatment, survival of RCC is lower than 10% at 5 years[7].

Other specific characteristic of RCC is the existence of documented late metastases (> 20 years from the primary diagnosis).

The first evidence of long survival after resection of a solitary lung lesion was published in 1939[8]. Since then, several retrospective series have confirmed the effectiveness of metastasectomy. However, there are no randomized or prospective studies available.

Some authors have reported 37.2%-42% 5-year survival rates in cases of mRCC with complete resection, in observational studies[9,10].

The best response has been found in resection of solitary lung metastases, with 56% 5-year survival compared to 28% for skin, 20% for visceral organs, 18% for peripheral bone, 13% brain and 9% for axial bone metastases[11].

General prognostic factors: Knowledge of prognostic factors is important for a correct selection of patients candidates to surgery.

A retrospective study of 278 cases treated with nephrectomy and a solitary metastasis treated with surgery found that the factors associated with favourable outcome were: solitary site and single metastatasis, complete resection, a long disease-free interval and metachronous presentation[12].

In a large series of clear cell mRCC from de Mayo Clinic (Rochester, MN, United States) of 727 cases, prognostic factors of poor survival were: constitutional symptoms at nephrectomy, metastases to the bone or liver, multiple metastases, metastases at time of nephrectomy or in the 2 year thereafter, caval thrombus, Fuhrman grade 4 and coagulative tumour necrosis. In this study, complete resection of metastatic sites improved survival significantly[13].

Eggener *et al*[14] have published that in mRCC patients, the risk score classification according to Motzer classical factors and metastasectomy were independent factors of good outcome. The best survival was observed in patients with favourable risk and metastasectomy (71% 5-year survival) compared to that with high risk, with no survival at 5 years, independently of metastasectomy.

Recently, Tosco *et al*[15] have published a predictive model based on the following independent prognostic factors: primary tumour T stage $\geqslant$ 3, primary tumour Fuhrman grade $\geqslant$ 3, nonpulmonary metastases, disease-free interval $\leqslant$ 12 mo and multiorgan metastases. The Leuven-Udine (LU) prognostic groups are: (1) Group A (0-1 risk factors) with 5-year cancer specific survival (CSS) of 83.1%; (2) Group B (2 risk factors) with 5-year CSS of 56.4%; (3) Group C (3 risk factors) with 5-year CSS of 32.6%; and (4) Grupo D (4-5 risk factors) with 5-year CSS of 0%.

Another multiinstitutional study of 556 patients with mRCC who underwent metastasectomy in 48 Japanese hospitals found four adverse prognostic factors: incomplete resection of metastases, brain metastases, C-reactive protein > 1.0 mg/dL and high grade[16].

In conclusion, the prognostic factors of poor survival in patients with mRCC treated with metastasectomy are: (1) Primary tumour T stage ≥ 3; (2) Primary tumour Fuhrman grade ≥ 3 or high grade according to Japanese classification (nuclei of tumour cells larger than nuclei of normal tubular cells); (3) Nonpulmonary metastases; (4) Disease-free interval ≤ 12 mo; (5) Multiorgan metastases; (6) Incomplete resection of metastases; (7) Brain metastases; (8) C-reactive protein > 1.0 mg/dL; and (9) Motzer Classification risk score for mRCC (MSKCC risk score).

There are studies that evaluate the role of metachronous multiple metastasectomies.

In 2010, Szendröi *et al*[17] reported a case of a patient with 11-year survival after multiple and successive metastatasectomies.

In a study of 141 cases[12], 5-year survival after complete resection of second and third metastases did not differ from patients with first complete metastasectomy [43% overall survival (OS) in first resection, 46% in second and 44% in third].

In a large cohort of 887 mRCC cases, 125 underwent complete resection of multiple metastases. 5-year OS of cases with complete resection of multiple non-lung-only metastases was 32.5% compared to 12.4% of those with incomplete resection[18].

Organ-specific surgery: Lung metastases resection has demonstrated to prolong survival, with 5-year OS of 37%-55%[10,18-20].

Some prognostic factors have been described in these studies: (1) Incomplete resection has a poorer outcome (0-22% 5-year OS); (2) The presence of multiple metastases. More then 7 pulmonary metastases had worse 5-year OS (14.5% *vs* 46.8% of those with less than 7 lesions)[21]; (3) The presence of mediastinic node plus lung metastases impacted in survival (19 mo of median survival *vs* 102 mo)[22]; (4) Short disease-free interval after nephrectomy[23]; (5) Synchronous lung metastases (0% 5-year OS in patients treated with nephrectomy and metastasectomy)[24]; and (6) Size of metastases, with 0.5 cm as the established limit[25].

A prognostic model has been created based in a study of 200 cases of a single institution[26]. In multivariate analysis, size more than 3 cm, N^+ at diagnosis, pleural invasion, synchronous metastases, tumor-infiltrated hiliar, incomplete resection (R1 or R2) and mediastinal nodes were independent prognostic factors. Munich score classified patients in three groups of low, intermediate and high risk, with different median OS (90, 31 and 14 mo respectively): (1) Munich Ⅰ (low): R0, no risk factor; (2) Munich Ⅱ (intermediate): R0, ≥ risk factor; and (3) Munich Ⅲ (high): R1 or R2.

Bone metastases are often symptomatic. The indications for surgical treatment are prolongation of survival and alleviation of pain or stabilization of the extremity.

In a retrospective series of 99 cases surgically treated, the factors of good outcome were: single metastasis, wide resection and cytoreductive nephrectomy[27].

One study included a literature review, with 5-year OS of 35.8%-55%, with the best outcome in cases of peripheral skeletal location and histological subtype clear cell[28].

In a large series of M.D. Anderson Cancer Center[29] of 295 patients with 368 metastases treated, the OS rates were: 47% 1st year, 30% 2nd year and 11% 5th year. Patients with solitary metastasis showed better results, with a 5-year OS of 35%.

Patients with liver metastases have a poor prognosis due to that only 5% of the cases have a solitary metachronous lesion[30].

A series of 31 cases showed that negative resection-margin was an independent prognostic factor in multivariate analysis. The 5-year OS was 38.9%[31].

The largest retrospective series (88 patients with only liver metastases) found that those patients with synchronous metastases and a high grade RCC did not show benefit from surgery. The morbidity was 20.1%[32].

Most of the cases of brain metastases (80%) are diagnosed by symptoms. Without treatment the prognostic is poor, with a survival of less of a few months. Treatment options are surgery and stereotactic radiosurgery.

In a series of 50 cases, resection of lung metastases and supratentorial (*vs* infratentorial) localization were good prognostic factors. Adjuvant radiotherapy showed no survival advantage[33].

A series of 69 cases published in 2003, with 146 lesions treated with radiosurgery achieved good local control. OS was 6 mo from treatment. Age, neurologic status and radiosurgery dose had an impact in OS[34].

A study of 46 cases with 99 brain lesions treated with radiosurgery achieved local control in 84.7% of patients. Median OS was 10 mo, but reached 18 mo when > 75% volume decrease[35].

There have been reported 411 patients with pancreatic metastases of RCC in 170 publications[36]. Of 411 cases, 321 were surgically treated; with 65.3% of solitary lesions in surgery group. The 5-year OS was 72.6%, and disease specific survival was 57%. In-hospital mortality was 2.8%, 35.8% of patients underwent pancreaticoduodenectomy and 19.9% total pancreatectomy.

There are reports of RCC metastases in other organs, like adrenal, bladder, vagina, thyroid gland, paranasal sinuses. These publications are case reports and no clear prognosis knowledge can be made.

The panel of European Association of Urology Guidelines has made a systematic review in accordance with Cochrane review methodology[37]. They concluded that all the studies were retrospective with a high risk of bias, but with the exception of brain and possibly bone metastases, surgery remains to be by default the best treatment for most sites.

In the last actualization of the Guidelines, the conclusion is that "no general recommendations can be made and the decision of metastasectomy has to be taken for each site, and on a case-by-case basis: performance status, risk profiles, patient preference and alternative techniques must be considered".

Rationale of multimode therapy in mRCC

mRCC is a complex entity that can be treated with cytokine treatment, sequential targeted therapies and metastasectomy.

The correct moment and sequence of each treatment is not clear, but we have some evidence that combination of surgery and systemic therapies can achieved excellent outcomes.

Cytoreductive nephrectomy: It is known that nephrectomy is curative if surgery can excise all tumour deposits.

In a metaanalysis of two randomized trials comparing immunotherapy only *vs* nephrectomy and immunotherapy, a long-term survival was reported in cases treated with nephrectomy and immunotherapy in patients with good performance status[38].

In a retrospective study[39], the previous advantage of cytoreductive nephrectomy was confirmed in patients treated with vascular endothelial growth factor-targeted therapy (VEGF-targeted therapy).

However, the value of cytoreductive nephrectomy followed by VEGF-targeted therapy has to be confirmed by ongoing trials.

Adjuvant therapy after nephrectomy in localized RCC: Adjuvant tumour vaccination might improve duration of PFS in patients with T3 RCC, but has not effect in OS. Adjuvant therapy with cytokines does not improve survival[40,41].

There are several ongoing phase Ⅲ trials of adjuvant sunitinib, sorafenib, pazopanib, axitinib and everolimus.

Presurgical treatment for locally advanced RCC: Neoadjuvant treatment could be used with the following objectives: (1) Decrease tumour size and facilitate surgery; (2) Allow the performance of nephronsparing surgery; (3) Improve survival acting against micrometastases; (4) Reduce morbidity of surgery by decreasing size and vascularisation of tumour; (5) Knowledge of response to systemic therapy before surgery; and (6) Future research.

Targeted therapies have been used in neoadjuvant/preoperatory settings in cases of locally advanced RCC (huge tumours, cases with large nodes near hilium and inferior vena cava thrombus) and in cases of T2 RCC with the aim to perform a nephronsparing procedure.

Sunitinib, sorafenib, axitinib, everolimus and temsirolimus are the 5 neoadjuvant therapies that have been used for locally advanced RCC treatment and before nephronsparing surgery.

First evidence of radiological downstaging effect of kinase inhibitors was reported in phase 2 and 3 trials[42,43].

In 2009, a complete histologic remission after sunitinib neoadjuvant therapy was reported in a case of T3b renal cell carcinoma[44].

The response of renal tumours to targeted therapies has been reported in small retrospective series and case reports. The most important series are summarized in Table 1[45-54].

Powles *et al*[55] reported that in cases of mRCC treated with sunitinib prior to nephrectomy, progression prior to planned nephrectomy, high Fuhrman grade and MSKCC poor risk at diagnosis were independent prognostic factors.

Another group made a systematic review[56] and concluded that downsizing of primary tumours with neoadjuvant sunitinib or sorafenib was related to size at presentation, being the major effect in tumours sized 5 to 7 cm.

Due to the high rate of surgical complications in IVC thrombus RCC, reduction of size of tumour thrombus with neoadjuvant sunitinib, sorafenib, axitinib and temsirolimus has been reported.

The majority of the published information are case reports[57-60]. The largest series reported 25 patients, 7 of which had level 3 or 4 IVC thrombus. 12% reduced thrombus size (only after sunitinib treatment), but the reduction (Median 1.5 cm) didn't have any impact on the surgery approach[61].

In 2010, Bex *et al*[62] reported two cases of IVC thrombi progression during neoadjuvant treatment with sunitinib.

Recently, Bigot *et al*[63], in a retrospective series of 14 cases treated with sunitinib or sorafenib, found that 43% of the patients had a measurable decrease while 14% had an increase in thrombus size. Only 1 case downstaged thrombus level. However, 50% of renal tumours experienced a significant reduction in size. They concluded that neoadjuvant therapy had limited impact on IVC thrombi RCC surgical management.

In conclusion, the response of primary tumour to targeted therapies is unpredictable, although 42%-100% cases show tumour shrinkage. The major effect reported was after sunitinib treatment and in smaller tumours (5 to 7 cm). Morbidity of these novel agents should be taken into account.

There are a few studies focused on the concept of neoadjuvant systemic therapy prior to metastasectomy.

Rini *et al*[64] described 2 patients with long-term response who were treated with adjuvant sunitinib and metastasectomy.

Thomas *et al*[65] reported 19 cases treated with surgery after targeted therapy, 3 of them with metastasectomy with partial response and good outcome.

In 2009, Daliani *et al*[66] reported 38 patients with mRCC, treated with targeted therapy and a partial response/stable disease who underwent metastasectomy

Table 1 Main series of neoadjuvant therapy in locally advanced renal cell carcinoma

Ref.	No.	Therapy	Median size	% median reduction	Partial response	< 30% reduction	% cases tumour shrinkage	Toxicity Garde 3-4
Thomas *et al*[45]	19	Sunitinib	10.5	24%	3	8	42%	37%
Hellenthal *et al*[46]	20	Sunitinib	7	27.90%	2	15	85%	30%
Silberstein *et al*[47]	14	Sunitinib	7	21%	4	10	100%	3 urine leaks
Kondo *et al*[48]	9	Sunitinib/sorafenib	-	9%-30%	3	6	100%	2 major surgery complications
Rini *et al*[49]	28	Sunitinib	-	22%	-	-	-	-
Powles *et al*[50]	52	Sunitinib	-	-	-	-	73%	27%
Bex *et al*[51]	10	Sunitinib	-	14%			60%	
Kats-Ugurlu *et al*[52]	10	Sorafenib	7.5	-	-	-	-	-
Cowey *et al*[53]	30	Sorafenib	8.7	9.60%	2	23	80%	-
Karam *et al*[54]	24	Axitinib	-	28.30%	11	-	100%	41.70%

(84% only one organ site). Ten percent of patients suffered complications. Twenty-one percent of patients were remained of disease. Absence of histological viable tumour in metastasectomy specimens and lung metastases had an OS of 5.6 years compared with those who did not (1.4 years).

In 2012, Karam *et al*[67] reported 22 cases with mRCC who received neoadjuvant treatment prior to metastasectomy with one of the following targeted therapies: sunitinib, sorafenib, bavacizumab, everolimus, pazopanib, Interleukin-2, ABT-510. 4 cases had multiple mestastases and 6 suffered complications. At 109 weeks, only one patient died from RCC. 11 (50%) cases experimented no recurrence.

Another study of 2012[68], reported 11 patients treated surgically after $\geqslant$ 3 mo of stable partial remission with sunitinib, bevacizumab or sunitinib plus temisrolimus. Seven cases had node retroperitoneal disease. Only 1 complication was reported. 5 cases showed no recurrence after a median follow-up of 12 mo.

In a series of 143 patients with mRCC treated with systemic therapy, those who were treated with metastasectomy too (n = 42) had a better OS (18.8 mo *vs* 15 mo, P = 0.07)[69].

A group of Japanese authors described two cases of large adrenal mestastases with liver and pancreas invasion that were successfully treated with sunitinib prior to surgery with a good outcome[70,71].

Johannsen *et al*[72] studied the discontinuation of targeted therapy after complete response to sunitinb. 12 cases were identified, 50% (6 cases) treated with sunitinib and consolidative metastasectomy (lungs, bone, skin and thyroid). No adjuvant treatment was prescribed. Only 5 of 11 patients experienced recurrence, with effective rescue after targeted therapy in all cases. In a recent actualization of the series, with 36 cases, 33.3% remained free of recurrence during follow-up. Factors that correlate with outcome, including metastasectomy, could not be identified[73].

Adjuvant targeted therapy after metastasectomy

It is known that neoadjuvant targeted therapy can be related with surgical complications, as mentioned above. Systemic treatment can obliterate normal tissues planes and make surgery more difficult and risky[74]. A recent review concluded that no general recommendations can be made about use of targeted therapy in preoperatory setting[75].

Based on evidence of effectiveness of multimodal treatment in different moments of mRCC, we try to answer the question of the title: Is there a role for systemic targeted therapy after surgical treatment for metastases of renal cell carcinoma?

In 2007, Kwak *et al*[76] reported 93 patients with mRCC treated with metastasectomy with or without adjuvant immunotherapy. Overall survival of group treated with surgery plus immunotherapy was 56.1 mo *vs* 21.3 mo in the only-surgery group. But when patients were stratified by time of metastases, no differences were found. In multivariate analysis only multiplicity of metastases and metastases sites were independent prognostic factors. Authors concluded that metastasectomy plus adjuvant immunotherapy did not render a higher overall survival.

Jacobsohn *et al*[77] reported no effect of adjuvant Interferon after lung metastasectomy.

Since then, some case reports suggest that adjuvant targeted therapy could be effective after metastasetomy.

In 2010, a study with 88 cases with liver metastases of RCC was published. Sixty-eight were treated with surgery and 78% of cases received adjuvant treatment in both groups (metastatasectomy yes/no). The 5-year overall survival rate after metastasectomy was 62.2% with a median survival of 142 mo compared with 29.3% and 27 mo in the control group. High-grade RCC as well as patients with synchronous metastases did not benefit from surgery[32].

A case report of a man with mRCC who was treated with metastasectomy for multiple organs deposits and adjuvant pazopanib showed 8-year survival[78].

In 2012, Gardini *et al*[79] described 8 cases of pancreatic metastases of RCC treated surgically and with adjuvant therapy (mostly immunotherapy), with disease free survival after 3 years of 30%.

In most of previous papers of neoadjuvant treatment after metastasectomy, adjuvant systemic therapies

are also used. For instance, in Karam *et al*[67] study, 9 of 22 patients received at least one adjuvant targeted therapy. Effect of this intervention in survival was not assessed. Daliani *et al*[66] also gave consolidative adjuvant systemic therapy.

A study of 106 cases with mRCC and brain metastases used combination of targeted therapy and local treatments. The patients were treated with sunitinib (n = 77), sorafenib (n = 23), bevacizumab (n = 5), and temsirolimus (n = 1). Local disease treatment included whole brain radiotherapy (81%), stereotactic radiosurgery (25%), and neurosurgery (25%). On multivariable analysis, surgery or radiosurgery failed to demonstrate to increase OS[80].

Two ongoing clinical trials published in Pubmed are studying adjuvant therapy after metastasectomy: (1) RESORT protocol[81]: a randomized, open-label, multicenter phase Ⅱ study to evaluate efficacy of sorafenib in patients with mRCC after complete metastasectomy. One hundred and thirty-two patients will be randomized to receive sorafenib or best supportive care, with a follow-up of 36 mo; and (2) SMAT-AN 20/04 of the Working Group of Urological Oncology (AUO)[82]: a prospective randomized multicenter phase Ⅱ study on resection of lung metastases in clear cell carcinoma ± adjuvant sunitinib over 1 year.

CONCLUSION

mRCC is a challenging disease. Despite the new targeted therapies, complete remissions occur only in 1%-3% of the cases, and the most effective first-line treatment drugs have reached a ceiling in OS (ranging from 9 to 49 mo)[5].

Metastasectomy remains to be the only curative option in most patients with mRCC. Prognostic models for general[15,16] and lung metastases[26] have been recently published, so we have tools to classify patients in risk groups, allowing us to detect the cases with the higher risk of recurrence after metastasectomy.

Although sparse, there is some evidence of effectiveness of neoadjuvant targeted therapy before metastasectomy; but with an increase in surgical complications due to the effects of these new drugs in tissue healing.

In 2007, Jacobsohn *et al*[77] concluded that metastasectomy plus adjuvant immunotherapy did not result in a higher overall survival and published a paper titled: "No role of adjuvant therapy after complete metastasectomy in metastatic renal cell carcinoma?"

Since then, mRCC treatment has dramatically changed after the approval of new drugs. We have aimed to answer the question: Is there a role for systemic targeted therapy after surgical treatment for metastases of renal cell carcinoma? As far as we know, evidence is low and it's based in case reports and small series of patients treated with adjuvant drugs after neoadjuvant therapy plus metastasectomy in cases of partial response to initial systemic treatment. Despite the limitations and high risk of bias, promising results and cases with long-term survival with this approach have been described[32,66,67,78-80].

Two ongoing clinical trials[81,82] may answer the question that concerns us. While we wait for the results, the recommendations of European Association of Urology Guidelines[37] are a rationale tool: "the decision of metastasectomy has to be taken for each site, and on a case-by-case basis: Performance status, risk profiles, patient preference and alternative techniques must be considered". From our point of view, adjuvant targeted therapy after metastasectomy combined or not with neoadjuvant treatment could be an effective multimodal approach in the future.

REFERENCES

1 European Network of Cancer Registries: Eurocim version 4.0. European incidence database V2.3, 730 entity dictionary (2001). Lyon: ENCR, 2001

2 **Lindblad P**. Epidemiology of renal cell carcinoma. *Scand J Surg* 2004; **93**: 88-96 [PMID: 15285559]

3 **Lam JS**, Shvarts O, Leppert JT, Figlin RA, Belldegrun AS. Renal cell carcinoma 2005: new frontiers in staging, prognostication and targeted molecular therapy. *J Urol* 2005; **173**: 1853-1862 [PMID: 15879764 DOI: 10.1097/01.ju.0000165693.68449.c3]

4 **Ruutu M,** Bono P, Taari K. Resection of renal cell cancer metastases: where do we stand in 2008? *Eur Urol Supl* 2008; **7**: 436-442 [DOI: 10.1016/j.eursup.2008.01.005]

5 **Bex A.** Integrating metastasectomy and stereotaxic radiosurgery in the treatment of metastatic renal cell carcinoma. *EJC Supplements* 2013; **2**: 192-203 [DOI: 10.1016/j.ejcsup.2013.07.017]

6 **Bianchi M**, Sun M, Jeldres C, Shariat SF, Trinh QD, Briganti A, Tian Z, Schmitges J, Graefen M, Perrotte P, Menon M, Montorsi F, Karakiewicz PI. Distribution of metastatic sites in renal cell carcinoma: a population-based analysis. *Ann Oncol* 2012; **23**: 973-980 [PMID: 21890909 DOI: 10.1093/annonc/mdr362]

7 **Ljungberg B**. Prognostic factors in renal cell carcinoma. *Scand J Surg* 2004; **93**: 118-125 [PMID: 15285563]

8 **Barney JD,** Churchill J. Adenocarcinoma of the kidney with mestatases to the lung. *J Urol* 1939; **42**: 271

9 **Piltz S**, Meimarakis G, Wichmann MW, Hatz R, Schildberg FW, Fuerst H. Long-term results after pulmonary resection of renal cell carcinoma metastases. *Ann Thorac Surg* 2002; **73**: 1082-1087 [PMID: 11996245 DOI: 10.1016/S0003-4975(01)03602-5]

10 **Assouad J**, Petkova B, Berna P, Dujon A, Foucault C, Riquet M. Renal cell carcinoma lung metastases surgery: pathologic findings and prognostic factors. *Ann Thorac Surg* 2007; **84**: 1114-1120 [PMID: 17888956 DOI: 10.1016/j.athoracsur.2007.04.118]

11 **Swanson DA**. Surgery for metastases of renal cell carcinoma. *Scand J Surg* 2004; **93**: 150-155 [PMID: 15285568]

12 **Kavolius JP**, Mastorakos DP, Pavlovich C, Russo P, Burt ME, Brady MS. Resection of metastatic renal cell carcinoma. *J Clin Oncol* 1998; **16**: 2261-2266 [PMID: 9626229]

13 **Leibovich BC**, Cheville JC, Lohse CM, Zincke H, Frank I, Kwon ED, Merchan JR, Blute ML. A scoring algorithm to predict survival for patients with metastatic clear cell renal cell carcinoma: a stratification tool for prospective clinical trials. *J Urol* 2005; **174**: 1759-1763; discussion 1763 [PMID: 16217278 DOI: 10.1097/01.ju.0000177487.64651.3a]

14 **Eggener SE**, Yossepowitch O, Kundu S, Motzer RJ, Russo P. Risk score and metastasectomy independently impact prognosis of patients with recurrent renal cell carcinoma. *J Urol* 2008; **180**: 873-878; discussion 878 [PMID: 18635225 DOI: 10.1016/j.juro.2008.05.006]

15 **Tosco L**, Van Poppel H, Frea B, Gregoraci G, Joniau S. Survival and impact of clinical prognostic factors in surgically treated metastatic renal cell carcinoma. *Eur Urol* 2013; **63**: 646-652 [PMID: 23041360 DOI: 10.1016/j.eururo.2012.09.037]

16 **Naito S**, Kinoshita H, Kondo T, Shinohara N, Kasahara T, Saito K, Takayama T, Masumori N, Takahashi W, Takahashi M, Terachi T, Ozono S, Naito S, Tomita Y. Prognostic factors of patients with metastatic renal cell carcinoma with removed metastases: a multicenter study of 556 patients. *Urology* 2013; **82**: 846-851 [PMID: 24074981 DOI: 10.1016/j.urology.2013.06.035]

17 **Szendröi A**, Szendröi M, Szücs M, Székely E, Romics I. 11-year survival of a renal cell cancer patient following multiple metastasectomy. *Can J Urol* 2010; **17**: 5475-5477 [PMID: 21172114]

18 **Alt AL**, Boorjian SA, Lohse CM, Costello BA, Leibovich BC, Blute ML. Survival after complete surgical resection of multiple metastases from renal cell carcinoma. *Cancer* 2011; **117**: 2873-2882 [PMID: 21692048 DOI: 10.1002/cncr.25836]

19 **Cerfolio RJ**, Allen MS, Deschamps C, Daly RC, Wallrichs SL, Trastek VF, Pairolero PC. Pulmonary resection of metastatic renal cell carcinoma. *Ann Thorac Surg* 1994; **57**: 339-344 [PMID: 8311594 DOI: 10.1016/0003-4975(94)90994-6]

20 **Kanzaki R**, Higashiyama M, Fujiwara A, Tokunaga T, Maeda J, Okami J, Nishimura K, Kodama K. Long-term results of surgical resection for pulmonary metastasis from renal cell carcinoma: a 25-year single-institution experience. *Eur J Cardiothorac Surg* 2011; **39**: 167-172 [PMID: 20591686 DOI: 10.1016/j.ejcts.2010.05.021]

21 **Yasujima M**, Abe K, Tanno M, Kohzuki M, Kasai Y, Kanazawa M, Omata K, Sato M, Takeuchi K, Yoshinaga K. No evidence on significant roles of the prostaglandin-thromboxane and kallikrein-kinin system in the antihypertensive effect of MK 421 in rats made hypertensive by norepinephrine or vasopressin. *Clin Exp Hypertens A* 1987; **9**: 323-328 [PMID: 2440625 DOI: 10.1016/S0003-4975(02)03803-1]

22 **Winter H**, Meimarakis G, Angele MK, Hummel M, Staehler M, Hoffmann RT, Hatz RA, Löhe F. Tumor infiltrated hilar and mediastinal lymph nodes are an independent prognostic factor for decreased survival after pulmonary metastasectomy in patients with renal cell carcinoma. *J Urol* 2010; **184**: 1888-1894 [PMID: 20846691 DOI: 10.1016/j.juro.2010.06.096]

23 **Friedel G**, Hürtgen M, Penzenstadler M, Kyriss T, Toomes H. Resection of pulmonary metastases from renal cell carcinoma. *Anticancer Res* 1999; **19**: 1593-1596 [PMID: 10365152]

24 **Hofmann HS**, Neef H, Krohe K, Andreev P, Silber RE. Prognostic factors and survival after pulmonary resection of metastatic renal cell carcinoma. *Eur Urol* 2005; **48**: 77-81; discussion 81-82 [PMID: 15967255 DOI: 10.1016/j.eururo.2005.03.004]

25 **Murthy SC**, Kim K, Rice TW, Rajeswaran J, Bukowski R, DeCamp MM, Blackstone EH. Can we predict long-term survival after pulmonary metastasectomy for renal cell carcinoma? *Ann Thorac Surg* 2005; **79**: 996-1003 [PMID: 15734422 DOI: 10.1016/j.athoracsur.2004.08.034]

26 **Meimarakis G**, Angele M, Staehler M, Clevert DA, Crispin A, Rüttinger D, Löhe F, Preissler G, Hatz RA, Winter H. Evaluation of a new prognostic score (Munich score) to predict long-term survival after resection of pulmonary renal cell carcinoma metastases. *Am J Surg* 2011; **202**: 158-167 [PMID: 21810496 DOI: 10.1016/j.amjsurg.2010.06.029]

27 **Fuchs B**, Trousdale RT, Rock MG. Solitary bony metastasis from renal cell carcinoma: significance of surgical treatment. *Clin Orthop Relat Res* 2005; **(431)**: 187-192 [PMID: 15685074]

28 **Althausen P**, Althausen A, Jennings LC, Mankin HJ. Prognostic factors and surgical treatment of osseous metastases secondary to renal cell carcinoma. *Cancer* 1997; **80**: 1103-1109 [PMID: 9305711 DOI: 10.1002/(SICI)1097-0142(19970915)80: 6<1103: : AID-CNCR13>3.0.CO; 2-C]

29 **Lin PP**, Mirza AN, Lewis VO, Cannon CP, Tu SM, Tannir NM, Yasko AW. Patient survival after surgery for osseous metastases from renal cell carcinoma. *J Bone Joint Surg Am* 2007; **89**: 1794-1801 [PMID: 17671020 DOI: 10.2106/JBJS.F.00603]

30 **Stief CG**, Jähne J, Hagemann JH, Kuczyk M, Jonas U. Surgery for metachronous solitary liver metastases of renal cell carcinoma. *J Urol* 1997; **158**: 375-377 [PMID: 9224306 DOI: 10.1016/S0022-5347(01)64483-5]

31 **Thelen A**, Jonas S, Benckert C, Lopez-Hänninen E, Rudolph B, Neumann U, Neuhaus P. Liver resection for metastases from renal cell carcinoma. *World J Surg* 2007; **31**: 802-807 [PMID: 17354021 DOI: 10.1007/s00268-007-0685-9]

32 **Staehler MD**, Kruse J, Haseke N, Stadler T, Roosen A, Karl A, Stief CG, Jauch KW, Bruns CJ. Liver resection for metastatic disease prolongs survival in renal cell carcinoma: 12-year results from a retrospective comparative analysis. *World J Urol* 2010; **28**: 543-547 [PMID: 20440505 DOI: 10.1007/s00345-010-0560-4]

33 **Wroński M**, Arbit E, Russo P, Galicich JH. Surgical resection of brain metastases from renal cell carcinoma in 50 patients. *Urology* 1996; **47**: 187-193 [PMID: 8607231 DOI: 10.1016/S0090-4295(99)80413-0]

34 **Sheehan JP**, Sun MH, Kondziolka D, Flickinger J, Lunsford LD. Radiosurgery in patients with renal cell carcinoma metastasis to the brain: long-term outcomes and prognostic factors influencing survival and local tumor control. *J Neurosurg* 2003; **98**: 342-349 [PMID: 12593621]

35 **Kim WH**, Kim DG, Han JH, Paek SH, Chung HT, Park CK, Kim CY, Kim YH, Kim JW, Jung HW. Early significant tumor volume reduction after radiosurgery in brain metastases from renal cell carcinoma results in long-term survival. *Int J Radiat Oncol Biol Phys* 2012; **82**: 1749-1755 [PMID: 21640509 DOI: 10.1016/j.ijrobp.2011.03.044]

36 **Tanis PJ**, van der Gaag NA, Busch OR, van Gulik TM, Gouma DJ. Systematic review of pancreatic surgery for metastatic renal cell carcinoma. *Br J Surg* 2009; **96**: 579-592 [PMID: 19434703 DOI: 10.1002/bjs.6606]

37 **Ljunberg B,** Bensalah K, Bex A. 2014 European Associaton of Urology Guidelines on renal cell carcinoma. Available from: URL: http: //www.uroweb.org/guidelines/online-guidelines/?no_cache=1

38 **Flanigan RC**, Mickisch G, Sylvester R, Tangen C, Van Poppel H, Crawford ED. Cytoreductive nephrectomy in patients with metastatic renal cancer: a combined analysis. *J Urol* 2004; **171**: 1071-1076 [PMID: 14767273 DOI: 10.1097/01.ju.0000110610.61545.ae]

39 **Choueiri TK**, Xie W, Kollmannsberger C, North S, Knox JJ, Lampard JG, McDermott DF, Rini BI, Heng DY. The impact of cytoreductive nephrectomy on survival of patients with metastatic renal cell carcinoma receiving vascular endothelial growth factor targeted therapy. *J Urol* 2011; **185**: 60-66 [PMID: 21074201 DOI: 10.1016/j.juro.2010.09.012]

40 **Galligioni E**, Quaia M, Merlo A, Carbone A, Spada A, Favaro D, Santarosa M, Sacco C, Talamini R. Adjuvant immunotherapy treatment of renal carcinoma patients with autologous tumor cells and bacillus Calmette-Guèrin: five-year results of a prospective randomized study. *Cancer* 1996; **77**: 2560-2566 [PMID: 8640706 DOI: 10.1002/(SICI)1097-0142(19960615)77: 12<2560: : AID-CNCR20>3.0.CO; 2-P]

41 **Clark JI**, Atkins MB, Urba WJ, Creech S, Figlin RA, Dutcher JP, Flaherty L, Sosman JA, Logan TF, White R, Weiss GR, Redman BG, Tretter CP, McDermott D, Smith JW, Gordon MS, Margolin KA. Adjuvant high-dose bolus interleukin-2 for patients with high-risk renal cell carcinoma: a cytokine working group randomized trial. *J Clin Oncol* 2003; **21**: 3133-3140 [PMID: 12810695 DOI: 10.1200/JCO.2003.02.014]

42 **Motzer RJ**, Rini BI, Bukowski RM, Curti BD, George DJ, Hudes GR, Redman BG, Margolin KA, Merchan JR, Wilding G, Ginsberg MS, Bacik J, Kim ST, Baum CM, Michaelson MD. Sunitinib in patients with metastatic renal cell carcinoma. *JAMA* 2006; **295**: 2516-2524 [PMID: 16757724 DOI: 10.1001/jama.295.21.2516]

43 **Escudier B**, Eisen T, Stadler WM, Szczylik C, Oudard S, Siebels M, Negrier S, Chevreau C, Solska E, Desai AA, Rolland F, Demkow T, Hutson TE, Gore M, Freeman S, Schwartz B, Shan M, Simantov R, Bukowski RM. Sorafenib in advanced clear-cell renal-cell carcinoma. *N Engl J Med* 2007; **356**: 125-134 [PMID: 17215530 DOI: 10.1056/NEJMoa060655]

44 **Robert G**, Gabbay G, Bram R, Wallerand H, Deminière C, Cornelis

F, Bernhard JC, Ravaud A, Ballanger P. Case study of the month. Complete histologic remission after sunitinib neoadjuvant therapy in T3b renal cell carcinoma. *Eur Urol* 2009; **55**: 1477-1480 [PMID: 19150171 DOI: 10.1016/j.eururo.2008.12.03]

45 **Thomas AA**, Rini BI, Lane BR, Garcia J, Dreicer R, Klein EA, Novick AC, Campbell SC. Response of the primary tumor to neoadjuvant sunitinib in patients with advanced renal cell carcinoma. *J Urol* 2009; **181**: 518-523; discussion 523 [PMID: 19100579 DOI: 10.1016/j.juro.2008.10.001]

46 **Hellenthal NJ**, Underwood W, Penetrante R, Litwin A, Zhang S, Wilding GE, Teh BT, Kim HL. Prospective clinical trial of preoperative sunitinib in patients with renal cell carcinoma. *J Urol* 2010; **184**: 859-864 [PMID: 20643461 DOI: 10.1016/j.juro.2010.05.041]

47 **Silberstein JL,** Millard F, Mehrazin R, Kopp R, Bazzi W, DiBlasio CJ, Patterson AL, Downs TM, Yunus F, Kane CJ, Derweesh IH. Feasibilty and efficacy of neiadjuvant sunitinib before nephron-sparing surgery. *BJU Int* 2010; **106**: 1270-1276 [PMID: 20394613 DOI: 10.1111/j.1464-410X.2010.09357.x]

48 **Kondo T**, Hashimoto Y, Kobayashi H, Iizuka J, Nishikawa T, Nakano M, Tanabe K. Presurgical targeted therapy with tyrosine kinase inhibitors for advanced renal cell carcinoma: clinical results and histopathological therapeutic effects. *Jpn J Clin Oncol* 2010; **40**: 1173-1179 [PMID: 20696817 DOI: 10.1093/jjco/hyq150]

49 **Rini BI**, Garcia J, Elson P, Wood L, Shah S, Stephenson A, Salem M, Gong M, Fergany A, Rabets J, Kaouk J, Krishnamurthi V, Klein E, Dreicer R, Campbell S. The effect of sunitinib on primary renal cell carcinoma and facilitation of subsequent surgery. *J Urol* 2012; **187**: 1548-1554 [PMID: 22425095 DOI: 10.1016/j.juro.2011.12.075]

50 **Powles T**, Kayani I, Blank C, Chowdhury S, Horenblas S, Peters J, Shamash J, Sarwar N, Boletti K, Sadev A, O'Brien T, Berney D, Beltran L, Haanen J, Bex A. The safety and efficacy of sunitinib before planned nephrectomy in metastatic clear cell renal cancer. *Ann Oncol* 2011; **22**: 1041-1047 [PMID: 21242586 DOI: 10.1093/annonc/mdq564]

51 **Bex A**, van der Veldt AA, Blank C, van den Eertwegh AJ, Boven E, Horenblas S, Haanen J. Neoadjuvant sunitinib for surgically complex advanced renal cell cancer of doubtful resectability: initial experience with downsizing to reconsider cytoreductive surgery. *World J Urol* 2009; **27**: 533-539 [PMID: 19145434 DOI: 10.1007/s00345-008-0368-7]

52 **Kats-Ugurlu G**, Oosterwijk E, Muselaers S, Oosterwijk-Wakka J, Hulsbergen-van de Kaa C, de Weijert M, van Krieken H, Desar I, van Herpen C, Maass C, de Waal R, Mulders P, Leenders W. Neoadjuvant sorafenib treatment of clear cell renal cell carcinoma and release of circulating tumor fragments. *Neoplasia* 2014; **16**: 221-228 [PMID: 24726142 DOI: 10.1016/j.neo.2014.03.007]

53 **Cowey CL**, Amin C, Pruthi RS, Wallen EM, Nielsen ME, Grigson G, Watkins C, Nance KV, Crane J, Jalkut M, Moore DT, Kim WY, Godley PA, Whang YE, Fielding JR, Rathmell WK. Neoadjuvant clinical trial with sorafenib for patients with stage II or higher renal cell carcinoma. *J Clin Oncol* 2010; **28**: 1502-1507 [PMID: 20159822 DOI: 10.1200/JCO.2009.24.7759]

54 **Karam JA,** Devine CE, Urbauer DL, Lozano M, Maity T, Ahrar K, Tamboli P, Tannir NM, Wood CG. Phase 2 Trial of Neoadjuvant Axitinib in Patients with Locally Advanced Nonmetastatic Clear Cell Renal Cell Carcinoma. *Eur Urol* 2014 Feb 7; Epub ahead of print [PMID: 24560330 DOI: 10.1016/j.eururo.2014.01.035]

55 **Powles T**, Blank C, Chowdhury S, Horenblas S, Peters J, Shamash J, Sarwar N, Boleti E, Sahdev A, O'Brien T, Berney D, Beltran L, Nathan P, Haanen J, Bex A. The outcome of patients treated with sunitinib prior to planned nephrectomy in metastatic clear cell renal cancer. *Eur Urol* 2011; **60**: 448-454 [PMID: 21612860 DOI: 10.1016/j.eururo.2011.05.028]

56 **Kroon BK**, de Bruijn R, Prevoo W, Horenblas S, Powles T, Bex A. Probability of downsizing primary tumors of renal cell carcinoma by targeted therapies is related to size at presentation. *Urology* 2013; **81**: 111-115 [PMID: 23153934 DOI: 10.1016/j.urology.2012.09.014]

57 **Karakiewicz PI**, Suardi N, Jeldres C, Audet P, Ghosn P, Patard JJ, Perrotte P. Neoadjuvant sutent induction therapy may effectively down-stage renal cell carcinoma atrial thrombi. *Eur Urol* 2008; **53**: 845-848 [PMID: 18053636 DOI: 10.1016/j.eururo.2007.11.006]

58 **Sano F**, Makiyama K, Tatenuma T, Sakata R, Yamanaka H, Fusayasu S, Nakayama T, Nakaigawa N, Yao M, Kubota Y. Presurgical downstaging of vena caval tumor thrombus in advanced clear cell renal cell carcinoma using temsirolimus. *Int J Urol* 2013; **20**: 637-639 [PMID: 23186017 DOI: 10.1111/iju.12012]

59 **Di Silverio F**, Sciarra A, Parente U, Andrea A, Von Heland M, Panebianco V, Passariello R. Neoadjuvant therapy with sorafenib in advanced renal cell carcinoma with vena cava extension submitted to radical nephrectomy. *Urol Int* 2008; **80**: 451-453 [PMID: 18587261 DOI: 10.1159/000132708]

60 **Sassa N**, Kato M, Funahashi Y, Maeda M, Inoue S, Gotoh M. Efficacy of pre-surgical axitinib for shrinkage of inferior vena cava thrombus in a patient with advanced renal cell carcinoma. *Jpn J Clin Oncol* 2014; **44**: 370-373 [PMID: 24571808 DOI: 10.1093/jjco/hyu014]

61 **Cost NG**, Delacroix SE, Sleeper JP, Smith PJ, Youssef RF, Chapin BF, Karam JA, Culp S, Abel EJ, Brugarolas J, Raj GV, Sagalowsky AI, Wood CG, Margulis V. The impact of targeted molecular therapies on the level of renal cell carcinoma vena caval tumor thrombus. *Eur Urol* 2011; **59**: 912-918 [PMID: 21367518 DOI: 10.1016/j.eururo.2011.02.032]

62 **Bex A**, Van der Veldt AA, Blank C, Meijerink MR, Boven E, Haanen JB. Progression of a caval vein thrombus in two patients with primary renal cell carcinoma on pretreatment with sunitinib. *Acta Oncol* 2010; **49**: 520-523 [PMID: 20105087 DOI: 10.3109/02841860903521111]

63 **Bigot P**, Fardoun T, Bernhard JC, Xylinas E, Berger J, Rouprêt M, Beauval JB, Lagabrielle S, Lebdai S, Ammi M, Baumert H, Escudier B, Grenier N, Hétet JF, Long JA, Paparel P, Rioux-Leclercq N, Soulié M, Azzouzi AR, Bensalah K, Patard JJ. Neoadjuvant targeted molecular therapies in patients undergoing nephrectomy and inferior vena cava thrombectomy: is it useful? *World J Urol* 2014; **32**: 109-114 [PMID: 23624719 DOI: 10.1007/s00345-013-1088-1]

64 **Rini BI**, Shaw V, Rosenberg JE, Kim ST, Chen I. Patients with metastatic renal cell carcinoma with long-term disease-free survival after treatment with sunitinib and resection of residual metastases. *Clin Genitourin Cancer* 2006; **5**: 232-234 [PMID: 17239278 DOI: 10.3816/CGC.2006.n.042]

65 **Thomas AA**, Rini BI, Stephenson AJ, Garcia JA, Fergany A, Krishnamurthi V, Novick AC, Gill IS, Klein EA, Zhou M, Campbell SC. Surgical resection of renal cell carcinoma after targeted therapy. *J Urol* 2009; **182**: 881-886 [PMID: 19616232 DOI: 10.1016/j.juro.2009.05.014]

66 **Daliani DD**, Tannir NM, Papandreou CN, Wang X, Swisher S, Wood CG, Swanson DA, Logothetis CJ, Jonasch E. Prospective assessment of systemic therapy followed by surgical removal of metastases in selected patients with renal cell carcinoma. *BJU Int* 2009; **104**: 456-460 [PMID: 19338544 DOI: 10.1111/j.1464-410X.2009.08490.x]

67 **Karam JA**, Rini BI, Varella L, Garcia JA, Dreicer R, Choueiri TK, Jonasch E, Matin SF, Campbell SC, Wood CG, Tannir NM. Metastasectomy after targeted therapy in patients with advanced renal cell carcinoma. *J Urol* 2011; **185**: 439-444 [PMID: 21167518 DOI: 10.1016/j.juro.2010.09.086]

68 **Firek P**, Richter S, Jaekel J, Brehmer B, Heidenreich A. [Metastasectomy in renal cell cancer after neoadjuvant therapy with multi-tyrosine kinase inhibitors]. *Urologe A* 2012; **51**: 398-402 [PMID: 22113553 DOI: 10.1007/s00120-011-2762-9]

69 **De Lichtenberg TH**, Hermann GG, Rørth M, Højer Larsen MJ, Mansourvar Z, Holm ML, Scheike T. Overall survival after immunotherapy, tyrosine kinase inhibitors and surgery in treatment of metastatic renal cell cancer: outcome of 143 consecutive patients from a single centre. *Scand J Urol* 2014; **48**: 379-386 [PMID: 24521185 DOI: 10.3109/21681805.2013.876550]

70 **Inoue T**, Murota T, Masuda T, Nishida T, Kawakita S, Kinoshita H, Matsuda T. [Pre- surgical therapy with sunitinib for adrenal

metastasis from renal cell carcinoma : a case report]. *Hinyokika Kiyo* 2013; **59**: 573-577 [PMID: 24113755]

71 **Hamada A**, Sunada T, Kato K, Sawada A, Kawanishi H, Okumura K. [A case of adrenal metastasectomy in renal cell carcinoma after neoadjuvant therapy with sunitinib]. *Hinyokika Kiyo* 2014; **60**: 79-82 [PMID: 24755818]

72 **Johannsen M**, Flörcken A, Bex A, Roigas J, Cosentino M, Ficarra V, Kloeters C, Rief M, Rogalla P, Miller K, Grünwald V. Can tyrosine kinase inhibitors be discontinued in patients with metastatic renal cell carcinoma and a complete response to treatment? A multicentre, retrospective analysis. *Eur Urol* 2009; **55**: 1430-1438 [PMID: 18950936 DOI: 10.1016/j.eururo.2008]

73 **Johannsen M**, Staehler M, Ohlmann CH, Flörcken A, Schmittel A, Otto T, Bex A, Hein P, Miller K, Weikert S, Grünwald V. Outcome of treatment discontinuation in patients with metastatic renal cell carcinoma and no evidence of disease following targeted therapy with or without metastasectomy. *Ann Oncol* 2011; **22**: 657-663 [PMID: 20870911 DOI: 10.1093/annonc/mdq437]

74 **Shuch B**, Riggs SB, LaRochelle JC, Kabbinavar FF, Avakian R, Pantuck AJ, Patard JJ, Belldegrun AS. Neoadjuvant targeted therapy and advanced kidney cancer: observations and implications for a new treatment paradigm. *BJU Int* 2008; **102**: 692-696 [PMID: 18410444 DOI: 10.1111/j.1464-410X.2008.07660.x]

75 **Bose D**, Meric-Bernstam F, Hofstetter W, Reardon DA, Flaherty KT, Ellis LM. Vascular endothelial growth factor targeted therapy in the perioperative setting: implications for patient care. *Lancet Oncol* 2010; **11**: 373-382 [PMID: 20171141 DOI: 10.1016/S1470-2045(09)70341-9]

76 **Kwak C**, Park YH, Jeong CW, Lee SE, Ku JH. No role of adjuvant systemic therapy after complete metastasectomy in metastatic renal cell carcinoma? *Urol Oncol* 2007; **25**: 310-316 [PMID: 17628297 DOI: 10.1016/j.urolonc.2006.08.022]

77 **Jacobsohn KM**, Wood CG. Adjuvant therapy for renal cell carcinoma. *Semin Oncol* 2006; **33**: 576-582 [PMID: 17045086 DOI: 10.1053/j.seminoncol.2006.06.005]

78 **Joshi S**, Eldefrawy A, Ciancio G. 8-year survival in a patient with several recurrences of renal cell carcinoma after radical nephrectomy. *Cent European J Urol* 2012; **65**: 242-243 [PMID: 24578974 DOI: 10.5173/ceju.2012.04.art16]

79 **Gardini A**, Morgagni P, Milandri C, Riccobon A, Ridolfi R, La Barba G, Saragoni L, Amadori D, Garcea D. Pancreatic resection for metastases from renal cancer: long term outcome after surgery and immunotherapy approach - single center experience. *Hepatogastroenterology* 2012; **59**: 687-690 [PMID: 22469709]

80 **Vickers MM**, Al-Harbi H, Choueiri TK, Kollmannsberger C, North S, MacKenzie M, Knox JJ, Rini BI, Heng DY. Prognostic factors of survival for patients with metastatic renal cell carcinoma with brain metastases treated with targeted therapy: results from the international metastatic renal cell carcinoma database consortium. *Clin Genitourin Cancer* 2013; **11**: 311-315 [PMID: 23684422 DOI: 10.1016/j.clgc.2013.04.012]

81 **Procopio G**, Verzoni E, Biondani P, Grassi P, Testa I, Garanzini E, de Braud F. Rationale and protocol of RESORT, a randomized, open-label, multicenter phase II study to evaluate the efficacy of sorafenib in patients with advanced renal cell carcinoma after radical resection of the metastases. *Tumori* 2014; **100**: e28-e30 [PMID: 24675507 DOI: 10.1700/1430.15834]

82 **Rexer H**. [Urgent patient admission to Working Group for Urological Oncology (AUO) studies on metastasized renal cell carcinoma needed: prospective randomized multicenter phase II study on resection of pulmonary metastases in clear cell renal cell carcinoma ± adjuvant sunitinib therapy over 1 year (SMAT - AN 20/04 of the AUO)]. *Urologe A* 2012; **51**: 252-253 [PMID: 22331073 DOI: 10.1007/s00120-012-2809-6]

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Primary glomerular diseases in the elderly

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Abstract

Primary glomerular diseases in the elderly population are a frustrating topic due to difficulties in both the diagnosis and decision making about treatment. The most frequent type of primary glomerular disease in elderly is membranous nephropathy; while its counterpart in younger population is IgA nephropathy. The most frequent cause of nephrotic syndrome in the elderly is also membranous nephropathy. Pauci-immune crescentic glomerulonephritis (GN) rate increases both in elderly and very elderly population. Pauci-immune crescentic GNs should be regarded as urgencies in elderly patients as in their younger counterparts due to potential for causing end-stage renal disease in case of delayed diagnosis and treatment, and also causing mortality due to alveolar hemorrhage in patients with pulmonary involvement. Renal biopsy is the inevitable diagnostic method in the elderly as in all other age groups. Renal biopsy prevents unnecessary treatments and provides prognostic data. So advanced age should not be the sole contraindication for renal biopsy. The course of primary glomerular diseases may differ in the elderly population. Acute kidney injury is more frequent in the course and renal functions may be worse at presentation. These patients are more prone to be hypertensive. The decision about adding immune suppressive therapies to conservative methods should be made considering many factors like co-morbidities, drug side effects and potential drug interactions, risk of infection, patient preference, life expectancy and renal functions at the time of diagnosis.

