ISSN 2220-6124 (online)

World Journal of *Nephrology*

World J Nephrol 2015 July 6; 4(3): 324-443

Published by Baishideng Publishing Group Inc

WJN World Journal of Nephrology

A peer-reviewed, online, open-access journal of nephrology

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WJN World Journal of Nephrology

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NAME OF JOURNAL
World Journal of Nephrology

ISSN
ISSN 2220-6124 (online)

LAUNCH DATE
February 6, 2012

FREQUENCY
Bimonthly

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PUBLICATION DATE
July 6, 2015

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DOI: 10.5527/wjn.v4.i3.324

World J Nephrol 2015 July 6; 4(3): 324-329
ISSN 2220-6124 (online)

EDITORIAL

Asymptomatic hyperuricemia following renal transplantation

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Author contributions: Bellomo G solely contributed to this paper.

Conflict-of-interest statement: The author hereby declares to hold no conflicts of interest whatsoever.

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Received: January 25, 2015
Peer-review started: January 26, 2015
First decision: March 6, 2015
Revised: March 19, 2015
Accepted: April 16, 2015
Article in press: April 20, 2015
Published online: July 6, 2015

Abstract

Evidence is accumulating indicating a role for uric acid in the genesis and progression of kidney disease, and a few studies are beginning to show a possible beneficial effect of urate-lowering therapy. Whether this holds true for renal allograft recipients is not clear. In this short review evidence from epidemiological as well as intervention studies is summarized and discussed, with some practical considerations presented at the end.

Key words: Uric acid; Renal transplant; Urate lowering therapy; Allopurinol; Febuxostat

Core tip: Hyperuricemia is a common finding following renal transplantation; its clinical, as well as prognostic, significance, however, is not known. We have summarized available evidence from human epidemiological and intervention studies and concluded that, in the absence of gout, evidence in support of treatment for this condition in renal graft recipients is insufficient at present, although, when required, treatment with low-dose allopurinol or febuxostat appears to be safe.

Bellomo G. Asymptomatic hyperuricemia following renal transplantation. *World J Nephrol* 2015; 4(3): 324-329 Available from: URL: http://www.wjgnet.com/2220-6124/full/v4/i3/324.htm DOI: http://dx.doi.org/10.5527/wjn.v4.i3.324

INTRODUCTION

A body of evidence, accumulated mainly in the last 15 years, based on animal and human experimental studies, as well as prospective observational and a few intervention studies, reviewed elsewhere[1-5], has lent support to the hypothesis that hyperuricemia may be linked to incident renal disease and the progression of chronic kidney disease (CKD). Fewer data are available regarding the effect of uric acid (UA) and hyperuricemia on graft function and survival following renal transplantation. Aim of this short review, which does not mean to be either exhaustive or comprehensive, is to summarize available evidence gathered from observational and intervention studies on the latter topic in adult patients; we will not cover incidence and treatment of gout after renal transplantation, referring the reader interested in a more detailed discussion to the excellent review by

Stamp *et al*[6].

Literature search was performed on the PubMed, EMBASE and Science Direct databases using the following search terms: Uric, Uric Acid, Urate, Hyperuricemia and Renal, Kidney Transplant, Transplantation, Graft, Allograft.

EPIDEMIOLOGY OF HYPERURICEMIA FOLLOWING RENAL TRANSPLANTATION

Hyperuricemia (serum UA greater or equal to 6.0 mg/dL in women and 7.0 mg/dL in men) is fairly common following renal transplantation. The prevalence of hyperuricemia in recipients of a renal allograft has been shown to range from 19% to 55% in patients whose immunosuppressive regimen did not include cyclosporin A (CsA) and from 30% to 84% in patients treated with CsA[7]. In the same series, incident gout was not observed in non-CsA treated patients, whereas it ranged from 2.0% to 28% following CsA therapy. More recently, Kalantar *et al*[8] have measured serum UA in 12767 samples from 2961 renal graft recipients; they detected hyperuricemia in 1553 patients (52.3%, 61% men, 39% women). In another study[9] of 302 patients with a well functioning kidney graft, at a median 7.6 years after transplantation, hyperuricemia was present in 42.1% of patients. Kim *et al*[10] investigated the prevalence of hyperuricemia in 356 transplanted patients with stable renal graft function (estimated GFR > 60 mL/min per 1.73 m^2). In this subgroup of patients they found raised UA levels in 55 (15.45%). Numakura *et al*[11] found hyperuricemia to be present in 38% of patients one year after transplantation; in their cohort male gender and dialytic vintage before transplantation were predictors of post-transplant hyperuricemia. According to the various studies, risk factors for hyperuricemia following renal transplantation include decreased glomerular filtration rate (GFR), diuretic use, pre-existent history of hyperuricemia, treatment with calcineurin inhibitors, in particular CsA, use of diuretics, male gender, diabetes mellitus, hypercalcemia, and higher body weight[7-11]; tacrolimus has been associated with lower odds of developing hyperuricemia, compared to CsA[9].