Key words: Elderly; Membranous nephropathy; Renal biopsy; Pauci-immune crescentic glomerulonephritis; Primary glomerular disease

Core tip: Primary glomerular diseases in the elderly population are a frustrating topic due to difficulties in both diagnosis and treatment. The most frequent type of primary glomerular disease and the most frequent cause of nephrotic syndrome is membranous nephropathy. The frequency of pauci-immune glomerulonephritides increases considerably in the very elderly population. Renal biopsy is the inevitable diagnostic method in the elderly as in all other age groups. The decision about adding immune suppressive therapies to conservative methods should be made considering many factors like co-morbidities, drug side effects, patient preference, life expectancy and renal functions at the time of diagnosis.

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INTRODUCTION

Primary glomerular diseases (PGD) in the elderly deserves mention under a heading separate from PGDs in young adults due to differences in epidemiological and clinical characteristics, and difficulties in diagnosis and decision making about diagnosis. Co-morbidities, shorter life expectancy, complications of renal biopsy and immunosuppressive medications are among the factors that challenge the clinicians about diagnosis and treatment. Paucity of clinical studies and so insufficiency of evidence and guidelines are other problems in the elderly population that increases progressively due to increased mean life expectancy[1]. First, general epidemiological and clinical characteristics of PGDs in the elderly will be mentioned followed by details about specific diseases.

The frequency of PGDs in the elderly may change in countries. Ethnic predisposition, different approaches about biopsy indications and differences in the methods and design of epidemiological studies are among the causes of this variability. We learn about epidemiological data about PGDs in the elderly, from glomerulonephritis or biopsy registries of countries. These studies may be classified as involving "elderly" (> 60-65 years) and "very elderly" (> 80-85 years) patients.

Some of the registries that you can gain information about epidemiological data in the elderly population in Europe are those of Italy, Spain, Czech Republic and Turkey[2-5]. Membranous nephropathy (MN) was reported in these studies as the most frequent PGD and the most frequent cause of nephrotic syndrome in patient older than 65 years. The PGD in the second order changes in different countries. The evaluation of pauci-immune crescentic glomerulonephritis (pauci-immune crescentic GN) within PGDs in some studies while within secondary glomerular diseases in the others leads to difficulties in evaluation of epidemiological studies. The most frequent biopsy indication is nephrotic syndrome as expected whether accompanied by acute kidney injury (AKI) or not. The manuscript by Yokoyama *et al*[6] who presented data of Japan Renal Biopsy Registry has a special place in the literature due the highest number of patients. Data of 2802 patients aged > 65 (group A) and 276 patients aged > 80 years (group B) were presented in this study. Forty-five percent of cases were PGDs. The most frequent PGDs in group A and B were MN, IgA nephropathy (IgAN) and minimal change disease (MCD) in order, while the most frequent diagnoses in elderly patients who had renal biopsy due to nephrotic syndrome were MN, MCD and focal segmental glomerulosclerosis (FSGS) with decreasing order. The most frequent biopsy indication was nephrotic syndrome in both groups, while rapidly progressive glomerulonephritis (RPGN) was the second most frequent cause in group B. When compared with patients aged less than 65 years, pauci-immune crescentic GN, MN, type 1 and 3 membranoproliferative glomerulonephritis (MPGN) were more frequent and IgAN was significantly less frequent in patients aged more than 65 years. The ratio of renal biopsies performed due to RPGN was higher in the elderly population compared to younger counterparts. There are also current studies presenting epidemiological data of elderly patients in a single center besides registry studies[7-10]. MN was again the most frequent diagnosis in these studies except in the study by Brown *et al*[10] in which pauci-immune crescentic GN was the most frequent PGD. Recent studies about the epidemiology of PGD in elderly are summarized in Table 1.

Recent articles have been published about epidemiological data of very elderly patients although the age limit is variable[11-15]. Although all cases are not PGDs in these studies, they provide important information about PGDs in the very elderly population. The most frequent PGD in studies originating from United States[11,14] was pauci-immune crescentic GN, while it was MN in other studies from European and Asian countries. Biopsy indications in these studies follow the same order, and provide clues about behavior regarding biopsy indication in this special age group in these countries. The most frequent biopsy indication is AKI in United States, while it is nephrotic syndrome in other European and Asian countries. Studies performed with very elderly patients are summarized in Table 2.

Although renal biopsy is the inevitable diagnostic method in glomerular diseases, it is not performed in some of the patients due to various factors including co-existing systemic diseases, shorter life expectancy, reluctance of the clinicians about biopsy and immunosuppressive treatment and patient preference. There are studies in the literature reporting that bleeding risk after renal biopsy in elderly patients is not different from other age groups[16,17]. But, the possibility that clinicians would have performed renal biopsy in elderly patients with lower risk in these studies in which data of biopsy series are presented, should be kept in mind. As well known, the most important predictor of bleeding complication is serum creatinine level[17]. This complication is more common in patients with renal failure compared to patients without. The concern of clinicians about this complication is not undue considering physiological changes related to age, co-existing systemic diseases (hypertension, atherosclerosis, diabetes mellitus, amyloidosis), and overestimation of glomerular filtration rate with creatinine level due to decreased muscle mass. When possible complications of immunosuppressive treatment add on these concerns, some clinicians prefer conservative methods without performing renal biopsy. Some other clinicians on the other hand try empiric

Table 1 Recent epidemiological studies in the elderly

Country	Ref.	Date	Number of cases	Age	The most frequent PGDs
Italy[1]	Vendemia *et al*[2]	2001	280	> 65	1. MN 2. Pauci-immune GN 3. MPGN
Turkey	Ozturk *et al*[5]	2014	150	> 60	1. MN 2. Pauci-immune GN 3. FSGS
Japan	Yokoyama *et al*[6]	2012	2802	> 65	1. MN 2. IgAN 3. MCD
Brasil	Carmo *et al*[7]	2010	113	> 60	1. MN 2. FSGS 3. MCD
South Africa	Okpechi *et al*[8]	2013	111	> 60	1. MN 2. IgAN 3. Pauci-immune GN
China	Jin *et al*[9]	2014	851	> 65	1. MN 2. IgAN 3. MCD
Ireland	Brown *et al*[10]	2012	236	> 65	1. Pauci-immune GN 2. MN 3. IgAN

[1]Only patients with PGDs were included in this study, while other studies included patients with secondary glomerular diseases also. FSGS: Focal segmental glomerulosclerosis; GN: Glomerulonephritis; IgAN: IgA nephropathy; MCD: Minimal change disease; MN: Membranous nephropathy; MPGN: Membranoproliferative glomerulonephritis; PGD: Primary glomerular disease.

immunosuppressive treatment without biopsy. Yoon *et al*[18] evaluated this subject in their study. They evaluated renal and patient survival rates of 99 patients (age > 60 years) presenting with nephrotic syndrome who were grouped as those who had renal biopsy (n = 64) and those who did not (n = 35). The major defect of this study was the lower mean age and better renal functions in the group who had renal biopsy. Although complete remission was more frequent (45% *vs* 26%, P = 0.013) in the biopsy group in which statistically significantly more patients had immunosuppressive therapy (P < 0.005), renal survival rates were similar. Patient survival was lower in the group without biopsy which was not a surprise considering significantly higher mean age.

On the other hand, there are factors that lead the clinician towards biopsy like need of urgent diagnosis for optimum treatment of pauci-immune glomerulonephritides presenting as RPGN; the risk of not giving specific treatment considering more susceptibility of elderly to infective and thrombotic complications of nephrotic syndrome[19,20]; prevention of unnecessary treatments by renal biopsy; and provision of prognostic data. Studies with very elderly patients revealed that therapeutic approach may change 40%-67% with renal biopsy[11,14]. So, advanced age should not be the sole contraindication for renal biopsy. The clinician has to decide respecting the preference of the patient within this multifactorial equation.

Renal biopsy in elderly has the potential to be problematic for pathologists as well as clinicians. Varying degrees of "background" glomerulosclerosis, tubular atrophy, arteriolar hyalinosis that may be seen as a result of both senility and co-morbidities may superimpose primary and secondary glomerular diseases[21].

Primary glomerular diseases in the elderly present as nephrotic syndrome, nephritic syndrome, RPGN, asymptomatic urine abnormalities or chronic glomerulonephritis as in other age groups. But nephrotic syndrome and acute nephritic syndrome including RPGN comprises most of the cases as can be understood from biopsy indications in reported by biopsy series. PGDs causing nephrotic syndrome are MN, FSGS and MDH, while MPGN, IgAN and pauci-immune crescentic GNs comprise the major causes of nephritic syndrome. But different and complex forms of presentation are not rare. As an example, AKI superimposed on nephrotic syndrome is more frequent in elderly population. Some of the authors consider AKI on the basis of nephrotic syndrome as idiopathic if there is no clear reason as drug use, exposure to radio contrast agent or interstitial nephritis[22].

The treatment of PGDs in the elderly causes difficulties as the diagnosis. Co-morbidities, the number of pills that the patients take, potential drug interactions, risk of infection, patient preference, expected life expectancy, renal functions at the time of diagnosis, increased drug toxicity risk due to age related decreased in drug metabolism and excretion[23,24] are some of the factors effective on the decision of the clinician about treatment. Moreover, disease specific secondary causes should be searched for promptly as well as

Table 2 Recent epidemiological studies in the very elderly population

Country	Ref.	Date	Number of cases	Age	The most frequent PGD
Japonya	Yokoyama *et al*[6]	2012	276	> 80	1. MN 2. IgAN 3. MCD
United States	Moutzouris *et al*[11]	2009	235	> 80	1. Pauci-immune GN 2. MN 3. IgAN
Italy	Rollino *et al*[12]	2014	131	> 75	1. MN 2. Pauci-immune GN 3. IgAN
Japan	Omokawa *et al*[13]	2012	73	> 80	1. MN 2. MCD
United States	Nair *et al*[14]	2004	100	> 80	1. Pauci-immune GN 2. MN
Spain	Verde *et al*[15]	2012	71	> 85	1. MN 2. Pauci-immune GN 3. IgAN

GN: Glomerulonephritis; IgAN: IgA nephropathy; MCD: Minimal change disease; MN: Membranous nephropathy; PGD: Primary glomerular disease.

any contraindication for treatment and screening for malignancy appropriate for the age group should be performed.

Conservative methods are the sine qua non of treatment of patients with nephrotic syndrome in this age group. Salt restriction, smoking cessation, diuretics, renin-angiotensin-aldosterone system blockers, statins, anticoagulant agents and pneumococcal vaccination are the components of conservative treatment[25,26]. Anticoagulation is recommended in patients with serum albumin level below 2 g/dL and co-existing risk factors if bleeding risk not high. But treatment decision should be individualized as in all cases. An article reporting the importance of forming a scaling system for thrombosis and bleeding before decision about anticoagulant use has been published recently[27].

Immunosuppressive therapy should be considered in cases with nephrotic proteinuria in spite of conservative methods, progressively declining renal functions, life threatening complications of nephrotic syndrome like thrombosis, and patients with RPGN. No guideline has been developed up to now for glomerulonephritides in the elderly. "Kidney Disease Improving Global Outcomes (KDIGO) Clinical Practice Guideline for Glomerulonephritis" published in 2012 helps the clinicians caring elderly patients. But it is difficult to adopt all recommendations to old patients. The clinician has to choose the correct treatment method considering both positive and negative sides together. All medications should be used in doses appropriate for the renal function of the patient.

MEMBRANOUS NEPHROPATHY

The most frequent PGD in adult population all over the world is IgAN as is well known[28,29]. But MN with its frequency increasing with aging, is the most common PGD and the most common reason of nephrotic syndrome in elderly. AKI is more frequent in the course of disease compared to other PGDs. Advanced age has been reported to be a risk factor for AKI in patients with MN[30]. Moreover, hypertension and worse renal functions at the time of presentation are expected to be more prevalent in elderly patients. There are studies reporting increased risk of thrombotic[31] and infectious[32] complications compared to adult patients.

The PGD that is most associated with malignancies is MN, and it is speculated that it accompanies tumors in 10% of cases[33,34]. M type anti phospholipase A2 antibodies that started a new era protects from unnecessary interventional investigations[35,36]. There is a tendency to screen patients with M type anti phospholipase A2 antibodies in accordance with age; while more complicated screening is necessary in those without anti phospholipase A2 antibody[37]. Another difference is in the subtypes of IgG on immune fluorescent microscopy, although not routinely studied. IgG4 predominates in primary MN, while IgG1 and/or IgG2 staining is expected to be positive in MN associated with malignancies[36]. Malignancies are usually clinically evident at the time of diagnosis of nephrotic syndrome. However, there are reported cases with malignancies reported late in the course. Some authors think that screening for cancer should be repeated within 5-10 years in cases with histological and serological testing resembling secondary MN[38,39]. History of medications, screening for infection (hepatitis B and malaria) and evaluation for systemic lupus erythematosus should not be forgotten. Nonsteroidal anti-inflammatory drugs (NSAID) are in the first order among drugs related with MN. NSAIDs may cause MN and MDH as well as non-glomerular diseases[40,41].

It has been shown that corticosteroid therapy alone in elderly patients with MN is not enough and actually, it is related with more complication[42,43]. Ponticelli

protocol (in which steroids are used in combination with either chlorambucil or cyclophosphamide) can be tried[44]. KDIGO guideline proposes immunosuppressive treatment in patients with severe life-threatening symptoms and findings, proteinuria more than 4 g/d in spite of conservative methods, or at least 30% increase in serum creatinine level within the last 6-12 mo[26]. However, there are no up-to-date randomized controlled trials about side effect profile and efficacy of steroid treatment in old patients. Besides, studies about the role of cyclosporine plus low dose steroid, and mycophenolate mofetil are not enough also. We can mention a study in which mizoribin was used in a few old patients. But the number of patients is not enough, and mizoribin group was not compared with patients receiving only steroid treatment[45].

MINIMAL CHANGE DISEASE

Minimal change disease which is one of the important causes of nephrotic syndrome in elderly presents withy hypertension and AKI more compared with younger population. Some authors believe that AKI superimposed on nephrotic syndrome in elderly is commonly associated with MCD, and elderly patients are more prone to acute tubular necrosis[46,47]. Relapses are rarer in patients older than 40 years compared to patients younger than 40 years[47,48]. All immunosuppressive medications used in the treatment of glomerulonephritis have been tried with considerable success, although steroids remain to be the mainstay of treatment[26].

FOCAL SEGMENTAL GLOMERULOSCLEROSIS

We do not have enough data in the literature about the clinical characteristics and treatment of FSGF in the elderly. Tip variant of FSGS has been reported to be the histologic type presenting with sudden onset of severe nephrotic syndrome and also the type which is the most sensitive to steroid treatment. Tip lesions ten de be more prevalent in older patients[49,50]. Important predictors of renal prognosis are the magnitude of proteinuria, the level of kidney function, and the amount of tubulointerstitial injury[51]. Corticosteroids are the first line treatment in appropriate patients while second line treatment with cyclosporine plus low dose steroid may be preferred in cases for which there is considerable risk for corticosteroid side effects[26]. Evaluation for secondary causes of FSGS should not be omitted. Interferons[52] and intravenous use of bisphosphonates (especially pamidronate)[53] which are commonly prescribed in this population are examples for causes of secondary FSGS. American Society of Clinical Oncology published an update for use of bisphosphonates in multiple myeloma including knowledge about dose reduction in case of decreased renal function[54].

IGA NEPHROPATHY

IgA nephropathy is associated with more severe renal manifestations at presentation in the elderly. It has been reported in Spanish Registry of Glomerulonephritis that 27.8% of patients with IgAN older than 65 years presented as AKI[3]. This ratio reached to 53% in another study with the emphasis that tubular injury is more prominent than glomerular damage in these patients[55]. Advanced age has been determines as a risk factor for progression to end-stage renal disease (ESRD) which was found to be 1.95 times more common compared to young adults[56]. An article has been published recently reporting that 70% of patients reach ESRD within 20 years[57]. The only immunosuppressive medication proved to be effective in IgAN is corticosteroids. Although persistent proteinuria in spite of conservative measures is an indication for corticosteroid treatment according to KDIGO guideline, it may not be wise to give corticosteroid treatment to elderly patients with normal renal functions, blood pressure and non-nephrotic range proteinuria, especially in the presence of comorbidities. However, IgAN presenting as crescentic glomerulonephritis should be treated as pauci-immune crescentic glomerulonephritis[26].

MEMBRANOPROLIFERATIVE GLOMERULONEPHRITIS

Primary MGPN is a rare disease. So, secondary causes, especially monoclonal gammopathies and hepatitis C infection, should be ruled out case of pathological diagnosis of MPGN[58,59]. Although usually not responsive, corticosteroid + mycophenolate mofetil or corticosteroid + oral cyclophosphamide may be tried in patients with MPGN type Ⅰ presenting with nephrotic syndrome and/or rapid increase in creatinine levels[26,60]. But patients and relatives should be informed thoroughly about the low response rates before deciding for immunosuppressive treatment.

PAUCI-IMMUNE CRESCENTIC GLOMERULONEPHRITIS

Pauci-immune crescentic GN is disease group with increased rate both in elderly and very elderly population[61]. This group represents renal involvement in anti-glomerular basement membrane disease and anti-neutrophil cytoplasmic autoantibody associated vasculitides. Renopulmonary syndrome is the more frequent type of presentation although isolated renal involvement may also be seen. The first explanation for increased frequency is the peak that the systemic vasculitides show between ages 65-74 years[62]. Moreover, presentation with RPGN increases the probability of performing renal biopsy in these patients for whom

the clinicians may prefer to remain conservative otherwise. Pauci-immune crescentic GNs should be regarded as urgencies in elderly patients as in their younger counterparts due to potential for causing ESRD in case of delayed diagnosis and treatment, and also causing mortality due to alveolar hemorrhage in patients with pulmonary involvement[63]. Renal biopsy should be scheduled immediately and serum samples should be taken for determination of anti-neutrophilic cytoplasmic antibody, anti-glomerular basal membrane antibody and then immunosuppressive treatment should be started as soon as possible. In the absence of absolute contraindications, pulse corticosteroid and cyclophosphamide treatment should be started together with plasma exchange in the presence of alveolar hemorrhage or rapid decline in renal functions[26]. In case of vasculitides limited to kidney, decision about treatment and its duration should be made regarding comorbidities and activity/chronicity of lesions on renal biopsy. Renal survival in anti-glomerular basal membrane disease is related with creatinine levels at the time of admission[64]. So, early diagnosis and treatment have prime importance. Independent determinants of mortality in anti neutrophil cytoplasmic autoantibody-associated vasculitides have been found to be advanced age and pulmonary infections[65]. KDIGO guideline recommends crescentic forms of any PGDs to be considered as pauci-immune GN and treated so[26].

As a conclusion, PGDs in elderly are a group of diseases that challenges the clinicians in both diagnosis and treatment. Although MN is the most common PGD in this age group, crescentic glomerulonephritides should always be considered due to irretrievable results.

REFERENCES

1 **Stevens LA**, Li S, Wang C, Huang C, Becker BN, Bomback AS, Brown WW, Burrows NR, Jurkovitz CT, McFarlane SI, Norris KC, Shlipak M, Whaley-Connell AT, Chen SC, Bakris GL, McCullough PA. Prevalence of CKD and comorbid illness in elderly patients in the United States: results from the Kidney Early Evaluation Program (KEEP). *Am J Kidney Dis* 2010; **55**: S23-S33 [PMID: 20172445 DOI: 10.1053/j.ajkd.2009.09.035]

2 **Vendemia F**, Gesualdo L, Schena FP, D'Amico G. Epidemiology of primary glomerulonephritis in the elderly. Report from the Italian Registry of Renal Biopsy. *J Nephrol* 2001; **14**: 340-352 [PMID: 11730266]

3 **Rivera F**, López-Gómez JM, Pérez-García R. Frequency of renal pathology in Spain 1994-1999. *Nephrol Dial Transplant* 2002; **17**: 1594-1602 [PMID: 12198210 DOI: 10.1093/ndt/17.9.1594]

4 **Rychlík I**, Jancová E, Tesar V, Kolsky A, Lácha J, Stejskal J, Stejskalová A, Dusek J, Herout V. The Czech registry of renal biopsies. Occurrence of renal diseases in the years 1994-2000. *Nephrol Dial Transplant* 2004; **19**: 3040-3049 [PMID: 15507479 DOI: 10.1093/ndt/gfh521]

5 **Ozturk S**, Sumnu A, Seyahi N, Gullulu M, Sipahioglu M, Artan S, Bicik Z, Kutlay S, Keles M, Oygar D, Odabas AR, Kayatas M, Dursun B, Sayarlioglu H, Trablus S, Taymez DG, Ozdemir AA, Sahin GM, Altun B, Azak A, Altintepe L, Suleymanlar G, Koc M, Selcuk Y, Kazancioglu R, Erkoc R, Gursu M, Kucuk M, Akcaoglu SA, Yıldız A, Unal A, Akarsu O, Ates K, Cankaya E, Turkmen A. Demographic and clinical characteristics of primary glomerular diseases in Turkey. *Int Urol Nephrol* 2014; **46**: 2347-2355 [PMID: 25269407 DOI: 10.1007/s11255-014-0838-3]

6 **Yokoyama H**, Sugiyama H, Sato H, Taguchi T, Nagata M, Matsuo S, Makino H, Watanabe T, Saito T, Kiyohara Y, Nishi S, Iida H, Morozumi K, Fukatsu A, Sasaki T, Tsuruya K, Kohda Y, Higuchi M, Kiyomoto H, Goto S, Hattori M, Hataya H, Kagami S, Yoshikawa N, Fukasawa Y, Ueda Y, Kitamura H, Shimizu A, Oka K, Nakagawa N, Ito T, Uchida S, Furuichi K, Nakaya I, Umemura S, Hiromura K, Yoshimura M, Hirawa N, Shigematsu T, Fukagawa M, Hiramatsu M, Terada Y, Uemura O, Kawata T, Matsunaga A, Kuroki A, Mori Y, Mitsuiki K, Yoshida H. Renal disease in the elderly and the very elderly Japanese: analysis of the Japan Renal Biopsy Registry (J-RBR). *Clin Exp Nephrol* 2012; **16**: 903-920 [PMID: 23053590 DOI: 10.1007/s10157-012-0673-8]

7 **Carmo PA**, Kirsztajn GM, Carmo WB, Franco MF, Bastos MG. Histopathological findings in elderly patients. *J Bras Nefrol* 2010; **32**: 286-291 [PMID: 21103693]

8 **Okpechi IG**, Ayodele OE, Rayner BL, Swanepoel CR. Kidney disease in elderly South Africans. *Clin Nephrol* 2013; **79**: 269-276 [PMID: 23195833 DOI: 10.5414/CN107746]

9 **Jin B**, Zeng C, Ge Y, Le W, Xie H, Chen H, Liang S, Xu F, Jiang S, Liu Z. The spectrum of biopsy-proven kidney diseases in elderly Chinese patients. *Nephrol Dial Transplant* 2014; **29**: 2251-2259 [PMID: 25034755 DOI: 10.1093/ndt/gfu239]

10 **Brown CM**, Scheven L, O'Kelly P, Dorman AM, Walshe JJ. Renal histology in the elderly: indications and outcomes. *J Nephrol* 2012; **25**: 240-244 [PMID: 21725922 DOI: 10.5301/JN.2011.8447]

11 **Moutzouris DA**, Herlitz L, Appel GB, Markowitz GS, Freudenthal B, Radhakrishnan J, D'Agati VD. Renal biopsy in the very elderly. *Clin J Am Soc Nephrol* 2009; **4**: 1073-1082 [PMID: 19443626 DOI: 10.2215/CJN.00990209]

12 **Rollino C**, Ferro M, Beltrame G, Quattrocchio G, Massara C, Quarello F, Roccatello D. Renal biopsy in patients over 75: 131 cases. *Clin Nephrol* 2014; **82**: 225-230 [PMID: 25161113 DOI: 10.5414/CN108258]

13 **Omokawa A**, Komatsuda A, Nara M, Fujiwara T, Sato R, Togashi M, Okuyama S, Sawada K, Wakui H. Renal biopsy in patients aged 80 years and older: a single-center experience in Japan. *Clin Nephrol* 2012; **77**: 461-467 [PMID: 22595388 DOI: 10.5414/CN107368]

14 **Nair R**, Bell JM, Walker PD. Renal biopsy in patients aged 80 years and older. *Am J Kidney Dis* 2004; **44**: 618-626 [PMID: 15384012 DOI: 10.1053/j.ajkd.2004.05.044]

15 **Verde E**, Quiroga B, Rivera F, López-Gómez JM. Renal biopsy in very elderly patients: data from the Spanish Registry of Glomerulonephritis. *Am J Nephrol* 2012; **35**: 230-237 [PMID: 22343659 DOI: 10.1159/000336307]

16 **Parrish AE**. Complications of percutaneous renal biopsy: a review of 37 years' experience. *Clin Nephrol* 1992; **38**: 135-141 [PMID: 1395165]

17 **Whittier WL**, Korbet SM. Timing of complications in percutaneous renal biopsy. *J Am Soc Nephrol* 2004; **15**: 142-147 [PMID: 14694166 DOI: 10.1097/01.ASN.0000102472.37947.14]

18 **Yoon HE**, Shin MJ, Kim YS, Choi BS, Kim BS, Choi YJ, Kim YO, Yoon SA, Kim YS, Yang CW. Clinical impact of renal biopsy on outcomes in elderly patients with nephrotic syndrome. *Nephron Clin Pract* 2011; **117**: c20-c27 [PMID: 20689321 DOI: 10.1159/000319643]

19 **Abrass CK**. Treatment of membranous nephropathy in the elderly. *Semin Nephrol* 2003; **23**: 373-378 [PMID: 12923725 DOI: 10.1016/S0270-9295(03)00053-6]

20 **Cameron JS**. Nephrotic syndrome in the elderly. *Semin Nephrol* 1996; **16**: 319-329 [PMID: 8829270]

21 **Rule AD**, Amer H, Cornell LD, Taler SJ, Cosio FG, Kremers WK, Textor SC, Stegall MD. The association between age and nephrosclerosis on renal biopsy among healthy adults. *Ann Intern Med* 2010; **152**: 561-567 [PMID: 20439574 DOI: 10.7326/0003-4819-152-9-201005040-00006]

22 **Tavares MB**, Chagas de Almeida Mda C, Martins RT, de Sousa AC, Martinelli R, dos-Santos WL. Acute tubular necrosis and renal failure in patients with glomerular disease. *Ren Fail* 2012; **34**:

1252-1257 [PMID: 23002699 DOI: 10.3109/0886022X.2012.723582]
23 **Mangoni AA**, Jackson SH. Age-related changes in pharmacokinetics and pharmacodynamics: basic principles and practical applications. *Br J Clin Pharmacol* 2004; **57**: 6-14 [PMID: 14678335 DOI: 10.1046/j.1365-2125.2003.02007.x]
24 **Shi S**, Klotz U. Age-related changes in pharmacokinetics. *Curr Drug Metab* 2011; **12**: 601-610 [PMID: 21495970 DOI: 10.2174/138920011796504527]
25 **Petrovichev NN**. [Change in the mitotic activity of hepatocytes and resorption of necrotic areas in the formation of liver cirrhosis]. *Biull Eksp Biol Med* 1976; **81**: 617-618 [PMID: 181102 DOI: 10.1111/j.1440-1797.2007.00890.x]
26 **International Society of Nephrology.** KDIGO Clinical Practice Guideline for Glomerulonephritis. *Kidney Int Suppl* 2012; **2**: 142 [DOI: 10.1038/kisup.2012.12]
27 **Lee T**, Biddle AK, Lionaki S, Derebail VK, Barbour SJ, Tannous S, Hladunewich MA, Hu Y, Poulton CJ, Mahoney SL, Charles Jennette J, Hogan SL, Falk RJ, Cattran DC, Reich HN, Nachman PH. Personalized prophylactic anticoagulation decision analysis in patients with membranous nephropathy. *Kidney Int* 2014; **85**: 1412-1420 [PMID: 24336031 DOI: 10.1038/ki.2013.476]
28 **Galla JH**. IgA nephropathy. *Kidney Int* 1995; **47**: 377-387 [PMID: 7723227 DOI: 10.1038/ki.1995.50]
29 **Donadio JV**, Grande JP. IgA nephropathy. *N Engl J Med* 2002; **347**: 738-748 [PMID: 12213946 DOI: 10.1056/NEJMra020109]
30 **Smith JD**, Hayslett JP. Reversible renal failure in the nephrotic syndrome. *Am J Kidney Dis* 1992; **19**: 201-213 [PMID: 1553965 DOI: 10.1016/S0272-6386(13)80001-7]
31 **O'Callaghan CA**, Hicks J, Doll H, Sacks SH, Cameron JS. Characteristics and outcome of membranous nephropathy in older patients. *Int Urol Nephrol* 2002; **33**: 157-165 [PMID: 12090324 DOI: 10.1023/A: 1014404006045]
32 **Yamaguchi M**, Ando M, Yamamoto R, Akiyama S, Kato S, Katsuno T, Kosugi T, Sato W, Tsuboi N, Yasuda Y, Mizuno M, Ito Y, Matsuo S, Maruyama S. Patient age and the prognosis of idiopathic membranous nephropathy. *PLoS One* 2014; **9**: e110376 [PMID: 25330372 DOI: 10.1371/journal.pone.0110376]
33 **Lefaucheur C**, Stengel B, Nochy D, Martel P, Hill GS, Jacquot C, Rossert J. Membranous nephropathy and cancer: Epidemiologic evidence and determinants of high-risk cancer association. *Kidney Int* 2006; **70**: 1510-1517 [PMID: 16941021 DOI: 10.1038/sj.ki.5001790]
34 **Beck LH**. Membranous nephropathy and malignancy. *Semin Nephrol* 2010; **30**: 635-644 [PMID: 21146128 DOI: 10.1016/j.semnephrol.2010.09.011]
35 **Beck LH**, Bonegio RG, Lambeau G, Beck DM, Powell DW, Cummins TD, Klein JB, Salant DJ. M-type phospholipase A2 receptor as target antigen in idiopathic membranous nephropathy. *N Engl J Med* 2009; **361**: 11-21 [PMID: 19571279 DOI: 10.1056/NEJMoa0810457]
36 **Larsen CP**, Messias NC, Silva FG, Messias E, Walker PD. Determination of primary versus secondary membranous glomerulopathy utilizing phospholipase A2 receptor staining in renal biopsies. *Mod Pathol* 2013; **26**: 709-715 [PMID: 23196797 DOI: 10.1038/modpathol.2012.207]
37 **Timmermans SA**, Ayalon R, van Paassen P, Beck LH, van Rie H, Wirtz JJ, Verseput GH, Frenken LA, Salant DJ, Cohen Tervaert JW. Anti-phospholipase A2 receptor antibodies and malignancy in membranous nephropathy. *Am J Kidney Dis* 2013; **62**: 1223-1225 [PMID: 24021909 DOI: 10.1053/j.ajkd.2013.07.019]
38 **Bjørneklett R**, Vikse BE, Svarstad E, Aasarød K, Bostad L, Langmark F, Iversen BM. Long-term risk of cancer in membranous nephropathy patients. *Am J Kidney Dis* 2007; **50**: 396-403 [PMID: 17720518 DOI: 10.1053/j.ajkd.2007.06.003]
39 **Beck LH,** Salant DJ. Causes and diagnosis of membranous nephropathy. In: UpToDate. Glassock RJ, Fervenza FC, editors. Waltham, MA: UpToDate. Available from: URL: http://www.uptodate.com/contents/causes-and-diagnosis-of-membranous-nephropathy
40 **Radford MG**, Holley KE, Grande JP, Larson TS, Wagoner RD, Donadio JV, McCarthy JT. Reversible membranous nephropathy associated with the use of nonsteroidal anti-inflammatory drugs. *JAMA* 1996; **276**: 466-469 [PMID: 8691554 DOI: 10.1001/jama.1996.03540060042033]
41 **Mihovilovic K**, Ljubanovic D, Knotek M. Safe administration of celecoxib to a patient with repeated episodes of nephrotic syndrome induced by NSAIDs. *Clin Drug Investig* 2011; **31**: 351-355 [PMID: 21271751 DOI: 10.2165/11586340-000000000-00000]
42 **Zent R**, Nagai R, Cattran DC. Idiopathic membranous nephropathy in the elderly: a comparative study. *Am J Kidney Dis* 1997; **29**: 200-206 [PMID: 9016890 DOI: 10.1016/S0272-6386(97)90030-5]
43 **Passerini P**, Como G, Viganò E, Melis P, Pozzi C, Altieri P, Ponticelli C. Idiopathic membranous nephropathy in the elderly. *Nephrol Dial Transplant* 1993; **8**: 1321-1325 [PMID: 8159299]
44 **Ponticelli C**, Zucchelli P, Passerini P, Cagnoli L, Cesana B, Pozzi C, Pasquali S, Imbasciati E, Grassi C, Redaelli B. A randomized trial of methylprednisolone and chlorambucil in idiopathic membranous nephropathy. *N Engl J Med* 1989; **320**: 8-13 [PMID: 2642605 DOI: 10.1056/NEJM198901053200102]
45 **Ito T**, Mochizuki K, Oka T, Hanada K, Tanabe K. Study of mizoribine therapy in elderly patients with membranous nephropathy: comparison with patients not receiving mizoribine. *Int Urol Nephrol* 2015; **47**: 131-135 [PMID: 25298141]
46 **Haas M**, Spargo BH, Wit EJ, Meehan SM. Etiologies and outcome of acute renal insufficiency in older adults: a renal biopsy study of 259 cases. *Am J Kidney Dis* 2000; **35**: 433-447 [PMID: 10692269 DOI: 10.1016/S0272-6386(00)70196-X]
47 **Waldman M**, Crew RJ, Valeri A, Busch J, Stokes B, Markowitz G, D'Agati V, Appel G. Adult minimal-change disease: clinical characteristics, treatment, and outcomes. *Clin J Am Soc Nephrol* 2007; **2**: 445-453 [PMID: 17699450 DOI: 10.2215/CJN.03531006]
48 **Nakayama M**, Katafuchi R, Yanase T, Ikeda K, Tanaka H, Fujimi S. Steroid responsiveness and frequency of relapse in adult-onset minimal change nephrotic syndrome. *Am J Kidney Dis* 2002; **39**: 503-512 [PMID: 11877569 DOI: 10.1053/ajkd.2002.31400]
49 **Chun MJ**, Korbet SM, Schwartz MM, Lewis EJ. Focal segmental glomerulosclerosis in nephrotic adults: presentation, prognosis, and response to therapy of the histologic variants. *J Am Soc Nephrol* 2004; **15**: 2169-2177 [PMID: 15284302 DOI: 10.1097/01.ASN.0000135051.62500.97]
50 **Thomas DB**, Franceschini N, Hogan SL, Ten Holder S, Jennette CE, Falk RJ, Jennette JC. Clinical and pathologic characteristics of focal segmental glomerulosclerosis pathologic variants. *Kidney Int* 2006; **69**: 920-926 [PMID: 16518352 DOI: 10.1038/sj.ki.5000160]
51 **Chitalia VC**, Wells JE, Robson RA, Searle M, Lynn KL. Predicting renal survival in primary focal glomerulosclerosis from the time of presentation. *Kidney Int* 1999; **56**: 2236-2242 [PMID: 10594800 DOI: 10.1038/sj.ki.4491164]
52 **Markowitz GS**, Nasr SH, Stokes MB, D'Agati VD. Treatment with IFN-{alpha}, -{beta}, or -{gamma} is associated with collapsing focal segmental glomerulosclerosis. *Clin J Am Soc Nephrol* 2010; **5**: 607-615 [PMID: 20203164 DOI: 10.2215/CJN.07311009]
53 **Markowitz GS**, Appel GB, Fine PL, Fenves AZ, Loon NR, Jagannath S, Kuhn JA, Dratch AD, D'Agati VD. Collapsing focal segmental glomerulosclerosis following treatment with high-dose pamidronate. *J Am Soc Nephrol* 2001; **12**: 1164-1172 [PMID: 11373339]
54 **Kyle RA**, Yee GC, Somerfield MR, Flynn PJ, Halabi S, Jagannath S, Orlowski RZ, Roodman DG, Twilde P, Anderson K. American Society of Clinical Oncology 2007 clinical practice guideline update on the role of bisphosphonates in multiple myeloma. *J Clin Oncol* 2007; **25**: 2464-2472 [PMID: 17515569 DOI: 10.1200/JCO.2007.12.1269]
55 **Wen YK**, Chen ML. Differences in new-onset IgA nephropathy between young adults and the elderly. *Ren Fail* 2010; **32**: 343-348 [PMID: 20370450 DOI: 10.3109/08860221003611687]
56 **Duan ZY**, Cai GY, Chen YZ, Liang S, Liu SW, Wu J, Qiu Q, Lin SP, Zhang XG, Chen XM. Aging promotes progression of IgA nephropathy: a systematic review and meta-analysis. *Am J Nephrol* 2013; **38**: 241-252 [PMID: 24021632 DOI: 10.1159/000354646]
57 **Oshima Y**, Moriyama T, Itabashi M, Takei T, Nitta K. Characteristics

of IgA nephropathy in advanced-age patients. *Int Urol Nephrol* 2015; **47**: 137-145 [PMID: 25388352]

58 **Sethi S**, Zand L, Leung N, Smith RJ, Jevremonic D, Herrmann SS, Fervenza FC. Membranoproliferative glomerulonephritis secondary to monoclonal gammopathy. *Clin J Am Soc Nephrol* 2010; **5**: 770-782 [PMID: 20185597 DOI: 10.2215/CJN.06760909]

59 **Johnson RJ**, Gretch DR, Yamabe H, Hart J, Bacchi CE, Hartwell P, Couser WG, Corey L, Wener MH, Alpers CE. Membranoproliferative glomerulonephritis associated with hepatitis C virus infection. *N Engl J Med* 1993; **328**: 465-470 [PMID: 7678440 DOI: 10.1056/NEJM199302183280703]

60 **Jones G**, Juszczak M, Kingdon E, Harber M, Sweny P, Burns A. Treatment of idiopathic membranoproliferative glomerulonephritis with mycophenolate mofetil and steroids. *Nephrol Dial Transplant* 2004; **19**: 3160-3164 [PMID: 15479745 DOI: 10.1093/ndt/gfh526]

61 **Abrass CK.** Glomerular Disease in the Elderly. In: Online Geriatric Nephrology Curriculum. Chicago: American Society of Nephrology, 2009. Available form: URL: https://www.asn-online.org/education/distancelearning/curricula/geriatrics/Chapter10.pdf

62 **Watts RA**, Lane SE, Bentham G, Scott DG. Epidemiology of systemic vasculitis: a ten-year study in the United Kingdom. *Arthritis Rheum* 2000; **43**: 414-419 [PMID: 10693883 DOI: 10.1002/1529-0131(200002)43: 2<414: : AID-ANR23>3.0.CO; 2-0]

63 **Ponticelli C,** Passerini P, Cresseri D. Primary glomerular diseases in the elderly. *Geriatr Nephrol Urol* 1996; **6**: 105-112 [DOI: 10.1007/BF00451114]

64 **Cui Z**, Zhao J, Jia XY, Zhu SN, Zhao MH. Clinical features and outcomes of anti-glomerular basement membrane disease in older patients. *Am J Kidney Dis* 2011; **57**: 575-582 [PMID: 21168945 DOI: 10.1053/j.ajkd.2010.09.022]

65 **Chen M**, Yu F, Zhang Y, Zhao MH. Antineutrophil cytoplasmic autoantibody-associated vasculitis in older patients. *Medicine* (Baltimore) 2008; **87**: 203-209 [PMID: 18626303 DOI: 10.1097/MD.0b013e31817c744b]

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Effect of urinary stone disease and its treatment on renal function

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Abstract

Urolithiasis is a common disease that affects urinary tract in all age groups. Both in adults and in children, stone size, location, renal anatomy, and other factors, can influence the success of treatment modalities. Recently, there has been a great advancement in technology for minimally invasive management of urinary stones. The epoch of open treatment modalities has passed and currently there are much less invasive treatment approaches, such as percutaneous nephrolithotomy, ureteroscopy, shockwave lithotripsy, and retrograde internal Surgery. Furthermore, advancement in imaging technics ensures substantial knowledge that permit physician to decide the most convenient treatment method for the patient. Thus, effective and rapid treatment of urinary tract stones is substantial for the preservation of the renal function. In this review, the effects of the treatment options for urinary stones on renal function have been reviewed.

Key words: Kidney stones; Chronic kidney disease; Estimated glomerular filtration rate; Renal function; Urinary stone disease

Core tip: In this article, urinary stone disease, treatment options and its effects on renal function are examined. Moreover, in the light of recent publications, effect of treatment options on functional state of the kidney in patients with renal impairment is investigated.

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INTRODUCTION

Urolithiasis is a widespread disease that affects the urinary system and a considerable, high-priced reason of morbidity. Urinary stone disease influences all age groups. The reported prevalence rate of stone disease is 5%-12% in men, 4%-7% in women[1]. Stone

formation is affected by gender, age and geography. Men's possibility of forming stones is more than women's. However, the ratio has decreased from a 3:1-male to female predominance to less than 1.3:1[2]. Recent studies indicate a raise in the prevalence of urinary stones, and this raise comprised in all gender, racial and ethnic ensemble in the United States[3].

Eating habits and environmental conditions also have a major act in the formation of urinary stones. Diabetes mellitus (DM), gout, and obesity are closely associated with urinary stone formation[4-6].

Children represent about 1% of all patients with urolithiasis, who have a almost 100% risk for recurrent stone formation. Both in adults and in children, stone size and location, other factors, including stone composition, patient factors, and renal anatomy, can influence the success of specific treatment modalities[7-9].

Over the years, there has been a great advancement in technology for minimally invasive treatment of urinary stones. The epoch of open pyelolithotomy has supplanted and currently there are much less invasive interventions, for instance, percutaneous nephrolithotomy (PCNL), ureteroscopy, shockwave lithotripsy (SWL), and RIRS (retrograde internal surgery). However, recurrent stone formation is still a major issue among patients with urolithiasis[10,11].

Chronic kidney disease (CKD) is a significant health issue that affects 13% of the adults in the United States. The potential reasons of renal failure in patients with urolithiasis are multifactorial and enclose hydronephrosis, infection, DM, hypertension (HT), repeated stone surgeries, eating habits, environmental, and genetic factors[12]. There are many studies that associate stone disease with varying degrees of renal insufficiency, patients with CKD present 0.8% to 17.5% of those presenting with urinary stone disease[13]. DM and HT, well-defined risk factors, were considerably related with renal impairment in patients with urinary stone[4,12].

PATHOGENESIS

The pathogenesis of renal calculi is a complicated process and similarly based on stone phenotype. Despite many investigations, the events that lead to kidney stone formation is as yet unknown. Several chemical theory has been proposed to explain the formation of stones[14]. These theories are supersaturation, nucleation and crystal formation, crystal retention, and effect of inhibitors and promoters on crystal growth.

In respect to the underlying reasons of kidney failure, previous researches have demonstrated that stone formation is related with the sedimentation of fragments in the peritubular field and in the medullary interstitium. A sedimentation like this can create inflammation and aid the advancement of fibrosis, which follows in tubular damage and detriment of kidney function. Moreover, fragment transition itself results in temporary occlusion, and occlusion is a well-defined risk factor for renal detriment in respect to the effect of fragments on kidney function, minor studies have contrasted the influences of the diverse stone types on kidney function. Two of the most widespread types of non-calcium stones were uric acid and struvite. The existence of struvite stones in some patients can reason the improvement of kidney insufficiency on account of recrudescent urinary tract infection (UTI). Patients with uric acid stone are mostly related with diseases which end up with renal insufficiency such as diabetes and gout[12].

DIAGNOSIS

Urinary stone formers generally complain with lumbar pain, vomitting, and occasionally fever, however, may not have any symptom as well. Routine assessment consists of an exhaustive history and physical examination. The preliminary diagnosis should be promoted by proper radiological technic. A wide variety of imaging methods are recently present to assist in the detection of urinary stones.

Imaging allows for the rapid and definitive diagnosis of stones, ensures significant knowledge that enables physicians to decide the most proper intervention for the patient. This knowledge contains the location and size of stone, and situation of kidney and collecting system[15]. Early on, abdominal plain films kidneys-ureters-bladder (KUB) and intravenous urography (IVU) were accepted as gold standards for the diagnosis of urolithiasis. However, with the advent of technologies such as ultrasound (US), noncontrast computed tomography (NCCT), a much larger assortment of imaging studies are now available to physicians appraising patients for stone disease.

Urinary stones can be categorized according to images on radiogram. Stones which contain calcium should be observable on radiogram (radiopaque). However, uric acid, ammonium urate, xanthine, and drug stones are not directly-visible (radiolucent). The declared sensitivity and specificity of radiogram in the determine of stone in patients with renal colic and no history of urolithiasis is limited. Further disadvantages of abdominal plain films in the detection of calculi include impaired image quality in obese patients and difficulty in differentiating pelvic vascular calcifications (phleboliths) from stones in the pelvic ureter. In addition, KUB generally will not generate useful information regarding the presence and/or degree of urinary tract obstruction[16].

Ultrasound is commonly performed during the evaluation of urolithiasis. The main advantage of ultrasound has over other imaging modalities such as NCCT or IVU is that is implemented without any radiation exposure. US can specify stones placed in the pelvis, calices, and proximal and distal ureter, as well as in patients with hydronephrosis[17].

Before the improvement of NCCT, IVU was considered the standard imaging technique for the assessment of

Table 1 Effects of shock wave lithotripsy on renal functions and glomerular filtration rate levels

Ref.	No. of patients	Patients' feature	Follow-up	Pre-SWL mean ± SD GFR (mL/min)	Post-SWL mean ± SD GFR (mL/min)	*P* value
Eassa *et al*[25]	108	Solitary kidney	3. 8 ± 3.5/yr	84.6 ± 24.7	82.5 ± 26.5	0.33
Fayad *et al*[27]	100	Children	6 mo	113.13 ± 4.51	113.01 ± 4.27	0.46

Difference is considered statistically significant at $P < 0.05$ and highly significant at $P < 0.01$. SWL: Shock wave lithotripsy; GFR: Glomerular filtration rate.

urinary stones. NCCT has higher sensitivity and specificity for detection of stones in urinary tract than IVU[18]. Uric acid and xanthine stones (radiolucent) can be determined by NCCT. Nonetheless, indinavir stones (radiolucent) cannot be specified by NCCT[19]. NCCT can define density and internal formation of the stone and the distance from skin to stone. IVU can provide information about renal function and whether a kidney is obstructed. Delayed images can be useful in evaluating ureteral anatomy for filling defects or strictures. It also provides detailed pelvicalyceal anatomy, which can be useful in planning surgical interventions, especially in those individuals with urinary tract anomalies. Therefore, IVU has largely been replaced by computed tomography (CT) with intravenous contrast or CT urograms. Low-dose NCCT (30 mAs) provides information close to those of standard NCCT (180 mAs) in demonstrating ureteral stone > 3 mm in patients with a BMI < 30[20]. It has been declared that low-dose NCCT provides significant information for the assessment of renal colic in pregnant patients[21].

TREATMENT OPTIONS AND EFFECTS ON RENAL FUNCTIONS

Extracorporeal SWL

Since its introduction, SWL has been a cornerstone for the management of the stone disease. SWL is the most common first line treatment for the majority of renal stones. Several studies have demonstrated stone-free rates as follows: renal pelvis 76%, upper calyx 69%, middle calyx 68%, and lower calyx 59%. Stone free rates were dependent on stone burden, with stones < 10 mm allowing excellent stone-free rates[2,22]. SWL success depends on many determinants, such as stone burden, position, composition of the stones, habitus of patient, and the efficacy of the lithotripter. SWL is a non-invasive treatment modality, nevertheless it might be related with some complications, for instance tissue injury, bleeding, adjacent organ injury, urinary tract obstruction, post treatment obstruction, and urinary tract infections, in early period. Clinically significant subcapsular and perirenal hematomas occur infrequently, with reported rates between 0.24% and 4.1%. Comorbidities for instance, HT, DM, obesity, coronary artery disease increase the risk of complication.

Moreover, new onset hypertension or diabetes mellitus after SWL treatment is controversial and previous studies are incoherent[23-25]. It has been investigated whether the influence of SWL on kidney function in long-term period. Cass reported that there was an average decline in eGFR of 22% in more than 24 mo of follow up. In contrast, it is stated that serum creatinine levels were not markedly affected after SWL in patients with a solitary kidney in approximately 4 years follow up[26]. In addition, it is stated that SWL treatment was not associated with a significant impact on kidney function or subsequent renal scaring, regardless of stone size or number of SWL seances in children[27]. The results of these two studies are shown in (Table 1).

SWL is an influential, proper, and noninvasive intervention in patients with urinary stones. Although the acute effects of SWL are well-known, it is accepted that treatment of renal stones with SWL does not affect kidney functions in the long term.

PERCUTANEOUS NEPHROLITHOTOMY

One of the most important factors in selecting the optimal surgical procedure for the patient with nephrolithiasis is stone burden because it has been shown to strongly influence stone-free rate, need for auxillary procedures, and complication rate for some treatment modalities[28]. PCNL is recommended for the treatment of all stones greater than or equal to 2 cm[29]. Deem *et al*[30] randomized 32 patients with moderate sized (1-2 cm) upper or middle calyceal or renal pelvis stones to PCNL or SWL and evaluated them at 3 mo with NCCT. PCNL stone-free rate was superior to SWL (85% *vs* 33%, respectively) and none of the PCNL patients required a secondary procedure, whereas 77% of the SWL patients required at least one other procedure and 17% required more than one[30].

The influence of PCNL on kidney function was evaluated in 81 patients with a solitary kidney. Mean eGFR increased from approximately 45 preoperatively to 52, 1 year after intervention[31]. However, achievement and complication rates of PCNL are different in patient with a solitary kidney. The goal of stone eradication in these patients is aimed at preserving nephrons, preventing stone-related complications, chronic renal failure, and dialysis. A recent global study recommends that the stone-free rate for PCNL in solitary kidneys is lower than in patients with bilateral

Table 2 Effects of percutaneous nephrolithotomy on estimated glomerular filtration rate

Ref.	*n*	Baseline eGFR (mL/min per 1.73 m²)	Postoperative eGFR	1 yr eGFR
Ozden *et al*[33]	67	37.9 ± 14.05	45.1 ± 16.8	51.3 ± 19.31
Canes *et al*[31]	81	44.9 ± 19.2	42.7 ± 18.0	51.7 ± 23.1
Bilen *et al*[36]	185	42.4	48.4	-

eGFR: Estimated glomerular filtration rate.

kidneys. Furthermore, it is concluded that kidney function deterioration and transfusion rates are greater in patients with a solitary kidney[32].

Besides, the existence of urinary stones in patient with CKD necessitates exclusive consideration. Hydronephrosis and infection are independent parameters for renal impairment in the patients with urinary stones. The alterations in the renal parenchyma created by infection become more evident with concomitant hydronephrosis. The duration of disease, repeated interventions, and stone recurrence also have unfavorable influences on renal function. Hence, the treatment of urinary stone disease in patients with CKD acts a significant role in improving renal function[33]. Kukreja *et al*[34] reviewed the influence of PCNL on renal function in 84 CKD patients with renal stone disease. They stated an overall improvement in renal function in 39%, stable function in 29%, and decreased function in 32% of patients. However, serum creatinine has been used instead of eGFR in this study. Factors predicting impairment in kidney function were proteinuria > 300 mg/d, cortical atrophy, recurrent UTI, stone burden > 1500 mm², time passed after surgical intervention < 15 years, and pediatric age group[34]. In another study, Kurien *et al*[35] studied 91 adult patients with serum creatinine level greater than 1.5 mg/dL who performed PCNL. Most patients had stage 3 or 4 CKD and most showed improvement or stabilization in renal function after PCNL. Postoperative complications and peak eGFR were the main factors predicting deterioration of kidney function during follow-up[35].

In another study, Bilen *et al*[36] evaluated 185 subjects with eGFR < 60 mL/min per 1.73 m² undergoing PCNL and found the average preoperative eGFR substantially improved from 42.4 to 48.4 at three months follow-up. None of the patients required dialysis during that relatively shorter follow-up. They also found that nearly all stage 5, half of stage 4, and one quarter of stage 3 subjects had some benefit from surgery. Renal function recovery was minimum in stage 2 subjects. They hypothesized that the effect of the calculi itself in a severely affected kidney is greater than the effect of PCNL and the opposite is probably true for moderately affected kidneys in which the detriment of surgery may be more significant, particularly if associated with UTI[36].

In our study, we analyzed 67 subjects, retrospectively. The eGFR was less than 60 mL/min per 1.73 m². The mean follow up was approximately 46 mo. The mean eGFR before and after PNL was approximately 38 and 46. DM and UTI were independent parameters for renal impairment at 1 year. Of the 67 subjects, 47% had downstaging. On the other hand, 3% of subjects had upstaging at the first year. During the 5-year study period, 1 of the subjects progressed to end-stage CKD. However, 6% of subjects with Stage 5 evolved to Stage 4[33]. The effects of PCNL on eGFR have shown in (Table 2).

Another important factor that has been proposed to affect renal function is the number of tracts. Animal and human studies have demonstrated that renal damage from nephrostomy tracts is minimal based on nuclear renography and has no effect on systemic renal function[37,38].

In summary, PCNL remains the primary modality for treatment of complex stones in patients with CKD. Nevertheless, it has important complications which are very important for in patients with CKD. The most common are hypotermia, bleeding, metabolic acidosis, serum electrolytes disturbances, urosepsis, and rarely death[39]. Anemia and underlying platelet dysfunction in patients with CKD may play important role in the high rate of transfusion[35,36].

RETROGRADE INTRARENAL SURGERY

Retrograde intrarenal surgery for the treatment of kidney stones has been more preferred approach owing to technological innovation, such as new model flexible ureteroscopies, laser fibers and baskets. The declared stone free rate of RIRS is about 97%, complication rates are lower than in other initiatives[40]. RIRS can be considered a proper alternative to PCNL in cases with significant comorbidity (anticoagulation, cardiopulmonary disease, advanced age)[41], and in cases with additional adverse anatomical factors such as obesity and renal malformations[42].

Giusti *et al*[43,44] stated that results of RIRS for stones up to 2 cm in diameter in 29 patients with solitary kidney. The primary stone free rate (SFR) was 72.4%, the secondary SFR was 93.1%. The mean number of applications per patient was 1.24. Median follow up time was 35.7 ± 19.3 (12-72) mo. Serum creatinine level, not eGFR, was used to evaluate effect of procedure on renal function and reported that there was no deterioration in kidney function[43]. Although RIRS has been related with repeated operations for the treatment of renal stones greater than 2 cm in diameter, it contributes to preservation of renal parenchyma, which might be substantial for patient with renal insufficiency[44,45].

Consequently, more comprehensive studies are needed for evaluating the effect of RIRS on renal function.

CONCLUSION

Urinary stone disease in patients with renal insufficiency can be caused to diverse clinical conditions. Patients with CKD can be a sufferer from other medical comorbidities. Urinary tract stone disease is the direct reason of ESRD in 3.2% of patients on dialysis. Coronary heart disease risk factors, such as obesity, hypertension, hyperuricemia, dyslipidemia and CKD, were related with urinary stone disease. Of these risk factors, hypertension and hyperuricemia demonstrated the most potential relation with urinary stones[46,47]. Therefore, to prevent morbidity and mortality of CKD, patients with urinary stone disease should be evaluated substantially and treated by an appropriate method.

REFERENCES

1 **Huang WY**, Chen YF, Carter S, Chang HC, Lan CF, Huang KH. Epidemiology of upper urinary tract stone disease in a Taiwanese population: a nationwide, population based study. *J Urol* 2013; **189**: 2158-2163 [PMID: 23313204 DOI: 10.1016/j.juro.2012.12.105]

2 **Schade GR**, Faerber GJ. Urinary tract stones. *Prim Care* 2010; **37**: 565-81, ix [PMID: 20705199 DOI: 10.1016/j.pop.2010.05.003]

3 **Scales CD**, Smith AC, Hanley JM, Saigal CS. Prevalence of kidney stones in the United States. *Eur Urol* 2012; **62**: 160-165 [PMID: 22498635 DOI: 10.1016/j.eururo.2012.03.052]

4 **Chung SD**, Chen YK, Lin HC. Increased risk of diabetes in patients with urinary calculi: a 5-year followup study. *J Urol* 2011; **186**: 1888-1893 [PMID: 21944094 DOI: 10.1016/j.juro.2011.07.011]

5 **Nowfar S**, Palazzi-Churas K, Chang DC, Sur RL. The relationship of obesity and gender prevalence changes in United States inpatient nephrolithiasis. *Urology* 2011; **78**: 1029-1033 [PMID: 21676441 DOI: 10.1016/j.urology.2011.04.011]

6 **Marchini GS**, Sarkissian C, Tian D, Gebreselassie S, Monga M. Gout, stone composition and urinary stone risk: a matched case comparative study. *J Urol* 2013; **189**: 1334-1339 [PMID: 23022002 DOI: 10.1016/j.juro.2013.02.2004]

7 **Straub M**, Gschwend J, Zorn C. Pediatric urolithiasis: the current surgical management. *Pediatr Nephrol* 2010; **25**: 1239-1244 [PMID: 20130924 DOI: 10.1007/s00467-009-1394-4]

8 **Méndez Probst CE**, Denstedt JD, Razvi H. Preoperative indications for percutaneous nephrolithotripsy in 2009. *J Endourol* 2009; **23**: 1557-1561 [PMID: 19630500 DOI: 10.1089/end.2009.1518]

9 **Smaldone MC**, Docimo SG, Ost MC. Contemporary surgical management of pediatric urolithiasis. *Urol Clin North Am* 2010; **37**: 253-267 [PMID: 20569803 DOI: 10.1016/j.ucl.2010.03.006]

10 **Pearle MS**, Calhoun EA, Curhan GC. Urologic diseases in America project: urolithiasis. *J Urol* 2005; **173**: 848-857 [PMID: 15711292 DOI: 10.1097/01.ju.0000152082.14384.d7]

11 **Srisubat A**, Potisat S, Lojanapiwat B, Setthawong V, Laopaiboon M. Extracorporeal shock wave lithotripsy (ESWL) versus percutaneous nephrolithotomy (PCNL) or retrograde intrarenal surgery (RIRS) for kidney stones. *Cochrane Database Syst Rev* 2009; **(4)**: CD007044 [PMID: 19821393]

12 **Chou YH**, Li CC, Hsu H, Chang WC, Liu CC, Li WM, Ke HL, Lee MH, Liu ME, Pan SC, Wang HS. Renal function in patients with urinary stones of varying compositions. *Kaohsiung J Med Sci* 2011; **27**: 264-267 [PMID: 21757143 DOI: 10.1016/j.kjms.2010.11.008]

13 **Kartha G**, Calle JC, Marchini GS, Monga M. Impact of stone disease: chronic kidney disease and quality of life. *Urol Clin North Am* 2013; **40**: 135-147 [PMID: 23177641 DOI: 10.1016/j.ucl.2012.09.004]

14 **Miller NL**, Evan AP, Lingeman JE. Pathogenesis of renal calculi. *Urol Clin North Am* 2007; **34**: 295-313 [PMID: 17678981 DOI: 10.1016/j.ucl.2007.05.007]

15 **Türk C,** Knoll T, Petrik A, Sarica K, Skolarikos A, Straub M, Seitz C. Guidelines on Urolithiasis 2014. Available from: URL: http://uroweb.org/fileadmin/guidelines/Guidelines_2014_5_June_2014.pdf

16 **Mandeville JA**, Gnessin E, Lingeman JE. Imaging evaluation in the patient with renal stone disease. *Semin Nephrol* 2011; **31**: 254-258 [PMID: 21784274 DOI: 10.1016/j.semnephrol.2011.05.006]

17 **Ray AA**, Ghiculete D, Pace KT, Honey RJ. Limitations to ultrasound in the detection and measurement of urinary tract calculi. *Urology* 2010; **76**: 295-300 [PMID: 20206970 DOI: 10.1016/j.urology.2009.12.015]

18 **Shine S**. Urinary calculus: IVU vs. CT renal stone? A critically appraised topic. *Abdom Imaging* 2008; **33**: 41-43 [PMID: 17786506 DOI: 10.1007/s00261-007-9307-0]

19 **Wu DS**, Stoller ML. Indinavir urolithiasis. *Curr Opin Urol* 2000; **10**: 557-561 [PMID: 11148725 DOI: 10.1097/00042307-200011000-00004]

20 **Poletti PA**, Platon A, Rutschmann OT, Schmidlin FR, Iselin CE, Becker CD. Low-dose versus standard-dose CT protocol in patients with clinically suspected renal colic. *AJR Am J Roentgenol* 2007; **188**: 927-933 [PMID: 17377025 DOI: 10.2214/AJR.06.0793]

21 **White WM**, Zite NB, Gash J, Waters WB, Thompson W, Klein FA. Low-dose computed tomography for the evaluation of flank pain in the pregnant population. *J Endourol* 2007; **21**: 1255-1260 [PMID: 18042011 DOI: 10.1089/end.2007.0017]

22 **Preminger GM**, Tiselius HG, Assimos DG, Alken P, Buck AC, Gallucci M, Knoll T, Lingeman JE, Nakada SY, Pearle MS, Sarica K, Türk C, Wolf JS. 2007 Guideline for the management of ureteral calculi. *Eur Urol* 2007; **52**: 1610-1631 [PMID: 18074433 DOI: 10.1016/j.eururo.2007.09.039]

23 **Krambeck AE**, Gettman MT, Rohlinger AL, Lohse CM, Patterson DE, Segura JW. Diabetes mellitus and hypertension associated with shock wave lithotripsy of renal and proximal ureteral stones at 19 years of followup. *J Urol* 2006; **175**: 1742-1747 [PMID: 16600747 DOI: 10.1016/S0022-5347(05)00989-4]

24 **Krambeck AE**, Rule AD, Li X, Bergstralh EJ, Gettman MT, Lieske JC. Shock wave lithotripsy is not predictive of hypertension among community stone formers at long-term followup. *J Urol* 2011; **185**: 164-169 [PMID: 21074794 DOI: 10.1016/j.juro.2010.09.033]

25 **Eassa WA**, Sheir KZ, Gad HM, Dawaba ME, El-Kenawy MR, Elkappany HA. Prospective study of the long-term effects of shock wave lithotripsy on renal function and blood pressure. *J Urol* 2008; **179**: 964-968; discussion 964-968 [PMID: 18207167 DOI: 10.1016/j.juro.2007.10.055]

26 **Atis G**, Gurbuz C, Arikan O, Kilic M, Pelit S, Canakci C, Gungor S, Caskurlu T. Retrograde intrarenal surgery for the treatment of renal stones in patients with a solitary kidney. *Urology* 2013; **82**: 290-294 [PMID: 23759378 DOI: 10.1016/j.urology.2013.04.013]

27 **Fayad A**, El-Sheikh MG, Abdelmohsen M, Abdelraouf H. Evaluation of renal function in children undergoing extracorporeal shock wave lithotripsy. *J Urol* 2010; **184**: 1111-1114 [PMID: 20650495 DOI: 10.1016/j.juro.2010.05.016]

28 **Antonelli JA**, Pearle MS. Advances in percutaneous nephrolithotomy. *Urol Clin North Am* 2013; **40**: 99-113 [PMID: 23177638 DOI: 10.1016/j.ucl.2012.09.012]

29 **Türk C**, Knoll T, Petrik A, Sarica K, Straub M, Seitz C. Guidelines on urolithiasis, 2012. Available from: http: //www.uroweb.org/gls/pdf/20_Urolithiasis.pdf

30 **Deem S**, Defade B, Modak A, Emmett M, Martinez F, Davalos J. Percutaneous nephrolithotomy versus extracorporeal shock wave lithotripsy for moderate sized kidney stones. *Urology* 2011; **78**: 739-743 [PMID: 21664653 DOI: 10.1016/j.urology.2011.04.010]

31 **Canes D**, Hegarty NJ, Kamoi K, Haber GP, Berger A, Aron M, Desai MM. Functional outcomes following percutaneous surgery in the solitary kidney. *J Urol* 2009; **181**: 154-160 [PMID: 19013616 DOI: 10.1016/j.juro.2008.09.023]

32 **Bucuras V**, Gopalakrishnam G, Wolf JS, Sun Y, Bianchi G, Erdogru T, de la Rosette J. The Clinical Research Office of the Endourological Society Percutaneous Nephrolithotomy Global Study: nephrolithotomy in 189 patients with solitary kidneys. *J Endourol*

2012; **26**: 336-341 [PMID: 22004844 DOI: 10.1089/end.2011.0169]
33 **Ozden E**, Mercimek MN, Bostanci Y, Yakupoglu YK, Sirtbas A, Sarikaya S. Long-term outcomes of percutaneous nephrolithotomy in patients with chronic kidney disease: a single-center experience. *Urology* 2012; **79**: 990-994 [PMID: 22309786 DOI: 10.1016/j.urology.2011.10.066]
34 **Kukreja R**, Desai M, Patel SH, Desai MR. Nephrolithiasis associated with renal insufficiency: factors predicting outcome. *J Endourol* 2003; **17**: 875-879 [PMID: 14744353 DOI: 10.1089/089277903772036181]
35 **Kurien A**, Baishya R, Mishra S, Ganpule A, Muthu V, Sabnis R, Desai M. The impact of percutaneous nephrolithotomy in patients with chronic kidney disease. *J Endourol* 2009; **23**: 1403-1407 [PMID: 19694531 DOI: 10.1089/end.2009.0339]
36 **Bilen CY**, Inci K, Kocak B, Tan B, Sarikaya S, Sahin A. Impact of percutaneous nephrolithotomy on estimated glomerular filtration rate in patients with chronic kidney disease. *J Endourol* 2008; **22**: 895-900 [PMID: 18393644 DOI: 10.1089/end.2007.0435]
37 **Handa RK**, Willis LR, Connors BA, Gao S, Evan AP, Kim SC, Tinmouth WW, Lingeman JE. Time-course for recovery of renal function after unilateral (single-tract) percutaneous access in the pig. *J Endourol* 2010; **24**: 283-288 [PMID: 20039833 DOI: 10.1089/end.2009.0454]
38 **Moskovitz B**, Halachmi S, Sopov V, Burbara J, Horev N, Groshar D, Nativ O. Effect of percutaneous nephrolithotripsy on renal function: assessment with quantitative SPECT of (99m)Tc-DMSA renal scintigraphy. *J Endourol* 2006; **20**: 102-106 [PMID: 16509791 DOI: 10.1089/end.2006.20.102]
39 **Michel MS**, Trojan L, Rassweiler JJ. Complications in percutaneous nephrolithotomy. *Eur Urol* 2007; **51**: 899-906; discussion 906 [PMID: 17095141 DOI: 10.1016/j.eururo.2006.10.020]
40 **Miernik A**, Wilhelm K, Ardelt PU, Adams F, Kuehhas FE, Schoenthaler M. Standardized flexible ureteroscopic technique to improve stone-free rates. *Urology* 2012; **80**: 1198-1202 [PMID: 23206763 DOI: 10.1016/j.urology.2012.08.042]
41 **Akman T**, Binbay M, Ugurlu M, Kaba M, Akcay M, Yazici O, Ozgor F, Muslumanoglu AY. Outcomes of retrograde intrarenal surgery compared with percutaneous nephrolithotomy in elderly patients with moderate-size kidney stones: a matched-pair analysis. *J Endourol* 2012; **26**: 625-629 [PMID: 22141372 DOI: 10.1089/end.2011.0526]
42 **Palmero JL**, Amoros A, Ramírez M, Pastor JC, Benedicto A. [Surgical therapy of lithiasis in horseshoe kidney]. *Actas Urol Esp* 2012; **36**: 439-443 [PMID: 22178345 DOI: 10.1016/j.acuro.2011.10.005]
43 **Giusti G**, Proietti S, Cindolo L, Peschechera R, Sortino G, Berardinelli F, Taverna G. Is retrograde intrarenal surgery a viable treatment option for renal stones in patients with solitary kidney? *World J Urol* 2014; **33**: 309-314 [PMID: 24756177 DOI: 10.1007/s00345-014-1305-6]
44 **Giusti G**, Proietti S, Luciani LG, Peschechera R, Giannantoni A, Taverna G, Sortino G, Graziotti P. Is retrograde intrarenal surgery for the treatment of renal stones with diameters exceeding 2 cm still a hazard? *Can J Urol* 2014; **21**: 7207-7212 [PMID: 24775573]
45 **Huang Z**, Fu F, Zhong Z, Zhang L, Xu R, Zhao X. Flexible ureteroscopy and laser lithotripsy for bilateral multiple intrarenal stones: is this a valuable choice? *Urology* 2012; **80**: 800-804 [PMID: 22743260 DOI: 10.1016/j.urology.2012.05.013]
46 **Jungers P**, Joly D, Barbey F, Choukroun G, Daudon M. ESRD caused by nephrolithiasis: prevalence, mechanisms, and prevention. *Am J Kidney Dis* 2004; **44**: 799-805 [PMID: 15492945 DOI: 10.1053/j.ajkd.2004.08.014]
47 **Ando R**, Nagaya T, Suzuki S, Takahashi H, Kawai M, Okada A, Yasui T, Kubota Y, Umemoto Y, Tozawa K, Kohri K. Kidney stone formation is positively associated with conventional risk factors for coronary heart disease in Japanese men. *J Urol* 2013; **189**: 1340-1346 [PMID: 23159273 DOI: 10.1016/j.juro.2012.11.045]

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Management of hepatorenal syndrome

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Abstract

Hepatorenal syndrome (HRS) is defined as development of renal dysfunction in patients with chronic liver diseases due to decreased effective arterial blood volume. It is the most severe complication of cirrhosis because of its very poor prognosis. In spite of several hypotheses and research, the pathogenesis of HRS is still poorly understood. The onset of HRS is a progressive process rather than a suddenly arising phenomenon. Since there are no specific tests for HRS diagnosis, it is diagnosed by the exclusion of other causes of acute kidney injury in cirrhotic patients. There are two types of HRS with different characteristics and prognostics. Type 1 HRS is characterized by a sudden onset acute renal failure and a rapid deterioration of other organ functions. It may develop spontaneously or be due to some precipitating factors. Type 2 HRS is characterized by slow and progressive worsening of renal functions due to cirrhosis and portal hypertension and it is accompanied by refractory ascites. The only definitive treatment for both Type 1 and Type 2 HRS is liver transplantation. The most suitable bridge treatment or treatment for patients who are not eligible for transplantation is a combination of terlipressin and albumin. For the same purpose, it is possible to try hemodialysis or renal replacement therapies in the form of continuous veno-venous hemofiltration. Artificial hepatic support systems are important for patients who do not respond to medical treatment. Transjugular intrahepatic portosystemic shunt may be considered as a treatment modality for unresponsive patients to medical treatment. The main goal of clinical surveillance in a cirrhotic patient is prevention of HRS before it develops. The aim of this article is to provide an updated review about the physiopathology of HRS and its treatment.

Key words: Hepatorenal syndrome; Cirrhosis; Renal failure; Vasoconstrictors; Transplantation

Core tip: Hepatorenal syndrome (HRS) is a severe complication of chronic liver diseases and is usually associated with a poor prognosis. It is not a renal disease but a renal dysfunction that develops as a result of a systemic condition associated with liver failure. To prevent HRS by taking some preventive measures is possible and although the definitive treatment is liver transplantation, a rapid diagnosis and prompt initiation of the treatment leads to an important improvement in the prognosis. In this review, we cover the physiopathology, diagnosis and treatment options of HRS.

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INTRODUCTİON

Hepatorenal syndrome (HRS) is defined as unexplainable progressively increasing serum creatinine in a patient with advanced liver disease. HRS is representative of the end stage of a process associated with progressive decrease in renal blood flow and glomerular filtration rate (GFR). Diagnosis is by exclusion of other causes of renal failure since there is no specific diagnostic test. In 1956, a special type of acute renal failure associated with low urinary output and very low urinary sodium excretion without proteinuria was defined[1]. In postmortem examination of these patients, it was observed that the kidney's histological structure was preserved. In 1969, the kidney taken from cadaveric donors with HRS functioned normally[2]. So, it is possible to conclude that HRS is not a renal disease but a renal dysfunction that occurs as a result of a systemic condition. The definitive treatment is liver transplantation (LT). HRS is an important risk factor since it increases the waiting list mortality and incidence of complications after LT[3] and renal function before LT is a predictor of survival[4]. Vasoconstrictive agents constitute the main part of pharmacological treatment, providing a bridge to LT. Hemodialysis, renal replacement therapies and artificial liver support systems may also be used as bridge treatment. The goal of treatment in HRS should be early diagnosis, effective and quick treatment and, most important of all, to take preventive measures. Despite all treatment options, likelihood of failure is still high.

DEFINITION

HRS is one of the most severe complications of cirrhosis and is defined as renal insufficiency emerging in chronic liver disease patients when all the other causes of renal failure are excluded[5]. Renal vasoconstriction, a result of progressive liver failure, is the main underlying reason for renal failure in HRS.

HRS was first classified into two groups, Type 1 and Type 2, by the International Ascites Club in 1994. According to this classification, Type 1 HRS is associated with doubling of initial serum creatinine to a level of more than 2.5 mg/dL or reduction in creatinine clearance because of a decreased glomerular filtration rate to a level less than 20 mL/min in a time period shorter than 2 wk[5-7]. Type 1 HRS usually occurs following a precipitating factor such as infectious conditions, particularly spontaneous bacterial peritonitis (SBP) which is considered the most important factor for HRS[8-11]. Type 2 HRS is a moderate and steady type of renal failure and serum creatinine level is higher than 1.5 mg/dL and often associated with sodium retention[5,7]. Type 2 HRS usually arises spontaneously as a result of refractory ascites[5].

In addition to this data, it is important to consider that the creatinine levels are not always increased in cases of renal failure in decompensated cirrhosis[12,13]. It is possible to say that even milder degrees of renal failure may be associated with a poor prognosis in cirrhotic patients[1,14]. According to the RIFLE (Risk, Injury, Failure, Loss, End stage renal disease) classification, it has been shown that even a small increase in serum creatinine level may be associated with clinically significant outcomes in patients with cirrhosis[15-17]. In accordance with this, the International Ascites Club and the Acute Dialysis Quality Initiative suggested a new definition for acute kidney injury. This new definition includes an increase in serum creatinine level to 0.3 mg/dL or more in a period less than 48 h or a 50% increase in serum creatinine level compared to the baseline levels recorded in previous 6 mo period, regardless of final serum creatinine levels[18].

PATHOPHYSIOLOGY

HRS is a sort of renal dysfunction which is generally reversible and occurs because of advanced liver disease. Although it is not completely ravelled, the most characteristic reason underlying renal dysfunction in HRS is renal vasoconstriction[19].

The four major factors considered to be responsible are: (1) decreased circulating blood volume and, as a result, decreased mean arterial blood pressure because of splanchnic vasodilatation; (2) renal vasoconstriction as a result of the activated renin-angiotensin-aldosterone system since the sympathetic nervous system has been activated; (3) cardiac dysfunction due to cirrhosis; and (4) release of several cytokines and vasoactive mediators which may affect blood flow to the kidneys and glomerular vascular bed[20,21].

The main pathophysiological mechanism in HRS is reduction of circulating blood volume due to increased resistance to blood flow in the cirrhotic liver, resulting in splanchnic blood pooling, which is in fact a multifactorial process[1]. Decreased circulating blood flow which means decreased mean arterial blood pressure causes stimulation of baroreceptors in the carotid body and consequently activation of the sympathetic nervous system. This is followed by activation of the renin-angiotensin-aldosterone system and nonosmotic release of vasopressin which causes a further decrease in systemic vascular resistance, hypotension and vasoconstriction in the renal vessels and glomerular vascular bed[22]. This vasoconstriction cannot be only explained by increased activity of endogenous vasoconstrictor systems. Because of the extreme hemodynamic changes in advanced liver diseases, renal vasodilatator systems become insufficient, creating a vicious cycle which contributes more and more to renal vasoconstriction[22-24].

Factors contributing to the persistence of renal vasoconstriction in spite of the vasodilatation of the

peripheral vasculature have been investigated in several studies. Iwao *et al*[25] investigated the contributing factors of hyperdynamic circulation in cirrhotic patients and found that mesenteric blood flow decreases as liver disease worsens. They concluded that splanchnic arterial vasodilatation plays an important role in the pathogenesis of decreased systemic vascular resistance in cirrhotic patients[25]. In accordance with this data, in some other human and animal studies, it has been shown that splanchnic circulation is the main vascular bed responsible for peripheral vasodilatation[26-29].

Advanced liver disease due to portal hypertension is characterized by a state of hyperdynamic circulation which is accompanied by increased cardiac output[30]. It is hard to understand how cardiac output is increased while myocardial function is usually impaired in cirrhotic patients. The heart in cirrhotic patients usually has several structural and functional abnormalities associated with alterations in ventricular wall size, systolic and diastolic function[31,32]. Although the reasons for these alterations are not known clearly, neurohumoral factors and continous mechanical stress may be responsible[33]. Ventricular function is inhibited due to circulating cytokines, such as tumor necrosis factor-alpha, and nitric oxide in cirrhotic patients. One of the contributing factors to the ventricular dysfunction is reduced beta adrenergic receptor signal transduction in the myocardium[34,35].

Whatever the cause, ventricular wall thickness is increased slightly and the diastolic function deteriorates, especially increasing with physical stress and with the presence of ascites and systolic dysfunction[30,34,36].

Sympathetic nervous system activity is shown to be increased in cases of portal hypertension as a result of the hepatorenal reflex[37,38]. Hepatorenal reflex activation occurs due to decreased sinusoidal blood flow or increased sinusoidal pressure in the liver, as shown in several animal models[38,39]. Increased renal sympathetic nervous system tone is held to be responsible for renal vasoconstriction together with thromboxanes, endotoxins, endothelins and neurotransmitters. Together with the activation of the sympathetic nervous system because of a low effective circulating volume stimulating baroreceptors in the carotid body and aortic arch, activation of the renin-angiotensin-aldosterone system and nonosmotic release of antidiuretic hormone occurs. Although all of these compensatory mechanisms help to provide an effective circulating volume and relatively normalize the mean arterial blood pressure, they also have important effects on renal function.

Vasoactive mediators and cytokines are the other actors in HRS, agents that affect both the systemic and renal circulation. The major ones studied include prostaglandins, endothelins, endotoxins, glucagon, nitric oxide and tumor necrosis factor-α. Among these, nitric oxide has a special role. Primary arterial vasodilatation in the splanchnic circulation, a result of portal hypertension, is the mainstay in explaining the development of renal insufficiency in cirrhotic patients[20]. The major cause of this arterial vasodilatation in the splanchnic circulation is increased synthesis and activity of nitric oxide and some other vasoactive agents[20]. The correlation between increased levels of nitric oxide and high plasma renin-angiotensin-aldosterone system activity and antidiuretic hormone levels accompanied by low urinary Na excretion in cirrhotic patients, especially with ascites, is remarkable[40,41]. It is thought that in the maintenance of hyperdynamic circulation, the hallmark of HRS, nitric oxide may be the primary factor[42]. However, increased nitric oxide levels are not able to prevent renal vasoconstriction. In the early stages of cirrhosis, renal perfusion is provided by increased synthesis and activity of renal vasodilatators, especially prostaglandins and kallikreins[43,44]. Vasodilating prostaglandins are the major actors in supplying glomerular blood flow at the beginning[45], but as the liver disease progresses, vasoconstrictor systems are further activated and synthesis and activity of renal vasodilating factors progressively decrease. The prostaglandin level in the urine of cirrhotic patients is high when compared with that of patients with HRS[46]. The reason for decreased prostaglandin production in HRS is a mystery but it is known that it is not the only factor in the development of HRS.

DIAGNOSIS

HRS is an important risk factor for renal failure in cirrhotic patients. In a prospective study, it was estimated that the 1 year probability of HRS in cirrhotic patients is 18% and the 5 year probability is 39%[47]. HRS was observed in 28% of alcoholic hepatitis cases without identifiable cirrhosis[48]. Major factors precipitating HRS are hyponatremia, high plasma renin-angiotensin-aldosterone system activity, gastrointestinal bleeding, bacterial infections, spontaneous bacterial peritonitis, large volume paracentesis without albumin infusion, some drugs, such as diuretics, aminoglycosides, non-steroid anti-inflammatory drugs, angiotensin converting enzyme inhibitors, surgical interventions and cholestasis[47,49,50]. Also, Doppler ultrasonography may be helpful to detect increased renal resistive index indicating renal vasoconstriction[19].

In chronic liver diseases, it may be difficult to diagnose renal failure since reduction in GFR is usually masked. This may be because urea and creatinine production is decreased due to chronic liver disease, the muscle mass is decreased due to chronic disease and the protein intake is decreased due to the loss of desire to eat. There are no specific diagnostic criteria for diagnosing HRS. The diagnosis is by exclusion of other causes of renal failure in cirrhotic patients. The major symptoms of HRS are decreased GFR (< 40 mL/min) and increased serum creatinine (> 1.5 mg/dL). Other symptoms defining functional characteristics

of HRS are decreased Na excretion (< 10 mmol/L), higher urine osmolality compared to plasma osmolality, hyponatremia (< 130 mmol/L) and decreased diuresis (< 500 mL).

The most widely accepted diagnostic criteria were developed in 1996 by the International Ascites Club when the major and minor criteria were defined. According to this diagnostic criteria, diagnosis of HRS requires the inclusion of all major criteria and the presence of minor criteria are thought to be suggestive for the diagnosis of HRS. Various new concepts have arisen since the first publication of the criteria for the diagnosis of HRS in 1996. These were modified in 2007 by the International Ascites Club[6]. According to current diagnostic criteria, minor criteria are omitted and concurrent bacterial infection is now not a factor that should be excluded in the diagnosis of HRS. Another important alteration is using albumin instead of 0.09% NaCl solution for plasma volume expansion.

The new diagnostic criteria defined by the International Ascites Club in 2007 are listed in Table 1.

Creatinine clearance is the most important diagnostic tool. It is important to exclude other causes of renal failure before the diagnosis of HRS. These include hypovolemia, parenchymal renal diseases, use of nephrotoxic drugs and shock. In cases of hematuria, severe proteinuria and increased renal size on USG, renal parenchymal disease should be considered in the differential diagnosis[51]. In such cases, renal biopsy is required so that the potential need for combined liver and kidney transplantation can be defined[51]. If there is an organic cause of renal insufficiency, urine analysis to see the Na concentration is clinically important. Since muscle mass and production of creatinine in the liver is decreased in chronic liver diseases, serum creatinine levels are not very reliable to evaluate renal function in liver diseases. Creatinine monitoring blood urea level is also insufficient in reflecting the GFR in cases of chronic liver disease[5,6]. So, investigations should be conducted to find more sensitive and specific markers. Some of these markers are cystatin-C, symmetric dimethylarginine, kidney injury molecule-1 and neutrophil gelatinase-associated lipocalin. Cystatin-C has been found to be more sensitive than creatinine in defining decreased GFR in cirrhotic patients[52,53]. Symmetric dimethylarginine, an endogenous inhibitor of nitric oxide synthase, has been shown to be increased in cases of HRS when compared with cirrhotic patients with normal kidney functions[54]. The investigations regarding kidney injury molecule-1 and neutrophil gelatinase-associated lipocalin which are very susceptible to ischemia and indicators of renal tubular injury are ongoing[55,56]. There are few studies about renal tubular damage markers. β2 microglobulin is one of them. It is especially increased in cases of aminoglycoside nephrotoxicity[57]. To make a differential diagnosis of HRS from acute tubular necrosis, gamma glutamyl transpeptidase, transaminase, neutrophil gelatinase-associated lipocalin, IL 8, liver type fatty acid binding protein and hepatitis virus cell receptor Type 1 are other markers which are of interest nowadays but their significance has not yet been evaluated[58].

TREATMENT

The main principle in the treatment of HRS is to bring back renal function until the patients undergo LT. So, all the therapeutic interventions for HRS are a sort of bridge therapy. During the treatment of HRS, etiology oriented treatment of liver diseases such as antiviral drug treatment should not be impeded. The choice of medical treatment depends upon several factors, including the availability of drugs, which is variable according to country and even region, whether the patient is admitted to an intensive care unit and if the patient is a candidate for LT. Cirrhotic patients with gastrointestinal system (GIS) bleeding, ascites, infections, arterial hypotension and dilutional hyponatremia should be monitored closely because of the increased risk of HRS.

Complications like GIS bleeding and spontaneous bacterial peritonitis should be prevented and urgently treated. Large volume paracentesis with plasma volume expansion decreases the incidence of HRS. Diuretic treatment may trigger HRS because of intravascular volume depletion so diuretic treatment should be stopped and electrolyte imbalances such as hyponatremia and hypocalcemia should be corrected. NSAIDs should also be stopped and appropriate infection treatment should be planned.

Effective circulating volume should be increased. Infusion of 0.9% NaCl and synthetic plasma expanders, even by monitoring central venous pressure, has not found to be helpful. It is proved that albumin is the most useful of all volume expanders. After albumin use, incidence of Type 1 HRS has been shown to be decreased. When albumin is used concomitantly with other agents, it has been observed that the effectiveness of these agents was also increased[23,51].

Prostaglandins, dopamine and endothelin receptor blockers were the first renal vasodilatators used in HRS treatment. Oral prostaglandin-E_1 analogue misoprostol or IV prostaglandin infusion did not provide a significant improvement in HRS[59,60]. Intravenous dopamine infusion has also been investigated in several studies but no improvements have been observed in renal function[61,62].

There is no specific vasoconstrictive agent used to increase systemic vascular resistance. Several vasoconstrictive agents such as norepinephrine, angiotensin 2 and vasopressin have been used for this purpose but alone they were not found to be effective. Vasoconstrictive agents, especially when used together with plasma expanders, are the most helpful pharmacological agents in the management of HRS[63,64]. Development of synthetic vasopressin analogues provides an important progression in HRS treatment. Ornipressin and terlipressin are vasoconstrictive agents that are effective on mesenteric circulation rather than renal and other vascular systems. Ornipressin is not

Table 1 Criteria for diagnosis of hepatorenal syndrome in cirrhosis

Cirrhosis with ascites
Serum creatinine > 1.5 mg/dL (133 μmol/L)
Absence of shock
Absence of hypovolemia as defined by no sustained improvement of renal function (creatinine decreasing to < 133 μmol/L) following at least 2 d of diuretic withdrawal (if on diuretics) and volume expansion with albumin at 1 g/kg per day up to a maximum of 100 g/d
No current or recent treatment with nephrotoxic drugs
Absence of parenchymal renal disease as defined by proteinuria < 0.5 g/d, no microhematuria (< 50 red cells/high powered field) and normal renal ultrasonography

being used because of its severe ischemic side effects.

Terlipressin and albumin infusion are the most important choices of treatment in Type 1 HRS[65]. It has been observed that terlipressin is effective in 40%-60% of patients with Type 1 HRS. Clinical response to terlipressin treatment is slow but the reduction in serum creatinine level is continuous[65,66]. To reverse HRS may take a long time and it has been observed that it recurred in 50% of patients. In cases of recurrence, the same treatment regimen is usually found to be successful[7]. When terlipressin and albumin treatment is successful, arterial blood pressure, urine amount and serum Na level increase. Systemic circulation improves and plasma renin and norepinephrine levels decrease significantly. Time required for recovery usually changes depending on the initial serum creatinine level but mean recovery time is 7 d. If the initial serum creatinine level is low, the recovery will be faster[23,51,67,68]. Terlipressin therapy is suggested to be used in combination with albumin. Terlipressin therapy may be given as an IV bolus (0.5-1 mg/4-6 h) or IV continuous infusion with an initial dose of 2 mg/d. During the follow-up period, if a 25% decrease is not observed in serum creatinine level, the IV bolus dose may be increased up to 2 mg/4 h or the IV continuous infusion dose may be increased up to a maximum of 12 mg/d. Monitoring CVP is essential and albumin infusion is required to retain CVP at a level of 10-15 cm H_2O. Albumin is given for 2 d in the form of IV bolus therapy with an initial dose of 1 g/kg (maximum 100 g/d) and the maintenance albumin dose should be 25-50 g/d until terlipressin therapy is ceased and serum creatinine level becomes normal[69]. There are some studies that showed nearly 75% improvement in HRS patients by using a continuous IV infusion of terlipressin. In these studies, how terlipressin is given was also found to be important[70-72]. The information about the treatment of Type 2 HRS by albumin and vasoconstrictive agents is limited. When albumin and vasoconstrictive agents are used in Type 2 HRS treatment, improvement in renal function has been observed but there is a 50% recurrence rate after the cessation of therapy[23,73,74]. The most common side effects of terlipressin treatment are cardiovascular and ischemic and are reported in nearly 12% of patients treated[20,75]. According to the 2012 Cochrane meta-analysis, GIS and infectious side effects did not increase significantly during terlipressin therapy, whereas cardiovascular side effects increased remarkably[76].

Other vasoconstrictive agents currently being used in HRS treatment are somatostatin analogues (octreotide), α-adrenergic agonists, midodrine and norepinephrine. Their effectiveness has been studied in several studies; some found that they are less effective than terlipressin[23,77,78] and some found that their effectiveness is similar to terlipressin[79,80]. Midodrine is an orally available α-adrenergic agonist. Its effect is systemic vasoconstriction. When it is used in combination with octreotide and albumin, systemic and renal hemodynamic status is improved[81]. Midodrine is given orally with an initial dose of 7.5 mg/8 h (maximum: 15 g/8 h) and octreotide may be given either as continuous infusion with a dose of 50 mcg/h or subcutaneously with a dose of 100-200 mcg/8 h. In combination with midodrine and octreotide, albumin is given as an IV bolus with an initial dose of 1 g/kg (maximum: 100 g) and a maintenance dose of 25-50 g/d. Using midodrine and octreotide in combination has been shown to decrease mortality[82]. Nevertheless, the number of patients reported using this therapy is not enough[78,83] so more trials are required for a more accurate conclusion.

Norepinephrine is a vasoconstrictive agent generally used in intensive care units since it is not convenient to use in general medical wards. It is given as an intravenous continuous infusion with a dose of 0.5-3 mg/h. The effectiveness of norepinephrine and terlipressin were shown to be similar, while norepinephrine was cheaper[80,84].

Since dopamine is known to decrease renal vascular resistance and increase renal blood flow, low doses of dopamine were tried in the past. Its clinical effectiveness could not be proved either alone or in combination with ornipressin and the results are controversial[23,78].

According to several studies, increasing mean arterial blood pressure is suggested to have favorable effects on the treatment process. The most used predictors of favorable treatment response are a serum bilirubin concentration of < 10 mg/dL and an increase in mean arterial blood pressure ⩾ 5 mmHg on the third day of treatment[68].

The principle is that the earlier the treatment has been started, the better the results are. If serum creatinine level is < 5 mg/dL when the treatment is started, the probability of a favorable response is increased.

In a study by Nazar *et al*[68] in patients with both decreased bilirubin level and increased mean arterial

blood pressure, treatment success was 100%. In patients with only a decreased bilirubin level, the success rate was found to be 53% and it was 25% in patients with only an increased mean arterial blood pressure. In the patient group, bilirubin levels did not decrease, mean arterial blood pressures did not increase and the success rate was 10%[68]. If there is treatment unresponsiveness, underlying renal disease other than HRS should be considered.

The goal of all vasopressor treatments is to achieve a 10-15 mmHg increase in the mean arterial blood pressure. Increased mean arterial blood pressure is usually associated with decreased serum creatinine levels[85].

For patients with HRS who are not admitted to an intensive care unit, combination therapy with terlipressin and albumin is suggested. If terlipressin is not available, combination therapy with midodrine, octreotide and albumin should be used in patients who are not in the intensive care unit. After two weeks of medical treatment, if there are no improvements in renal function, medical treatment is considered to be useless.