OBSERVATIONAL STUDIES EXPLORING THE ASSOCIATION BETWEEN HYPERURICEMIA AND GRAFT FUNCTION/SURVIVAL

Table 1 summarizes the most relevant studies exploring this relationship[10,12-32]. Although most studies tend to favour an influence of UA on graft function and survival, there are notable exceptions: for instance, Meier-Kriesche *et al*[20], reviewing their data from the SYMPHONY Study, in which a cohort of 1645 was followed-up for 3 years, found that the association of baseline UA with follow-up eGFR, disappeared when adjusting for baseline eGFR. Conversely, in the study by Haririan *et al*[21], after a mean 68 mo follow-up, hyperuricemia was associated with a 1.26 (95%CI: 1.03-1.53) hazard ratio (HR) of graft loss. Kim *et al*[24] recently reported their review of patients transplanted between 1990 and 2009, and they observed that hyperuricemia conferred an 1.45 ($P < 0.001$) HR of graft loss; however the same group, in a study enrolling transplanted patients with preserved renal function[10] found hyperuricemia to be associated with decreased renal function, but not with graft survival. Choi *et al*[27] have investigated the effect of hyperuricemia on graft survival in recipients of living-donor kidney transplants, and found a nearly double incidence of graft loss in hyperuricemic patients (22.2% *vs* 11.4%). Hart *et al*[29], in a post-hoc analysis of patients participating in the ABCAN Trial, undergoing protocol biopsies of the graft, found an association between serum UA levels and the degree of interstitial fibrosis and tubular atrophy. In patients undergoing biopsy for acute allograft dysfunction, Weng *et al*[30] found hyperuricemia to be associated with a greater cumulative incidence at one year of the combined end-point of doubling serum creatinine or graft loss (29.8% *vs* 14.9%, $P = 0.02$) compared to normouricemia. As far as cardiovascular outcomes are concerned, Dahle *et al*[28] after a 7.4 year follow-up of 2200 patients found a J-shaped association between serum UA levels and cardiovascular as well as all-cause mortality, with a significant increased HR in the 5th UA centile, and a similar tendency (though not reaching statistical significance) for the lowest UA quintile. Other studies have yielded conflicting results, although those with a longer follow-up, and those assessing graft survival (rather than eGFR) as an end-point, tend to favour an adverse effect of hyperuricemia. The reason for the discrepancies among studies are not completely clear, however differences in the definition of hyperuricemia, duration of follow-up, end-points evaluated, populations studied and in adjustment for confounders and comorbidities may have played a role. Recently Huang *et al*[33] have published a meta-analysis, including 12 studies judged to be of medium-high quality according to the Newcastle-Ottawa quality assessment scale; the results of the meta-analysis showed that hyperuricemia was a risk factor for chronic allograft nephropathy [unadjusted Odds ratio (OR) = 2.85, 95%CI: 1.84-4.38, adjusted HR = 1.65, 95%CI: 1.02-2.65] and graft loss (Unadjusted OR = 2.29, 95%CI: 1.55-3.39; adjusted HR = 2.01, 95%CI: 1.39-2.94). The authors of this meta-analysis concluded that hyperuricemia may be an independent risk factor of allograft dysfunction and may increase slightly the risk of poor outcomes.

At the moment, the evidence supporting a causative or prognostic role for serum UA in renal transplant recipients is not conclusive.