Transjugular intrahepatic portosystemic shunts (TIPS) have been reported to improve renal function in patients with Type 1 HRS[86-88] and it has also been used for the treatment of refractory ascites in patients with Type 2 HRS[89-91]. However, TIPS therapy is possible under limited circumstances because of its contraindications and complications. Major complications associated with TIPS are hepatic encephalopathy which is a common and treatable condition, worsening of hepatic function, bleeding due to the procedure and acute kidney injury because of intravenous contrast injection during the procedure[92]. The underlying mechanism explaining how renal function is improved after TIPS is not known completely. TIPS provides portal decompression in cirrhotic patients, portal pressure decreases and blood pooling in the splanchnic vascular bed returns to the systemic circulation. As a result of this, RAAS and SNS activity is suppressed and renal vasoconstriction improves. In a study investigating renal function after TIPS in seven patients with Type 1 HRS, a significant decrease in serum creatinine level and increase in urine volume was observed in six of the patients in a one month period. This was accompanied by significant improvement in renal blood flow and GFR[86]. However, amelioration of renal function may take as long as six months in some cases after TIPS[89]. Also, the effect of TIPS on survival in Type 1 HRS patients is appreciable. HRS improved in nearly 50% of patients and survival increased more than three months after TIPS[78,87]. In cases of Type 2 HRS when TIPS is applied to control ascites, it was found to be successful and 70% of patients survived the following year[87,93]. The average survival after TIPS was approximately five months which was longer than the expected survival for such patients[86]. Unfortunately, mostly it is too late for patients with HRS to undergo TIPS and so it is suggested as a choice of treatment for only a selected group of patients. Before the decision to undergo TIPS, the high incidence of complications and especially encephalopathy should be considered.

Although LT is the most effective and definitive treatment of liver failure and HRS, supportive treatment modalities are required until LT is carried out. Non biological liver support systems have been developed for this purpose. The mechanism of action is provided by detoxification through a semi-permeable membrane in these non biological support systems. During the course of liver failure, according to the dominant clinical presentation (HRS, hepatopulmonary syndrome, hyperbilirubinemia), the most appropriate type of support system, each with different prominent features, will be chosen. Whereas in HRS venovenous hemodiafiltration is the first choise of treatment in cases of treatment unresponsiveness, a molecular adsorbents recirculating system should be considered. High-flux dialysis provides effective elimination of water soluble substances such as ammonia and lactate but it is insufficient in eliminating substances binding to proteins such as bile acids. Plasma exchange is no longer being used as it is too risky since large volumes are required to be exchanged in this procedure. For that reason, nowadays continuous venovenous hemodiafiltration may be useful in patients with HRS if there is a reversible precipitating factor such as infection[71]. Hemodialysis or continuous hemofiltration is used in the treatment of acute renal failure in cirrhotic patients[94,95]. In a study by Witzke *et al*[96], thirty-day survival was reported as 50% after renal replacement therapy but it is obvious that long term survival is usually poor. According to the Acute Dialysis Quality Initiative Group, renal support therapies should be suggested for patients who are candidates for LT[71]. There are several studies reporting that albumin dialysis has been beneficial in HRS[23,97]. In a randomized controlled trial, a molecular adsorbents recirculating system was observed to be more effective and safer when compared to standard medical treatment in the management of type 3-4 hepatic encephalopathy[23,97]. However, the data about this subject in the literature is limited.

Renal transplantation is the best treatment choice for both Type 1 and Type 2 HRS patients[98]. If liver transplantation is performed after the HRS is improved, posttransplantation morbidity and mortality is decreased. Three year survival after liver transplantation in patients with HRS is 60%, while it is 70%-80% if it is performed before HRS has developed[98,99].

PREVENTION

Prevention of HRS is important since it develops with a constant frequency in cases of SBP and alcoholic hepatitis. It is possible to prevent HRS if SBP is urgently diagnosed and treated. Albumin infusion may help to prevent HRS when SBP develops. Albumin infusion is started together with antibiotherapy with an initial dose of 1.5 g/kg at the time of diagnosis of infection

and albumin infusion is repeated after 48 h with a dose of 1 g/kg[23,100]. The incidence of renal dysfunction is decreased when compared to patients who are not treated with albumin (8% *vs* 31%) and mortality is also decreased (16% *vs* 35%)[100]. Norfloxacin is recommended in selected patients with cirrhosis and ascites. Four hundred mg/day dose of oral norfloxacin in a one year time period was found to decrease SBP development (7% *vs* 61%), decrease HRS development (28% *vs* 41%) and improve survival at three months (94% *vs* 62%) and one year (60% *vs* 48%)[100,101]. In a study investigating whether pentoxifylline is beneficial or not, significant benefit with 1200 mg/d pentoxifylline was observed when compared with placebo[102] but a meta-analysis revealed that pentoxifylline has no benefit in HRS[103].

REFERENCES

1 **Arroyo V**. Hecker R, Sherlock S. Electrolyte and circulatory changes in terminal liver failure [Lancet 1956; 2: 1221-1225]. *J Hepatol* 2002; **36**: 315-320 [PMID: 11867173]

2 **Koppel MH**, Coburn JW, Mims MM, Goldstein H, Boyle JD, Rubini ME. Transplantation of cadaveric kidneys from patients with hepatorenal syndrome. Evidence for the functionalnature of renal failure in advanced liver disease. *N Engl J Med* 1969; **280**: 1367-1371 [PMID: 4890476]

3 **Meltzer J**, Brentjens TE. Renal failure in patients with cirrhosis: hepatorenal syndrome and renal support strategies. *Curr Opin Anaesthesiol* 2010; **23**: 139-144 [PMID: 20124895 DOI: 10.1097/ACO.0b013e32833724a8]

4 **Weismüller TJ**, Prokein J, Becker T, Barg-Hock H, Klempnauer J, Manns MP, Strassburg CP. Prediction of survival after liver transplantation by pre-transplant parameters. *Scand J Gastroenterol* 2008; **43**: 736-746 [PMID: 18569992 DOI: 10.1080/00365520801932944]

5 **Arroyo V**, Ginès P, Gerbes AL, Dudley FJ, Gentilini P, Laffi G, Reynolds TB, Ring-Larsen H, Schölmerich J. Definition and diagnostic criteria of refractory ascites and hepatorenal syndrome in cirrhosis. International Ascites Club. *Hepatology* 1996; **23**: 164-176 [PMID: 8550036]

6 **Salerno F**, Gerbes A, Ginès P, Wong F, Arroyo V. Diagnosis, prevention and treatment of hepatorenal syndrome in cirrhosis. *Postgrad Med J* 2008; **84**: 662-670 [PMID: 19201943 DOI: 10.1136/gut.2006.107789]

7 **Wadei HM**, Mai ML, Ahsan N, Gonwa TA. Hepatorenal syndrome: pathophysiology and management. *Clin J Am Soc Nephrol* 2006; **1**: 1066-1079 [PMID: 17699328]

8 **Sort P**, Navasa M, Arroyo V, Aldeguer X, Planas R, Ruiz-del-Arbol L, Castells L, Vargas V, Soriano G, Guevara M, Ginès P, Rodés J. Effect of intravenous albumin on renal impairment and mortality in patients with cirrhosis and spontaneous bacterial peritonitis. *N Engl J Med* 1999; **341**: 403-409 [PMID: 10432325]

9 **Fasolato S**, Angeli P, Dallagnese L, Maresio G, Zola E, Mazza E, Salinas F, Donà S, Fagiuoli S, Sticca A, Zanus G, Cillo U, Frasson I, Destro C, Gatta A. Renal failure and bacterial infections in patients with cirrhosis: epidemiology and clinical features. *Hepatology* 2007; **45**: 223-229 [PMID: 17187409]

10 **Thabut D**, Massard J, Gangloff A, Carbonell N, Francoz C, Nguyen-Khac E, Duhamel C, Lebrec D, Poynard T, Moreau R. Model for end-stage liver disease score and systemic inflammatory response are major prognostic factors in patients with cirrhosis and acute functional renal failure. *Hepatology* 2007; **46**: 1872-1882 [PMID: 17972337]

11 **Terra C**, Guevara M, Torre A, Gilabert R, Fernández J, Martín-Llahí M, Baccaro ME, Navasa M, Bru C, Arroyo V, Rodés J, Ginès P. Renal failure in patients with cirrhosis and sepsis unrelated to spontaneous bacterial peritonitis: value of MELD score. *Gastroenterology* 2005; **129**: 1944-1953 [PMID: 16344063]

12 **Orlando R**, Floreani M, Padrini R, Palatini P. Evaluation of measured and calculated creatinine clearances as glomerular filtration markers in different stages of liver cirrhosis. *Clin Nephrol* 1999; **51**: 341-347 [PMID: 10404694]

13 **Sherman DS**, Fish DN, Teitelbaum I. Assessing renal function in cirrhotic patients: problems and pitfalls. *Am J Kidney Dis* 2003; **41**: 269-278 [PMID: 12552488]

14 **Belcher JM**, Garcia-Tsao G, Sanyal AJ, Bhogal H, Lim JK, Ansari N, Coca SG, Parikh CR. Association of AKI with mortality and complications in hospitalized patients with cirrhosis. *Hepatology* 2013; **57**: 753-762 [PMID: 22454364 DOI: 10.1002/hep.25735]

15 **Tsien CD**, Rabie R, Wong F. Acute kidney injury in decompensated cirrhosis. *Gut* 2013; **62**: 131-137 [PMID: 22637695 DOI: 10.1136/gutjnl-2011-301255]

16 **Jenq CC**, Tsai MH, Tian YC, Lin CY, Yang C, Liu NJ, Lien JM, Chen YC, Fang JT, Chen PC, Yang CW. RIFLE classification can predict short-term prognosis in critically ill cirrhotic patients. *Intensive Care Med* 2007; **33**: 1921-1930 [PMID: 17605129]

17 **Cholongitas E**, Calvaruso V, Senzolo M, Patch D, Shaw S, O'Beirne J, Burroughs AK. RIFLE classification as predictive factor of mortality in patients with cirrhosis admitted to intensive care unit. *J Gastroenterol Hepatol* 2009; **24**: 1639-1647 [PMID: 19788604 DOI: 10.1111/j.1440-1746.2009.05908.x]

18 **Wong F**, Nadim MK, Kellum JA, Salerno F, Bellomo R, Gerbes A, Angeli P, Moreau R, Davenport A, Jalan R, Ronco C, Genyk Y, Arroyo V. Working Party proposal for a revised classification system of renal dysfunction in patients with cirrhosis. *Gut* 2011; **60**: 702-709 [PMID: 21325171 DOI: 10.1136/gut.2010.236133]

19 **Platt JF**, Ellis JH, Rubin JM, Merion RM, Lucey MR. Renal duplex Doppler ultrasonography: a noninvasive predictor of kidney dysfunction and hepatorenal failure in liver disease. *Hepatology* 1994; **20**: 362-369 [PMID: 8045497]

20 **Ginès P**, Schrier RW. Renal failure in cirrhosis. *N Engl J Med* 2009; **361**: 1279-1290 [PMID: 19776409 DOI: 10.1056/NEJMra0809139]

21 **Dagher L**, Moore K. The hepatorenal syndrome. *Gut* 2001; **49**: 729-737 [PMID: 11600480]

22 **Schrier RW**, Arroyo V, Bernardi M, Epstein M, Henriksen JH, Rodés J. Peripheral arterial vasodilation hypothesis: a proposal for the initiation of renal sodium and water retention in cirrhosis. *Hepatology* 1988; **8**: 1151-1157 [PMID: 2971015]

23 **Arroyo V**, Fernández J. Management of hepatorenal syndrome in patients with cirrhosis. *Nat Rev Nephrol* 2011; **7**: 517-526 [PMID: 21826080 DOI: 10.1038/nrneph.2011.96]

24 **Arroyo V**, Clària J, Saló J, Jiménez W. Antidiuretic hormone and the pathogenesis of water retention in cirrhosis with ascites. *Semin Liver Dis* 1994; **14**: 44-58 [PMID: 8016662]

25 **Iwao T**, Oho K, Sakai T, Tayama C, Sato M, Nakano R, Yamawaki M, Toyonaga A, Tanikawa K. Splanchnic and extrasplanchnic arterial hemodynamics in patients with cirrhosis. *J Hepatol* 1997; **27**: 817-823 [PMID: 9382968]

26 **Fernandez-Seara J**, Prieto J, Quiroga J, Zozaya JM, Cobos MA, Rodriguez-Eire JL, Garcia-Plaza A, Leal J. Systemic and regional hemodynamics in patients with liver cirrhosis and ascites with and without functional renal failure. *Gastroenterology* 1989; **97**: 1304-1312 [PMID: 2676683]

27 **Maroto A**, Ginès P, Arroyo V, Ginès A, Saló J, Clària J, Jiménez W, Bru C, Rivera F, Rodés J. Brachial and femoral artery blood flow in cirrhosis: relationship to kidney dysfunction. *Hepatology* 1993; **17**: 788-793 [PMID: 8491446]

28 **Vorobioff J**, Bredfeldt JE, Groszmann RJ. Increased blood flow through the portal system in cirrhotic rats. *Gastroenterology* 1984; **87**: 1120-1126 [PMID: 6479534]

29 **Sato S**, Ohnishi K, Sugita S, Okuda K. Splenic artery and superior mesenteric artery blood flow: nonsurgical Doppler US measurement in healthy subjects and patients with chronic liver disease. *Radiology* 1987; **164**: 347-352 [PMID: 2955448]

30 **Torregrosa M**, Aguadé S, Dos L, Segura R, Gónzalez A, Evangelista A, Castell J, Margarit C, Esteban R, Guardia J, Genescà J. Cardiac alterations in cirrhosis: reversibility after liver transplantation. *J Hepatol* 2005; **42**: 68-74 [PMID: 15629509]

31 **Ma Z**, Lee SS. Cirrhotic cardiomyopathy: getting to the heart of the matter. *Hepatology* 1996; **24**: 451-459 [PMID: 8690419]

32 **Møller S**, Henriksen JH. Cirrhotic cardiomyopathy: a pathophysiological review of circulatory dysfunction in liver disease. *Heart* 2002; **87**: 9-15 [PMID: 11751653]

33 **Myers RP**, Lee SS. Cirrhotic cardiomyopathy and liver transplantation. *Liver Transpl* 2000; **6**: S44-S52 [PMID: 10915191]

34 **Pozzi M**, Carugo S, Boari G, Pecci V, de Ceglia S, Maggiolini S, Bolla GB, Roffi L, Failla M, Grassi G, Giannattasio C, Mancia G. Evidence of functional and structural cardiac abnormalities in cirrhotic patients with and without ascites. *Hepatology* 1997; **26**: 1131-1137 [PMID: 9362352]

35 **Saba S**, Janczewski AM, Baker LC, Shusterman V, Gursoy EC, Feldman AM, Salama G, McTiernan CF, London B. Atrial contractile dysfunction, fibrosis, and arrhythmias in a mouse model of cardiomyopathy secondary to cardiac-specific overexpression of tumor necrosis factor-{alpha}. *Am J Physiol Heart Circ Physiol* 2005; **289**: H1456-H1467 [PMID: 15923312]

36 **Bernardi M**, Rubboli A, Trevisani F, Cancellieri C, Ligabue A, Baraldini M, Gasbarrini G. Reduced cardiovascular responsiveness to exercise-induced sympathoadrenergic stimulation in patients with cirrhosis. *J Hepatol* 1991; **12**: 207-216 [PMID: 2050999]

37 **Henriksen JH**, Ring-Larsen H. Hepatorenal disorders: role of the sympathetic nervous system. *Semin Liver Dis* 1994; **14**: 35-43 [PMID: 8016660]

38 **Moncrief K**, Kaufman S. Splenic baroreceptors control splenic afferent nerve activity. *Am J Physiol Regul Integr Comp Physiol* 2006; **290**: R352-R356 [PMID: 16210416]

39 **Kostreva DR**, Castaner A, Kampine JP. Reflex effects of hepatic baroreceptors on renal and cardiac sympathetic nerve activity. *Am J Physiol* 1980; **238**: R390-R394 [PMID: 7377377]

40 **Guarner C**, Soriano G, Tomas A, Bulbena O, Novella MT, Balanzo J, Vilardell F, Mourelle M, Moncada S. Increased serum nitrite and nitrate levels in patients with cirrhosis: relationship to endotoxemia. *Hepatology* 1993; **18**: 1139-1143 [PMID: 8225220]

41 **Lluch P**, Torondel B, Medina P, Segarra G, Del Olmo JA, Serra MA, Rodrigo JM. Plasma concentrations of nitric oxide and asymmetric dimethylarginine in human alcoholic cirrhosis. *J Hepatol* 2004; **41**: 55-59 [PMID: 15246208]

42 **Bomzon A**, Blendis LM. The nitric oxide hypothesis and the hyperdynamic circulation in cirrhosis. *Hepatology* 1994; **20**: 1343-1350 [PMID: 7927270]

43 **Ginès P**, Guevara M, Arroyo V, Rodés J. Hepatorenal syndrome. *Lancet* 2003; **362**: 1819-1827 [PMID: 14654322]

44 **Laffi G**, La Villa G, Pinzani M, Ciabattoni G, Patrignani P, Mannelli M, Cominelli F, Gentilini P. Altered renal and platelet arachidonic acid metabolism in cirrhosis. *Gastroenterology* 1986; **90**: 274-282 [PMID: 3079716]

45 **Boyer TD**, Zia P, Reynolds TB. Effect of indomethacin and prostaglandin A1 on renal function and plasma renin activity in alcoholic liver disease. *Gastroenterology* 1979; **77**: 215-222 [PMID: 447034]

46 **Rimola A**, Ginés P, Arroyo V, Camps J, Pérez-Ayuso RM, Quintero E, Gaya J, Rivera F, Rodés J. Urinary excretion of 6-keto-prostaglandin F1 alpha, thromboxane B2 and prostaglandin E2 in cirrhosis with ascites. Relationship to functional renal failure (hepatorenal syndrome). *J Hepatol* 1986; **3**: 111-117 [PMID: 3462243]

47 **Ginès A**, Escorsell A, Ginès P, Saló J, Jiménez W, Inglada L, Navasa M, Clària J, Rimola A, Arroyo V. Incidence, predictive factors, and prognosis of the hepatorenal syndrome in cirrhosis with ascites. *Gastroenterology* 1993; **105**: 229-236 [PMID: 8514039]

48 **Akriviadis E**, Botla R, Briggs W, Han S, Reynolds T, Shakil O. Pentoxifylline improves short-term survival in severe acute alcoholic hepatitis: a double-blind, placebo-controlled trial. *Gastroenterology* 2000; **119**: 1637-1648 [PMID: 11113085]

49 **Follo A**, Llovet JM, Navasa M, Planas R, Forns X, Francitorra A, Rimola A, Gassull MA, Arroyo V, Rodés J. Renal impairment after spontaneous bacterial peritonitis in cirrhosis: incidence, clinical course, predictive factors and prognosis. *Hepatology* 1994; **20**: 1495-1501 [PMID: 7982650]

50 **Cárdenas A**, Ginès P, Uriz J, Bessa X, Salmerón JM, Mas A, Ortega R, Calahorra B, De Las Heras D, Bosch J, Arroyo V, Rodés J. Renal failure after upper gastrointestinal bleeding in cirrhosis: incidence, clinical course, predictive factors, and short-term prognosis. *Hepatology* 2001; **34**: 671-676 [PMID: 11584362]

51 **European Association for the Study of the Liver.** EASL clinical practice guidelines on the management of ascites, spontaneous bacterial peritonitis, and hepatorenal syndrome in cirrhosis. *J Hepatol* 2010; **53**: 397-417 [PMID: 20633946 DOI: 10.1016/j.jhep.2010.05.004]

52 **Orlando R**, Mussap M, Plebani M, Piccoli P, De Martin S, Floreani M, Padrini R, Palatini P. Diagnostic value of plasma cystatin C as a glomerular filtration marker in decompensated liver cirrhosis. *Clin Chem* 2002; **48**: 850-858 [PMID: 12029000]

53 **Gerbes AL**, Gülberg V, Bilzer M, Vogeser M. Evaluation of serum cystatin C concentration as a marker of renal function in patients with cirrhosis of the liver. *Gut* 2002; **50**: 106-110 [PMID: 11772976]

54 **Lluch P**, Mauricio MD, Vila JM, Segarra G, Medina P, Del Olmo JA, Rodrigo JM, Serra MA. Accumulation of symmetric dimethylarginine in hepatorenal syndrome. *Exp Biol Med* (Maywood) 2006; **231**: 70-75 [PMID: 16380646]

55 **Vaidya VS**, Ramirez V, Ichimura T, Bobadilla NA, Bonventre JV. Urinary kidney injury molecule-1: a sensitive quantitative biomarker for early detection of kidney tubular injury. *Am J Physiol Renal Physiol* 2006; **290**: F517-F529 [PMID: 16174863]

56 **Mishra J**, Dent C, Tarabishi R, Mitsnefes MM, Ma Q, Kelly C, Ruff SM, Zahedi K, Shao M, Bean J, Mori K, Barasch J, Devarajan P. Neutrophil gelatinase-associated lipocalin (NGAL) as a biomarker for acute renal injury after cardiac surgery. *Lancet* 1982; **365**: 1231-1238 [PMID: 15811456]

57 **Cabrera J**, Arroyo V, Ballesta AM, Rimola A, Gual J, Elena M, Rodes J. Aminoglycoside nephrotoxicity in cirrhosis. Value of urinary beta 2-microglobulin to discriminate functional renal failure from acute tubular damage. *Gastroenterology* 1982; **82**: 97-105 [PMID: 6171479]

58 **Malyszko J**. Biomarkers of acute kidney injury in different clinical settings: a time to change the paradigm? *Kidney Blood Press Res* 2010; **33**: 368-382 [PMID: 20924195 DOI: 10.1159/000319505]

59 **Ginès A**, Salmerón JM, Ginès P, Arroyo V, Jiménez W, Rivera F, Rodés J. Oral misoprostol or intravenous prostaglandin E2 do not improve renal function in patients with cirrhosis and ascites with hyponatremia or renal failure. *J Hepatol* 1993; **17**: 220-226 [PMID: 8445236]

60 **Clewell JD**, Walker-Renard P. Prostaglandins for the treatment of hepatorenal syndrome. *Ann Pharmacother* 1994; **28**: 54-55 [PMID: 8123962]

61 **Barnardo DE**, Baldus WP, Maher FT. Effects of dopamine on renal function in patients with cirrhosis. *Gastroenterology* 1970; **58**: 524-531 [PMID: 5438003]

62 **Bennett WM**, Keeffe E, Melnyk C, Mahler D, Rösch J, Porter GA. Response to dopamine hydrochloride in the hepatorenal syndrome. *Arch Intern Med* 1975; **135**: 964-971 [PMID: 1156055]

63 **Gülberg V**, Bilzer M, Gerbes AL. Long-term therapy and retreatment of hepatorenal syndrome type 1 with ornipressin and dopamine. *Hepatology* 1999; **30**: 870-875 [PMID: 10498636]

64 **Durkin RJ**, Winter SM. Reversal of hepatorenal syndrome with the combination of norepinephrine and dopamine. *Crit Care Med* 1995; **23**: 202-204 [PMID: 8001372]

65 **Runyon BA**. Management of adult patients with ascites due to cirrhosis: an update. *Hepatology* 2009; **49**: 2087-2107 [PMID: 19475696 DOI: 10.1002/hep.22853]

66 **Solanki P**, Chawla A, Garg R, Gupta R, Jain M, Sarin SK. Beneficial effects of terlipressin in hepatorenal syndrome: a prospective, randomized placebo-controlled clinical trial. *J*

Gastroenterol Hepatol 2003; **18**: 152-156 [PMID: 12542598]
67 **Gluud LL**, Christensen K, Christensen E, Krag A. Systematic review of randomized trials on vasoconstrictor drugs for hepatorenal syndrome. *Hepatology* 2010; **51**: 576-584 [PMID: 19885875 DOI: 10.1002/hep.23286]
68 **Nazar A**, Pereira GH, Guevara M, Martín-Llahi M, Pepin MN, Marinelli M, Solá E, Baccaro ME, Terra C, Arroyo V, Ginès P. Predictors of response to therapy with terlipressin and albumin in patients with cirrhosis and type 1 hepatorenal syndrome. *Hepatology* 2010; **51**: 219-226 [PMID: 19877168 DOI: 10.1002/hep.23283]
69 **Runyon BA,** Sterns RH, Forman JP. Hepatorenal syndrome. Uptodate [Serial online] Version 19.3. Available from: URL: http://uptodate.com/contents/hepatotrnal-syndrom
70 **Lata J**. Hepatorenal syndrome. *World J Gastroenterol* 2012; **18**: 4978-4984 [PMID: 23049205 DOI: 10.3748/wjg.v18.i36.4978]
71 **Nadim MK**, Kellum JA, Davenport A, Wong F, Davis C, Pannu N, Tolwani A, Bellomo R, Genyk YS. Hepatorenal syndrome: the 8th International Consensus Conference of the Acute Dialysis Quality Initiative (ADQI) Group. *Crit Care* 2012; **16**: R23 [PMID: 22322077 DOI: 10.1186/cc11188]
72 **Ginès P**. Pharmacological management of hepatorenal syndrome: lessons from non-responders. *J Hepatol* 2011; **55**: 268-269 [PMID: 21349296 DOI: 10.1016/j.jhep.2011.02.006]
73 **Martín-Llahí M**, Pépin MN, Guevara M, Díaz F, Torre A, Monescillo A, Soriano G, Terra C, Fábrega E, Arroyo V, Rodés J, Ginès P. Terlipressin and albumin vs albumin in patients with cirrhosis and hepatorenal syndrome: a randomized study. *Gastroenterology* 2008; **134**: 1352-1359 [PMID: 18471512 DOI: 10.1053/j.gastro.2008.02.024]
74 **Alessandria C**, Venon WD, Marzano A, Barletti C, Fadda M, Rizzetto M. Renal failure in cirrhotic patients: role of terlipressin in clinical approach to hepatorenal syndrome type 2. *Eur J Gastroenterol Hepatol* 2002; **14**: 1363-1368 [PMID: 12468959]
75 **Moreau R**, Lebrec D. The use of vasoconstrictors in patients with cirrhosis: type 1 HRS and beyond. *Hepatology* 2006; **43**: 385-394 [PMID: 16496352]
76 **Gluud LL**, Christensen K, Christensen E, Krag A. Terlipressin for hepatorenal syndrome. *Cochrane Database Syst Rev* 2012; **9**: CD005162 [PMID: 22972083 DOI: 10.1002/14651858.CD005162.pub3]
77 **Sharma P**, Kumar A, Shrama BC, Sarin SK. An open label, pilot, randomized controlled trial of noradrenaline versus terlipressin in the treatment of type 1 hepatorenal syndrome and predictors of response. *Am J Gastroenterol* 2008; **103**: 1689-1697 [PMID: 18557715 DOI: 10.1111/j.1572-0241.2008.01828.x]
78 **Wong F**, Pantea L, Sniderman K. Midodrine, octreotide, albumin, and TIPS in selected patients with cirrhosis and type 1 hepatorenal syndrome. *Hepatology* 2004; **40**: 55-64 [PMID: 15239086]
79 **Singh V**, Ghosh S, Singh B, Kumar P, Sharma N, Bhalla A, Sharma AK, Choudhary NS, Chawla Y, Nain CK. Noradrenaline vs. terlipressin in the treatment of hepatorenal syndrome: a randomized study. *J Hepatol* 2012; **56**: 1293-1298 [PMID: 22322237 DOI: 10.1016/j.jhep.2012.01.012]
80 **Alessandria C**, Ottobrelli A, Debernardi-Venon W, Todros L, Cerenzia MT, Martini S, Balzola F, Morgando A, Rizzetto M, Marzano A. Noradrenalin vs terlipressin in patients with hepatorenal syndrome: a prospective, randomized, unblinded, pilot study. *J Hepatol* 2007; **47**: 499-505 [PMID: 17560680]
81 **Kalambokis G**, Economou M, Fotopoulos A, Al Bokharhii J, Pappas C, Katsaraki A, Tsianos EV. The effects of chronic treatment with octreotide versus octreotide plus midodrine on systemic hemodynamics and renal hemodynamics and function in nonazotemic cirrhotic patients with ascites. *Am J Gastroenterol* 2005; **100**: 879-885 [PMID: 15784036]
82 **Esrailian E**, Pantangco ER, Kyulo NL, Hu KQ, Runyon BA. Octreotide/Midodrine therapy significantly improves renal function and 30-day survival in patients with type 1 hepatorenal syndrome. *Dig Dis Sci* 2007; **52**: 742-748 [PMID: 17235705]
83 **Angeli P**, Volpin R, Gerunda G, Craighero R, Roner P, Merenda R, Amodio P, Sticca A, Caregaro L, Maffei-Faccioli A, Gatta A. Reversal of type 1 hepatorenal syndrome with the administration of midodrine and octreotide. *Hepatology* 1999; **29**: 1690-1697 [PMID: 10347109]
84 **Duvoux C**, Zanditenas D, Hézode C, Chauvat A, Monin JL, Roudot-Thoraval F, Mallat A, Dhumeaux D. Effects of noradrenalin and albumin in patients with type I hepatorenal syndrome: a pilot study. *Hepatology* 2002; **36**: 374-380 [PMID: 12143045]
85 **Velez JC**, Nietert PJ. Therapeutic response to vasoconstrictors in hepatorenal syndrome parallels increase in mean arterial pressure: a pooled analysis of clinical trials. *Am J Kidney Dis* 2011; **58**: 928-938 [PMID: 21962618 DOI: 10.1053/j.ajkd.2011.07.017]
86 **Guevara M**, Ginès P, Bandi JC, Gilabert R, Sort P, Jiménez W, Garcia-Pagan JC, Bosch J, Arroyo V, Rodés J. Transjugular intrahepatic portosystemic shunt in hepatorenal syndrome: effects on renal function and vasoactive systems. *Hepatology* 1998; **28**: 416-422 [PMID: 9696006]
87 **Brensing KA**, Textor J, Perz J, Schiedermaier P, Raab P, Strunk H, Klehr HU, Kramer HJ, Spengler U, Schild H, Sauerbruch T. Long term outcome after transjugular intrahepatic portosystemic stent-shunt in non-transplant cirrhotics with hepatorenal syndrome: a phase II study. *Gut* 2000; **47**: 288-295 [PMID: 10896924]
88 **Brensing KA**, Textor J, Strunk H, Klehr HU, Schild H, Sauerbruch T. Transjugular intrahepatic portosystemic stent-shunt for hepatorenal syndrome. *Lancet* 1997; **349**: 697-698 [PMID: 9078203]
89 **Ochs A**, Rössle M, Haag K, Hauenstein KH, Deibert P, Siegerstetter V, Huonker M, Langer M, Blum HE. The transjugular intrahepatic portosystemic stent-shunt procedure for refractory ascites. *N Engl J Med* 1995; **332**: 1192-1197 [PMID: 7700312]
90 **Somberg KA**, Lake JR, Tomlanovich SJ, LaBerge JM, Feldstein V, Bass NM. Transjugular intrahepatic portosystemic shunts for refractory ascites: assessment of clinical and hormonal response and renal function. *Hepatology* 1995; **21**: 709-716 [PMID: 7875668]
91 **Ginès P**, Uriz J, Calahorra B, Garcia-Tsao G, Kamath PS, Del Arbol LR, Planas R, Bosch J, Arroyo V, Rodés J. Transjugular intrahepatic portosystemic shunting versus paracentesis plus albumin for refractory ascites in cirrhosis. *Gastroenterology* 2002; **123**: 1839-1847 [PMID: 12454841]
92 **Rössle M**, Gerbes AL. TIPS for the treatment of refractory ascites, hepatorenal syndrome and hepatic hydrothorax: a critical update. *Gut* 2010; **59**: 988-1000 [PMID: 20581246 DOI: 10.1136/gut.2009.193227]
93 **Testino G**, Ferro C, Sumberaz A, Messa P, Morelli N, Guadagni B, Ardizzone G, Valente U. Type-2 hepatorenal syndrome and refractory ascites: role of transjugular intrahepatic portosystemic stent-shunt in eighteen patients with advanced cirrhosis awaiting orthotopic liver transplantation. *Hepatogastroenterology* 2002; **50**: 1753-1755 [PMID: 14696397]
94 **Capling RK**, Bastani B. The clinical course of patients with type 1 hepatorenal syndrome maintained on hemodialysis. *Ren Fail* 2004; **26**: 563-568 [PMID: 15526916]
95 **Keller F**, Heinze H, Jochimsen F, Passfall J, Schuppan D, Büttner P. Risk factors and outcome of 107 patients with decompensated liver disease and acute renal failure (including 26 patients with hepatorenal syndrome): the role of hemodialysis. *Ren Fail* 1995; **17**: 135-146 [PMID: 7644764]
96 **Witzke O**, Baumann M, Patschan D, Patschan S, Mitchell A, Treichel U, Gerken G, Philipp T, Kribben A. Which patients benefit from hemodialysis therapy in hepatorenal syndrome? *J Gastroenterol Hepatol* 2004; **19**: 1369-1373 [PMID: 15610310]
97 **Hassanein TI**, Tofteng F, Brown RS, McGuire B, Lynch P, Mehta R, Larsen FS, Gornbein J, Stange J, Blei AT. Randomized controlled study of extracorporeal albumin dialysis for hepatic encephalopathy in advanced cirrhosis. *Hepatology* 2007; **46**: 1853-1862 [PMID: 17975845]
98 **Gonwa TA**, Morris CA, Goldstein RM, Husberg BS, Klintmalm GB. Long-term survival and renal function following liver transplantation in patients with and without hepatorenal syndrome--experience in 300 patients. *Transplantation* 1991; **51**: 428-430 [PMID: 1994538]

99 **Gonwa TA**, McBride MA, Anderson K, Mai ML, Wadei H, Ahsan N. Continued influence of preoperative renal function on outcome of orthotopic liver transplant (OLTX) in the US: where will MELD lead us? *Am J Transplant* 2006; **6**: 2651-2659 [PMID: 16939515]

100 **Salerno F**, Navickis RJ, Wilkes MM. Albumin infusion improves outcomes of patients with spontaneous bacterial peritonitis: a meta-analysis of randomized trials. *Clin Gastroenterol Hepatol* 2013; **11**: 123-130.e1 [PMID: 23178229 DOI: 10.1016/j.cgh.2012.11.007]

101 **Fernández J**, Navasa M, Planas R, Montoliu S, Monfort D, Soriano G, Vila C, Pardo A, Quintero E, Vargas V, Such J, Ginès P, Arroyo V. Primary prophylaxis of spontaneous bacterial peritonitis delays hepatorenal syndrome and improves survival in cirrhosis. *Gastroenterology* 2007; **133**: 818-824 [PMID: 17854593]

102 **Tyagi P**, Sharma P, Sharma BC, Puri AS, Kumar A, Sarin SK. Prevention of hepatorenal syndrome in patients with cirrhosis and ascites: a pilot randomized control trial between pentoxifylline and placebo. *Eur J Gastroenterol Hepatol* 2011; **23**: 210-217 [PMID: 21285885 DOI: 10.1097/MEG.0b013e3283435d76]

103 **Whitfield K**, Rambaldi A, Wetterslev J, Gluud C. Pentoxifylline for alcoholic hepatitis. *Cochrane Database Syst Rev* 2009; **4**: CD007339 [PMID: 19821406 DOI: 10.1002/14651858.CD007339.pub2]

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MINIREVIEWS

Evidence-based medicine: An update on treatments for peritoneal dialysis-related peritonitis

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Abstract

Peritonitis continues to be a major complication of peritoneal dialysis (PD), and adequate treatment is crucial for a favorable outcome. There is no consensus regarding the optimal therapeutic regimen, and few prospective controlled studies have been published. The objective of this manuscript is to review the results of PD peritonitis treatment reported in narrative reviews, systematic reviews, and proportional meta-analyses. Two narrative reviews, the only existing systematic review and its update published between 1991 and 2014 were included. In addition, we reported the results of a proportional meta-analysis published by our group. Results from systematic reviews of randomized control trials (RCT) and quasi-RCT were not able to identify any optimal antimicrobial treatment, but glycopeptide regimens were more likely to achieve a complete cure than a first generation cephalosporin. Compared to urokinase, simultaneous catheter removal and replacement resulted in better outcomes. Continuous and intermittent IP antibiotic use had similar outcomes. Intraperitoneal antibiotics were superior to intravenous antibiotics in reducing treatment failure. In the proportional meta-analysis of RCTs and the case series, the resolution rate (86%) of ceftazidime plus glycopeptide as initial treatment was significantly higher than first generation cephalosporin plus aminoglycosides (66%) and glycopeptides plus aminoglycosides (75%). Other comparisons of regimens used for either initial treatment or treatment of gram-positive rods or gram-negative rods did not show statistically significant differences. The superiority of a combination of a glycopeptide and a third generation cephalosporin was also reported by a narrative review study published in 1991, which reported an 88% resolution rate.

Key words: Peritonitis; Peritoneal dialysis; Antibiotic; Treatment

Core tip: This manuscript revised the data from narrative and systematic review, as well as those from a proportional meta-analysis study, regarding comparisons between antibiotic regimens used to peritoneal dialysis-related treatment, empathizing protocols for initial treatment.

There is no consensus on the best treatment and the only published systematic review and its recent update have failed to find superiority of any regimen. This type of analysis, commonly excludes several studies, some of them with a great number of cases. Therefore, this review intends to contribute in this issue analyzing the results from different types of reviews.

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INTRODUCTION

Since the introduction of peritoneal dialysis (PD) in routine clinical practice, peritonitis has been the main complication influencing patient mortality. Peritonitis continues to be the most frequent cause of technique failure[1], despite technological improvement. The choice of initial treatment for PD-related peritonitis remains a challenge to nephrologists who perform PD, particularly because of the lack of evidence to indicate the best therapeutic protocols, beyond temporal changes in the bacterial antibiotic susceptibility profile.

Coagulase negative staphylococci (CNS) are the most common etiological agents of PD-related peritonitis. In most PD centers[2], these microorganisms cause approximately one-third of the episodes. Over the last two decades, *Staphylococcus aureus* has lost its status as a PD-related peritonitis etiology, possibly because of technological advances in connection systems and the routine use of antibiotic prophylaxis at the catheter exit site[3]. However, the proportion of cases due to gram-negative bacilli has increased in several centers[4]. In addition, a gradual increase in the frequency of methicillin-resistant CNS and gram-negative species resistant to commonly used antibiotics has been reported[5,6].

Historically, the choice of initial antimicrobial regimen for PD-related peritonitis has been based on the recommendations of the International Society for Peritoneal Dialysis (ISPD), which published six documents between 1989 and 2010[7-12]. According to these guidelines, the initial treatment of peritonitis (prior to the results of microbiological tests) should be based on a combination of drugs for coverage of gram-positive cocci and gram-negative bacilli. The recommendations regarding the class of antimicrobials have varied over time. In general, for coverage of gram-positive cocci, the use of a first generation cephalosporin or vancomycin has been proposed, while for gam-negative bacilli an aminoglycoside or ceftazidime has been recommended. However, based on the available literature there is no consensus regarding the best antimicrobial therapy for the initial treatment of these infections, and few prospective and controlled studies have been published.

This manuscript intends to review the results from evidence-based medicine, comparing different treatment protocols for PD-related peritonitis in narrative reviews, systematic reviews and proportional meta-analysis.

NARRATIVE REVIEWS

Since the introduction of ambulatory PD as a modality of renal substitutive therapy as part of the clinical routine, several reviews have been published discussing general and specific aspects of this therapy, including peritonitis and its management; however, few of these articles have focused on comparing the therapeutic regimens.

In 1991, Millikin *et al*[13] published the first robust review compiling existing data on antimicrobial treatment of PD-related peritonitis. That study reported on studies of antimicrobial treatment for peritonitis published in the medical literature before January 1990. According to the review, the regimens most frequently used for empirical therapy were a combination of two antimicrobial drugs; the majority of the regimens involved an aminoglycoside associated with an antibiotic to gram positive organism coverage. An aminoglycoside with a first-generation cephalosporin was used in 165 episodes, with an overall resolution rate of 83%, while the combination of an aminoglycoside with a glycopeptide resulted in a clinical response in 88% of 286 cases. When a glycopeptide associated with a third generation cephalosporin was used, the resolution rate reached 93% as reported by three studies in a total of 197 peritonitis episodes.

The efficacy of drugs used for treatment of infections due to gram positive cocci was proven in 413 peritonitis episodes. The resolution rate was 90% for a first generation cephalosporin, used in 164 episodes. A similar clinical response was observed whether intraperitoneal (IP) cefazolin was prescribed for intermittent or continuous administration. However, the results from second-generation cephalosporins, used for treatment in 29 episodes, showed a resolution rate of 76%. In turn, the prescription of a glycopeptide, particularly vancomycin, resulted in a resolution rate of 94% in 220 cases.

For gram negative peritonitis episodes, aminoglycoside monotherapy produced a clinical response in 48% of the 58 episodes, while a monobactam (aztreonam) resolved 22 of 27 cases (81%), and a quinolone resolved 13 of 17 cases (76.4%). In 97% of cases involving *pseudomonas* peritonitis, an aminoglycoside was used either as monotherapy or in combination with anti-*pseudomonas* penicillin. When the peritonitis episode was at the exit site or was catheter related (n = 47), the response rate was only 32%. *Pseudomonas* peritonitis that was not associated with catheter infection, however, responded to these agents in 73% of 44 cases.

In 2000, our group published a literature review analyzing the therapeutic response from the empirical antimicrobial regimen proposed in the first, second, and third report of the Ad Hoc Committee on Peritonitis

Management of the International Society of Nephrology ("ISPD guidelines"), published between 1985 and 2000[14].

From 1985 to 1990, covering the period from the first report by The Ad Hoc Committee on Peritonitis Management[7], a total of six publications with 204 peritonitis episodes, a resolution rate higher than 80% was observed with the combination of a first generation cephalosporin and an aminoglycoside. In 1993, the second report by The Ad Hoc Committee on Peritonitis Management[8] recommended the initial use of vancomycin plus an aminoglycoside, both by an intermittent IP route, or IP injection of vancomycin combined with a third generation cephalosporin.

Results from the empirical prescription of vancomycin plus an aminoglycoside were reported in 23 publications between 1985 and 2000, corresponding to more than 1300 peritonitis episodes. A clinical response above 80% was reported in almost all of the series. In the series with the largest number of consecutive episodes (241 cases), the authors observed a resolution rate of 86%.

Vancomycin associated with ceftazidime was used in four studies, with a total of 302 episodes, resulting in a resolution rate above 90%. In the study with the largest number of cases (102 episodes) a cure rate of 92% was reported[15].

The third report of The Ad Hoc Committee on Peritonitis Management was published in 1996[9]. Based on the emergence of vancomycin-resistant enterococci and the possibility of gene transfer or resistance to *Staphylococcus aureus*, that document recommended the non-use of vancomycin in the empirical treatment of peritonitis. The combination of a first generation cephalosporin with an aminoglycoside again became the recommended empirical treatment for PD-related peritonitis.

Between the publication of the third report of The Ad Hoc Committee on Peritonitis Management and its fourth version in 2000[10], the results obtained with this protocol were reported in six publications[14]. In five of these reports, the resolution rate was over 75%. In our center, a study reporting 34 peritonitis episodes demonstrated complete cure in only 55% of the cases[16].

SYSTEMATIC REVIEWS

Wiggins *et al*[17] published a systematic review of randomized controlled trials (RCTs) on PD-related peritonitis in 2007. The study included 36 trials published from 1985 to 2006. The results indicated that there was no superior antimicrobial agent or regimen, although glycopeptide-based regimens achieved a significantly higher complete cure rate (three studies, 370 episodes) than first-generation cephalosporin-based regimens. Vancomycin and teicoplanin resulted in similar treatment failure and relapse rates (two trials, 178 participants). Equivalent treatment failure rates and risk of relapse were observed between IP intermittent or continuous antibiotic administration (four trials, 338 participants), while one trial with 75 patients showed an advantages of IP antibiotics over intravenous therapy. Based on one trial with 37 patients with relapsing or persistent peritonitis, simultaneous catheter removal/replacement was demonstrated to be superior to urokinase at reducing treatment failure rates. Catheter removal was not decreased by urokinase treatment compared with placebo (two trials, 168 participants). Based on one trial with 36 patients, there was no statistically significant difference in clinical response within a 24-h period of peritoneal lavage when compared to non-lavage.

Recently, Ballinger *et al*[18], from the same group of investigators, published an update of this systematic review. The authors included RCTs and quasi-RCTs to assess the treatment of peritonitis in adults and children. In total, there were 42 studies published up to March 5 2014, with 3013 episodes of peritonitis. Their results were similar to the previous analysis; the authors did not identify any optimal antibiotic agent or combination of agents. The advantages of a glycopeptide-based regimen over those based on a first generation cephalosporin regarding complete cure rate were demonstrated (three studies, 370 participants). However, no differences between these regimens have been found when the endpoints were primary treatment failure (two studies, 305 participants), relapse (3 studies, 350 participants), catheter removal (two studies, 305 participants), and microbiological eradication (one study, 45 participants). Similarities between vancomycin and teicoplanin in the treatment failure and relapse were shown, although the authors provided new information, showing that the primary treatment failure rate was lower with teicoplanin than vancomycin (two studies, 138 participants). Similar to the previous systematic review, comparisons between IP intermittent or continuous antibiotic administration showed no difference in the complete cure and relapse rates (four studies, 338 participants). The results were updated for primary treatment failure (five studies, 522 participants) and the catheter removal rate (1 study, 20 participants); no differences between the two forms of antibiotics were found. A preference for IP antibiotics (vancomycin and tobramycin) over intravenous administration was newly stated based on one study with 75 patients. In addition, based on one study, comparisons of the adverse effects of these antibiotic administration routes were included. No significant differences were observed in the incidence of hypotension (76 participants), cutaneous rash (20 participants), and infusion pain (20 participants). The advantage of simultaneous catheter removal/replacement over urokinase at reducing treatment failure rate was rewritten (one study, 37 participants), but the authors presented new information on comparisons between fibrinolytic agents and non-urokinase or

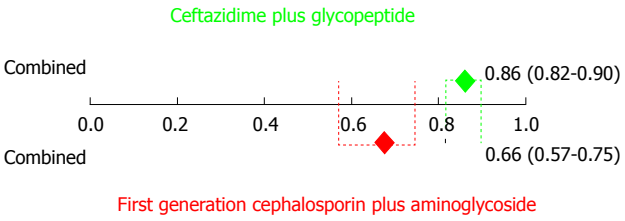

Figure 1 Combined resolution rate and 95%CIs of studies on initial treatment of peritoneal dialysis-related peritonitis with ceftazidime plus a glycopeptide *vs* a first generation cephalosporin plus an aminoglycoside.

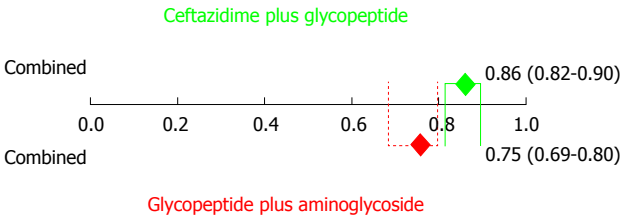

Figure 2 Combined resolution rate and 95%CIs of studies on initial treatment of peritoneal dialysis-related peritonitis with ceftazidime plus a glycopeptide *vs* a glycopeptide plus an aminoglycoside.

placebo. No significant differences were found in the following outcomes: complete cure rate (one study, 88 participants), primary treatment failure (two studies, 99 participants), relapse in persistent peritonitis (2 studies, 101 patients), relapse when fibrinolytic therapy was initiated at the time peritonitis was diagnosed (one study, 80 participants), catheter removal (2 studies, 116 participants), and all-cause mortality (1 study, 88 participants). Finally, the study found that there is no advantage to a 24-h period of peritoneal lavage compared to non-lavage (one study, 36 participants).

PROPORTIONAL META-ANALYSIS

One limitation of systematic review studies is the exclusion of a large number of publications with a large number of patients and episodes of peritonitis. Most of these excluded studies were case series. In turn, their authors have noted the inclusion of many trials with small patient numbers as a limitation[17,18]. In an attempt to overcome these limitations, our center is employing an alternative methodology: the proportional meta-analysis to examine possible differences among therapeutic protocols. This method has been used in other clinical settings[19,20], and it is possible to perform a meta-analysis of results from case series. Accordingly, a review of case series and RCTs concerning the treatment of PD-related peritonitis has been developed, focusing on comparing peritonitis resolution with antibiotics or antibiotic combinations more frequently recommended by the ISPD guidelines for empirical treatment of peritonitis and peritonitis due to gram positive or gram negative bacteria[21].

Studies were obtained between 1966 and January 2013, using the following sources: United States National Library of Medicine, Excerpta Medica database, and Literatura Latino-Americana e do Caribe em Ciências da Saúde. Peritonitis was defined according to the authors in accordance with the contemporary ISPD guidelines[7-12]. The criterion for peritonitis resolution was based on definitions used by authors and can vary greatly; the outcome resolution rate was treated as a dichotomous variable (peritonitis resolution *vs* non-resolution).

For first generation cephalosporins, we included the following: cefazolin, cephalotin, and cephaloridine. The only third generation cephalosporin we analyzed was ceftazidime. For aminoglycosides, we included gentamicin, amikacin, netilmicin and tobramycin. Vancomycin and teicoplanin were considered in the analysis as glycopeptides. Finally, ciprofloxacin, levofloxacin and ofloxacin were the fluoroquinolones included.

After screening by title and abstract, we obtained full paper copies of 140 eligible studies reporting antibiotic therapy for PD-related peritonitis. However, after applying the inclusion and exclusion criteria, only 43 studies (26 case series and 17 RCT) were acceptable for a proportional meta-analysis.

Initial treatment with ceftazidime plus a glycopeptide was used in five[15,22-25] studies with a total of 443 episodes; the pooled resolution rate was 86% (95%CI: 0.82-0.89). This resolution rate was significantly higher than initial treatment with a first generation cephalosporin plus aminoglycosides (pooled proportion of 66%, 95%CI: 0.57-0.75) from 14 studies[25-38] with a total of 1438 total episodes (Figure 1). Initial treatment with ceftazidime plus a glycopeptide also showed a higher resolution rate than a glycopeptide plus aminoglycosides (pooled proportion of 75%, 95%CI: 0.69-0.80), which was used in 16 studies[29-31,38-50] with a total of 574 episodes (Figure 2).

The following comparisons showed no statistically significant differences because their CIs overlapped: a first generation cephalosporin plus aminoglycosides [resolution rate (RR) = 66%, 95%CI: 0.57-0.75] *vs* glycopeptides plus aminoglycosides (RR = 75%, 95%CI: 0.69-0.80); a first generation cephalosporin plus aminoglycosides (RR = 66%, 95%CI: 0.57-0.75) *vs* a first generation cephalosporin plus ceftazidime (RR = 59%, 95%CI: 0.32-0.83); glycopeptides plus aminoglycosides (RR = 75%, 95%CI: 0.69-0.80) *vs* first generation cephalosporin plus ceftazidime (RR = 59%, 95%CI: 0.32-0.83), and a first generation cephalosporin plus ceftazidime (RR = 59%, 95%CI: 0.32-0.83) *vs* ceftazidime plus a glycopeptide (RR = 86%, 95%CI: 0.82-0.89).

For treatment of episodes due to gram-positive rods, the pooled resolution rate from 13[23,39,40,48,49,51-58] studies with a total of 917 episodes was 78% (95%CI: 0.66-0.88) for a glycopeptide, while the rates from five studies[26,37,53,58,59] with a total of 532 episodes for a first generation cephalosporin were 73% (95%CI: 0.55-0.88). There were no significant differences

Table 1 Recommendations for antibiotics choice in peritoneal dialysis-related peritonitis

	Monitoring the etiologies and antimicrobial resistance profile	
	Yes	**No**
Initial (empirical) protocol	Start intraperitoneal antibiotics to cover gram-positive and gram roads, according to local microbiologic profile	Start a glycopeptide (gram-positive coverage) plus ceftazidime (gram-negative coverage), both by intraperitoneal route[1]
After results of culture and *in vitro* susceptibility tests	Culture positive: adjust the treatment according to bacterial susceptibility. If *Pseudomonas spp* on culture, add a second anti-*pseudomonas* drug acting in different ways that organism is sensitive to[2]	Culture positive: adjust the treatment according to bacterial susceptibility. If *Pseudomonas spp* on culture, add a second anti-*pseudomonas* drug acting in different ways that organism is sensitive to[2]
Therapy duration	Culture negative: continue initial antibiotics *Pseudomonas spp*, *Enterococcus*/*Streptococcus spp* = 21 d Non-*pseudomonas* single gram-negative = 14-21 d Culture negative, coagulase negative staphylococcus, other gram-positive roads = 14 d	Culture negative: Continue initial antibiotics

[1]Evidence-based medicine; [2]*E.g.*, quinolone, ceftazidime, cefepime, amiglycoside, piperacillin.

between the schemes.

Comparisons of episodes due to gram-negative rods showed that the pooled proportion resolution rate from nine studies[39,40,49,57,60-63] with a total of 138 episodes was 68% (95%CI: 0.50-0.85) for a quinolone. For ceftazidime, the resolution rate was 61% (95%CI: 0.53-0.70) from three studies[33,63,64] with a total of 117 episodes, and for aminoglycosides the resolution rate was 65% (95%CI: 0.51-0.77) from nine studies[23,26,31,39,40,49,55,60,61] with a total of 211 episodes. There were no significant differences among these antibiotics.

LIMITATIONS

The limitations of narrative reviews are those inherent to this type of publication, which include the use of different types of studies, such as RCTs, case series, and others without a statistical tool for comparisons among the treatments. Moreover, they refer to data published many years ago and may be influenced by an era effect.

Regarding the systematic reviews, their authors emphasize inadequate randomization and concealment methods. In addition, the definitions of peritonitis, successful treatment, and relapse varied among trials[17]. Finally, many trials had small patient numbers, which reduces their statistical power.

The most important limitation of our proportional meta-analysis is the low evidence level of case series included with the RCTs. In addition, there is significant heterogeneity among the studies, which differed considerably in their patient selection, baseline renal disease, number of subjects, antibiotic administration routes, and definition of peritonitis and resolution.

CONCLUSION

According to the results of the systematic reviews, there is no superior antimicrobial agent to treat PD-related peritonitis, although glycopeptide-based regimens achieved a significantly higher complete cure rate. Similar treatment failure rates were found with vancomycin and teicoplanin, while the primary treatment failure rate was lower with teicoplanin. Intermittent or continuous IP antibiotic administration had similar complete cure, primary treatment failure, relapse, and catheter removal rates. The advantages of IP antibiotics over intravenous therapy were reported. In cases of persistent or relapsing peritonitis, catheter removal is associated with better outcomes than with IP urokinase. Finally, no advantages were found to be associated with adjunctive therapies, such as fibrinolytic drugs and peritoneal lavage.

A narrative review of antimicrobial treatment for patients with PD-related peritonitis published in 1991[13] concluded that the optimal empirical treatment was weekly vancomycin and ceftazidime.

Our proportional meta-analysis[21] was able to identify that the combination of a glycopeptide plus ceftazidime in the initial treatment of PD-related peritonitis was superior to a glycopeptide plus an aminoglycoside or the combination of a first generation cephalosporin plus an aminoglycoside. This result strongly suggests that the differences found may be related to better coverage of gram-negative bacilli with third generation cephalosporins than with aminoglycosides. Bacterial resistance of gram-negative bacilli, particularly *Pseudomonas* species, to commonly prescribed antimicrobials has been reported in recent years[6]; this finding may explain the superiority of the protocols employing ceftazidime. This review showed that a treatment regimen with a glycopeptide plus ceftazidime could be a promising initial therapy in patients with PD-related peritonitis. However, this result should be carefully analyzed, because this treatment was only used in four cases series[15,22-24] and one RCT[25] for a total of 443 peritonitis episodes. Moreover, an emphasis should be placed on the necessity of monitoring the local microbiologic profile in each dialysis center to determine the initial therapeutic protocol. Recommendations for antibiotics choice in peritoneal dialysis-related peritonitis are expressed in the Table 1.

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REFERENCES

1 **Davenport A**. Peritonitis remains the major clinical complication of peritoneal dialysis: the London, UK, peritonitis audit 2002-2003. *Perit Dial Int* 2009; **29**: 297-302 [PMID: 19458302]
2 **Camargo CH**, Cunha Mde L, Caramori JC, Mondelli AL, Montelli AC, Barretti P. Peritoneal dialysis-related peritonitis due to coagulase-negative Staphylococcus: a review of 115 cases in a Brazilian center. *Clin J Am Soc Nephrol* 2014; **9**: 1074-1081 [PMID: 24677560 DOI: 10.2215/CJN.09280913]
3 **Piraino B**, Bernardini J, Florio T, Fried L. Staphylococcus aureus prophylaxis and trends in gram-negative infections in peritoneal dialysis patients. *Perit Dial Int* 2003; **23**: 456-459 [PMID: 14604197]
4 **Bender FH**, Bernardini J, Piraino B. Prevention of infectious complications in peritoneal dialysis: best demonstrated practices. *Kidney Int Suppl* 2006; **(103)**: S44-S54 [PMID: 17080111 DOI: 10.1038/sj.ki.5001915]
5 **Kim DK**, Yoo TH, Ryu DR, Xu ZG, Kim HJ, Choi KH, Lee HY, Han DS, Kang SW. Changes in causative organisms and their antimicrobial susceptibilities in CAPD peritonitis: a single center's experience over one decade. *Perit Dial Int* 2004; **24**: 424-432 [PMID: 15490981]
6 **Barretti P**, Pereira D, Brasil MA, de Lourdes Cunha M, Caramori J, Montelli A. Evolution of gram-negative bacilli susceptibility in peritoneal dialysis-related peritonitis in Brazil: a single center's experience over nine years. *Perit Dial Int* 2009; **29**: 230-233 [PMID: 19293362]
7 Continuous ambulatory peritoneal dialysis (CAPD) peritonitis treatment recommendations: 1989 update. The Ad Hoc Advisory Committee on Peritonitis Management. *Perit Dial Int* 1989; **9**: 247-256 [PMID: 2488376]
8 **Keane WF**, Everett ED, Golper TA, Gokal R, Halstenson C, Kawaguchi Y, Riella M, Vas S, Verbrugh HA. Peritoneal dialysis-related peritonitis treatment recommendations. 1993 update. The Ad Hoc Advisory Committee on Peritonitis Management. International Society for Peritoneal Dialysis. *Perit Dial Int* 1993; **13**: 14-28 [PMID: 8443273]
9 **Keane WF**, Alexander SR, Bailie GR, Boeschoten E, Gokal R, Golper TA, Holmes CJ, Huang CC, Kawaguchi Y, Piraino B, Riella M, Schaefer F, Vas S. Peritoneal dialysis-related peritonitis treatment recommendations: 1996 update. *Perit Dial Int* 1996; **16**: 557-573 [PMID: 8981523]
10 **Keane WF**, Bailie GR, Boeschoten E, Gokal R, Golper TA, Holmes CJ, Kawaguchi Y, Piraino B, Riella M, Vas S. Adult peritoneal dialysis-related peritonitis treatment recommendations: 2000 update. *Perit Dial Int* 2000; **20**: 396-411 [PMID: 11007371]
11 **Piraino B**, Bailie GR, Bernardini J, Boeschoten E, Gupta A, Holmes C, Kuijper EJ, Li PK, Lye WC, Mujais S, Paterson DL, Fontan MP, Ramos A, Schaefer F, Uttley L; ISPD Ad Hoc Advisory Committee. Peritoneal dialysis-related infections recommendations: 2005 update. *Perit Dial Int* 2005; **25**: 107-131 [PMID: 15796137]
12 **Li PK**, Szeto CC, Piraino B, Bernardini J, Figueiredo AE, Gupta A, Johnson DW, Kuijper EJ, Lye WC, Salzer W, Schaefer F, Struijk DG; International Society for Peritoneal Dialysis. Peritoneal dialysis-related infections recommendations: 2010 update. *Perit Dial Int* 2010; **30**: 393-423 [PMID: 20628102 DOI: 10.3747/pdi.2010.00049]
13 **Millikin SP**, Matzke GR, Keane WF. Antimicrobial treatment of peritonitis associated with continuous ambulatory peritoneal dialysis. *Perit Dial Int* 1991; **11**: 252-260 [PMID: 1832968]
14 **Barretti P,** Montelli AC, Cunha MLRS, Caramori JCT. Tratamento atual das peritonites em em diálise peritoneal ambulatorial contínua. *J Bras Nefrol* 2001; **23**: 114-120
15 **Beaman M**, Solaro L, McGonigle RJ, Michael J, Adu D. Vancomycin and ceftazidime in the treatment of CAPD peritonitis. *Nephron* 1989; **51**: 51-55 [PMID: 2644565 DOI: 10.1159/000185242]
16 **Onozato ML**, Caramori JC, Barretti P. Initial treatment of CAPD peritonitis: poor response with association of cefazolin and amikacin. *Perit Dial Int* 1999; **19**: 88-89 [PMID: 10201353]
17 **Wiggins KJ,** Johnson DW, Craig JC, Strippoli GF. Treatment of peritoneal dialysis-associated peritonitis: a systematic review of randomized controlled trials. *Am J Kidney Dis* 2007; **50**: 967-988 [PMID: 18037098 DOI: 10.1053/j.ajkd.2007.08.015]
18 **Ballinger AE**, Palmer SC, Wiggins KJ, Craig JC, Johnson DW, Cross NB, Strippoli GF. Treatment for peritoneal dialysis-associated peritonitis. *Cochrane Database Syst Rev* 2014; **4**: CD005284 [PMID: 24771351 DOI: 10.1002/14651858.CD005284.pub3]
19 **El Dib R**, Touma NJ, Kapoor A. Cryoablation vs radiofrequency ablation for the treatment of renal cell carcinoma: a meta-analysis of case series studies. *BJU Int* 2012; **110**: 510-516 [PMID: 22304329 DOI: 10.1111/j.1464-410X.2011.10885.x]
20 **Gurgel SJ**, El Dib R, do Nascimento P. Enhanced recovery after elective open surgical repair of abdominal aortic aneurysm: a complementary overview through a pooled analysis of proportions from case series studies. *PLoS One* 2014; **9**: e98006 [PMID: 24887022 DOI: 10.1371/journal.pone.0098006]
21 **Barretti P**, Doles JV, Pinotti DG, El Dib R. Efficacy of antibiotic therapy for peritoneal dialysis-associated peritonitis: a proportional meta-analysis. *BMC Infect Dis* 2014; **14**: 445 [PMID: 25135487 DOI: 10.1186/1471-2334-14-445]
22 **Ludlam HA**, Price TN, Berry AJ, Phillips I. Laboratory diagnosis of peritonitis in patients on continuous ambulatory peritoneal dialysis. *J Clin Microbiol* 1988; **26**: 1757-1762 [PMID: 3183023]
23 **Blunden M**, Zeitlin D, Ashman N, Fan SL. Single UK centre experience on the treatment of PD peritonitis--antibiotic levels and outcomes. *Nephrol Dial Transplant* 2007; **22**: 1714-1719 [PMID: 17369615 DOI: 10.1093/ndt/gfm079]
24 **Quintanar Lartundo JA**, Palomar R, Dominguez-Diez A, Salas C, Ruiz-Criado J, Rodrigo E, Martinez De Francisco AL, Arias M. Microbiological profile of peritoneal dialysis peritonitis and predictors of hospitalization. *Adv Perit Dial* 2011; **27**: 38-42 [PMID: 22073826]
25 **Gucek A**, Bren AF, Hergouth V, Lindic J. Cefazolin and netilmycin versus vancomycin and ceftazidime in the treatment of CAPD peritonitis. *Adv Perit Dial* 1997; **13**: 218-220 [PMID: 9360685]
26 **Leung CB**, Szeto CC, Chow KM, Kwan BC, Wang AY, Lui SF, Li PK. Cefazolin plus ceftazidime versus imipenem/cilastatin monotherapy for treatment of CAPD peritonitis--a randomized controlled trial. *Perit Dial Int* 2004; **24**: 440-446 [PMID: 15490983]
27 **Chan MK**, Cheng IK, Ng WS. A randomized prospective trial of three different regimens of treatment of peritonitis in patients on continuous ambulatory peritoneal dialysis. *Am J Kidney Dis* 1990; **15**: 155-159 [PMID: 2405653 DOI: 10.1016/S0272-6386(12)80513-0]
28 **Weber J**, Kuhlmann U. Intraperitoneal cefazolin and gentamicin in the management of CAPD-related peritonitis. *Contrib Nephrol* 1991; **89**: 108-118 [PMID: 1893715]
29 **Lupo A**, Rugiu C, Bernich P, Laudon A, Marcantoni C, Mosconi G, Cantaluppi MC, Maschio G. A prospective, randomized trial of two antibiotic regimens in the treatment of peritonitis in CAPD patients: teicoplanin plus tobramycin versus cephalothin plus tobramycin. *J Antimicrob Chemother* 1997; **40**: 729-732 [PMID: 9421325 DOI: 10.1093/jac/40.5.729]
30 **Vas S**, Bargman J, Oreopoulos D. Treatment in PD patients of peritonitis caused by gram-positive organisms with single daily dose of antibiotics. *Perit Dial Int* 1997; **17**: 91-94 [PMID: 9068032]
31 **Goldberg L**, Clemenger M, Azadian B, Brown EA. Initial treatment of peritoneal dialysis peritonitis without vancomycin with a once-daily cefazolin-based regimen. *Am J Kidney Dis* 2001; **37**: 49-55 [PMID: 11136167 DOI: 10.1053/ajkd.2001.20581]
32 **Silva MM**, Pecoits-Filho R, Rocha CS, Stinghen AE, Pachaly MA,

Nascimento MM, Campos RP, Sauthier S, Fuerbringer R, Riella MC. The recommendations from the International Society for Peritoneal Dialysis for Peritonitis Treatment: a single-center historical comparison. *Adv Perit Dial* 2004; **20**: 74-77 [PMID: 15384800]

33 **Toussaint N**, Mullins K, Snider J, Murphy B, Langham R, Gock H. Efficacy of a non-vancomycin-based peritoneal dialysis peritonitis protocol. *Nephrology* (Carlton) 2005; **10**: 142-146 [PMID: 15877673 DOI: 10.1111/j.1440-1797.2005.00379.x]

34 **Chen KH,** Chang CT, Weng SM, Yu CC, Fang JT, Huang JY, Yang CW, Hung CC. Culture-negative peritonitis: a fifteen-year review. *Ren Fail* 2007; **29**: 177-181[PMID: 17365933 DOI: 10.1080/08860220601098847]

35 **Barretti P**, Montelli AC, Batalha JE, Caramori JC, Cunha Mde L. The role of virulence factors in the outcome of staphylococcal peritonitis in CAPD patients. *BMC Infect Dis* 2009; **9**: 212 [PMID: 20028509 DOI: 10.1186/1471-2334-9-212]

36 **Lee CC**, Sun CY, Chang KC, Wu MS. Positive dialysate gram stain predicts outcome of empirical antibiotic therapy for peritoneal dialysis-associated peritonitis. *Ther Apher Dial* 2010; **14**: 201-208 [PMID: 20438543 DOI: 10.1111/j.1744-9987.2009.00784.x]

37 **Huang ST**, Chuang YW, Cheng CH, Wu MJ, Chen CH, Yu TM, Shu KH. Evolution of microbiological trends and treatment outcomes in peritoneal dialysis-related peritonitis. *Clin Nephrol* 2011; **75**: 416-425 [PMID: 21543021 DOI: 10.5414/CNP75416]

38 **Oliveira LG**, Luengo J, Caramori JC, Montelli AC, Cunha Mde L, Barretti P. Peritonitis in recent years: clinical findings and predictors of treatment response of 170 episodes at a single Brazilian center. *Int Urol Nephrol* 2012; **44**: 1529-1537 [PMID: 22302733 DOI: 10.1007/s11255-011-0107-7]

39 **Lye WC**, Lee EJ, van der Straaten J. Intraperitoneal vancomycin/oral pefloxacin versus intraperitoneal vancomycin/gentamicin in the treatment of continuous ambulatory peritoneal dialysis peritonitis. *Perit Dial Int* 1993; **13** Suppl 2: S348-S350 [PMID: 8399607]

40 **Bennett-Jones DN**, Russell GI, Barrett A. A comparison between oral ciprofloxacin and intra-peritoneal vancomycin and gentamicin in the treatment of CAPD peritonitis. *J Antimicrob Chemother* 1990; **26** Suppl F: 73-76 [PMID: 2292547 DOI: 10.1093/jac/26.suppl_F.73]

41 **Friedland JS**, Iveson TJ, Fraise AP, Winearls CG, Selkon JB, Oliver DO. A comparison between intraperitoneal ciprofloxacin and intraperitoneal vancomycin and gentamicin in the treatment of peritonitis associated with continuous ambulatory peritoneal dialysis (CAPD). *J Antimicrob Chemother* 1990; **26** Suppl F: 77-81 [PMID: 2292548 DOI: 10.1093/jac/26.suppl_F.77]

42 **Tapson JS**, Orr KE, George JC, Stansfield E, Bint AJ, Ward MK. A comparison between oral ciprofloxacin and intraperitoneal vancomycin and netilmicin in CAPD peritonitis. *J Antimicrob Chemother* 1990; **26** Suppl F: 63-71 [PMID: 2292546 DOI: 10.1093/jac/26.suppl_F.63]

43 **Merchant MR**, Anwar N, Were A, Uttley L, Tooth JA, Gokal R. Imipenem versus netilmicin and vancomycin in the treatment of CAPD peritonitis. *Adv Perit Dial* 1992; **8**: 234-237 [PMID: 1361795]

44 **Were AJ**, Marsden A, Tooth A, Ramsden R, Mistry CD, Gokal R. Netilmycin and vancomycin in the treatment of peritonitis in CAPD patients. *Clin Nephrol* 1992; **37**: 209-213 [PMID: 1582059]

45 **Anwar N**, Merchant M, Were T, Tooth A, Uttley L, Gokal R. A prospective, randomized study of the comparative safety and efficacy of intraperitoneal imipenem versus vancomycin and netilmicin in the treatment of peritonitis on CAPD. *Perit Dial Int* 1995; **15**: 167-171 [PMID: 7612739]

46 **Guest SS**, Erickson LJ. Combination therapy involving ciprofloxacin for peritonitis. *Perit Dial Int* 1996; **16**: 316-318 [PMID: 8761547]

47 **Lai MN**, Kao MT, Chen CC, Cheung SY, Chung WK. Intraperitoneal once-daily dose of cefazolin and gentamicin for treating CAPD peritonitis. *Perit Dial Int* 1997; **17**: 87-89 [PMID: 9068030]

48 **Bowley JA**, Pickering SJ, Scantlebury AJ, Ackrill P, Jones DM. Intraperitoneal teicoplanin in the treatment of peritonitis associated with continuous ambulatory peritoneal dialysis. *J Antimicrob Chemother* 1988; **21** Suppl A: 133-139 [PMID: 2965125 DOI: 10.1093/jac/21.suppl_A.133]

49 **Cheng IK**, Fang GX, Chau PY, Chan TM, Tong KL, Wong AK, Li CS, Lo WK, Cheung KO, Kumana CR. A randomized prospective comparison of oral levofloxacin plus intraperitoneal (IP) vancomycin and IP netromycin plus IP vancomycin as primary treatment of peritonitis complicating CAPD. *Perit Dial Int* 1998; **18**: 371-375 [PMID: 10505557]

50 **Wong KM**, Chan YH, Cheung CY, Chak WL, Choi KS, Leung SH, Leung J, Chau KF, Tsang DN, Li CS. Cefepime versus vancomycin plus netilmicin therapy for continuous ambulatory peritoneal dialysis-associated peritonitis. *Am J Kidney Dis* 2001; **38**: 127-131 [PMID: 11431192 DOI: 10.1053/ajkd.2001.25205]

51 **Al-Wali W,** Baillod RA, Brumfitt W, Hamilton-Miller JMT. Teicoplanin in the treatment of peritonitis in patients receiving continuous ambulatory peritoneal dialysis: A comparative trial against vancomycin. *Int J Antimicrob Agents* 1992; **1**: S1-S6

52 **Vargemezis V**, Pasadakis P, Thodis H, Coucudis P, Peihaberis P, Jafer H, Jara F, Kartali S. Vancomycin therapy for gram-positive peritonitis in patients on CAPD. *Adv Perit Dial* 1989; **5**: 128-129 [PMID: 2577394]

53 **Flanigan MJ,** Lim VS. Initial treatment of dialysis associated peritonitis: a controlled trial of vancomycin versus cefazolin. *Perit Dial Int* 1991; **11**: 31-37

54 **Bailie GR**, Haqqie SS, Eisele G, Gorman T, Low CL. Effectiveness of once-weekly vancomycin and once-daily gentamicin, intraperitoneally, for CAPD peritonitis. *Perit Dial Int* 1995; **15**: 269-271 [PMID: 7578507]

55 **Chadwick DH**, Agarwal S, Vora BJ, Hair M, McKewan A, Gokal R. Outcome of peritonitis treated with intraperitoneal (i.p.) weekly vancomycin and i.p. daily netilmicin. *J Nephrol* 1999; **12**: 318-321 [PMID: 10630696]

56 **Li PK**, Ip M, Law MC, Szeto CC, Leung CB, Wong TY, Ho KK, Wang AY, Lui SF, Yu AW, Lyon DJ, Cheng AF, Lai KN. Use of intraperitoneal cefepime as monotherapy in treatment of CAPD peritonitis. *Perit Dial Int* 2000; **20**: 232-234 [PMID: 10809249]

57 **Goffin E**, Herbiet L, Pouthier D, Pochet JM, Lafontaine JJ, Christophe JL, Gigi J, Vandercam B. Vancomycin and ciprofloxacin: systemic antibiotic administration for peritoneal dialysis-associated peritonitis. *Perit Dial Int* 2004; **24**: 433-439 [PMID: 15490982]

58 **Szeto CC**, Kwan BC, Chow KM, Lau MF, Law MC, Chung KY, Leung CB, Li PK. Coagulase negative staphylococcal peritonitis in peritoneal dialysis patients: review of 232 consecutive cases. *Clin J Am Soc Nephrol* 2008; **3**: 91-97 [PMID: 18032790 DOI: 10.2215/CJN.03070707]

59 **Percival A**, Cohen SL. The treatment of peritoneal infections in patients on peritoneal dialysis. *Postgrad Med J* 1967; **43** Suppl 43: 160-165 [PMID: 6052327]

60 **Ludlam HA**, Barton I, White L, McMullin C, King A, Phillips I. Intraperitoneal ciprofloxacin for the treatment of peritonitis in patients receiving continuous ambulatory peritoneal dialysis (CAPD). *J Antimicrob Chemother* 1990; **25**: 843-851 [PMID: 2197264 DOI: 10.1093/jac/25.5.843]

61 **Cheng IK**, Chan CY, Wong WT. A randomised prospective comparison of oral ofloxacin and intraperitoneal vancomycin plus aztreonam in the treatment of bacterial peritonitis complicating continuous ambulatory peritoneal dialysis (CAPD). *Perit Dial Int* 1991; **11**: 27-30 [PMID: 2049419]

62 **Dryden M**, Eykyn SJ. Short-course gentamicin in gram-negative CAPD peritonitis. *Lancet* 1993; **341**: 497 [PMID: 8094518 DOI: 10.1016/0140-6736(93)90252-C]

63 **Brown MC**, Simpson K, Kerssens JJ, Mactier RA. Peritoneal dialysis-associated peritonitis rates and outcomes in a national cohort are not improving in the post-millennium (2000-2007). *Perit Dial Int* 2011; **31**: 639-650 [PMID: 21804138 DOI: 10.3747/pdi.2010.00185]

64 **de Fijter CW,** ter Wee PM, Oe LP, Verbrugh HA. Intraperitoneal ciprofloxacin and rifampicin versus cephradine as initial treatment

of (C)APD-related peritonitis: a prospective randomized multicenter comparison (CIPPER trial). *Perit Dial Int* 2001; **21**: 480-486 [PMID: 11757832]

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African origins and chronic kidney disease susceptibility in the human immunodeficiency virus era

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Abstract

Chronic kidney disease (CKD) is a major public health problem worldwide with the estimated incidence growing by approximately 6% annually. There are striking ethnic differences in the prevalence of CKD such that, in the United States, African Americans have the highest prevalence of CKD, four times the incidence of end stage renal disease when compared to Americans of European ancestry suggestive of genetic predisposition. Diabetes mellitus, hypertension and human immunodeficiency virus (HIV) infection are the major causes of CKD. HIV-associated nephropathy (HIVAN) is an irreversible form of CKD with considerable morbidity and mortality and is present predominantly in people of African ancestry. The APOL1 G1 and G2 alleles were more strongly associated with the risk for CKD than the previously examined MYH9 E1 risk haplotype in individuals of African ancestry. A strong association was reported in HIVAN, suggesting that 50% of African Americans with two APOL1 risk alleles, if untreated, would develop HIVAN. However these two variants are not enough to cause disease. The prevailing belief is that modifying factors or second hits (including genetic hits) underlie the pathogenesis of kidney disease. This work reviews the history of genetic susceptibility of CKD and outlines current theories regarding the role for APOL1 in CKD in the HIV era.

Key words: Chronic kidney disease; Genetics; African ancestry; Human immunodeficiency virus; APOL1; MYH9; Human immunodeficiency virus-associated nephropathy

Core tip: There are striking ethnic differences in the prevalence of chronic kidney disease, including human immunodeficiency virus (HIV)-associated nephropathy (HIVAN), in people of African ancestry suggestive of genetic predisposition. The APOL1 G1 and G2 alleles

are more strongly associated with the risk for HIVAN than the previously reported MYH9 E1 risk haplotype in individuals of African ancestry. The high prevalence of HIVAN among individuals of African ancestry could be a result of high frequencies of APOL1 risk variants as well as the prevalence of HIV-1 subtypes and modifying factors or second hits underlying the pathogenesis of kidney disease.

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INTRODUCTION

Chronic kidney disease (CKD) is a major public health problem worldwide[1]. Mortality due to CKD nearly doubled worldwide between 1990 and 2010, and is now positioned at 18th as a cause of death in the Global Burden of Disease Study[2] and at 5th position in South Africa[3]. An estimated 3.2 million people were on renal replacement therapy by the end of 2013, approximately 2522000 people undergoing dialysis treatment (haemodialysis or peritoneal dialysis) and 678000 people living with renal transplants[4] and it is also estimated that CKD incidence grows by approximately 6% annually[4]. There are striking ethnic differences in the prevalence of CKD such that, in the United States, African Americans have the highest prevalence of CKD[5]. Diabetes and hypertension, which have been considered the two leading causes of CKD, together with differences in clinical, social-demographic or lifestyle factors, are insufficient to account satisfactorily for the excess risk of end stage renal disease (ESRD) in African Americans[6,7]. Africa, the second largest and the world's second most populous continent, is approximately 30.2 million square km^2 and composed of 54 countries[8] and more than 1.1 billion people as of 2013, accounting for 15% of the world's population[9]. It has been postulated that, by 2030, approximately 70% of the patients with ESRD will be living in low income countries such as those in sub-Saharan Africa where majority of people live on less than one dollar-a-day[10,11]. The increased burden of CKD in Africa could be as a result of various communicable diseases such as leishmaniasis, schistosomiasis, infectious glomerulonephritis and importantly, human immunodeficiency virus (HIV) infection superimposed on non-communicable diseases such as hypertension and diabetes mellitus. These factors have resulted in the increase in CKD; several studies have shown that there is a four-fold increase in CKD in HIV uninfected individuals, compared to 18-50 fold increase in CKD in HIV positive individuals of African descent[12,13].

HIV AND CHRONIC KIDNEY DISEASE

Acquired immune deficiency syndrome (AIDS)-associated nephropathy was originally reported in AIDS patients in the United States in 1984. Subsequently, asymptomatic HIV-infected individuals showed similar clinical and histological features, and the name was later changed to HIV-associated nephropathy (HIVAN)[14]. HIVAN is an irreversible form of CKD which is a pathologically distinct complication of HIV infection with considerable morbidity and mortality[15,16]. The odds of developing HIVAN have increased in recent years to fifty according to the United States Renal Data System[17]. There is a huge regional variation in the prevalence of HIV infection; globally, an estimated 35.3 million people were living with HIV as at 2012; in North Africa, approximately 260000 people are living with HIV; while in sub-Saharan Africa, which comprises two thirds of all people living with HIV, an estimated 25 million people are living with HIV[18]. CKD occurs in approximately 6.0%-48.5% of HIV positive patients in Africa[19]. About 24%-83% of these cases are classic HIVAN in South Africa[20-22]. HIVAN is a clinicopathological condition characterized by the presence of focal glomerulosclerosis with collapsing glomerulopathy and glomerular epithelial cell proliferation, together with microcystic tubular dilatation and interstitial inflammation[23]. Risk factors for HIVAN are older age, lower CD4 counts, high viral load, co-morbidity (such as diabetes mellitus, hypertension and hepatitis C co-infection)[24]. HIVAN is present predominantly in people of African ancestry, indicating a possible genetic predisposition[25,26]. Renal histology in HIV infected patients in South Africa is shown in Table 1. A 30-year review of 1848 renal biopsies by Vermeulen at Chris Hani Baragwanath Hospital in Johannesburg, South Africa found that focal segmental glomerulosclerosis (FSGS) comprised 29.6% of primary glomerulonephritis (GN), 24.4% of membranous GN, 23.8% of membranoproliferative GN, 10.3% of minimal change disease, 4.1% of mesangial proliferative GN and 2.7% of IgA nephritis; 19.7% of the biopsies were in HIV positive individuals (Vermeulen A, MMed, University of the Witwatersrand, 2014).

The mechanism by which HIV induces glomerular injury leading to the pathologic syndrome of HIVAN is not well understood, hence a number of theories have been postulated as to how HIV causes renal injury. First, a direct viral infection of podocytes, renal parenchymal cells, especially the visceral epithelial cells of the glomerulus, and the tubular epithelial cells and as a result, this elicits cytopathic effects including proliferation and apoptosis[27]. Secondly, HIV infects the lymphocytes and macrophages that enter the kidney, resulting in the release of inflammatory lymphokines or cytokines which promote injury and fibrosis[27]. In addition, there are studies that have demonstrated that *CCR5* and *CXCR4,* the two main HIV co-receptors, that mediate entry of HIV strains into susceptible

Table 1 Spectrum of renal histology in human immunodeficiency virus in South Africa

Histology	Durban[21]	JHB[20]	Cape Town[22]	JHB[1]
Biopsy numbers	**30**	**99**	**192**	**364**
Classic HIVAN (%)	83	27	24.4	32.7
FSGS		3	32.8	11.3
HIV Immune Complex Disease (%) (mostly with hepatitis B or C co-infection)		21	30.2	11.8
Mesangial proliferative		6		
Membranoproliferative (type I and III) (%)	7			2.7
Lupus-like (%)				4.4
IgA				
Membranous (%)	13.3	13	5.2	7.7
Exudative-proliferative				
HIV TTP/HUS (thrombotic microangiopathy)				
Various glomerulonephropathies (%) (heterogenous group with different aetiologies)	7	41	24	29.4
Minimal change (%)		2		3.3
Immunotactoid				
Amyloidosis				

[1]Adapted from Vermeulen Alda, MMed Research report, University of the Witwatersrand, 2014[83]. JHB: Johannesburg; HIVAN: Human immunodeficiency virus-associated nephropathy; TTP: Thrombotic thrombocytopaenic purpura; HUS: Haemolytic syndrome.

cells, are not expressed by intrinsic renal cells, but are expressed in circulating and infiltrating leukocytes at sites of tubulo-interstitial inflammation[28].

GENETIC PREDISPOSITION TO CHRONIC KIDNEY DISEASE IN PATIENTS OF AFRICAN ANCESTRY

Genetic variation plays an important role in susceptibility to common forms of disease such as diabetes, hypertension and kidney disease, with marked differences in the prevalence and sometimes the presentation, according to ethnicity and ancestry. African Americans have four times the incidence of ESRD when compared to Americans of European ancestry, supporting a causal role for genetics in the aetiology of kidney disease[12,13,29]. These observations led to the use of ancestry informative population variation data to help explain this disparity. In 2008, two groups published papers back to back in Nature Genetics, heralding the discovery of genetic association of markers in the non-muscle myosin heavy chain 9 (*MYH9*) gene on chromosome 22 with non-diabetic ESRD[30] and FSGS[31] in African Americans (Figure 1 and Table 2). Both groups used genome wide admixture mapping approaches in their analysis, showing that increased African ancestry was correlated with increased susceptibility.

The transatlantic slave trade in the 16th to 19th centuries brought in an estimated 12 million individuals from Africa (mainly West Africa) to enslavement in America[32,33] and this was the driver for the introduction of African genetic variation to America. As a consequence of this population relocation, admixture occurred with Native Americans (Amerindians) and Europeans leading to mixed genomic profiles among the group now referred to as African Americans. African Americans have, on average, about 80% African ancestry, although there are regional differences across the country[34]. Differences in allele frequencies of common and rare variants have occurred as a result of random genetic drift, selection and other forces over thousands of years of separation of the ancestral populations, with Europeans having separated roughly 40000 years ago from African populations[35]. Computational approaches take advantage of ancestry informative markers (AIMs), which are single nucleotide polymorphisms (SNPs) that show marked allele frequency differences among the ancestral populations to infer the global ancestry of individuals. Studies utilizing AIMs have shown that American populations with African, Hispanic and Caribbean origins are admixed with varying substantial components of African continental ancestry[36]. This effect of admixture helped in identifying genetic regions that affect one ancestral population and not others, which drive phenotypic associations[37] and can be measured using mapping by admixture linkage disequilibrium (MALD), which quantifies the degree of ancestry of each locus[37-39]. MALD studies were used to identify genomic regions where admixed African American patients with CKD had an excess of African genomic markers compared to unaffected individual controls[30,31]. These studies identified CKD susceptibility loci in African Americans localised to a specific genomic region on chromosome 22q12 that contains more than 21 genes, and proceeded to pinpoint the association with non-diabetic and hypertensive CKD, to markers in non-muscle myosin heavy chain 9 (*MYH9*) gene.

The *MYH9* gene was an excellent biologically plausible candidate as it has a direct link to the structure of podocytes since it codes for a 1960 amino acid protein (Myosin IIA) expressed in the podocytes and widely-distributed cellular motor protein that is essential for cytoskeleton rearrangement, cell motility, division, and

Table 2 Summary of the studies of *MYH9* and *APOL1* variants

Year	Population (ancestry)	Disease	Variant	Freq.	OR (95%CI)	Ref.
2008	African Americans	Hypertensive ESRD	*MYH9* E1	0.67	1.9 (1.25-2.87)	Kopp *et al*[31]
		HIVAN	*MYH9* E1	0.67	5.3 (2.40-12.90)	
		FSGS	*MYH9* E1	0.67	4.5 (2.92, 7.19)	
	European Americans	T2DM ESRD	*MYH9* E1	0.04	NS	
		FSGS	*MYH9* E1	0.04	9.7 (1.07, 463)	
2008	African Americans	Hypertensive ESRD	*MYH9* E1	0.3	2.1 (1.56, 2.74)	Kao *et al*[30]
		Non-diabetic ESRD	*MYH9* E1	0.3	2.2 (1.73, 2.73)	
		FSGS	*MYH9* E1	0.3	3.7 (2.11, 6.34)	
2009	African Americans	Hypertensive ESRD	*MYH9* E1	0.75	2.4 (NS)	Freedman *et al*[47]
		Non-diabetic ESRD	*MYH9* E1	0.76	2.5 (NS)	
2009	African Americans	T2DM ESRD	*MYH9* E1	0.67	1.4 (NS)	Freedman *et al*[44]
2010	African Americans	Non-diabetic ESRD	*MYH9* E1	NS	2.0 (1.37, 2.92)	Behar *et al*[46]
	Hispanic Americans	Non-diabetic ESRD	*MYH9* E1	NS	3.7 (1.67, 8.20)	
2010	American Indians	Kidney dysfunction	*MYH9* SNPs	0.43	1.04 (0.79, 1.36)	Franceschini *et al*[43] Strong Heart Family Study
2010	African Americans	Non-diabetic ESRD	*APOL1* G1	0.46	4.86 (2.35, 10.06)	Tzur *et al*[29]
	Hispanic Americans	Non-diabetic ESRD	*APOL1* G1	0	15.48 (4.00, 60.00)	
2010	African Americans	Hypertensive ESRD	*APOL1* G1/G2	0.41/0.21	7.3 (5.60, 9.50)	Genovese *et al*[13]
		FSGS	*APOL1* G1/G2	0.47/0.25	10.5 (6.0, 18.4)	
2011	African Americans	HIVAN	*APOL1* G1/G2	0.54/0.28	29.2 (13.10, 68.50)	Kopp *et al*[12]
		FSGS	*APOL1* G1/G2	0.55/0.25	16.9 (11.00, 26.50)	
2014	South African blacks	HIVAN	*MYH9* E1	0.83	2.10 (0.07-60.99)	Kasembeli *et al* (Unpublished observations)
			APOL1 G1/G2	0.56/0.34	89.10 (17.68, 911.72)	

NS: Not stated; SNPs: Single nucleotide polymorphisms; OR: Odds ratio; Freq: Frequencies; HIVAN: Human immunodeficiency virus-associated nephropathy; FSGS: Focal segmental glomerulosclerosis; T2DM: Type 2 diabetes mellitus; ESRD: End stage renal disease; APOL1: Apolipoprotein L1; MYH9: Non-muscle myosin heavy chain 9.

cell-cell adhesion[40]. As a result of these observations, researchers concluded that *MYH9* variants that are associated with susceptibility to CKD likely play a causal role in disease pathology[41]. The high frequency of the *MYH9* associated haplotypes in African populations led to speculations of selection in Africa (Figure 2)[42]. The ethnic specificity of the association was explored with different phenotypes and different populations (Table 2) and subsequent studies provided evidence for a contribution of *MYH9* variants in early stages of CKD as well as diabetic and hypertensive-related CKD in both African Americans and Europeans, but not in Native Americans[43-45]. However, biopsy-proven forms of CKD were lacking in some of these studies and therefore, as in the case of diabetic and hypertensive nephropathies, researchers suggested that the association with *MYH9* could also reflect the presence of non-diabetic and non-hypertensive CKD. This hypothesis was supported by association with *MYH9* SNPs that were strongly associated with these types of CKD[46].

The *MYH9* SNPs with the strongest associations were categorised into three haplotype groups termed as E, S and F[42,46] and E1 was defined as the risk haplotype (rs4821480, rs2032487, rs4821481 and rs3752462), being highly associated with CKD in individuals of African descent. E1 was then further found to be highly distributed in Africa as compared to other regions of the world (Figure 2)[42]. The *MYH9* E1 haplotype explained nearly the entire excess burden of major forms of CKD in African Americans with attributable risks of 100% and 70% for HIVAN and FSGS, respectively, and a significant percentage for hypertensive nephrosclerosis risk[31,40,47]. However, despite major scientific efforts, including *MYH9* re-sequencing experiments and detailed-intense genotyping, no mutations with a clear predicted functional effect could be identified that would impact kidney function and the field began to shift toward exploring neighbouring genes on chromosome 22.

Two research groups re-analyzed the chromosome 22q12 genomic region using data from International HapMap and 1000 Genomes Projects[29,48]. The data from these studies played a vital role in the discovery of candidate SNPs in the neighbouring apolipoprotein L1 (*APOL1*) gene, approximately 20 kb downstream from the 3' end of *MYH9,* that were statistically powered to explain an increased risk of CKD in individuals of African ancestry[13,29]. The studies yielded 7479 SNPs, four of which were non-synonymous mutations in the coding region of the genes in high linkage disequilibrium with the *MYH9* E1 risk haplotype. Two of these (rs73885319 and rs60910145) were missense mutations in the last exon (exon 7) of the *APOL1* gene, which result in amino acid substitutions: Ser342Gly and Ile384Met. These two missense mutations are referred to as the G1 alleles since they were in almost complete linkage disequilibrium (r^2 = 1.0) with each other, and are both highly associated with CKD susceptibility. Another SNP, rs71785313, was also found in exon 7 of *APOL1* and represents a six base pair deletion resulting in loss of two amino acids (Asn388-Tyr389del), and is referred to as the G2 allele. These three codon-changing variants in the *APOL1* gene, encoding apolipoprotein L1, were found to be in strong association with HIVAN odds

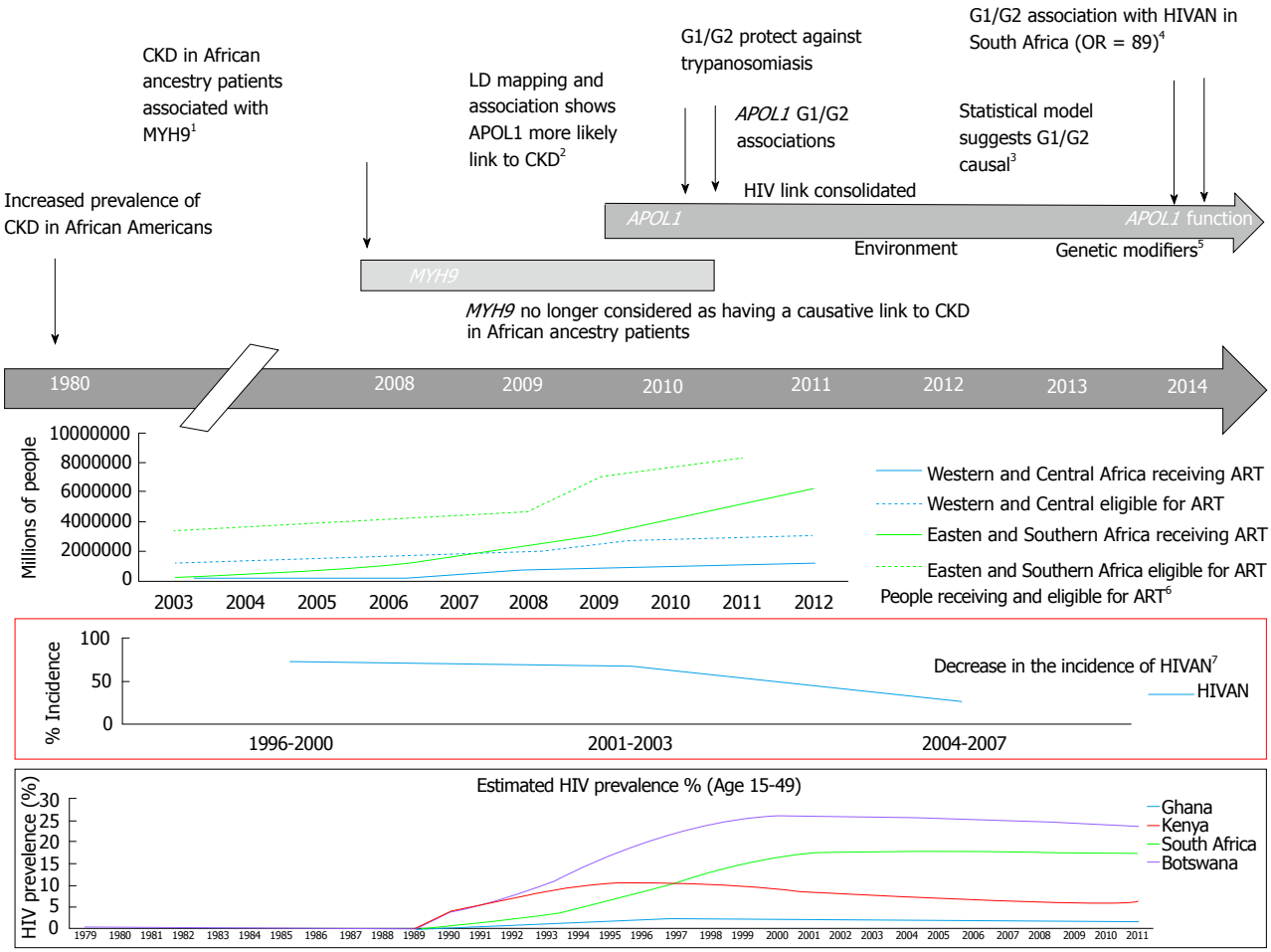

Figure 1 Historical timeline reflecting the discovery of genetic association to chronic kidney disease in populations with African ancestry. [1]Adapted from Kopp *et al*[31] and Kao *et al*[30]; [2]Adapted from Freedman *et al*[49], Genovese *et al*[13], Tzur *et al*[29]; [3]Adapted from Genovese *et al*[84]; [4]Adapted from Kasembeli *et al* (2014 unpublished observations); [5]Adapted from Freedman *et al*[64]; [6]Adapted from UNAIDS report on global AIDS epidemic[18]; [7]Adapted from USRDS 2012 Annual Data Report. APOL1: Apolipoprotein L1; MYH9: Non-muscle myosin heavy chain 9; ART: Antiretroviral therapy; CKD: Chronic kidney disease.

ratio (OR = 29), FSGS (OR = 17) and ESRD (OR = 7) in African Americans, for homozygotes or compound heterozygotes carrying two risk alleles[12,13,29]. They were also absent in individuals of European ancestry but common in African populations. The G1 and G2 alleles were strongly associated with the risk for CKD than the previously examined *MYH9* E1 risk haplotype in a sample of individuals of African ancestry. They are in perfect negative linkage disequilibrium, never occurring on the same parental chromosome, suggesting that these variants arose independently and due to their proximity and high linkage disequilibrium, they have remained mutually exclusive in almost all haplotypes observed[12,13]. The historical timeline reflecting the discovery of genetic association to CKD in populations with African ancestry is shown in Figure 1. The link with increased susceptibility to kidney dysfunction in the presence of HIV infection[15,16] is now well established and the association of *APOL1* risk alleles with HIVAN is of particular concern in sub-Saharan Africa, where the risk allele frequency is high (Figure 2). *APOL1* association with CKD and the postulated mechanism of action and the functional role of *APOL1* in kidney disease are explored further in the next sections.

APOL1 ASSOCIATION WITH CKD IN INDIVIDUALS OF AFRICAN ANCESTRY

The coding variants (G1 and G2) are suggested to be causally related to CKD and provide an explanation for selection of *APOL1*-associated CKD risk polymorphisms as a protective measure against Trypanosomiasis, an infectious disease that was common in Africa. A study by Genovese *et al*[13], (2010), comparing 205 African Americans with biopsy-proven FSGS with 180 African Americans without kidney disease as controls was performed, using SNPs from the 1000 Genomes Project belonging to Yoruba as proxies to the African American population. These SNPs revealed evidence of a strong association within a 10 kb region in the exon 7 of the *APOL1* gene. The strong signal was found to be at non-synonymous coding variants, rs73885319 (S342G) and rs60910145 (I384M) which were in perfect linkage disequilibrium (r^2 = 1.0). The frequency of these variants was 52% in patients and 18% in

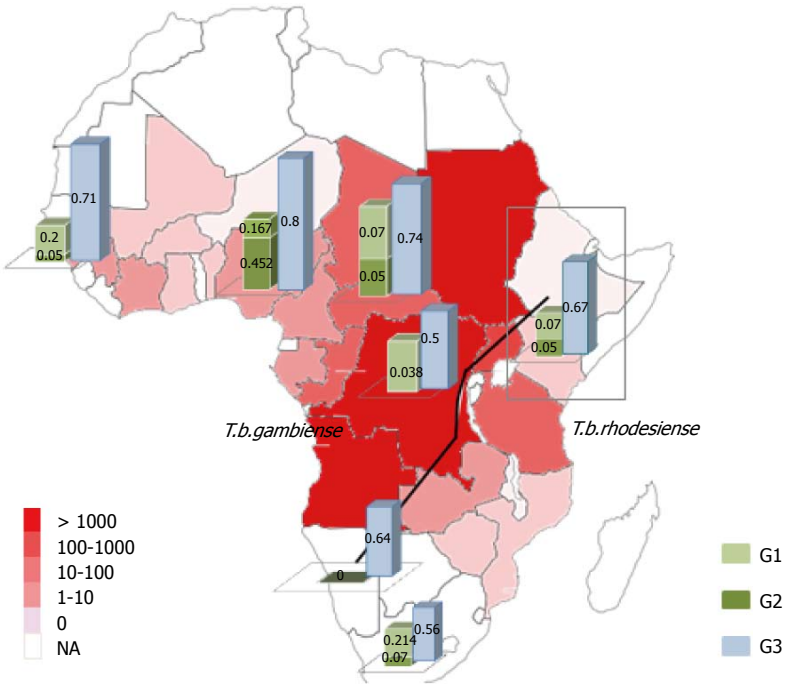

Figure 2 Distribution of Human African Trypanosomiasis (T.b *gambiense* and T.b *rhodesiense*), MYH9 E1 and APOL1 G1 and G2 risk alleles in Africa[12,42]. The frequency of distribution of *APOL1* risk variants in Africa are represented by bar charts and overlap the areas distribution of Human African Trypanosomiasis. The numbers reflect the reported cases of Trypanosomiasis from the WHO, 2010. T.b: *Trypanosoma brucei*.

controls. They further controlled for the effects of these two variants and found a second strong *APOL1* signal, 12 base pairs from I384M. This signal is a 6-base pair deletion represented by rs71785313 which removes two amino acid residues (Asparagine-N and Tyrosine-Y). The frequency of this variant was 23% in patients and 15% in controls. The odds ratio (OR) of association for carrying at least one risk (G1-G2) allele was 10.5 (95%CI: 6.0-18.4). Controlling for both G1 and G2 did not result in significant association with *MYH9.* However, controlling for *MYH9* variants maintained significant *APOL1* signal at G1 and G2. Kopp *et al*[12], (2011), in a larger FSGS and HIVAN cohort confirmed the association but this time, a greater association was observed in HIVAN (OR = 29.2, 95%CI: 13.1-68.5, $P = 6 \times 10^{-22}$) compared to FSGS (OR = 16.9, 95%CI: 11.0 to 26.5, $P = 1.3 \times 10^{-48}$). The authors reported that 50% of African Americans with two *APOL1* risk alleles would develop HIVAN if not on antiretroviral therapy. A study in an indigenous South African black cohort showed an independent high association with HIVAN susceptibility for these G1 and G2 variants of 89-fold, 95%CI: 17.68-911.72, $P = 1.2 \times 10^{-14}$ (Kasembeli *et al*, unpublished observations). In all these studies, there was strong evidence for a contribution of *APOL1* variants to CKD (Figure 1 and Table 2).

The observed mode of inheritance of the *APOL1* risk variants was fully recessive in both FSGS and HIVAN cohorts. However, there have been instances where mild dominant effects (OR of 1.26 for one risk allele and OR of 7.3 for 2 risk alleles) have been observed in larger cohorts of hypertensive-associated CKD and geographically matched control subjects[13]. We therefore cannot fully exclude this mild dominant inheritance because this could be explained by yet "undiscovered variants" or by sporadic mutations that might occur in patients with recessive model. Furthermore, there could be a possibility of additional rare variants in *APOL1, MYH9* or other neighbouring genes that could be involved in CKD susceptibility since extended linkage disequilibrium exists in this region as a result of selective pressure[49].

POSITIVE SELECTION FOR *APOL1*-ASSOCIATED CKD RISK VARIANTS AS A RESULT OF TRYPANOSOMIASIS EPIDEMIC IN AFRICA

It has been shown that harbouring of *APOL1* risk variants protects against Trypanosomiasis disease [Human African Trypanosomiasis (H.A.T)], otherwise known as sleeping sickness, that was epidemic in Africa many years ago and still affects millions of Africans today. This effect explains the high frequencies of these variants in the general African American and indigenous African population (Figure 2)[12,42]. The *APOL1* G1 and G2 alleles show distinct distributions among various African and African-derived populations and evidence shows these mutations to be maintained in these populations. In Yoruba from Nigeria in West Africa, the frequency is greater than 45% for G1 (Figure 2) while in African Americans the G1 frequency is approximately 20%. The battle between host and pathogen (*Trypanosoma* species), resulted in development of *APOL1* mutations (G1 and G2) that provided positive selective advantage to carriers at the expense of increased risk for CKD (Figure 3).

There are three main *Trypanosoma* species; *Trypanosoma brucei brucei, Trypanosoma brucei rhodesiense* and *Trypanosoma brucei gambiense*.

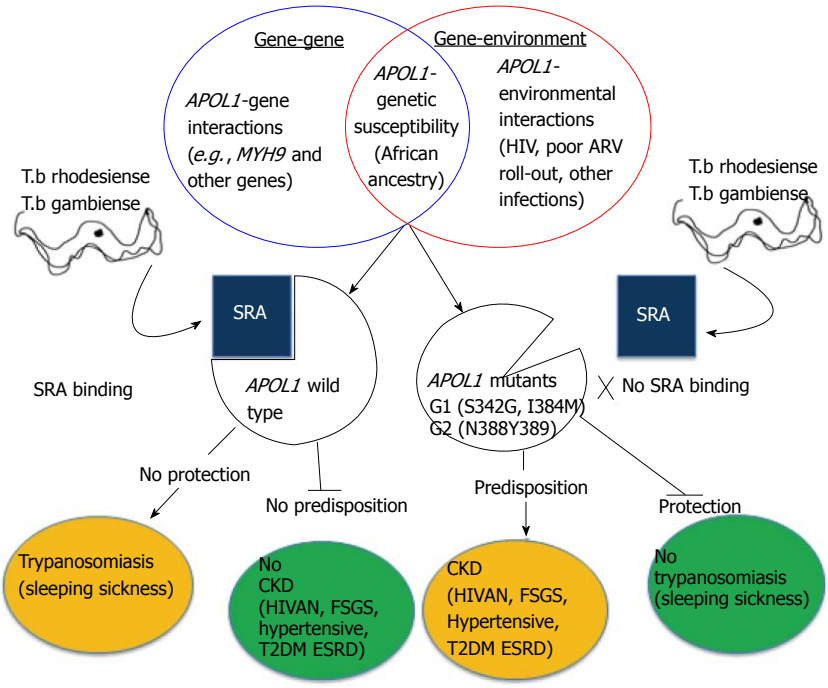

Figure 3 Gene-Gene, Gene-Environment steering contribution to APOL1 associated CKD and the positive selection of APOL1 associated CKD variants as a result of Trypanosomiasis. SRA: Serum resistant associated protein; HIV: Human immunodeficiency virus; T.b: Trypanosoma brucei; APOL1: Apolipoprotein L1; MYH9: Non-muscle myosin heavy chain 9; HIVAN: Human immunodeficiency virus-associated nephropathy; FSGS: Focal segmental glomerulosclerosis; T2DM: Type 2 diabetes mellitus; ESRD: End stage renal disease; CKD: Chronic kidney disease.

Trypanosoma brucei brucei is unable to infect humans because of the complex, trypanolytic factor (TLF) comprising of apolipoprotein L1, high density lipoprotein (HDL) particles, haptoglobin-related protein and apoliprotein A1 that is present in the human serum. This confers innate protection against *Trypanosoma brucei brucei.* However, both *Trypanosoma brucei rhodesiense* and *Trypanosoma brucei gambiense* have evolved a mechanism to evade lysis by the TLF leading to infection, hence sleeping sickness (Figure 3)[50,51]. Apolipoprotein L1, a protein product of *APOL1* gene is usually part of TLF circulating in the blood. The *APOL1* gene is a member of the *APOL* gene family which is composed of six genes in humans (*APOL1, APOL2, APOL3, APOL4, APOL5* and *APOL6*), grouped within 619kb on chromosome 22[52]. This protein has five functional and structural domains: a secretory domain, pore forming domain, B-cell lymphoma 2 homology domain 3, membrane addressing domain, leucine zipper domain and serum resistant-associated interacting domain (SRA), listed from the N-terminal to C-terminal respectively.

The trypanolytic function of apolipoprotein L1 is the most widely studied function of apolipoprotein L1[13,53]. The secretory domain allows it to be expressed as a circulating protein, which makes it the only circulating *APOL* protein[53-56]. *Trypanosoma brucei rhodesiense* and *Trypanosoma brucei gambiense* evade the TLF lysis by expressing SRA protein that binds to the C-terminus domain of the apolipoprotein L1, in the process neutralizing its lytic activity[57,58]. However, *APOL1* variants G1 and G2, powerfully associated with CKD arose to modify the C-terminus SRA binding site of the *APOL1* gene resulting in a mutated apolipoprotein L1 that evades neutralization by the *Trypanosoma* SRA protein[58]. By so doing, apolipoprotein L1 exercised its trypanolytic activity, conferring an adaptive advantage in the endemic regions of Africa (Figure 3). This explains the distribution of G1 and G2 risk variants in Africa.

Genovese *et al*[13] reported that both G1 and G2 variants restored the lytic activity of human serum and this provides the selective advantage to carriers of two *APOL1* risk variants against sleeping sickness. These findings corroborated the evidence of the recent evolution of *APOL1* which occurred in the last 10000 years and also suggesting that these variants were selected for within Africa because they conferred protection against lethal trypanosomiasis while at the same time increasing susceptibility to CKD (Figure 3)[49]. A more recent study in a South African black population (Kasembeli *et al*, unpublished data, 2014) has found the odds to have almost doubled. The prevalence of HIVAN in Africa is variable, 24%-83% in South Africa, while in the United States, it is highest in the African American population (15.5%). This is eight-fold greater than that of HIV-infected European Americans[59]. This high prevalence of HIVAN among individuals of African ancestry could be, not only as a result of high frequencies of *APOL1* risk variants, but also the prevalence of HIV-1 subtypes circulating in Africa. For instance, HIV-1 subtype C is highly virulent, accounting for approximately 50% of all HIV infections worldwide and 98% of HIV infections in South, West and East Africa, with corresponding higher viral loads[60,61]. Another more important reason could be because sub-Saharan African countries are resource limited, and therefore roll-out of antiretroviral therapy (ART) may have been delayed, giving more room for the development of HIVAN among individuals carrying two *APOL1* risk variants. An effective roll-out of ART has been shown to reduce the occurrence of HIVAN[22,62,63]; Figure 1. Therefore, there is need for HIV screening,

surveillance, and strict implementation of World Health Organization (WHO) recommendations for ART initiation to reduce the burden of HIVAN and other forms of HIV-related CKD in Africa. At present, WHO ART guidelines 2013 for the treatment of HIV infection in Africa suggest that ART be instituted for individuals with WHO clinical stage 3 and 4 disease and in all HIV positive individuals with CD4 counts < 500 cells/μL.

GENE-ENVIRONMENTAL MODIFIERS OF *APOL1* SUSCEPTIBILITY

APOL1-environmental interactions play a vital role in CKD susceptibility in individuals of African ancestry (Figure 3). These could be social demographic status, lifestyle or presence of other communicable diseases and most importantly, HIV infection coupled with poor ART roll-out. Environmental exposure to HIV was initially thought to trigger HIVAN. However, observations in African American family studies showed that relatives of HIVAN patients, in the absence of HIV infection, suffered ESRD due to other aetiologies[64]. This illustrates that there could be other environmental factors that drive the process. As described, patients with HIVAN harbouring two *APOL1* risk alleles that are untreated or undertreated for HIV infection will suffer rapid progression to ESRD[12]. In striking contrast, HIV patients without the *APOL1* risk genotype are protected from HIVAN even when HIV infection is not properly controlled. This effect is well illustrated in the Ethiopian population who appear to be protected from HIVAN since their genomes lack the *APOL1* risk variants. A study by Behar *et al*[65], in HIV infected individuals of Ethiopian origin reported complete absence of HIVAN. This led to the emphasis of skewed ethnic distribution, inter-individual variability and/or familial aggregation of HIVAN suggesting that host genetic susceptibility plays a major contributing factor. This study genotyped 676 African individuals from 12 populations, including 304 Ethiopians, for mutations in the *MYH9* and *APOL1* risk clusters. The frequency of the G1 and G2 *APOL1* risk variants was zero[65]. However, there was an increasing trend in frequency of the risk variants proceeding towards the west and south in Africa (Figure 2). This led researchers to conclude that the risk of developing HIVAN is not a African-wide problem but rather restricted to Western, Central and Southern Africa, and absent in regions of the North and North-East parts of Africa including Ethiopia. Since sleeping sickness was not an epidemic in Southern Africa, a possible explanation for the increase in prevalence of *APOL1* risk variants could be the result of migration of the bantu-speaking populations from West Africa and East Africa[66,67].

In the United States there has been a steady decline in the incidence of HIVAN with the introduction of HAART, in spite of stable frequencies of the risk variants[68]. Risk factors for progression to ESRD in HIVAN are severity of renal dysfunction, percentage of sclerotic glomeruli[25,69], lack of viral suppression[26,70], 2 *APOL1* risk alleles[63,71], while use of renin angiotensin system blockers were reported to be protective[25]. HIV-infected individuals with non-HIVAN pathology and two *APOL1* risk alleles had an almost 3-fold risk of ESRD, in spite of effective ART-suppression of viral load and use of renin-angiotensin aldosterone blockers; baseline kidney function was the strongest predictor of progression to ESRD in this study[71]. Investigators reviewing the African American study on Kidney Disease and Hypertension (AASK) and The Chronic Renal Insufficiency Cohort (CRIC) found that *APOL1* risk variants in black patients were associated with higher rates of ESRD and progression of CKD[72].

Thus HIV is considered a risk factor for HIVAN when presented with the appropriate genetic susceptibility. Either genetic risk or viral infections alone do not cause the kidney disease. Instead it is the geneenvironment interaction that is fundamental for the pathogenesis. The rapidly changing natural history of *APOL1*-associated HIVAN provides further support that HIV is an environmental risk factor[68]. Additional viral environmental modifiers have been proposed. The John Cunningham (JC) polyoma virus has been shown to maintain a reservoir in the uroepithelium of the kidney after infection and has been proposed to interact with the genetic risk posed by *APOL1* variants[73]. Divers *et al*[73] studied the relationship between the JC virus and genetic risk for kidney disease hypothesising that the presence of the *APOL1* risk variants may predispose individuals to JC infection and that this second hit may act as an additional environmental factor increasing kidney disease risk. However, paradoxically, the reverse scenario was observed where the presence of the high risk *APOL1* variants in the presence of JC virus resulted in less kidney disease. The JC virus in the kidney was postulated to either protect against other nephropathic viruses or alter cellular function as protection against other sources of glomerular injury.

GENE-GENE INTERACTIONS AS MODIFIERS OF *APOL1*-ASSOCIATED NEPHROPATHY

Whilst nephrology research in African ancestry populations has been hampered by the lack of large genome wide- association studies, a number of gene-gene interaction studies have been conducted on pooled GWAS data in non-diabetic ESRD in African Americans and non-nephropathy controls. Results of a gene-gene interaction analysis identified several SNPs that interacted with *APOL1* risk variants (Figure 3)[74]. *MYH9* has been shown to be one of the genes linked to *APOL1* to cause CKD susceptibility. Other studies have also shown a possibility of other genes interaction with *APOL1* gene. In a replication study, eleven SNPs were validated and three genes, podocin (NPHS2; rs16854341); serologically defined colon cancer

antigen 8 (SDCCAG8; rs2802723) and SNP "near bone morphogenetic protein 4" (BMP4; rs8014363) were significant. These interactions were quantified and all show effects on *APOL1* association[75,76]. These three genes show expression in podocytes and are linked to renal disease characterised by FSGS. It has thus been postulated that they play a role in inducing podocyturia and glomerular damage.

APOL1-ASSOCIATED CKD: THE FUTURE

Since there is evidence of *APOL1* association with non-diabetic forms of CKD and the role of selection in the increase in frequencies of the risk variants, it is necessary to move beyond the statistical tests of association to molecular cellular characterization to evaluate the effects of these risk variants in CKD. It has been postulated that cellular and physiologic activities of apolipoprotein L1 include involvement in autophagic and apoptosis pathways[52,77-79]. There is a general agreement that *APOL1* expression occurs in podocytes[80]. But whether there is apolipoprotein L1 expression in the tubular cells, glomerular endothelial cells, and the tunica intima and media of the renal blood vessels is uncertain. Currently, there is no definitive mechanism by which *APOL1* variants cause kidney injury but several possibilities have been proposed. Firstly, apolipoprotein L1 isoform expressed in the kidney cells may be retained in the cells and cause cell destruction *via* the apoptotic pathway since they share structural and functional similarities with proteins from the BcL2 family[77,78]. Secondly, *APOL1* as part of TLF, is directed to lysosomes to induce programmed cell death *via* the autophagic response[13,56,81]. Thirdly, circulating apolipoprotein L1 may also be important in the pathogenesis of CKD since the presence of G1 and G2 could lead to dysfunctional HDL particles leading to inflammation of vascular endothelial cells, with arteriolar nephrosclerosis[49]. There is a proven race specific relationship between *APOL1* genotype and HDL cholesterol concentration and kidney function[82]. In Han Chinese and European American populations, there was a higher HDL level in association with higher eGFR. The inverse association was observed in West Africans and African Americans. However, a significant effect was observed only in African Americans with *APOL1* risk variants (but not in West Africans). These observations have led to the view that the mechanism underlying *APOL1* nephropathy most likely involves HDL cholesterol. In addition, circulating *APOL1*, may be available for uptake by podocytes after passage across the glomerular filtration barrier and exercising their effect on the podocytes. Future studies should define the role of *APOL1* in the pathogenesis of kidney disease.

LESSONS LEARNED FROM CKD POPULATION GENETICS

The remarkable advances in molecular genetics have enabled researchers to unravel the underlying genetic susceptibility to kidney disease in African Ancestry populations. Identification of region of 22q12.3 using MALD studies and identification of *APOL1* risk variants have raised the possibility of a personalised approach to treat several forms of kidney disease that are prevalent in African populations. As regards HIVAN, the priority for the African continent should be targeting of the modifying trigger of the disease, HIV infection, through effective treatment and prevention campaigns. Greater advances in understanding the mechanisms underlying *APOL1* pathogenesis, the identification of modifiable environmental factors and interacting genes offer the promise of novel preventive, prognostic and therapeutic measures to treat *APOL1* associated forms of kidney disease in the genetically susceptible and therefore vulnerable African descent individual.

REFERENCES

1 **United Nations General Assembly.** Political declaration of the high-level meeting of the general assembly on the prevention and control of non-communicable diseases. Cited 2014-06-13. Available from URL: http://www.who.int/nmh/events/un_ncd_summit2011/political_declaration_en.pdf

2 **Lozano R,** Naghavi M, Foreman K, Lim S, Shibuya K, Aboyans V, Abraham J, Adair T, Aggarwal R, Ahn SY, Alvarado M, Anderson HR, Anderson LM, Andrews KG, Atkinson C, Baddour LM, Barker-Collo S, Bartels DH, Bell ML, Benjamin EJ, Bennett D, Bhalla K, Bikbov B, Bin Abdulhak A, Birbeck G, Blyth F, Bolliger I, Boufous S, Bucello C, Burch M, Burney P, Carapetis J, Chen H, Chou D, Chugh SS, Coffeng LE, Colan SD, Colquhoun S, Colson KE, Condon J, Connor MD, Cooper LT, Corriere M, Cortinovis M, de Vaccaro KC, Couser W, Cowie BC, Criqui MH, Cross M, Dabhadkar KC, Dahodwala N, De Leo D, Degenhardt L, Delossantos A, Denenberg J, Des Jarlais DC, Dharmaratne SD, Dorsey ER, Driscoll T, Duber H, Ebel B, Erwin PJ, Espindola P, Ezzati M, Feigin V, Flaxman AD, Forouzanfar MH, Fowkes FG, Franklin R, Fransen M, Freeman MK, Gabriel SE, Gakidou E, Gaspari F, Gillum RF, Gonzalez-Medina D, Halasa YA, Haring D, Harrison JE, Havmoeller R, Hay RJ, Hoen B, Hotez PJ, Hoy D, Jacobsen KH, James SL, Jasrasaria R, Jayaraman S, Johns N, Karthikeyan G, Kassebaum N, Keren A, Khoo JP, Knowlton LM, Kobusingye O, Koranteng A, Krishnamurthi R, Lipnick M, Lipshultz SE, Ohno SL, Mabweijano J, MacIntyre MF, Mallinger L, March L, Marks GB, Marks R, Matsumori A, Matzopoulos R, Mayosi BM, McAnulty JH, McDermott MM, McGrath J, Mensah GA, Merriman TR, Michaud C, Miller M, Miller TR, Mock C, Mocumbi AO, Mokdad AA, Moran A, Mulholland K, Nair MN, Naldi L, Narayan KM, Nasseri K, Norman P, O'Donnell M, Omer SB, Ortblad K, Osborne R, Ozgediz D, Pahari B, Pandian JD, Rivero AP, Padilla RP, Perez-Ruiz F, Perico N, Phillips D, Pierce K, Pope CA 3rd, Porrini E, Pourmalek F, Raju M, Ranganathan D, Rehm JT, Rein DB, Remuzzi G, Rivara FP, Roberts T, De León FR, Rosenfeld LC, Rushton L, Sacco RL, Salomon JA, Sampson U, Sanman E, Schwebel DC, Segui-Gomez M, Shepard DS, Singh D, Singleton J, Sliwa K, Smith E, Steer A, Taylor JA, Thomas B, Tleyjeh IM, Towbin JA, Truelsen T, Undurraga EA, Venketasubramanian N, Vijayakumar L, Vos T, Wagner GR, Wang M, Wang W, Watt K, Weinstock MA, Weintraub R, Wilkinson JD, Woolf AD, Wulf S, Yeh PH, Yip P, Zabetian A, Zheng ZJ, Lopez AD, Murray CJ, AlMazroa MA, Memish ZA. Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the Global Burden of Disease Study 2010. *Lancet* 2012; **380**: 2095-2128 [PMID: 23245604 DOI: 10.1016/S0140-6736(12)61728-0]

3 **Mayosi BM**, Flisher AJ, Lalloo UG, Sitas F, Tollman SM, Bradshaw D. The burden of non-communicable diseases in South Africa.

Lancet 2009; **374**: 934-947 [PMID: 19709736 DOI: 10.1016/S0140-6736(09)61087-4]
4 ESRD Patients in 2013: A Global Perspective. Cited 2014-07-07. Available from: URL: http: //www.vision-fmc.com/files/ESRD_Patients_in_2013.pdf
5 **Rostand SG**, Kirk KA, Rutsky EA, Pate BA. Racial differences in the incidence of treatment for end-stage renal disease. *N Engl J Med* 1982; **306**: 1276-1279 [PMID: 7040967 DOI: 10.1056/NEJM198205273062106]
6 **Shlush LI**, Bercovici S, Wasser WG, Yudkovsky G, Templeton A, Geiger D, Skorecki K. Admixture mapping of end stage kidney disease genetic susceptibility using estimated mutual information ancestry informative markers. *BMC Med Genomics* 2010; **3**: 47 [PMID: 20955568 DOI: 10.1186/1755-8794-3-47]
7 **Klag MJ**, Whelton PK, Randall BL, Neaton JD, Brancati FL, Stamler J. End-stage renal disease in African-American and white men. 16-year MRFIT findings. *JAMA* 1997; **277**: 1293-1298 [PMID: 9109467 DOI: 10.1001/jama.1997.03540400043029]
8 **Sayre AP.** Africa: The Seven Continents. Cited 2014-05-20. Available from: URL: http: //www.amazon.com/Africa-Seven-Continents-April-Pulley/dp/0761313672
9 World Population Data Sheet. Cited 2014-06-13. Available from: URL: http: //www.prb.org/Publications/Datasheets/2013/2013-world-population-data-sheet/world-map.aspx#map/world/population/2013
10 **Population Division of the Department of Economic and Social Affairs of the United Nations Secretariat.** World population prospects: the 2007 revision and world urbanization prospects. Cited 2014-06-13. Available from: URL: http://www.un.org/en/development/desa/index.html
11 **Barsoum RS.** Epidemiology of ESRD: a world-wide perspective. In: El Nahas M, editor. Kidney diseases in the developing world and ethnic minorities. London, UK: Taylor & Francis, 2005: 1-13 [DOI: 10.1201/b14128]
12 **Kopp JB**, Nelson GW, Sampath K, Johnson RC, Genovese G, An P, Friedman D, Briggs W, Dart R, Korbet S, Mokrzycki MH, Kimmel PL, Limou S, Ahuja TS, Berns JS, Fryc J, Simon EE, Smith MC, Trachtman H, Michel DM, Schelling JR, Vlahov D, Pollak M, Winkler CA. APOL1 genetic variants in focal segmental glomerulosclerosis and HIV-associated nephropathy. *J Am Soc Nephrol* 2011; **22**: 2129-2137 [PMID: 21997394 DOI: 10.1681/ASN.2011040388]
13 **Genovese G**, Friedman DJ, Ross MD, Lecordier L, Uzureau P, Freedman BI, Bowden DW, Langefeld CD, Oleksyk TK, Uscinski Knob AL, Bernhardy AJ, Hicks PJ, Nelson GW, Vanhollebeke B, Winkler CA, Kopp JB, Pays E, Pollak MR. Association of trypanolytic ApoL1 variants with kidney disease in African Americans. *Science* 2010; **329**: 841-845 [PMID: 20647424 DOI: 10.1126/science.1193032]
14 **Rao TK**, Filippone EJ, Nicastri AD, Landesman SH, Frank E, Chen CK, Friedman EA. Associated focal and segmental glomerulosclerosis in the acquired immunodeficiency syndrome. *N Engl J Med* 1984; **310**: 669-673 [PMID: 6700641 DOI: 10.1056/NEJM198403153101101]
15 **Estrella MM,** Fine DM, Atta MG. Recent developments in HIV-related kidney disease. *HIV Ther* 2010; **4**: 589-603 [PMID: 21331321 DOI: 10.2217/hiv.10.42]
16 **Estrella MM**, Parekh RS, Abraham A, Astor BC, Szczech LA, Anastos K, Dehovitz JA, Merenstein DJ, Pearce CL, Tien PC, Cohen MH, Gange SJ. The impact of kidney function at highly active antiretroviral therapy initiation on mortality in HIV-infected women. *J Acquir Immune Defic Syndr* 2010; **55**: 217-220 [PMID: 20581688 DOI: 10.1097/QAI.0b013e3181e674f4]
17 USRDS 2013 Annual Data Report: Atlas of Chronic Kidney Disease and End-Stage Renal Disease in the United States. *American Journal of Kidney Disease* 2014; **63** Suppl: e1-e478
18 UNAIDS report on the global AIDS epidemic 2013. Cited 2014-05-20. Available from: URL: http: //www.unaids.org/en/media/unaids/contentassets/documents/epidemiology/2013/gr2013/UNAIDS_Global_Report_2013_en.pdf
19 **Fabian J**, Naicker S. HIV and kidney disease in sub-Saharan Africa. *Nat Rev Nephrol* 2009; **5**: 591-598 [PMID: 19776781 DOI: 10.1038/nrneph.2009.141]
20 **Gerntholtz TE**, Goetsch SJ, Katz I. HIV-related nephropathy: a South African perspective. *Kidney Int* 2006; **69**: 1885-1891 [PMID: 16625149 DOI: 10.1038/sj.ki.5000351]
21 **Han TM**, Naicker S, Ramdial PK, Assounga AG. A cross-sectional study of HIV-seropositive patients with varying degrees of proteinuria in South Africa. *Kidney Int* 2006; **69**: 2243-2250 [PMID: 16672914 DOI: 10.1038/sj.ki.5000339]
22 **Wearne N**, Swanepoel CR, Boulle A, Duffield MS, Rayner BL. The spectrum of renal histologies seen in HIV with outcomes, prognostic indicators and clinical correlations. *Nephrol Dial Transplant* 2012; **27**: 4109-4118 [PMID: 22200584 DOI: 10.1093/ndt/gfr702]
23 **D'Agati V**, Appel GB. HIV infection and the kidney. *J Am Soc Nephrol* 1997; **8**: 138-152 [PMID: 9013459]
24 **Ray PE**. HIV-associated nephropathy: a diagnosis in evolution. *Nephrol Dial Transplant* 2012; **27**: 3969-3972 [PMID: 22584790 DOI: 10.1093/ndt/gfs114]
25 **Bigé N**, Lanternier F, Viard JP, Kamgang P, Daugas E, Elie C, Jidar K, Walker-Combrouze F, Peraldi MN, Isnard-Bagnis C, Servais A, Lortholary O, Noël LH, Bollée G. Presentation of HIV-associated nephropathy and outcome in HAART-treated patients. *Nephrol Dial Transplant* 2012; **27**: 1114-1121 [PMID: 21745806 DOI: 10.1093/ndt/gfr376]
26 **Szczech LA**, Gupta SK, Habash R, Guasch A, Kalaylian R, Appel R, Fields TA, Svetkey LP, Flanagan KH, Klotman PE, Winston JA. The clinical epidemiology and course of the spectrum of renal diseases associated with HIV infection. *Kidney Int* 2004; **66**: 1145-1152 [PMID: 15327410 DOI: 10.1111/j.1523-1755.2004.00865.x]
27 **Ray PE**, Liu XH, Henry D, Dye L, Xu L, Orenstein JM, Schuztbank TE. Infection of human primary renal epithelial cells with HIV-1 from children with HIV-associated nephropathy. *Kidney Int* 1998; **53**: 1217-1229 [PMID: 9573536 DOI: 10.1046/j.1523-1755.1998.00900.x]
28 **Eitner F**, Cui Y, Hudkins KL, Stokes MB, Segerer S, Mack M, Lewis PL, Abraham AA, Schlöndorff D, Gallo G, Kimmel PL, Alpers CE. Chemokine receptor CCR5 and CXCR4 expression in HIV-associated kidney disease. *J Am Soc Nephrol* 2000; **11**: 856-867 [PMID: 10770963]
29 **Tzur S**, Rosset S, Shemer R, Yudkovsky G, Selig S, Tarekegn A, Bekele E, Bradman N, Wasser WG, Behar DM, Skorecki K. Missense mutations in the APOL1 gene are highly associated with end stage kidney disease risk previously attributed to the MYH9 gene. *Hum Genet* 2010; **128**: 345-350 [PMID: 20635188 DOI: 10.1007/s00439-010-0861-0]
30 **Kao WH**, Klag MJ, Meoni LA, Reich D, Berthier-Schaad Y, Li M, Coresh J, Patterson N, Tandon A, Powe NR, Fink NE, Sadler JH, Weir MR, Abboud HE, Adler SG, Divers J, Iyengar SK, Freedman BI, Kimmel PL, Knowler WC, Kohn OF, Kramp K, Leehey DJ, Nicholas SB, Pahl MV, Schelling JR, Sedor JR, Thornley-Brown D, Winkler CA, Smith MW, Parekh RS. MYH9 is associated with nondiabetic end-stage renal disease in African Americans. *Nat Genet* 2008; **40**: 1185-1192 [PMID: 18794854 DOI: 10.1038/ng.232]
31 **Kopp JB**, Smith MW, Nelson GW, Johnson RC, Freedman BI, Bowden DW, Oleksyk T, McKenzie LM, Kajiyama H, Ahuja TS, Berns JS, Briggs W, Cho ME, Dart RA, Kimmel PL, Korbet SM, Michel DM, Mokrzycki MH, Schelling JR, Simon E, Trachtman H, Vlahov D, Winkler CA. MYH9 is a major-effect risk gene for focal segmental glomerulosclerosis. *Nat Genet* 2008; **40**: 1175-1184 [PMID: 18794856 DOI: 10.1038/ng.226]
32 **Salas A**, Carracedo A, Richards M, Macaulay V. Charting the ancestry of African Americans. *Am J Hum Genet* 2005; **77**: 676-680 [PMID: 16175514 DOI: 10.1086/491675]
33 **Tishkoff SA**, Reed FA, Friedlaender FR, Ehret C, Ranciaro A, Froment A, Hirbo JB, Awomoyi AA, Bodo JM, Doumbo O, Ibrahim M, Juma AT, Kotze MJ, Lema G, Moore JH, Mortensen H, Nyambo TB, Omar SA, Powell K, Pretorius GS, Smith MW, Thera MA, Wambebe C, Weber JL, Williams SM. The genetic structure and history of Africans and African Americans. *Science* 2009; **324**: 1035-1044 [PMID: 19407144 DOI: 10.1126/science.1172257]

34 **Zakharia F**, Basu A, Absher D, Assimes TL, Go AS, Hlatky MA, Iribarren C, Knowles JW, Li J, Narasimhan B, Sidney S, Southwick A, Myers RM, Quertermous T, Risch N, Tang H. Characterizing the admixed African ancestry of African Americans. *Genome Biol* 2009; **10**: R141 [PMID: 20025784 DOI: 10.1186/gb-2009-10-12-r141]

35 **Macaulay V**, Hill C, Achilli A, Rengo C, Clarke D, Meehan W, Blackburn J, Semino O, Scozzari R, Cruciani F, Taha A, Shaari NK, Raja JM, Ismail P, Zainuddin Z, Goodwin W, Bulbeck D, Bandelt HJ, Oppenheimer S, Torroni A, Richards M. Single, rapid coastal settlement of Asia revealed by analysis of complete mitochondrial genomes. *Science* 2005; **308**: 1034-1036 [PMID: 15890885 DOI: 10.1126/science.1109792]

36 **Tang H**, Peng J, Wang P, Risch NJ. Estimation of individual admixture: analytical and study design considerations. *Genet Epidemiol* 2005; **28**: 289-301 [PMID: 15712363 DOI: 10.1002/gepi.20064]

37 **Tian C**, Gregersen PK, Seldin MF. Accounting for ancestry: population substructure and genome-wide association studies. *Hum Mol Genet* 2008; **17**: R143-R150 [PMID: 18852203 DOI: 10.1093/hmg/ddn268]

38 **Hoggart CJ**, Shriver MD, Kittles RA, Clayton DG, McKeigue PM. Design and analysis of admixture mapping studies. *Am J Hum Genet* 2004; **74**: 965-978 [PMID: 15088268 DOI: 10.1086/420855]

39 **Patterson N**, Hattangadi N, Lane B, Lohmueller KE, Hafler DA, Oksenberg JR, Hauser SL, Smith MW, O'Brien SJ, Altshuler D, Daly MJ, Reich D. Methods for high-density admixture mapping of disease genes. *Am J Hum Genet* 2004; **74**: 979-1000 [PMID: 15088269 DOI: 10.1086/420871]

40 **Winkler CA**, Nelson G, Oleksyk TK, Nava MB, Kopp JB. Genetics of focal segmental glomerulosclerosis and human immunodeficiency virus-associated collapsing glomerulopathy: the role of MYH9 genetic variation. *Semin Nephrol* 2010; **30**: 111-125 [PMID: 20347641 DOI: 10.1016/j.semnephrol.2010.01.003]

41 **Kopp JB**. Glomerular pathology in autosomal dominant MYH9 spectrum disorders: what are the clues telling us about disease mechanism? *Kidney Int* 2010; **78**: 130-133 [PMID: 20588287 DOI: 10.1038/ki.2010.82]

42 **Oleksyk TK**, Nelson GW, An P, Kopp JB, Winkler CA. Worldwide distribution of the MYH9 kidney disease susceptibility alleles and haplotypes: evidence of historical selection in Africa. *PLoS One* 2010; **5**: e11474 [PMID: 20634883 DOI: 10.1371/journal.pone.0011474]

43 **Franceschini N**, Voruganti VS, Haack K, Almasy L, Laston S, Goring HH, Umans JG, Lee ET, Best LG, Fabsitz RR, MacCluer JW, Howard BV, North KE, Cole SA. The association of the MYH9 gene and kidney outcomes in American Indians: the Strong Heart Family Study. *Hum Genet* 2010; **127**: 295-301 [PMID: 19921264 DOI: 10.1007/s00439-009-0769-8]

44 **Freedman BI**, Hicks PJ, Bostrom MA, Comeau ME, Divers J, Bleyer AJ, Kopp JB, Winkler CA, Nelson GW, Langefeld CD, Bowden DW. Non-muscle myosin heavy chain 9 gene MYH9 associations in African Americans with clinically diagnosed type 2 diabetes mellitus-associated ESRD. *Nephrol Dial Transplant* 2009; **24**: 3366-3371 [PMID: 19567477 DOI: 10.1093/ndt/gfp316]

45 **Freedman BI**, Kopp JB, Winkler CA, Nelson GW, Rao DC, Eckfeldt JH, Leppert MF, Hicks PJ, Divers J, Langefeld CD, Hunt SC. Polymorphisms in the nonmuscle myosin heavy chain 9 gene (MYH9) are associated with albuminuria in hypertensive African Americans: the HyperGEN study. *Am J Nephrol* 2009; **29**: 626-632 [PMID: 19153477 DOI: 10.1159/000194791]

46 **Behar DM**, Rosset S, Tzur S, Selig S, Yudkovsky G, Bercovici S, Kopp JB, Winkler CA, Nelson GW, Wasser WG, Skorecki K. African ancestry allelic variation at the MYH9 gene contributes to increased susceptibility to non-diabetic end-stage kidney disease in Hispanic Americans. *Hum Mol Genet* 2010; **19**: 1816-1827 [PMID: 20144966 DOI: 10.1093/hmg/ddq040]

47 **Freedman BI**, Hicks PJ, Bostrom MA, Cunningham ME, Liu Y, Divers J, Kopp JB, Winkler CA, Nelson GW, Langefeld CD, Bowden DW. Polymorphisms in the non-muscle myosin heavy chain 9 gene (MYH9) are strongly associated with end-stage renal disease historically attributed to hypertension in African Americans. *Kidney Int* 2009; **75**: 736-745 [PMID: 19177153 DOI: 10.1038/ki.2008.701]

48 **Abecasis GR**, Altshuler D, Auton A, Brooks LD, Durbin RM, Gibbs RA, Hurles ME, McVean GA. A map of human genome variation from population-scale sequencing. *Nature* 2010; **467**: 1061-1073 [PMID: 20981092 DOI: 10.1038/nature09534]

49 **Freedman BI**, Kopp JB, Langefeld CD, Genovese G, Friedman DJ, Nelson GW, Winkler CA, Bowden DW, Pollak MR. The apolipoprotein L1 (APOL1) gene and nondiabetic nephropathy in African Americans. *J Am Soc Nephrol* 2010; **21**: 1422-1426 [PMID: 20688934 DOI: 10.1681/ASN.2010070730]

50 **Brun R**, Blum J. Human African trypanosomiasis. *Infect Dis Clin North Am* 2012; **26**: 261-273 [PMID: 22632638 DOI: 10.1016/j.idc.2012.03.003]

51 **Brun R**, Blum J, Chappuis F, Burri C. Human African trypanosomiasis. *Lancet* 2010; **375**: 148-159 [PMID: 19833383 DOI: 10.1016/S0140-6736(09)60829-1]

52 **Smith EE**, Malik HS. The apolipoprotein L family of programmed cell death and immunity genes rapidly evolved in primates at discrete sites of host-pathogen interactions. *Genome Res* 2009; **19**: 850-858 [PMID: 19299565 DOI: 10.1101/gr.085647.108]

53 **Vanhamme L**, Paturiaux-Hanocq F, Poelvoorde P, Nolan DP, Lins L, Van Den Abbeele J, Pays A, Tebabi P, Van Xong H, Jacquet A, Moguilevsky N, Dieu M, Kane JP, De Baetselier P, Brasseur R, Pays E. Apolipoprotein L-I is the trypanosome lytic factor of human serum. *Nature* 2003; **422**: 83-87 [PMID: 12621437 DOI: 10.1038/nature01461]

54 **Madhavan SM**, O'Toole JF. The biology of APOL1 with insights into the association between APOL1 variants and chronic kidney disease. *Clin Exp Nephrol* 2014; **18**: 238-242 [PMID: 24233469 DOI: 10.1007/s10157-013-0907-4]

55 **Vanhamme L**, Pays E. The trypanosome lytic factor of human serum and the molecular basis of sleeping sickness. *Int J Parasitol* 2004; **34**: 887-898 [PMID: 15217727 DOI: 10.1016/j.ijpara.2004.04.008]

56 **Vanhollebeke B**, Pays E. The trypanolytic factor of human serum: many ways to enter the parasite, a single way to kill. *Mol Microbiol* 2010; **76**: 806-814 [PMID: 20398209 DOI: 10.1111/j.1365-2958.2010.07156.x]

57 **De Greef C**, Chimfwembe E, Kihang'a Wabacha J, Bajyana Songa E, Hamers R. Only the serum-resistant bloodstream forms of Trypanosoma brucei rhodesiense express the serum resistance associated (SRA) protein. *Ann Soc Belg Med Trop* 1992; **72** Suppl 1: 13-21 [PMID: 1417165]

58 **Lecordier L**, Vanhollebeke B, Poelvoorde P, Tebabi P, Paturiaux-Hanocq F, Andris F, Lins L, Pays E. C-terminal mutants of apolipoprotein L-I efficiently kill both Trypanosoma brucei brucei and Trypanosoma brucei rhodesiense. *PLoS Pathog* 2009; **5**: e1000685 [PMID: 19997494 DOI: 10.1371/journal.ppat.1000685]

59 **Ikpeme EE**, Ekrikpo UE, Akpan MU, Ekaidem SI. Determining the prevalence of human immunodeficiency virus-associated nephropathy (HIVAN) using proteinuria and ultrasound findings in a Nigerian paediatric HIV population. *Pan Afr Med J* 2012; **11**: 13 [PMID: 22368756]

60 **Hemelaar J**, Gouws E, Ghys PD, Osmanov S. Global and regional distribution of HIV-1 genetic subtypes and recombinants in 2004. *AIDS* 2006; **20**: W13-W23 [PMID: 17053344 DOI: 10.1097/01.aids.0000247564.73009.bc]

61 **Novitsky V**, Ndung'u T, Wang R, Bussmann H, Chonco F, Makhema J, De Gruttola V, Walker BD, Essex M. Extended high viremics: a substantial fraction of individuals maintain high plasma viral RNA levels after acute HIV-1 subtype C infection. *AIDS* 2011; **25**: 1515-1522 [PMID: 21505307 DOI: 10.1097/QAD.0b013e3283471eb2]

62 **Hays T,** Wyatt CM. APOL1 variants in HIV-associated nephropathy: just one piece of the puzzle. *Kidney Int* 2012; **82**: 259-260 [PMID: 22791322 DOI: 10.1038/ki.2012.129]

63 **Kalayjian RC**. The treatment of HIV-associated nephropathy. *Adv Chronic Kidney Dis* 2010; **17**: 59-71 [PMID: 20005490 DOI:

10.1053/j.ackd.2009.08.013]
64 **Freedman BI**, Skorecki K. Gene-gene and gene-environment interactions in apolipoprotein L1 gene-associated nephropathy. *Clin J Am Soc Nephrol* 2014; **9**: 2006-2013 [PMID: 24903390 DOI: 10.2215/CJN.01330214]
65 **Behar DM**, Shlush LI, Maor C, Lorber M, Skorecki K. Absence of HIV-associated nephropathy in Ethiopians. *Am J Kidney Dis* 2006; **47**: 88-94 [PMID: 16377389 DOI: 10.1053/j.ajkd.2005.09.023]
66 **de Filippo C**, Barbieri C, Whitten M, Mpoloka SW, Gunnarsdóttir ED, Bostoen K, Nyambe T, Beyer K, Schreiber H, de Knijff P, Luiselli D, Stoneking M, Pakendorf B. Y-chromosomal variation in sub-Saharan Africa: insights into the history of Niger-Congo groups. *Mol Biol Evol* 2011; **28**: 1255-1269 [PMID: 21109585 DOI: 10.1093/molbev/msq312]
67 **Tishkoff SA**, Williams SM. Genetic analysis of African populations: human evolution and complex disease. *Nat Rev Genet* 2002; **3**: 611-621 [PMID: 12154384 DOI: 10.1038/nrg865]
68 USRDS 2012 Annual Data Report. Cited 2014-05-10. Available from: URL: http: //www.usrds.org/atlas12.aspx
69 **Post FA**, Campbell LJ, Hamzah L, Collins L, Jones R, Siwani R, Johnson L, Fisher M, Holt SG, Bhagani S, Frankel AH, Wilkins E, Ainsworth JG, Larbalestier N, Macallan DC, Banerjee D, Baily G, Thuraisingham RC, Donohoe P, Hendry BM, Hilton RM, Edwards SG, Hangartner R, Howie AJ, Connolly JO, Easterbrook PJ. Predictors of renal outcome in HIV-associated nephropathy. *Clin Infect Dis* 2008; **46**: 1282-1289 [PMID: 18444868 DOI: 10.1086/529385]
70 **Lucas GM**, Eustace JA, Sozio S, Mentari EK, Appiah KA, Moore RD. Highly active antiretroviral therapy and the incidence of HIV-1-associated nephropathy: a 12-year cohort study. *AIDS* 2004; **18**: 541-546 [PMID: 15090808 DOI: 10.1097/00002030-200402200-00022]
71 **Fine DM**, Wasser WG, Estrella MM, Atta MG, Kuperman M, Shemer R, Rajasekaran A, Tzur S, Racusen LC, Skorecki K. APOL1 risk variants predict histopathology and progression to ESRD in HIV-related kidney disease. *J Am Soc Nephrol* 2012; **23**: 343-350 [PMID: 22135313 DOI: 10.1681/ASN.2011060562]
72 **Parsa A**, Kao WH, Xie D, Astor BC, Li M, Hsu CY, Feldman HI, Parekh RS, Kusek JW, Greene TH, Fink JC, Anderson AH, Choi MJ, Wright JT, Lash JP, Freedman BI, Ojo A, Winkler CA, Raj DS, Kopp JB, He J, Jensvold NG, Tao K, Lipkowitz MS, Appel LJ. APOL1 risk variants, race, and progression of chronic kidney disease. *N Engl J Med* 2013; **369**: 2183-2196 [PMID: 24206458 DOI: 10.1056/NEJMoa1310345]
73 **Divers J**, Núñez M, High KP, Murea M, Rocco MV, Ma L, Bowden DW, Hicks PJ, Spainhour M, Ornelles DA, Kleiboeker SB, Duncan K, Langefeld CD, Turner J, Freedman BI. JC polyoma virus interacts with APOL1 in African Americans with nondiabetic nephropathy. *Kidney Int* 2013; **84**: 1207-1213 [PMID: 23677244 DOI: 10.1038/ki.2013.173]
74 **Bostrom MA**, Freedman BI. The spectrum of MYH9-associated nephropathy. *Clin J Am Soc Nephrol* 2010; **5**: 1107-1113 [PMID: 20299374 DOI: 10.2215/CJN.08721209]
75 **Divers J**, Freedman BI. Genetics in kidney disease in 2013: Susceptibility genes for renal and urological disorders. *Nat Rev Nephrol* 2014; **10**: 69-70 [PMID: 24296626 DOI: 10.1038/nrneph.2013.259]
76 **Divers J**, Palmer ND, Lu L, Langefeld CD, Rocco MV, Hicks PJ, Murea M, Ma L, Bowden DW, Freedman BI. Gene-gene interactions in APOL1-associated nephropathy. *Nephrol Dial Transplant* 2014; **29**: 587-594 [PMID: 24157943 DOI: 10.1093/ndt/gft423]
77 **Zhaorigetu S**, Wan G, Kaini R, Jiang Z, Hu CA. ApoL1, a BH3-only lipid-binding protein, induces autophagic cell death. *Autophagy* 2008; **4**: 1079-1082 [PMID: 18927493 DOI: 10.4161/auto.7066]
78 **Wan G**, Zhaorigetu S, Liu Z, Kaini R, Jiang Z, Hu CA. Apolipoprotein L1, a novel Bcl-2 homology domain 3-only lipid-binding protein, induces autophagic cell death. *J Biol Chem* 2008; **283**: 21540-21549 [PMID: 18505729 DOI: 10.1074/jbc.M800214200]
79 **Pérez-Morga D**, Vanhollebeke B, Paturiaux-Hanocq F, Nolan DP, Lins L, Homblé F, Vanhamme L, Tebabi P, Pays A, Poelvoorde P, Jacquet A, Brasseur R, Pays E. Apolipoprotein L-I promotes trypanosome lysis by forming pores in lysosomal membranes. *Science* 2005; **309**: 469-472 [PMID: 16020735 DOI: 10.1126/science.1114566]
80 **Madhavan SM**, O'Toole JF, Konieczkowski M, Ganesan S, Bruggeman LA, Sedor JR. APOL1 localization in normal kidney and nondiabetic kidney disease. *J Am Soc Nephrol* 2011; **22**: 2119-2128 [PMID: 21997392 DOI: 10.1681/ASN.2011010069]
81 **Pays E**, Vanhollebeke B, Vanhamme L, Paturiaux-Hanocq F, Nolan DP, Pérez-Morga D. The trypanolytic factor of human serum. *Nat Rev Microbiol* 2006; **4**: 477-486 [PMID: 16710327 DOI: 10.1038/nrmicro1428]
82 **Bentley AR**, Doumatey AP, Chen G, Huang H, Zhou J, Shriner D, Jiang C, Zhang Z, Liu G, Fasanmade O, Johnson T, Oli J, Okafor G, Eghan BA, Agyenim-Boateng K, Adeleye J, Balogun W, Adebamowo C, Amoah A, Acheampong J, Adeyemo A, Rotimi CN. Variation in APOL1 Contributes to Ancestry-Level Differences in HDLc-Kidney Function Association. *Int J Nephrol* 2012; **2012**: 748984 [PMID: 22973513 DOI: 10.1155/2012/748984]
83 **Vermeulen A.** A Review of Patterns of Renal Disease at Chris Hani Baragwanath Academic Hospital from 1982 to 2011. University of the Witwatersrand: MMED Research report. Cited 2014-06-30. Available from: URL: http: //wiredspace.wits.ac.za/jspui/bitstream/10539/15463/2/Vermeulen_ResReport.pdf
84 **Genovese G,** Handsaker RE, Li H, Kenny EE, McCarroll SA. Mapping the human reference genome's missing sequence by three-way admixture in Latino genomes. *Am J Hum Genet* 2013; **93**: 411-421 [PMID: 23932108 DOI: 10.1016/j.ajhg.2013.07.002]

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Species differences in regulation of renal proximal tubule transport by certain molecules

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Abstract

Renal proximal tubules (PTs) play important roles in the regulation of acid/base, plasma volume and blood pressure. Recent studies suggest that there are substantial species differences in the regulation of PT transport. For example, thiazolidinediones (TZDs) are widely used for the treatment of type 2 diabetes mellitus, but the use of TZDs is associated with fluid overload. In addition to the transcriptional enhancement of sodium transport in distal nephrons, TZDs rapidly stimulate PT sodium transport *via* a non-genomic mechanism depending on peroxisome proliferator activated receptor γ/Src/epidermal growth factor receptor (EGFR)/MEK/ERK. In mouse PTs, however, TZDs fail to stimulate PT transport probably due to constitutive activation of Src/EGFR/ERK pathway. This unique activation of Src/ERK may also affect the effect of high concentrations of insulin on mouse PT transport. On the other hand, the effect of angiotensin Ⅱ (Ang Ⅱ) on PT transport is known to be biphasic in rabbits, rats, and mice. However, Ang Ⅱ induces a concentration-dependent, monophasic transport stimulation in human PTs. The contrasting responses to nitric oxide/guanosine 3',5'-cyclic monophosphate pathway may largely explain these different effects of Ang Ⅱ on PT transport. In this review, we focus on the recent findings on the species differences in the regulation of PT transport, which may help understand the species-specific mechanisms underlying edema formation and/or hypertension occurrence.

Key words: Renal proximal tubule; Thiazolidinediones; Peroxisome proliferator activated receptor γ; Insulin; Angiotensin Ⅱ; Nitric oxide

Core tip: Renal proximal tubule (PT) transport is essential for the regulation of plasma volume and blood pressure. Several species differences are found as to the stimulatory effects of thiazolidinediones, insulin, and angiotensin Ⅱ on PT sodium transport. This review focuses on this topic, which may be relevant to species-specific mechanisms underlying edema formation and/or hypertension occurrence.

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INTRODUCTION

The kidney plays an essential role in the homeostatic regulation of electrolytes, acid-base and plasma volume in the body. Some species differences are known to exist in the distribution patterns of solute transporters as well as the hormonal actions in distal nephron[1,2]. In proximal tubules (PTs), on the other hand, there are no major species differences in the distribution patterns and functions of main sodium transporters such as the apical Na^+/H^+ exchanger type 3 NHE3 and the basolateral electrogenic Na^+-HCO_3^- cotransporter NBCe1[3,4]. While the transport stoichiometry of NBCe1 was reported to be $1Na^+$ to $3HCO_3^-$ in rat PTs *in vivo*[5], it was found to be $1Na^+$ to $2HCO_3^-$ in rabbit PTs *in vitro*[6]. However, this difference was turned out to be due to the differences in experimental conditions[7,8].

Nevertheless, recent studies identified substantial species differences in the regulation of PT transport, which might be important for uncovering the species-specific causes for edema and/or hypertension. Species differences in renal physiologic and/or metabolic responses may be also potentially important for understanding mechanisms for the different effects of several agents against diabetic nephropathy between animal models and human patients. For example, inhibition of advanced glycosylation end products by aminoguanidine or pyridoxamine was reported to be protective in rodent models of diabetic nephropathy[9-12]. However, these agents were not found effective in initial human trials in diabetic patients[13,14]. Effectiveness of a selective PKC-inhibitor Ruboxistaurin found in rodent models of diabetic nephropathy[15,16] was also not confirmed in human type 2 diabetic patients[17]. Indeed, rodent models of diabetic nephropathy exhibit proteinuria and pathological glomerular changes, but do not exhibit progressive renal failure[18]. Dogs, pigs and other non-human primates have been used for toxicology testing. However, ideal animal models have not been established for revealing drug side effects[19]. We focus on species differences in the regulation of PT transport in this review.

EFFECTS OF THIAZOLIDINEDIONES

Thiazolidinediones (TZDs) activate a nuclear receptor peroxisome proliferator activated receptor γ (PPARγ), thereby improving insulin resistance through the transcriptional modulation of the relevant genes[20]. TZDs have been widely used for the treatment of type 2 diabetes. However, fluid retention is a serious clinical problem, which may exert an adverse effect on the cardiovascular system[21].

Initially, PPARγ-mediated transcriptional enhancement of the epithelial Na channel ENaCγ subunit in collecting ducts was proposed to be a main mechanism for TZDs-induced volume expansion[22,23]. Subsequent analysis in renal principal cell culture models, however, did not support the central role of ENaC in TZDs-induced volume expansion[24]. Moreover, TZDs-induced volume expansion was preserved in collecting ducts specific ENaC deficient mice[25]. These results indicate that mechanism(s) other than the activation of ENaC in collecting ducts may be also involved in TZDs-induced volume expansion. Interestingly, Muto and colleagues reported the rapid stimulation of NBCe1 activity by troglitazone in isolated rabbit PTs[26]. Because TDZs were reported to stimulate PT transport also in humans[27], we performed the detailed analysis on the mechanism of TZDs-induced stimulation of PT sodium transport.

We found that TZDs rapidly induced transport stimulation within minutes in isolated PTs from rabbits, rats, and humans. Our subsequent analysis revealed that TZDs-induced PT transport stimulation was dependent on the non-genomic signaling cascade consisting of PPARγ/Src/epidermal growth factor receptor (EGFR)/MEK/ERK[28]. Notably, however, TZDs failed to induce transport stimulation in isolated mouse PTs, despite the definite expression of PPARγ in these tubules. We speculated that some factor(s) specific for mouse PTs might interfere with TZDs-induced rapid signaling. Consistent with this view, Kiley *et al*[29] reported the constitutive activation of Src/EGFR pathway that was found only in mouse PTs. This unique constitutive activation of Src/EGFR, which could potentially explain the different effects of exogenous EGF on unilateral ureteral obstruction in rats and mice[30], was confined to mouse PTs and not found in other mouse tissues[29]. Because TZDs rapidly activated the Src/ERK pathway in kidney cortex of rabbits and rats but not mice, we concluded that the constitutive activation of Src/EGFR prevented TZDs-induced transport stimulation in mouse PTs[28]. Consistent with this conclusion, the constitutive activation of Src was also reported to interfere with the non-genomic signaling of another nuclear receptor for estrogen[31].

Most likely, TZDs-induced volume expansion is multifactorial, depending on both genomic and non-genomic actions of PPARγ on tubular sodium transport. One of potential targets of non-genomic actions of PPARγ may be distal nephron, where WNK kinases regulate the balance between renal NaCl absorption and K^+ secretion[32,33]. WNK4 is also known to affect Cl^- transport in extrarenal epithelia[34]. It remains to be determined whether PPARγ regulates the WNK kinase system.

EFFECTS OF INSULIN

Insulin is thought to enhance renal sodium retention by activating sodium transport in several nephron segments[35]. In PTs, insulin stimulates the major sodium transporters NHE3, NBCe1, and the Na^+/K^+-ATPase[36-38]. In isolated rabbit PTs, Baum found that insulin, at the concentrations between 10^{-10} mol/L and 10^{-8} mol/L, dose-dependently stimulates volume and bicarbonate absorption[39]. Recently, we also found the similar dose-dependent stimulation of NBCe1 by up to 10^{-8} mol/L insulin in isolated PTs from rats

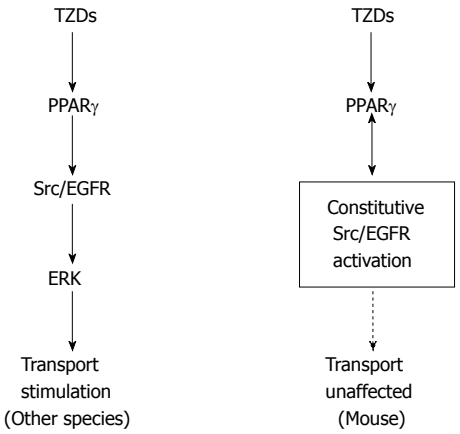

Figure 1 Species differences in thiazolidinediones effects on proximal tubule transport. Non-genomic activation of ERK by TZDs *via* PPARγ/Src/EGFR pathway is suppressed in mouse proximal tubule probably due to constitutive activation of Src/EGFR. TZDs: Thiazolidinediones; PPAR: Peroxisome proliferator activated receptor; Src: Sarcoma; EGFR: Epidermal growth factor receptor; ERK: Extracellular signal-regulated kinase.

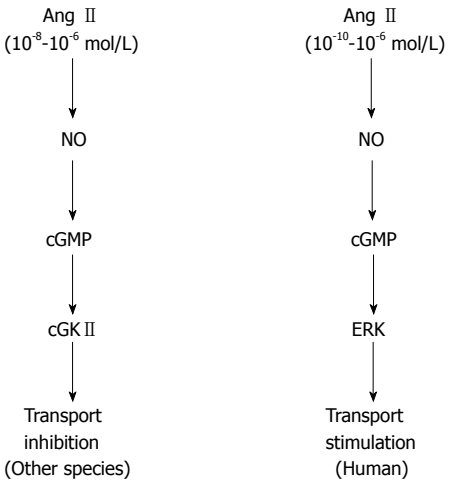

Figure 2 Species differences in angiotensin Ⅱ effects on proximal tubule transport. In human, NO/cGMP pathway mediates the concentration-dependent stimulatory effect of Ang Ⅱ. In other species, NO/cGMP pathway mediates the inhibitory effect of high concentrations of Ang Ⅱ. Ang Ⅱ: Angiotensin Ⅱ; NO: Nitrox oxide; cGMP: Guanosine 3',5'-cyclic monophosphate; cGKⅡ: cGMP-dependent kinase Ⅱ; ERK: Extracellular signal-regulated kinase.

and humans[40]. This stimulatory effect of insulin on PT sodium transport, which is completely preserved in insulin resistant rats and humans, may play an important role in the pathogenesis of hypertension associated with insulin resistance[40].

Insulin actions are initiated by the activation of tyrosine kinase in the cell membrane receptor, which induces a series of phosphorylation in multiple insulin receptor substrates (IRSs). The two major IRS proteins, IRS1 and IRS2 often mediate distinct insulin actions, and defects at the levels of IRS1 or IRS2 may be responsible for the occurrence of selective insulin resistance in several tissues[41]. We have shown that IRS2 but not IRS1 mediates the stimulatory effect of insulin on PT transport in both mice and rats. Moreover, we have confirmed that insulin activates Akt in kidney cortex of both mice and rats[40,42]. We found that up to 10^{-9} mol/L insulin stimulated mouse PT sodium transport. Unlike in rats and humans, however, the higher concentrations of insulin (*i.e.,* more than 10^{-8} mol/L) failed to stimulate PT transport in mice[42]. In isolated mouse collecting ducts, by contrast, insulin at the high concentrations up to 10^{-7} mol/L was reported to activate ENaC[43]. Importantly, insulin is known to activate both Akt and ERK pathways, and these two pathways are interconnected with each other[44,45]. It is therefore possible that the constitutive activation of Src/ERK may somehow interfere with the effects of high concentrations of insulin in mouse PTs.

EFFECTS OF ANGIOTENSIN Ⅱ

The stimulation of PT sodium transport by Angiotensin Ⅱ (Ang Ⅱ) may be essential for Ang Ⅱ-induced hypertension[46,47]. Actually, however, Ang Ⅱ is known to regulate PT transport in a biphasic way: transport is stimulated by low (picomolar to nanomolar) concentrations of Ang Ⅱ, but inhibited by high (nanomolar to micromolar) concentrations of Ang Ⅱ. This biphasic regulation of PT transport by Ang Ⅱ has been confirmed in rats, mice, and rabbits[48-51]. Regarding the receptor subtype(s) responsible for the biphasic effects of Ang Ⅱ, controversial results had been reported[52-54]. However, the analyses on isolated PTs from type 1A Ang Ⅱ receptor (AT_{1A}) deficient mice have clearly shown that AT_{1A} mediates both the stimulatory and inhibitory effects of Ang Ⅱ[49,51].

The stimulatory effect of Ang Ⅱ is dependent on the activation of protein kinase C and/or the decrease in the intracellular cAMP concentration, which may result in the activation of ERK[55,56]. On the other hand, the inhibitory effect of Ang Ⅱ is dependent on the activation of phospholipase A_2/arachidonic acid/5,6-epoxyeicosatrienoic acid (EET) pathway and/or nitric oxide (NO)/guanosine 3',5'-cyclic monophosphate (cGMP) pathway[53,55,57]. Because little had been known about the direct effects of Ang Ⅱ on human PT transport, we tried to clarify this issue using isolated, intact human PTs obtained from nephrectomy surgery.

Surprisingly, we found that the inhibitory effect of Ang Ⅱ is lost in human PTs. Actually, up to 10^{-5} mol/L Ang Ⅱ dose-dependently stimulated human PT transport. In view of high intrarenal concentrations of Ang Ⅱ, these data suggest that Ang Ⅱ may play an even more important role in the regulation of plasma volume and blood pressure in humans than in other species[58].

The detailed analysis revealed that the contrasting responses to NO/cGMP could explain the different modes of PT transport regulation in humans and other species. While the NO/cGMP/ERK pathway mediates the dose-dependent stimulatory effect of Ang Ⅱ in

humans, the NO/cGMP/cGMP-dependent kinase Ⅱ (cGKⅡ) pathway mediates the inhibitory effect of high concentrations of Ang Ⅱ in mice[58]. In cGKⅡ-deficient mice, the inhibitory effect of Ang Ⅱ was lost, but the NO/cGMP pathway failed to stimulate PT transport. These results indicate that the loss of cGKⅡ alone in mice cannot reproduce the NO/cGMP/ERK-dependent stimulatory effect of Ang Ⅱ in humans.

Currently, the reason why the NO/cGMP pathway exerts different effects on PT transport in humans and other species remains unknown. Nevertheless, several previous reports supported the existence of such species differences. For example, renal NO production was enhanced by salt loading in rodents, which might facilitate sodium diuresis and prevent blood pressure elevation[59,60]. Thus, renal NO is thought to induce a natriuretic response to salt loading in rodents[61]. However, renal NO production was not significantly enhanced by salt loading in humans[62,63], and an adaptive role of renal NO to salt loading is less clear in humans[64,65]. In any case, the stimulatory effect of NO/cGMP pathway on PT transport may represent a human-specific target for hypertension.

The NO/cGMP pathway is thought to be inhibitory on sodium transport in thick ascending limb and collecting ducts[61]. Therefore, it will be interesting to examine whether the similar species differences may also exist in the effects of NO/cGMP pathway on these nephron segments. Such knowledge may help understand the mechanism of fluid overload by selective endothelin receptor antagonism[66].

CONCLUSION

In summary, non-genomic stimulation of PT transport by TZDs is uniquely absent in mice probably because of the constitutive activation of Src/EGFR as shown in Figure 1. As shown in Figure 2, on the other hand, the inhibitory effect of Ang Ⅱ is lost in human PTs, where the NO/cGMP pathway is stimulatory unlike in other species. These species differences may be at least partially responsible for the species-specific mechanisms underlying edema formation and/or hypertension occurrence.

REFERENCES

1 **Biner HL**, Arpin-Bott MP, Loffing J, Wang X, Knepper M, Hebert SC, Kaissling B. Human cortical distal nephron: distribution of electrolyte and water transport pathways. *J Am Soc Nephrol* 2002; **13**: 836-847 [PMID: 11912242]
2 **Morel F**. Sites of hormone action in the mammalian nephron. *Am J Physiol* 1981; **240**: F159-F164 [PMID: 6259951]
3 **Alpern RJ**. Cell mechanisms of proximal tubule acidification. *Physiol Rev* 1990; **70**: 79-114 [PMID: 2404291]
4 **Boron WF**. Acid-base transport by the renal proximal tubule. *J Am Soc Nephrol* 2006; **17**: 2368-2382 [PMID: 16914536 DOI: 10.1681/ASN.2006060620]
5 **Yoshitomi K**, Burckhardt BC, Frömter E. Rheogenic sodium-bicarbonate cotransport in the peritubular cell membrane of rat renal proximal tubule. *Pflugers Arch* 1985; **405**: 360-366 [PMID: 3936018]
6 **Seki G**, Coppola S, Frömter E. The Na(+)-HCO3- cotransporter operates with a coupling ratio of 2 HCO3- to 1 Na+ in isolated rabbit renal proximal tubule. *Pflugers Arch* 1993; **425**: 409-416 [PMID: 8134258 DOI: 10.1007/BF00374866]
7 **Müller-Berger S**, Nesterov VV, Frömter E. Partial recovery of in vivo function by improved incubation conditions of isolated renal proximal tubule. II. Change of Na-HCO3 cotransport stoichiometry and of response to acetazolamide. *Pflugers Arch* 1997; **434**: 383-391 [PMID: 9211803 DOI: 10.1007/s004240050411]
8 **Seki G**, Coppola S, Yoshitomi K, Burckhardt BC, Samarzija I, Müller-Berger S, Frömter E. On the mechanism of bicarbonate exit from renal proximal tubular cells. *Kidney Int* 1996; **49**: 1671-1677 [PMID: 8743474 DOI: 10.1038/ki.1996.244]
9 **Alderson NL**, Chachich ME, Youssef NN, Beattie RJ, Nachtigal M, Thorpe SR, Baynes JW. The AGE inhibitor pyridoxamine inhibits lipemia and development of renal and vascular disease in Zucker obese rats. *Kidney Int* 2003; **63**: 2123-2133 [PMID: 12753299 DOI: 10.1046/j.1523-1755.2003.00027.x]
10 **Bucala R**, Vlassara H. Advanced glycosylation end products in diabetic renal and vascular disease. *Am J Kidney Dis* 1995; **26**: 875-888 [PMID: 7503061]
11 **Degenhardt TP**, Alderson NL, Arrington DD, Beattie RJ, Basgen JM, Steffes MW, Thorpe SR, Baynes JW. Pyridoxamine inhibits early renal disease and dyslipidemia in the streptozotocin-diabetic rat. *Kidney Int* 2002; **61**: 939-950 [PMID: 11849448 DOI: 10.1046/j.1523-1755.2002.00207.x]
12 **Friedman EA**, Distant DA, Fleishhacker JF, Boyd TA, Cartwright K. Aminoguanidine prolongs survival in azotemic-induced diabetic rats. *Am J Kidney Dis* 1997; **30**: 253-259 [PMID: 9261037]
13 **Bolton WK**, Cattran DC, Williams ME, Adler SG, Appel GB, Cartwright K, Foiles PG, Freedman BI, Raskin P, Ratner RE, Spinowitz BS, Whittier FC, Wuerth JP. Randomized trial of an inhibitor of formation of advanced glycation end products in diabetic nephropathy. *Am J Nephrol* 2004; **24**: 32-40 [PMID: 14685005 DOI: 10.1159/000075627]
14 **Lewis EJ**, Greene T, Spitalewiz S, Blumenthal S, Berl T, Hunsicker LG, Pohl MA, Rohde RD, Raz I, Yerushalmy Y, Yagil Y, Herskovits T, Atkins RC, Reutens AT, Packham DK, Lewis JB. Pyridorin in type 2 diabetic nephropathy. *J Am Soc Nephrol* 2012; **23**: 131-136 [PMID: 22034637 DOI: 10.1681/ASN.2011030272]
15 **Ishii H**, Jirousek MR, Koya D, Takagi C, Xia P, Clermont A, Bursell SE, Kern TS, Ballas LM, Heath WF, Stramm LE, Feener EP, King GL. Amelioration of vascular dysfunctions in diabetic rats by an oral PKC beta inhibitor. *Science* 1996; **272**: 728-731 [PMID: 8614835]
16 **Koya D**, Haneda M, Nakagawa H, Isshiki K, Sato H, Maeda S, Sugimoto T, Yasuda H, Kashiwagi A, Ways DK, King GL, Kikkawa R. Amelioration of accelerated diabetic mesangial expansion by treatment with a PKC beta inhibitor in diabetic db/db mice, a rodent model for type 2 diabetes. *FASEB J* 2000; **14**: 439-447 [PMID: 10698958]
17 **Tuttle KR**, Bakris GL, Toto RD, McGill JB, Hu K, Anderson PW. The effect of ruboxistaurin on nephropathy in type 2 diabetes. *Diabetes Care* 2005; **28**: 2686-2690 [PMID: 16249540 DOI: 10.2337/diacare.28.11.2686]
18 **Breyer MD**, Böttinger E, Brosius FC, Coffman TM, Fogo A, Harris RC, Heilig CW, Sharma K. Diabetic nephropathy: of mice and men. *Adv Chronic Kidney Dis* 2005; **12**: 128-145 [PMID: 15822049 DOI: 10.1053/j.ackd.2005.01.004]
19 **Helke KL**, Swindle MM. Animal models of toxicology testing: the role of pigs. *Expert Opin Drug Metab Toxicol* 2013; **9**: 127-139 [PMID: 23216131 DOI: 10.1517/17425255.2013.739607]
20 **Yki-Järvinen H**. Thiazolidinediones. *N Engl J Med* 2004; **351**: 1106-1118 [PMID: 15356308 DOI: 10.1056/NEJMra041001]
21 **Mudaliar S**, Chang AR, Henry RR. Thiazolidinediones, peripheral edema, and type 2 diabetes: incidence, pathophysiology, and clinical implications. *Endocr Pract* 2003; **9**: 406-416 [PMID: 14583425 DOI: 10.4158/EP.9.5.406]
22 **Guan Y**, Hao C, Cha DR, Rao R, Lu W, Kohan DE, Magnuson

MA, Redha R, Zhang Y, Breyer MD. Thiazolidinediones expand body fluid volume through PPARgamma stimulation of ENaC-mediated renal salt absorption. *Nat Med* 2005; **11**: 861-866 [PMID: 16007095 DOI: 10.1038/nm1278]

23 **Zhang H**, Zhang A, Kohan DE, Nelson RD, Gonzalez FJ, Yang T. Collecting duct-specific deletion of peroxisome proliferator-activated receptor gamma blocks thiazolidinedione-induced fluid retention. *Proc Natl Acad Sci USA* 2005; **102**: 9406-9411 [PMID: 15956187 DOI: 10.1073/pnas.0501744102]

24 **Nofziger C**, Chen L, Shane MA, Smith CD, Brown KK, Blazer-Yost BL. PPARgamma agonists do not directly enhance basal or insulin-stimulated Na(+) transport via the epithelial Na(+) channel. *Pflugers Arch* 2005; **451**: 445-453 [PMID: 16170524 DOI: 10.1007/s00424-005-1477-4]

25 **Vallon V**, Hummler E, Rieg T, Pochynyuk O, Bugaj V, Schroth J, Dechenes G, Rossier B, Cunard R, Stockand J. Thiazolidinedione-induced fluid retention is independent of collecting duct alphaENaC activity. *J Am Soc Nephrol* 2009; **20**: 721-729 [PMID: 19158355 DOI: 10.1681/ASN.2008040415]

26 **Muto S**, Miyata Y, Imai M, Asano Y. Troglitazone stimulates basolateral rheogenic Na+/HCO3- cotransport activity in rabbit proximal straight tubules. *Exp Nephrol* 2001; **9**: 191-197 [PMID: 11340303 DOI: 10.1159/000052611]

27 **Zanchi A**, Chiolero A, Maillard M, Nussberger J, Brunner HR, Burnier M. Effects of the peroxisomal proliferator-activated receptor-gamma agonist pioglitazone on renal and hormonal responses to salt in healthy men. *J Clin Endocrinol Metab* 2004; **89**: 1140-1145 [PMID: 15001599 DOI: 10.1210/jc.2003-031526]

28 **Endo Y**, Suzuki M, Yamada H, Horita S, Kunimi M, Yamazaki O, Shirai A, Nakamura M, Iso-O N, Li Y, Hara M, Tsukamoto K, Moriyama N, Kudo A, Kawakami H, Yamauchi T, Kubota N, Kadowaki T, Kume H, Enomoto Y, Homma Y, Seki G, Fujita T. Thiazolidinediones enhance sodium-coupled bicarbonate absorption from renal proximal tubules via PPARγ-dependent nongenomic signaling. *Cell Metab* 2011; **13**: 550-561 [PMID: 21531337 DOI: 10.1016/j.cmet.2011.02.015]

29 **Kiley SC**, Chevalier RL. Species differences in renal Src activity direct EGF receptor regulation in life or death response to EGF. *Am J Physiol Renal Physiol* 2007; **293**: F895-F903 [PMID: 17626154 DOI: 10.1152/ajprenal.00227.2007]

30 **Kiley SC**, Thornhill BA, Belyea BC, Neale K, Forbes MS, Luetteke NC, Lee DC, Chevalier RL. Epidermal growth factor potentiates renal cell death in hydronephrotic neonatal mice, but cell survival in rats. *Kidney Int* 2005; **68**: 504-514 [PMID: 16014027 DOI: 10.1111/j.1523-1755.2005.00428.x]

31 **Li L**, Hisamoto K, Kim KH, Haynes MP, Bauer PM, Sanjay A, Collinge M, Baron R, Sessa WC, Bender JR. Variant estrogen receptor-c-Src molecular interdependence and c-Src structural requirements for endothelial NO synthase activation. *Proc Natl Acad Sci USA* 2007; **104**: 16468-16473 [PMID: 17921256 DOI: 10.1073/pnas.0704315104]

32 **Kahle KT**, Wilson FH, Leng Q, Lalioti MD, O'Connell AD, Dong K, Rapson AK, MacGregor GG, Giebisch G, Hebert SC, Lifton RP. WNK4 regulates the balance between renal NaCl reabsorption and K+ secretion. *Nat Genet* 2003; **35**: 372-376 [PMID: 14608358 DOI: 10.1038/ng1271]

33 **Yang CL**, Angell J, Mitchell R, Ellison DH. WNK kinases regulate thiazide-sensitive Na-Cl cotransport. *J Clin Invest* 2003; **111**: 1039-1045 [PMID: 12671053 DOI: 10.1172/JCI17443]

34 **Kahle KT**, Gimenez I, Hassan H, Wilson FH, Wong RD, Forbush B, Aronson PS, Lifton RP. WNK4 regulates apical and basolateral Cl- flux in extrarenal epithelia. *Proc Natl Acad Sci USA* 2004; **101**: 2064-2069 [PMID: 14769928 DOI: 10.1073/pnas.0308434100]

35 **Horita S**, Seki G, Yamada H, Suzuki M, Koike K, Fujita T. Insulin resistance, obesity, hypertension, and renal sodium transport. *Int J Hypertens* 2011; **2011**: 391762 [PMID: 21629870]

36 **Féraille E**, Carranza ML, Rousselot M, Favre H. Insulin enhances sodium sensitivity of Na-K-ATPase in isolated rat proximal convoluted tubule. *Am J Physiol* 1994; **267**: F55-F62 [PMID: 8048565]

37 **Gesek FA**, Schoolwerth AC. Insulin increases Na(+)-H+ exchange activity in proximal tubules from normotensive and hypertensive rats. *Am J Physiol* 1991; **260**: F695-F703 [PMID: 1674643]

38 **Ruiz OS**, Qiu YY, Cardoso LR, Arruda JA. Regulation of the renal Na-HCO3 cotransporter: IX. Modulation by insulin, epidermal growth factor and carbachol. *Regul Pept* 1998; **77**: 155-161 [PMID: 9809810 DOI: 10.1016/S0167-0115(98)00115-3]

39 **Baum M**. Insulin stimulates volume absorption in the rabbit proximal convoluted tubule. *J Clin Invest* 1987; **79**: 1104-1109 [PMID: 3549779]

40 **Nakamura M,** Yamazaki O, Shirai A, Horita S, Satoh N, Suzuki M, Hamasaki Y, Noiri E, Kume H, Enomoto Y, Homma Y, Seki G. Preserved Na/HCO3 cotransporter sensitivity to insulin may promote hypertension in metabolic syndrome. *Kidney Int* 2014; **87**: 535-542 [PMID: 25354240 DOI: 10.1038/ki.2014.351]

41 **Thirone AC**, Huang C, Klip A. Tissue-specific roles of IRS proteins in insulin signaling and glucose transport. *Trends Endocrinol Metab* 2006; **17**: 72-78 [PMID: 16458527 DOI: 10.1016/j.tem.2006.01.005]

42 **Zheng Y**, Yamada H, Sakamoto K, Horita S, Kunimi M, Endo Y, Li Y, Tobe K, Terauchi Y, Kadowaki T, Seki G, Fujita T. Roles of insulin receptor substrates in insulin-induced stimulation of renal proximal bicarbonate absorption. *J Am Soc Nephrol* 2005; **16**: 2288-2295 [PMID: 15975995 DOI: 10.1681/ASN.2005020193]

43 **Pavlov TS**, Ilatovskaya DV, Levchenko V, Li L, Ecelbarger CM, Staruschenko A. Regulation of ENaC in mice lacking renal insulin receptors in the collecting duct. *FASEB J* 2013; **27**: 2723-2732 [PMID: 23558339]

44 **Taniguchi CM**, Emanuelli B, Kahn CR. Critical nodes in signalling pathways: insights into insulin action. *Nat Rev Mol Cell Biol* 2006; **7**: 85-96 [PMID: 16493415 DOI: 10.1038/nrm1837]

45 **Vigneri R**, Squatrito S, Sciacca L. Insulin and its analogs: actions via insulin and IGF receptors. *Acta Diabetol* 2010; **47**: 271-278 [PMID: 20730455 DOI: 10.1007/s00592-010-0215-3]

46 **Crowley SD**, Gurley SB, Herrera MJ, Ruiz P, Griffiths R, Kumar AP, Kim HS, Smithies O, Le TH, Coffman TM. Angiotensin II causes hypertension and cardiac hypertrophy through its receptors in the kidney. *Proc Natl Acad Sci USA* 2006; **103**: 17985-17990 [PMID: 17090678 DOI: 10.1073/pnas.0605545103]

47 **Gurley SB**, Riquier-Brison AD, Schnermann J, Sparks MA, Allen AM, Haase VH, Snouwaert JN, Le TH, McDonough AA, Koller BH, Coffman TM. AT1A angiotensin receptors in the renal proximal tubule regulate blood pressure. *Cell Metab* 2011; **13**: 469-475 [PMID: 21459331 DOI: 10.1016/j.cmet.2011.03.001]

48 **Harris PJ,** Young JA. Dose-dependent stimulation and inhibition of proximal tubular sodium reabsorption by angiotensin II in the rat kidney. *Pflugers Arch* 1977; **367**: 295-297 [PMID: 556854 DOI: 10.1007/BF00581370]

49 **Horita S,** Zheng Y, Hara C, Yamada H, Kunimi M, Taniguchi S, Uwatoko S, Sugaya T, Goto A, Fujita T, Seki G. Biphasic regulation of Na -HCO3- cotransporter by angiotensin II type 1A receptor. *Hypertension* 2002; **40**: 707-712 [PMID: 12411466 DOI: 10.1161/01.HYP.0000036449.70110.DE]

50 **Schuster VL,** Kokko JP, Jacobson HR. Angiotensin II directly stimulates sodium transport in rabbit proximal convoluted tubules. *J Clin Invest* 1984; **73**: 507-515 [PMID: 6699174 DOI: 10.1172/JCI111237]

51 **Zheng Y**, Horita S, Hara C, Kunimi M, Yamada H, Sugaya T, Goto A, Fujita T, Seki G. Biphasic regulation of renal proximal bicarbonate absorption by luminal AT(1A) receptor. *J Am Soc Nephrol* 2003; **14**: 1116-1122 [PMID: 12707382]

52 **Haithcock D,** Jiao H, Cui XL, Hopfer U, Douglas JG. Renal proximal tubular AT2 receptor: signaling and transport. *J Am Soc Nephrol* 1999; **10** Suppl 11: S69-S74 [PMID: 9892143]

53 **Houillier P,** Chambrey R, Achard JM, Froissart M, Poggioli J, Paillard M. Signaling pathways in the biphasic effect of angiotensin II on apical Na/H antiport activity in proximal tubule. *Kidney Int* 1996; **50**: 1496-1505 [PMID: 8914015 DOI: 10.1038/ki.1996.464]

54 **Poggioli J,** Lazar G, Houillier P, Gardin JP, Achard JM, Paillard M. Effects of angiotensin II and nonpeptide receptor antagonists on transduction pathways in rat proximal tubule. *Am J Physiol* 1992;

263: C750-C758 [PMID: 1329542]
55 **Li Y**, Yamada H, Kita Y, Kunimi M, Horita S, Suzuki M, Endo Y, Shimizu T, Seki G, Fujita T. Roles of ERK and cPLA2 in the angiotensin II-mediated biphasic regulation of Na+-HCO3(-) transport. *J Am Soc Nephrol* 2008; **19**: 252-259 [PMID: 18094367 DOI: 10.1681/ASN.2007030289]
56 **Liu FY,** Cogan MG. Angiotensin II stimulates early proximal bicarbonate absorption in the rat by decreasing cyclic adenosine monophosphate. *J Clin Invest* 1989; **84**: 83-91 [PMID: 2544631 DOI: 10.1172/JCI114174]
57 **Zhang C**, Mayeux PR. NO/cGMP signaling modulates regulation of Na+-K+-ATPase activity by angiotensin II in rat proximal tubules. *Am J Physiol Renal Physiol* 2001; **280**: F474-F479 [PMID: 11181409]
58 **Shirai A**, Yamazaki O, Horita S, Nakamura M, Satoh N, Yamada H, Suzuki M, Kudo A, Kawakami H, Hofmann F, Nishiyama A, Kume H, Enomoto Y, Homma Y, Seki G. Angiotensin II dose-dependently stimulates human renal proximal tubule transport by the nitric oxide/guanosine 3',5'-cyclic monophosphate pathway. *J Am Soc Nephrol* 2014; **25**: 1523-1532 [PMID: 24511122 DOI: 10.1681/ASN.2013060596]
59 **Shultz PJ**, Tolins JP. Adaptation to increased dietary salt intake in the rat. Role of endogenous nitric oxide. *J Clin Invest* 1993; **91**: 642-650 [PMID: 7679414 DOI: 10.1172/JCI116244]
60 **Tolins JP**, Shultz PJ. Endogenous nitric oxide synthesis determines sensitivity to the pressor effect of salt. *Kidney Int* 1994; **46**: 230-236 [PMID: 7523754 DOI: 10.1038/ki.1994.264]
61 **Ortiz PA**, Garvin JL. Role of nitric oxide in the regulation of nephron transport. *Am J Physiol Renal Physiol* 2002; **282**: F777-F784 [PMID: 11934686]
62 **Facchini FS**, DoNascimento C, Reaven GM, Yip JW, Ni XP, Humphreys MH. Blood pressure, sodium intake, insulin resistance, and urinary nitrate excretion. *Hypertension* 1999; **33**: 1008-1012 [PMID: 10205239 DOI: 10.1161/01.HYP.33.4.1008]
63 **Schmidt RJ**, Beierwaltes WH, Baylis C. Effects of aging and alterations in dietary sodium intake on total nitric oxide production. *Am J Kidney Dis* 2001; **37**: 900-908 [PMID: 11325670]
64 **Parker JD**, Farrell B, Fenton T, Cohanim M, Parker JO. Counterregulatory responses to continuous and intermittent therapy with nitroglycerin. *Circulation* 1991; **84**: 2336-2345 [PMID: 1835676 DOI: 10.1161/01.CIR.84.6.2336]
65 **White WB**, Halley SE. Comparative renal effects of intravenous administration of fenoldopam mesylate and sodium nitroprusside in patients with severe hypertension. *Arch Intern Med* 1989; **149**: 870-874 [PMID: 2565105 DOI: 10.1001/archinte.1989.00390040080016]
66 **Sarafidis PA**, Lasaridis AN. Diabetic nephropathy: Endothelin antagonism for diabetic nephropathy. *Nat Rev Nephrol* 2010; **6**: 447-449 [PMID: 20664627 DOI: 10.1038/nrneph.2010.86]

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ORIGINAL ARTICLE

Case Control Study

Histopathology of renal asphyxia in newborn piglets: Individual susceptibility to tubular changes

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Informed consent: The research complies with the guidelines for human studies and animal welfare regulations. The authors declare that subjects have given their informed consent.
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Abstract

AIM: To analyze the effects on the kidney of hypoxia-reoxygenation in an experimental model of normocapnic asphyxia.

METHODS: To this end, 40 newborn Landrace/Large-White piglets aged 1-4 d were studied in this work. Hypoxia was induced by decreasing the inspired fiO2 to 0.06-0.08. Animals were resuscitated with different fiO_2 and subdivided into 4 groups: group 1, 2, 3 and 4 received 18%, 21%, 40% and 100% O_2 respectively. Macroscopic examination was carried out to evidence possible pathological features. Tissue sample were obtained from both kidneys. Four or five micron paraffin sections were stained with H-E and PAS stain and examined under an optical microscope.

RESULTS: Pathological changes, mainly affecting tubular cells, were observed in the vast majority of kidneys of asphyxiated piglets. The most frequent tubular changes were: tubular casts (95%), tubular dilatation (87.5%), tubular vacuolization (70%), tubular eosinophilia (52.5%), sloughing (50%), fragmentation of the brush border (50%), oedema (32.5%), apoptosis (15%) and glomerular changes (meningeal cell proliferation, capsular adhesion between the flocculus and Bowman's capsule, glomerulosclerosis and fibrous or cellular crescents associated with collapse of the glomerular tuft). Statistical analysis was carried out on changes observed when the animals were allocated in the 4 groups (χ^2-test 0.05). The statistical analysis showed no evidence of differences regarding kidney lesions among the animals groups.

CONCLUSION: Our data show that renal pathology in newborn piglets is characterized by interindividual variability to hypoxia and is not associated with oxygen

concentration.

Key words: Asphyxia; Kidney; Tubular eosinophilia; Tubular dilatation; Vacuolization; Sloughing; Apoptosis; Brush border fragmentation

Core tip: This work studied pathological renal changes following hypoxia-reoxygenation using an established experimental model of normocapnic asphyxia. Tubular dilatation, vacuolization, tubular eosinophilia, sloughing, fragmentation of the brush border and apoptosis were the most frequent changes detected in proximal tubules. Tubular dilatation, vacuolization and sloughing were the earliest lesions, interstitial oedema and apoptosis the late ones. In newborn piglets undergoing asphyxia, renal pathology was not associated with oxygen concentration used during resuscitation.

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INTRODUCTION

Perinatal asphyxia is generally considered an important aetiological factor at the basis of neonatal mortality and morbidity in survivors, with involvement of all organs, including the kidney[1-3]. The kidney is particularly susceptible to hypoxia[4], since the renal tubular cells are characterized by a high metabolism rate, due to high demand and consumption of oxygen[5-7].

Neonates on the first or second day post-asphyxia, often present with symptoms of acute renal impairment or failure, including oliguria, anuria, oedema, and hyperkalemia[8]. Acute renal injury is a common condition encountered in neonates admitted to Neonatal Intensive Care Units[9,10], occurring in as many as 8% of them, and carrying a mortality rate of around 40%[11,12]. Acute kidney injury is generally considered to be related to severe pathological changes in proximal tubular cells, which are considered the typical diagnostic signs of the disease[13,14].

The most severe renal damage has been described to occur 2 h post-asphyxia. It is mainly attributed to the reperfusion injury of the tubular epithelial cells by free oxidant radicals accumulation and the influx of Ca^{2+}[15]. This overload may activate the cysteine protease calpain in tubular cells, leading to hydrolysis of integrins and cytoskeleton components such as F-actin, talin, alpha-actin, and filamin, resulting in tubular cell membrane damage[16].

Acute renal failure has been reported to cause structural changes in tubular renal cells, including loss of brush border, vacuolization of tubular cells, apoptosis and cast formation of necrotic epithelial cells[6]. In experimental animal models of hypoxia, only minor histological changes, including mild brush border loss and vacuolization of tubular cells, are present in the kidney at 22 h post-peritonitis-induced septic shock in pigs[17]. Piglets subjected to mild hypothermic cardiopulmonary bypass exhibit tubular dilatation, vacuoles, leukocyte infiltration, epithelial destruction and interstitial oedema[18]. In cultured glomerular endothelial cells, hypoxia has been shown to induce apoptosis[19].

Resuscitation strategies modify the relationship between the inhibitors and the factors that promote angiogenesis. In particular, reoxygenation using 100% oxygen of neonatal piglets has been shown to decrease serum levels of angiostatin[20].

On clinical grounds, several questions remain unanswered at the moment: how does asphyxia cause acute renal tubular injury in neonates and what histological change corresponds to tubular dysfunction? What is the role of oxygen concentration used during resuscitation in determining kidney injury?

In the present study we investigated the renal injury caused at the histological level by hypoxia-reoxygenation in an experimental neonatal swine model previously described by our group[21].

MATERIALS AND METHODS

Forty male Landrace/Large White newborn piglets, weighing 2.3-3.8 kg and aged 1-4 d were studied in this work. All experimental piglets came from the same breeding unit. Experimental procedures were previously approved by the General Directorate of Veterinary Services (Permit no. 404/21-04-09)[21].

Briefly, following sedation with 10 mg/kg ketamine and 0.5 mg/kg midazolam, anesthesia was induced with 1 mg/kg propofol and 10 mg/kg fentanyl, administered through a peripheral vein. Hypoxia was induced by decreasing the inspired fiO_2 to 0.06-0.08; resuscitation efforts were carried out according to the Newborn Life Support (NLS) algorithm[1]. Animals were resuscitated with different fiO_2 and subdivided into 4 groups: groups 1, 2, 3 and 4 received 18%-21%-40% and 100% O_2 respectively (Table 1). Surviving animals were euthanized by intravenous infusion of 30 mg/kg sodium thiopental (Pentothal, Hospira Enterprises BV, The Netherlands). A macroscopic examination was carried out to evidence possible pathological features. Tissue samples were obtained from both kidneys, fixed in 10% formalin, routinely processed, paraffin embedded. Four or five micron paraffin sections were stained with H-E and PAS stain and examined under an optical microscope. Depending on recovery time, the experimental animals were subdivided into 5 groups (Table 2): group A: fast recovery (< 15 min); group

Table 1 Animal groups according to oxygen concentration used for resuscitation

	F. O_2	Deaths	Survivors	Mean time of resuscitation (min)
Group 1	18%	3	7	5-26
Group 2	21%	1	9	7-75
Group 3	40%	2	8	12-142
Group 4	100%	3	7	30-120

B: medium (15-45 min); group C: slow (45-90 min); group D: very slow recovery (> 90 min), and group E: dead animals.

Kidney samples from 4 male Landrace/large White newborn piglets not submitted to hypoxia served as subjects for the control group. Statistical analyses were based on the use of the χ^2 test.

RESULTS

Kidneys from the control group preserved their architecture. In the subcapsular regions, active glomerulogenesis was detected, represented by the tubule-glomerular nodules recently reported by our group as the typical developing unit in piglets[21]. Moreover, in the deep cortex, some senescent glomeruli with different degrees of sclerosis were detected and were occasionally associated with cellular or fibrotic crescents. Few scattered PAS-positive tubular casts were also observed in all control kidneys.

Pathological changes, mainly affecting tubular cells, were observed in the vast majority of kidneys from asphyxiated piglets. The most frequent tubular changes are illustrated in Figure 1.

Tubular dilatation, mainly affecting distal tubules, was observed in 35/40 cases (87.5%); in 7 of the 35 positive cases, tubular dilatation was marked and diffuse. Dilatation was found to occur even in the subcapsular regions, in developing nephrons and, in particular, in renal vesicles and S-shaped bodies.

Tubular vacuolization was detected in 28/40 (70%) cases with an incidence similar to that of tubular dilatation. In the majority of cases, tubular vacuolization was focal, whereas in 7 cases it was diffuse. In kidneys with focal tubular vacuolization the lesions were mainly observed in the deep cortex at the cortico-medullary boundary. Only in rare cases did vacuolization affect tubular structures in the subcapsular regions. Vacuolization was mainly observed in proximal tubules. In the majority of affected kidneys, the size of vacuoles varied widely among cases: multiple microvacuoli were observed but, in few cases, larger vacuoles were found, sometimes occupying entirely the cytoplasm of affected tubular cells (Figure 2).

Apoptosis: this was diagnosed on the basis of morphological changes. It was detected only in 6/40 (15%) cases, was mainly focal and occurred in proximal tubular cells. Cells undergoing apoptosis showed cell fragmentation with the formation of multiple eosinophilic globules, some of which contained nuclear remnants (Figure 3). Apoptotic bodies often appeared strongly PAS-positive.

Tubular eosinophilia was observed in 21/40 (52.5%) cases. Eosinophilic changes were mainly focal, localized in tubular structures of single nephrons with their cytoplasm intensely stained by eosin. Eosinophilia was detected in the superficial and deep cortical regions: subcapsular areas with active nephrogenesis were frequently affected. At high power, tubules affected by eosinophilia often showed associated degenerative changes, including vacuolization.

Tubular casts were observed in 38/40 cases (95%), as well as in the kidneys from the control group. Casts were mainly hyaline, detected in areas affected by interstitial oedema and tubular cell vacuolization. Occasionally, granular casts were found in areas with tubular cells apoptosis.

Oedema was observed in the interstitial space of the cortical region in 13/40 cases (32.5%): in 10 cases it was focal and mainly localized in the subcortical regions; in 3 cases it was diffuse in the whole cortex, including the deep areas.

Sloughing. Detachment of tubular cells from the basal membrane was observed in 20/40 cases (50%) mainly in proximal tubuli. Inside the affected tubules, sloughing was focal, with scattered cells detaching from the basal lamina of tubuli. Sloughing was unevenly distributed throughout the entire cortex, with no preference for superficial or deep cortical zones.

Fragmentation of the brush border. In 20 out of 40 cases (50%), proximal tubules at high power revealed a previously underestimated lesion: the brush border had lost its integrity and appeared fragmented. Brush border fragmentation was often associated with sloughing and with other tubular changes, including dilatation and cytoplasmic vacuolization.

Glomerular changes were found in all samples examined, most frequently in the deep cortex and at the cortico-medullary junction. Mesangial cell proliferation, capsular adhesion between the flocculus and Bowman' s capsule, glomerulosclerosis and fibrous or cellular crescents associated with collapse of the glomerular tuft (Figure 4) were detected. In some cases, these glomerular lesions were also detected in the subcapsular regions, affecting glomeruli during the initial phases of their development. Focal glomerulosclerosis and hyalinization were observed even in controls, but always restricted to the deep cortex.

As for the glomerular changes, these lesions will not be discussed, given their presence in all the control kidneys examined. They might represent physiological senescence of "not well developed" glomeruli.

Statistical analysis was carried out on changes observed when the animals were allocated in the 4 groups depending on the concentration of oxygen used

Table 2 Animal groups according to time of resuscitation

	Resuscitation				
	Group A Fast < 15′ (%)	Group B Middle 16-45′ (%)	Group C Slow 45-90′ (%)	Group D Very slow > 90′ (%)	Group E Death (%)
Oedema	12.5	50	28.50	0	44.40
Eosinophilia	50	50	42.80	100	44.40
Tubular dilatation	87.50	83.30	85.70	75	100
Casts	100	100	85.70	100	88.80
Tubular vacuoles	75	83.30	42.80	75	66.60
Sloughing	75	41.60	42.80	25	55.50
Brush border fragmentation	75	41.60	42.80	25	55.50
Apoptosis	25	8.30	0	25	22.20

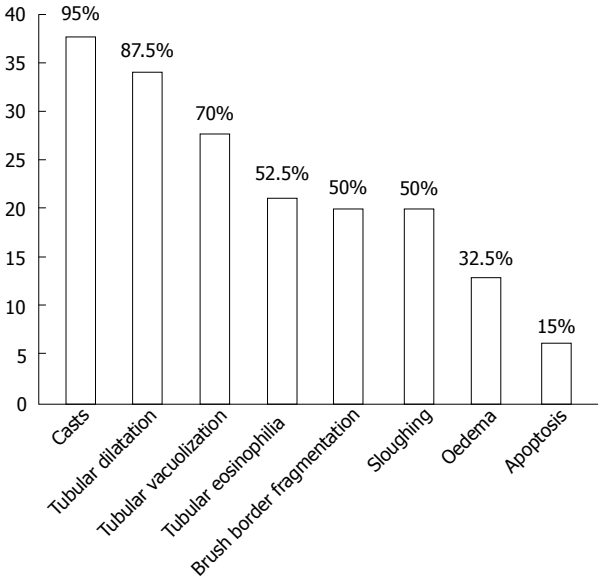

Figure 1 Percentage of the elementary lesions in the kidney.

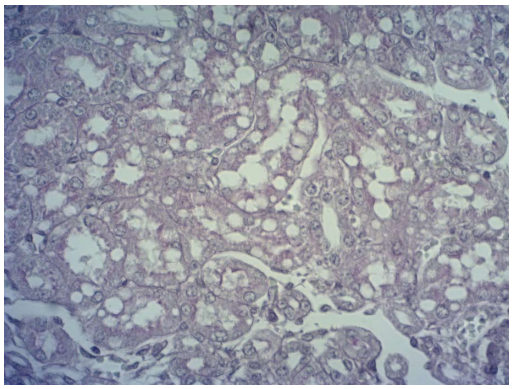

Figure 2 Vacuolation of the proximal tubules.

during resuscitation and when they were allocated in 5 groups on the basis of the time of resuscitation. Statistical analysis showed no evidence of differences regarding kidney lesions among animals groups.

DISCUSSION

Despite improvement in knowledge over the last

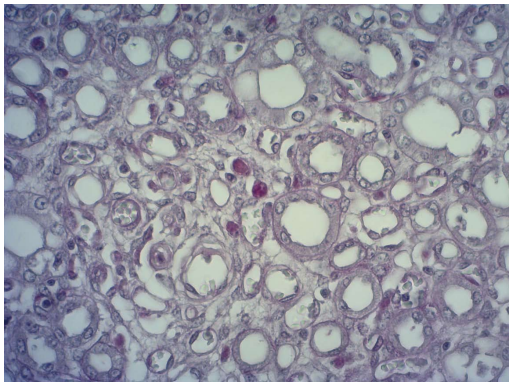

Figure 3 Apoptosis.

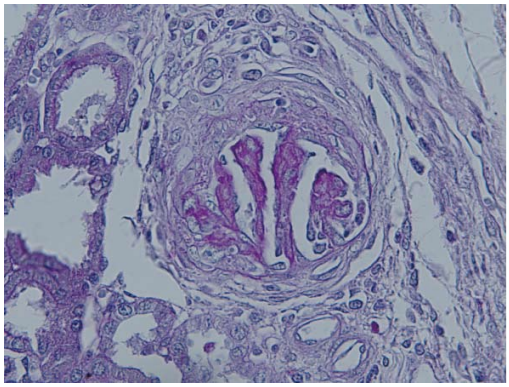

Figure 4 Fibrous and cellular crescents associated with collapse of the glomerular tuft.

decade[5] regarding the molecular mechanisms by which perinatal asphyxia causes tissue damage, the insult leading to acute kidney injury is partly unknown and still under discussion. Some authors claim that the predominant role in tubular cell death is played by hypoxia *per se*, while others attribute this to dysoxia 4 or reoxygenation[20].

This study presents data on histological changes occurring in kidneys of newborn piglets exposed to acute hypoxia-reoxygenation. The degenerative and necrotic changes were predominantly localized in the proximal tubules, followed by distal tubules, in absence of significant pathological changes in glomerular cells

and vascular structures. These data confirm that kidney proximal tubular cells are the main target of hypoxia, due to their high physiological metabolic rate[4] and high rate of aerobic glycolysis[5]. Distal tubular cells, despite their lower metabolism rate, were however not devoid of pathological changes in this study, with tubular dilatation being the main one. Tubular vacuolization was detected both in proximal and distal tubules.

As for the role of oxygen concentration used for resuscitation in acute kidney injury, the present data suggest that it did not play a significant role in acute kidney injury following hypoxia-reoxygenation in our experimental model. No significant difference in the incidence of renal pathological changes among animals of groups 1-4 were detected (Table 1). However, we cannot exclude long-term effects of different oxygen concentrations in the kidney.

Interesting data on the changes of renal cells were obtained when piglets were subdivided into groups A-E depending on the time required for resuscitation (Table 2). Interstitial oedema appeared to be a "late" lesion, detected in only 12.5% of animals in group A. On the contrary, eosinophilia of tubular cells should be considered an "early" lesion as it was found in 50% of piglets in group A, and a similar percentage was found in deceased animals. Tubular dilatation appeared frequently in group A (87.5%) thus suggesting that it is a "very early" response of kidney structures to hypoxia. The observation of dilated renal tubules in 100% of deceased piglets reinforces the hypothesis of a major role of this early pathological lesion in the pathogenesis of acute kidney injury following hypoxia. Tubular casts were observed in the majority of animals, ranging from 85.7% up to 100% of cases (Figure 1). This association with their detection in control kidneys suggests a minor, if any, role of casts in acute kidney injury.

As for the vacuolization occurring in the cytoplasm of proximal and distal tubular cells, it appears to be an "early" lesion, observed in 75% of piglets in group A. Contrary to other lesions, the incidence of this pathological change had not significantly increased in animals with a longer time of resuscitation and was found in 66.6% of deceased animals, thus suggesting that some of them probably did not survive long enough after asphyxia for cytoplasmic vacuoles to appear. Sloughing and brush border changes appeared to be "early" lesions observed in 75% of animals in group A and decreased in piglets with late and very late (> 90 min) resuscitation, finally to be observed in about 50% of deceased animals (Table 2).

The rare presence of apoptotic globules with nuclear condensation and fragmentation in animals of group A may be attributed to the short time to complete the whole process of apoptotic cell death. The same explanation can be applied to deceased animals: as apoptosis is an active process, we can speculate that the majority of deceased piglets did not survive long enough to allow apoptosis to occur. On the contrary, the incidence of apoptotic globules in kidneys of animals characterized by a very late resuscitation time (> 90 min) suggests an important role of apoptosis in asphyxia-related acute kidney injury. We may also speculate that sloughing should be considered the morphological sign of tubular cell apoptosis: the detachment of tubular cells from each other and from the basal lamina and their removal by the urinary flow may be a limit for the development of the complete sequence of the apoptotic process, thus limiting their finding to few kidney samples.

Our data clearly show that the Landrace/Large White piglet model provides useful data for the study of hypoxia-induced kidney injury. Tubular dilatation, tubular cell vacuolization, sloughing and brush border changes are the main, earliest and most severe lesions in the post-asphyxia kidney. A strong interindividual variability in the severity of renal changes to asphyxia was observed. The extent of kidney lesions was not associated with the concentration of oxygen used during resuscitation, thus suggesting a previously unreported individual susceptibility to hypoxia. Further studies on pathological kidney changes from human and non-human models are needed to verify their role in the short- and long-term kidney damage following hypoxia-reoxygenation.

COMMENTS

Background

The study focuses on the histological renal change following asphyxia in a experimental model of neonatal asphyxia. On the basis of recent data suggesting a role of reoxygenation in the development of renal lesions, the authors evaluated the presence and degree of renal lesions in piglets submitted to different oxygen percentages following asphyxia.

Research frontiers

The study is mainly related to the research field of tissue damage following asphyxia and reoxygenation in the perinatal period.

Innovations and breakthroughs

The most important data of the study regard the usefulness of the Landrace/Large White piglet model for the study of hypoxia-induced kidney injury. The strong interindividual variability in the severity of renal changes to asphyxia described in the study represents a new finding that may lead the neonatologist towards an individualized sartorial approach in each newborn affected by perinatal asphyxia.

Applications

In the study, tubular dilatation, tubular cell vacuolization, sloughing and brush border changes are the main, earliest and most severe lesions observed in the post-asphyxia kidney. These data may help pathologists involved in the study of asphyxia-induced renal pathology.

Peer-review

The study is interesting and well-conducted.

REFERENCES

1 **Bryce J**, Boschi-Pinto C, Shibuya K, Black RE. WHO estimates of the causes of death in children. *Lancet* 2005; **365**: 1147-1152 [PMID: 15794969]

2 **Huang CC**. Perinatal hypoxic/ischemic encephalopathy: clinical challenge and experimental implications. *Zhonghua Minguoxiaoerke Yixuehui Zazhi* 1998; **39**: 157-165 [PMID: 9684520]

3 **Martín-Ancel A**, García-Alix A, Gayá F, Cabañas F, Burgueros M, Quero J. Multiple organ involvement in perinatal asphyxia. *J Pedi-*

atr 1995; **127**: 786-793 [PMID: 7472837]
4 **Legrand M**, Mik EG, Johannes T, Payen D, Ince C. Renal hypoxia and dysoxia after reperfusion of the ischemic kidney. *Mol Med* 2008; **14**: 502-516 [PMID: 18488066 DOI: 10.2119/2008-00006]
5 **Fanos V**, Dessì A, D'Aloja E, Finco G, Iacovidou N, Faa G. The kidney in post asphytic syndrome: state of the art. In: Fanos V, Chevalier RL, Faa G, Cataldi L, editors. Development nephrology: from embryology to metabolomics. Quartu S. Elena: Hygeia Press, 2011: 159-179
6 **Baricos WH**. Protease mediated tubular injury: a new direction in acute renal failure? *Kidney Int* 2002; **61**: 1174-1175 [PMID: 11849473]
7 **Rosen S**, Epstein FH, Brezis M. Determinants of intrarenal oxygenation: factors in acute renal failure. *Ren Fail* 1992; **14**: 321-325 [PMID: 1509164]
8 **Subramanian PT**. Acute renal failure in the neonate. *Saudi J Kidney Dis Transpl* 1998; **9**: 320 [PMID: 18408309]
9 **Andreoli SP**. Acute kidney injury in children. *Pediatr Nephrol* 2009; **24**: 253-263 [PMID: 19083019 DOI: 10.1007/s00467-008-1074-9]
10 **Nouri S**, Mahdhaoui N, Beizig S, Zakhama R, Salem N, Ben Dhafer S, Methlouthi J, Seboui H. [Acute renal failure in full term neonates with perinatal asphyxia. Prospective study of 87 cases]. *Arch Pediatr* 2008; **15**: 229-235 [PMID: 18329254 DOI: 10.1016/j.arcped.2008.01.011]
11 **Putensen C**. [Acute renal failure. Still a cause of increased mortality]. *Anaesthesist* 2007; **56**: 1103-1104 [PMID: 17932628]
12 **Stapleton FB**, Jones DP, Green RS. Acute renal failure in neonates: incidence, etiology and outcome. *Pediatr Nephrol* 1987; **1**: 314-320 [PMID: 3153295]
13 **Hoste EA**, Kellum JA. Acute kidney injury: epidemiology and diagnostic criteria. *Curr Opin Crit Care* 2006; **12**: 531-537 [PMID: 17077682]
14 **Rosen S**, Heyman SN. Difficulties in understanding human "acute tubular necrosis": limited data and flawed animal models. *Kidney Int* 2001; **60**: 1220-1224 [PMID: 11576335]
15 **Yu B**, Li S, Yao Y, Lin Z. Changes in beta(1) integrin in renal tubular epithelial cells after intrauterine asphyxia of rabbit pups. *J Perinat Med* 2009; **37**: 59-65 [PMID: 18767988]
16 **Elliget KA**, Phelps PC, Trump BF. Cytosolic Ca2+ elevation and calpain inhibitors in HgCl2 injury to rat kidney proximal tubule epithelial cells. *Pathobiology* 1994; **62**: 298-310 [PMID: 7598799]
17 **Chvojka J**, Sykora R, Krouzecky A, Radej J, Varnerova V, Karvunidis T, Hes O, Novak I, Radermacher P, Matejovic M. Renal haemodynamic, microcirculatory, metabolic and histopathological responses to peritonitis-induced septic shock in pigs. *Crit Care* 2008; **12**: R164 [PMID: 19108740 DOI: 10.1186/cc7164]
18 **Tirilomis T**, Tempes T, Waldmann-Beushausen R, Ballat C, Bensch M, Schoendube FA. Histological changes in neonatal kidneys after cardiopulmonary bypass and deep hypothermic circulatory arrest. *Thorac Cardiovasc Surg* 2009; **57**: 7-9 [PMID: 19169989 DOI: 10.1055/s-2008-1039061]
19 **Tanaka T**, Miyata T, Inagi R, Kurokawa K, Adler S, Fujita T, Nangaku M. Hypoxia-induced apoptosis in cultured glomerular endothelial cells: involvement of mitochondrial pathways. *Kidney Int* 2003; **64**: 2020-2032 [PMID: 14633124]
20 **Emara M**, Obaid L, Johnson S, Bigam DL, Cheung PY. Angiostatins decrease in the kidney of newborn piglets after hypoxia-reoxygenation. *Eur J Pharmacol* 2010; **644**: 203-208 [PMID: 20621087 DOI: 10.1016/j.ejphar.2010.06.051]
21 **Aroni F**, Xanthos T, Varsami M, Argyri I, Alexaki A, Stroumpoulis K, Lelovas P, Papalois A, Faa G, Fanos V, Iacovidou N. An experimental model of neonatal normocapnic hypoxia and resuscitation in Landrace/Large White piglets. *J Matern Fetal Neonatal Med* 2012; **25**: 1750-1754 [PMID: 22489565 DOI: 10.3109/14767058.2012.663823]

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CASE REPORT

Prolonged hypernatremia triggered by hyperglycemic hyperosmolar state with coma: A case report

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Ethics approval: NA.
Informed consent: Approval for this case report was obtained from the human research committee of the Raymond G Murphy VA Medical Center.
Conflict-of-interest: The authors have no conflicts of interest to declare.

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Abstract

A man with past lithium use for more than 15 years, but off lithium for two years and not carrying the diagnosis of diabetes mellitus or nephrogenic diabetes insipidus (NDI), presented with coma and hyperglycemic hyperosmolar state (HHS). Following correction of HHS, he developed persistent hypernatremia accompanied by large volumes of urine with low osmolality and no response to desmopressin injections. Urine osmolality remained < 300 mOsm/kg after injection of vasopressin. Improvement in serum sodium concentration followed the intake of large volumes of water plus administration of amiloride and hydrochlorothiazide. Severe hyperglycemia may trigger symptomatic lithium-induced NDI years after cessation of lithium therapy. Patients with new-onset diabetes mellitus who had been on prolonged lithium therapy in the past require monitoring of their serum sodium concentration after hyperglycemic episodes regardless of whether they do or do not carry the diagnosis of NDI.

Key words: Hypertonicity; Lithium; Hypernatremia; Hyperglycemia; Nephrogenic diabetes insipidus

Core tip: Hyperglycemic coma with large losses of body water may aggravate lithium-induced nephrogenic diabetes insipidus (NDI) which had been asymptomatic and undiagnosed for years after cessation of lithium therapy. The development of conditions leading to loss of water and consciousness in patients who were on long term lithium therapy should trigger surveillance for NDI even when they were asymptomatic in the past.

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INTRODUCTION

Hypertonicity resulting from excessive losses of body water through the kidneys, the respiratory tract, the skin, the gastrointestinal tract and/or gain in body solute, causes neurological manifestations that may become life threatening[1,2]. Hypernatremia[3] and hyperglycemia[4] are the two common causes of hypertonicity. Severe hyperglycemia developing on the ground of another condition potentially causing hypernatremia may lead to extreme hypertonicity. We present a patient who developed coma from hyperglycemic hyperosmolar state (HHS) followed by prolonged hypernatremia. Nephrogenic diabetes insipidus (NDI) secondary to chronic lithium intake was diagnosed during the period of hypernatremia. NDI had apparently persisted despite discontinuation of lithium two years prior to the HHS, but had not been diagnosed because of absence of hypernatremia and lack of symptoms of hypertonicity.

CASE REPORT

Calculated values, summary statistics

Calculated values: Serum tonicity (effective osmolarity), mOsm/L[5] = 2 × serum sodium concentration ([Na]) + serum glucose concentration ([Glu])/18. Corrected serum sodium concentration[6]: [Na] at hyperglycemia corrected to a [Glu] value of 100 mg/dL by the use of Katz's[7] correction factor, which computes that a 100 mg/dL rise in [Glu] causes a 1.6 mmol/L depression in [Na]:

$[Na]_{Corrected}$, mmol/L = [Na] + 0.016 × ([Glu] - 100)

Calculated serum osmolarity, mOsm/L[5] = 2 × [Na] + [Glu]/18 + blood urea nitrogen ([BUN])/2.8

Summary statistics: Parametric variables are presented as mean ± SD.

Patient report

A 58-year-old man with bipolar disorder was admitted with HHS and coma. He had been treated in the past with lithium carbonate for more than 15 years. During that period, serum lithium level was 0.72 ± 0.27 mmol/L (36 determinations) with two values, 1.3 and 1.4 mmol/L above the therapeutic range (0.5-1.2 mmol/L); in 22 determinations, average [Na] and [Glu] values were within the normal range (Table 1), with one [Na] value, at 146 mmol/L, above the upper normal limit of 145 mmol/L and one [Glu] value was in the hyperglycemic range (171 mg/dL); and in 15 determinations urine specific gravity was 1.008 ± 0.004. The urine specific gravity of all five urinalyses obtained in the last five years of this period was ⩽ 1.005.

Two years prior to the admission he moved to another town and discontinued the intake of lithium. Two months prior to admission with HHS, he resumed his visits to the outpatient clinics of this hospital after a large left lung mass was diagnosed. Positron emission tomography (PET) study showed a left lung mass, 10.9 cm in diameter invading the left main bronchus and the wall of the left pulmonary artery and involvement of several lymph nodes. Lung biopsy revealed squamous cell carcinoma.

He refused treatment for his tumor and opted for palliative management. He did not carry the diagnosis of diabetes mellitus or diabetes insipidus up to that time. During subsequent outpatient visits, progressive hyperglycemia was noted in. Three successive blood samples (Table 1). He refused admission when [Glu] was 809 mg/dL, but was admitted in deep coma three days later. On admission, blood pressure was 147/87 mmHg and heart rate 87 beats per minute. His mucosae were dry. Initial serum chemistries revealed hyperglycemia and profound hypertonicity (Table 1). In addition, BUN was 67 mg/dL, and serum potassium 3.7 mmol/L, total carbon dioxide 16 mmol/L, creatinine (previously in the normal range) 2.49 mg/dL, phosphorus 6.2 mg/dL, magnesium 4.2 mg/dL, lactate 3.4 mmol/L and calculated serum osmolarity 428.6 mOsm/L. The urine had a specific gravity of 1.016 and contained > 500 mg/dL of glucose, but no acetone. Arterial blood pH was 7.01, P_aO_2 102 mmHg (on nasal oxygen supplementation), P_aCO_2 71 mmHg and calculated bicarbonate 13.2 mEq/L. Chest X-ray showed a large mass in the left lung displacing the trachea to the right and several enlarged noncalcified lymph nodes in both lung fields. These findings were unchanged from those in recent earlier chest X-rays.

He received endotracheal intubation with mechanical ventilation, continuous infusion of insulin and large volumes of hypotonic saline containing potassium chloride. Large urine output was noted from the onset of treatment. Progressive decline in [Glu] was documented (Table 1). In a blood sample obtained four hours after onset of treatment, BUN was 66 mg/dL, serum creatinine 2.33 mg/dL, and calculated osmolarity 418.2 mOsm/L, while a simultaneously measured serum osmolality was 424 mOsm/kg. Following these measurements he received larger volumes of water in his infusions and through a gastric tube.

Hyperglycemia, hypokalemia, and hyperphosphatemia were corrected by 48 h after initiation of treatment. At that time, BUN was 48 mg/dL and calculated serum osmolarity 451.5 mOsm/L. Serum creatinine and magnesium declined progressively and reached normal levels by 72 h after the start of treatment. He was extubated on the fourth hospital day. However,

Table 1 Serum chemistries and calculated values related to tonicity

Time	[Glu]	[Na]	Tonicity	[Na]Corrected
	mg/dL	mmol/L	mOsm/L	mmol/L
-15 to -2 yr[1a]	99.6 ± 22.0	141.1 ± 2.7	287.6 ± 5.6	141.0 ± 2.7
-21 d[1]	235	139	291.1	141.6
-14 d[1]	304	141	298.9	144.3
-3 d[1]	809	135	314.9	146.3
Admission	1236	168	404.7	186.2
+2 h[2]	1141	169	401.4	185.7
+4 h[2]	982	170	394.6	184.1
+10 h[2]	771	165	372.8	175.7
+14 h[2]	650	163	362.1	171.8
+18 h[2]	611	160	353.9	168.2
+22 h[2]	548	165	360.4	172.2
+24-48 h[2]	233.5 ± 151.7	164.0 ± 1.9	341.0 ± 9.7	166.0 ± 3.3
+48-72 h[2]	176.6 ± 72.3	168.0 ± 1.9	345.8 ± 7.6	169.2 ± 3.0
+72-96 h[2]	151.8 ± 23.0	165.3 ± 2.6	338.9 ± 4.7	166.1 ± 2.1
+96-120 h[2]	185.0 ± 99.0	176.5 ± 2.1	363.3 ± 1.3	177.9 ± 0.5
+120-144 h[2]	131	172	351.3	172.5
+144-168 h[2]	135	157	321.5	157.6
+168-192 h[2]	186.5 ± 139.3	158.0 ± 9.9	326.4 ± 27.5	159.4 ± 12.2
+9-14 d[2]	175.5 ± 53.7	146.8 ± 1.8	303.5 ± 4.4	147.9 ± 1.8
+14-21 d[2]	241.3 ± 55.6	143.0 ± 3.9	299.4 ± 7.0	145.2 ± 3.7
+3 mo[2]	240	155	323.3	157.2

[1]Time before admission; [2]Time after onset of treatment; [a]Period of lithium intake; [Glu]: Serum glucose concentration; [Na]: Serum sodium concentration; [Na]Corrected: Serum sodium concentration corrected to a serum glucose level of 100 mg/dL; Values reported as mean ± SD represent 2-22 measurements.

production of copious volumes of dilute urine, confusion and severe hypernatremia persisted despite the combined administration of up to 400 mL per hour of 5% dextrose intravenously and free water by nasogastric tube. Over the four days following normalization of glycemia, he received progressively larger injections of desmopressin (from 1 to 4 mcg), but post-injection urine osmolality values ranged between 139 and 180 mOsm/kg, while [Na] ranged between 164 and 171 mmol/L, [Glu] between 84 and 281 mg/dL and [Na]Corrected between 164.7 and 173.7 mmol/L.

On day seven of admission, simultaneous serum and urine measurements revealed the following values: [Na] 161 mmol/L, serum osmolality 330 mOsm/kg, serum vasopressin 4.6 pg/mL, serum lithium undetectable and urine osmolality 279 mOsm/kg. Immediately following these measurements, he received an injection of 5 units of vasopressin. One hour post-injection, urine osmolality was 290 mOsm/kg. Over the next 20 d, his mental status improved slowly, [Glu] ranged between 69 and 304 mg/dL, while [Na] and [Na]Corrected remained elevated (Table 1).

Hypernatremia improved slowly after increase in water intake and administration of amiloride and hydrochlorothiazide. His last two [Na] values were in the normal range. He left the hospital against medical advice after he was declared competent to make treatment decisions by a Psychiatrist. He was advised to continue the medications for hypernatremia and to have a liberal water intake. Two months later he returned with progressive dyspnea. Computed chest tomography revealed increases in the size of lymph nodes and a large clot in the right pulmonary artery. [Na] was elevated (Table 1). He expired in respiratory failure within 48 h of his last admission. Table 1 shows tonicity values throughout his follow-up.

DISCUSSION

This report of a patient developing protracted hypernatremia following treatment of severe HHS illustrates the following clinical points: (1) the level of hypertonicity can become extreme in patients with HHS that remains untreated for several days; (2) Lithium-induced NDI that remained asymptomatic and undiagnosed for years after cessation of lithium therapy can cause severe hypernatremia in patients who encounter difficulties in consuming adequate volumes of water.

Tonicity of the serum is its property to cause osmotic transfers of water into or out of cells suspended in it. [Na] is, in general, an accurate indicator of serum tonicity[8]. Gain in extracellular solutes other than sodium salts, such as glucose, is the main exception to this rule. Hypertonicity in hyperglycemia should be evaluated in two steps: (1) At presentation, the degree of hypertonicity, which results from extracellular accumulation of solute (glucose)[9] and loss of water through osmotic diuresis[10,11], determines the severity of the presenting clinical manifestations is calculated by the tonicity formula[1,5]; (2) The prescription of the tonicity (*i.e.*, sodium plus potassium concentration) of the replacement solutions should be based on [Na]Corrected[12], reflecting the fact that correction of hyperglycemia without any further changes in the external balances of water and monovalent cations leads to rise in [Na], but decrease in serum effective osmolality[12]. Monitoring of the clinical status and serum chemistries is imperative during treatment of severe HHS[12].

Both serum tonicity and [Na]Corrected were at admission extremely high, indicating profound water deficit, in the patient of this report, who despite infusions of large volumes of hypotonic fluids exhibited subsequently protracted hypernatremia and was eventually diagnosed with NDI by formal testing[13]. Persistently low urine specific gravity values during and after lithium therapy identified lithium as the probable cause of NDI.

Lithium use is associated with a variety of renal functional and structural abnormalities[14,15]. NDI is the most prevalent lithium-induced disorder. Lithium enters the principal cells of the collecting ducts through luminal (apical) epithelial sodium channels (ENaC) and inhibits the signaling pathways that involve glycogen synthase 3-beta causing disruption of the aquaporin-2 structure and function and NDI[16,17].

Amiloride is effective in the prevention and treatment of lithium-induced NDI in part because it is an inhibitor of ENaC, while hydrochlorothiazide affects several transport

proteins[18].

Lithium-induced NDI may persist for years after cessation of lithium therapy[19]. Most available reports have found an association between the duration of lithium use and reduced renal concentrating ability supporting a progressive deficit[20]. Movig *et al*[21] reported that 37% of 75 patients receiving lithium developed polyuria (> 3 L/24 h). Polyuria was strongly associated with simultaneous use of serotonergic antidepressants and duration of lithium therapy. Although lithium-induced NDI is often reversible with median duration of therapy (< 6 years), the renal concentrating defect may be permanent after prolonged (> 15 years) therapy with lithium[22]. In large studies with long term follow-up, approximately 15% of patients using lithium demonstrate an irreversible impairment of renal concentration[22]. Several cases of NDI persistence after discontinuation of lithium therapy have been reported[23-29]. Special care is required for patients with this syndrome when they develop medical conditions preventing spontaneous fluid consumption[28].

Another characteristic of lithium-induced NDI is that it may go undiagnosed for years. Patients are able to compensate for this form of NDI, in which the defect in urinary concentration is usually partial, by consuming large fluid volumes. For example the urine volume that is needed for excretion of a solute load of 900 mOsm at a urine osmolality of 300 mOsm/kg is 3 L and can easily be achieved without the development of hypernatremia by patients with normal thirst mechanism.

Lithium-induced NDI can cause severe hypernatremia[30,31] especially after the development of stressful conditions leading to inability of the patients to drink adequate amounts of fluid. We found three reports of four patients on lithium who developed severe hypernatremia secondary to previously undiagnosed lithium-induced NDI in the immediate post- operative period[32-34]. In contrast to these subjects, our patient had stopped lithium intake two years before his admission.

Finally, our patient illustrates the association of manifestations of diabetes mellitus and lithium-induced NDI. Two patients presenting with clinical manifestations of lithium-induced NDI and diabetic ketoacidosis[35] or severe hyperglycemia[36] have been reported. Potential mechanisms of induction of glucose intolerance by lithium were discussed[36]. In addition to the possibility that lithium triggered the development of diabetes mellitus, it is probable that lithium-induced NDI aggravated the water loss secondary to osmotic diuresis in our patient. In osmotic diuresis osmolality values are higher in urine than plasma in all patients except those with diabetes insipidus who exhibit osmolality values lower in urine than in plasma. Thus, water losses from osmotic diuresis are comparatively larger and the hypertonic state that ensues is comparatively more severe in the patients with diabetes insipidus.

Lithium-induced NDI that remained asymptomatic and undiagnosed for years after cessation of lithium therapy may cause severe clinical manifestations of hypertonicity during clinical episodes affecting the patients' access to fluid intake. If these episodes consist of hyperglycemic emergencies, water loss trough combination of hyperglycemic osmotic diuresis and NDI may be massive leading to severe hypertonicity. Patients with severe hyperglycemia who had been on long-term lithium therapy require prolonged attention to their fluid balance after correction of the hyperglycemic episode.

COMMENTS

Case characteristics

Development of hyperglycemic hyperosmolar state (HHS) with profound coma followed by protracted hypernatremia in a patient who had stopped lithium therapy two years in the past.

Clinical diagnosis

Lithium-induced nephrogenic diabetes insipidus (NDI) diagnosed after correction of the HHS by lack of response of the urinary concentration to a formal vasopressin infusion test.

Differential diagnosis

Other causes of hypernatremia including central diabetes insipidus, persistent osmotic diuresis, and inadequate water intake were excluded by appropriate testing.

Laboratory diagnosis

Extreme hyperglycemia and serum effective osmolarity at presentation was followed by protracted hypernatremia which was shown to be the result of NDI by lack of response of urine osmolality to vasopressin infusion.

Imaging diagnosis

Inoperable lung malignant tumor found in chest X-rays, and computed tomography and positron emission tomography scans.

Pathological diagnosis

Squamous cell carcinoma of the lung found on a biopsy of the tumor.

Treatment

Insulin infusion, large volumes of hypotonic fluids given parenterally, by nasogastric tube, and later by mouth, amiloride and hydrochlorothiazide for the HHS and later the NDI, refusal of the patient to receive treatment for his lung tumor.

Related reports

Reports in the literature suggest that lithium-induced NDI may be permanent after cessation of lithium treatment when the duration of lithium therapy exceeded 15 years, while other reports suggest that lithium-induced NDI may cause severe hyponatremia following episodes of severe hyperglycemia.

Experience and lessons

Patients who had been in the past on long-term lithium therapy are at risk of developing severe hypernatremia during episodes that limit their ability to drink water and should have their serum sodium concentration closely monitored during these episodes even if they had not been diagnosed with nephrogenic diabetes insipidus in the past.

Peer-review

This reviewer thinks that it is worth sharing this case with "prolonged hypernatremia triggered by hyperglycemic hyperosmolar state after discontinuation of lithium therapy" by physicians.

REFERENCES

1 **Feig PU**, McCurdy DK. The hypertonic state. *N Engl J Med* 1977; **297**: 1444-1454 [PMID: 337143 DOI: 10.1056/NEJM197712292972608]
2 **Pollock AS**, Arieff AI. Abnormalities of cell volume regulation and their functional consequences. *Am J Physiol* 1980; **239**: F195-F205 [PMID: 7435558]
3 **Adrogué HJ**, Madias NE. Hypernatremia. *N Engl J Med* 2000; **342**:

1493-1499 [PMID: 10816188 DOI: 10.1056/NEJM200005183422006]
4 **Arieff AI**, Carroll HJ. Nonketotic hyperosmolar coma with hyperglycemia: clinical features, pathophysiology, renal function, acid-base balance, plasma-cerebrospinal fluid equilibria and the effects of therapy in 37 cases. *Medicine* (Baltimore) 1972; **51**: 73-94 [PMID: 5013637 DOI: 10.1097/00005792-197203000-00001]
5 **McCurdy DK**. Hyperosmolar hyperglycemic nonketotic diabetic coma. *Med Clin North Am* 1970; **54**: 683-699 [PMID: 4987277]
6 **Al-Kudsi RR**, Daugirdas JT, Ing TS, Kheirbek AO, Popli S, Hano JE, Gandhi VC. Extreme hyperglycemia in dialysis patients. *Clin Nephrol* 1982; **17**: 228-231 [PMID: 7047032]
7 **Katz MA**. Hyperglycemia-induced hyponatremia--calculation of expected serum sodium depression. *N Engl J Med* 1973; **289**: 843-844 [PMID: 4763428 DOI: 10.1056/NEJM197310182891607]
8 **Kapsner CO**, Tzamaloukas AH. Understanding serum electrolytes. How to avoid mistakes. *Postgrad Med* 1991; **90**: 151-154, 151-154, 161 [PMID: 1749730]
9 **Kitabchi AE**, Umpierrez GE, Miles JM, Fisher JN. Hyperglycemic crises in adult patients with diabetes. *Diabetes Care* 2009; **32**: 1335-1343 [PMID: 19564476 DOI: 10.2337/dc09-9032]
10 **Seldin DW**, Tarail R. Effect of hypertonic solutions on metabolism and excretion of electrolytes. *Am J Physiol* 1949; **159**: 160-174 [PMID: 15391092]
11 **Gennari FJ**, Kassirer JP. Osmotic diuresis. *N Engl J Med* 1974; **291**: 714-720 [PMID: 4604866 DOI: 10.1056/NEJM197410032911408]
12 **Tzamaloukas AH,** Sun Y, Konstantinov NK, Ing TS, Dorin RI, Malhotra D, Murata GH, Shapiro JI. Principles of quantitative fluid and cation replacement in extreme hyperglycemia. *Cureus* 2013; **5:** e110 [DOI: 10.7759/cureus.110]
13 **Robertson GL.** Thirst and vasopressin. In: Alpern RJ, Hebert SC. Seldin and Giebisch's The Kidney. Physiology and Pathophysiology. 4th ed. Amsterdam: Elsevier, 2008: 1123-1141
14 **Markowitz GS**, Radhakrishnan J, Kambham N, Valeri AM, Hines WH, D'Agati VD. Lithium nephrotoxicity: a progressive combined glomerular and tubulointerstitial nephropathy. *J Am Soc Nephrol* 2000; **11**: 1439-1448 [PMID: 10906157]
15 **Marples D**, Christensen S, Christensen EI, Ottosen PD, Nielsen S. Lithium-induced downregulation of aquaporin-2 water channel expression in rat kidney medulla. *J Clin Invest* 1995; **95**: 1838-1845 [PMID: 7535800 DOI: 10.1172/JCI117863]
16 **Grünfeld JP**, Rossier BC. Lithium nephrotoxicity revisited. *Nat Rev Nephrol* 2009; **5**: 270-276 [PMID: 19384328 DOI: 10.1038/nrneph.2009.43]
17 **Kim GH**, Lee JW, Oh YK, Chang HR, Joo KW, Na KY, Earm JH, Knepper MA, Han JS. Antidiuretic effect of hydrochlorothiazide in lithium-induced nephrogenic diabetes insipidus is associated with upregulation of aquaporin-2, Na-Cl co-transporter, and epithelial sodium channel. *J Am Soc Nephrol* 2004; **15**: 2836-2843 [PMID: 15504936 DOI: 10.1097/01.ASN.0000134476.93376.94]
18 **Stone KA**. Lithium-induced nephrogenic diabetes insipidus. *J Am Board Fam Pract* 1999; **12**: 43-47 [PMID: 10050642 DOI: 10.3122/15572625-12-1-43]
19 **Shaikh BS**, Nicholas GG, Miller FJ. Persistent nephrogenic diabetes insipidus after lithium carbonate. *Ann Intern Med* 1977; **86**: 446-447 [PMID: 848808 DOI: 10.7326/0003-4819-86-4-446]
20 **Gitlin M**. Lithium and the kidney: an updated review. *Drug Saf* 1999; **20**: 231-243 [PMID: 10221853 DOI: 10.2165/00002018-199920030-00004]
21 **Movig KL**, Baumgarten R, Leufkens HG, van Laarhoven JH, Egberts AC. Risk factors for the development of lithium-induced polyuria. *Br J Psychiatry* 2003; **182**: 319-323 [PMID: 12668407 DOI: 10.1192/bjp.182.4.319]
22 **Grandjean EM**, Aubry JM. Lithium: updated human knowledge using an evidence-based approach: part III: clinical safety. *CNS Drugs* 2009; **23**: 397-418 [PMID: 19453201 DOI: 10.2165/00023210-200923050-00004]
23 **Price TR**, Beisswenger PJ. Persistent lithium-induced nephrogenic diabetes insipidus. *Am J Psychiatry* 1978; **135**: 1247-1248 [PMID: 696918]
24 **Rabin EZ**, Garston RG, Weir RV, Posen GA. Persistent nephrogenic diabetes insipidus associated with long-term lithium carbonate treatment. *Can Med Assoc J* 1979; **121**: 194-198 [PMID: 519597]
25 **Cairns SR**, Wolman R, Lewis JG, Thakker R. Persistent nephrogenic diabetes insipidus, hyperparathyroidism, and hypothyroidism after lithium treatment. *Br Med J* (Clin Res Ed) 1985; **290**: 516-517 [PMID: 3918654 DOI: 10.1136/bmj.290.6467.516]
26 **Neithercut WD**, Spooner RJ, Hendry A, Dagg JH. Persistent nephrogenic diabetes insipidus, tubular proteinuria, aminoaciduria, and parathyroid hormone resistance following longterm lithium administration. *Postgrad Med J* 1990; **66**: 479-482 [PMID: 2170960 DOI: 10.1136/pgmj.66.776.479]
27 **Thompson CJ**, France AJ, Baylis PH. Persistent nephrogenic diabetes insipidus following lithium therapy. *Scott Med J* 1997; **42**: 16-17 [PMID: 9226773]
28 **Guirguis AF**, Taylor HC. Nephrogenic diabetes insipidus persisting 57 months after cessation of lithium carbonate therapy: report of a case and review of the literature. *Endocr Pract* 2000; **6**: 324-328 [PMID: 11242610 DOI: 10.4158/EP.7.4.324]
29 Hyperosmolar coma due to lithium-induced diabetes insipidus. *Lancet* 1995; **346**: 413-417 [PMID: 7623573]
30 **Sze L**, Ulrich B, Brändle M. Severe hypernatraemia due to nephrogenic diabetes insipidus - a life-threatening side effect of chronic lithium therapy. *Exp Clin Endocrinol Diabetes* 2006; **114**: 596-598 [PMID: 17177143 DOI: 10.1055/s-2006-924124]
31 **Johnson MA**, Ogorman J, Golembiewski GH, Paluzzi MW. Nephrogenic diabetes insipidus secondary to lithium therapy in the postoperative patient: a case report. *Am Surg* 1994; **60**: 836-839 [PMID: 7978676]
32 **Waise A**, Fisken RA. Unsuspected nephrogenic diabetes insipidus. *BMJ* 2001; **323**: 96-97 [PMID: 11451787 DOI: 10.1136/bmj.323.7304.96]
33 **Paw H**, Slingo ME, Tinker M. Late onset nephrogenic diabetes insipidus following cessation of lithium therapy. *Anaesth Intensive Care* 2007; **35**: 278-280 [PMID: 17444321]
34 **Lee RV**, Jampol LM, Brown WV. Nephrogenic diabetes insipidus and lithium intoxication--complications of lithium carbonate therapy. *N Engl J Med* 1971; **284**: 93-94 [PMID: 5538641 DOI: 10.1056/NEJM197101142840209]
35 **Martinez-Maldonado M**, Terrell J. Lithium carbonate-induced nephrogenic diabetes insipidus and glucose intolerance. *Arch Intern Med* 1973; **132**: 881-884 [PMID: 4757260 DOI: 10.1001/archinte.1973.03650120081015]
36 **de Wardener HE.** The Kidney. An Outline of Normal and Abnormal Structure and Function. 2nd ed. London: JA Churchill Ltd, 1961: 45-48

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