Table 1 Studies investigating the association between serum uric acid and renal function/graft survival in patients with kidney transplantation

Author	Numerosity	Average follow-up	Major findings	Ref.
Gerhardt *et al* (1999)	375	5 yr	Hyperuricemia (> 8.0 mg/dL in men and > 6.2 mg/dL in women), associated with reduced graft survival	[12]
Armstrong *et al* (2005)	90	2.2 yr	UA independent predictor of follow-up eGFR, but not of eGFR change over time	[13]
Akgul *et al* (2007)	133	3 yr	No association found between serum UA and the development of chronic allograft nephropathy	[14]
Saglam *et al* (2008)	34	Not reported	Serum UA associated to development of cyclosporine A nephropathy (biopsy proven)	[15]
Akalin *et al* (2008)	307	4.3 yr	Hyperuricemia 6 mo after transplantation significantly associated with new cardiovascular events and graft dysfunction	[16]
Bandukwala *et al* (2009)	405	2 yr	Hyperuricemia associated with cardiovascular events, and, inversely with eGFR	[17]
Meyer-Kriesche *et al* (2009)	1645	3 yr	UA levels one month after transplantation not associated with follow-up eGFR, after adjustment for baseline renal function	[20]
Karbowska *et al* (2009)	78	Not reported	Hyperuricemia associated with markers of endothelial dysfunction and inflammation	[19]
Min *et al* (2009)	368	58 ± 23 mo	Early-onset moderate-to-severe hyperuricaemia (serum UA ⩾ 8.0 mg/dL) was found to be a significant risk factor for chronic allograft nephropathy (P = 0.035) and a poorer graft survival (P = 0.026) by multivariate analysis, whereas mild hyperuricaemia was not	[18]
Haririan *et al* (2010)	212	68 ± 27 mo	Serum UA during the first 6 mo postransplant, is an independent predictor of graft survival	[21]
Kim *et al* (2010)	356	102.6 ± 27.2 mo	Patients with eGFR> 60 mL/min per 1.73 m^2. Hyperuricemia associated with decreased eGFR	[10]
Boratyńska *et al* (2010)	100	34 ± 12 mo	Serum UA not associated to graft survival during 30 mo of follow-up	[22]
Chung *et al* (2011)	351	10 yr	Hyperuricemia increased risk of cardiovascular complication; graft survival at 5 and 10 yr lower in hyperuricemic *vs* normouricemic patients (89% *vs* 96% and 81% *vs* 93% respectively, P = 0.02)	[23]
Kim *et al* (2011)	556	Not reported	Serum UA levels affect graft function, even after adjustment for baseline eGFR	[24]
Wang *et al* (2011)	524	10 yr	Retrospective study: UA significantly lower in patients with longer graft survival	[25]
Park *et al* (2013)	428	120 ± 58 mo	Serum UA associated with allograft loss, but rate of eGFR decline more potent predictor	[26]
Choi *et al* (2013)	378	10 yr	Graft survival (living donor renal transplantation) 88.6% in normouricemic *vs* 78.8% in hyperuricemic patients	[27]
Dahle *et al* (2014)	2200	7.4 yr	Highest serum UA quintile independently associated with increased HR (2.87, 95%CI: 1.55-5.32) of cardiovascular and all-cause (1.55, 95%CI: 1.09-2.25) mortality	[28]
Hart *et al* (2014)	149	5 yr	Post-hoc study of the ABCAN trial. Serum UA independently associated with increased odds of composite outcome of doubling of interstitium or ESRD from Interstitial Fibrosis/Tubular Atrophy, after adjusting for eGFR	[29]
Weng *et al* (2014)	880	43.3 ± 26.3 mo	Hyperuricemia associated with poorer graft survival (60.5% *vs* 75.8%, P = 0.007), no difference in all-cause mortality	[30]
Boratyńska *et al* (2014)	637	10 yr	Retrospective study. Hyperuricemia associated with chronic allograft dysfunction	[31]
Weng *et al* (2014)	124	14.3 mo	Patients undergoing biopsies for acute allograft dysfunction. Hyperuricemia associated with a greater cumulative incidence at one year of doubling serum creatinine or graft loss (29.8% *vs* 14.9%, P = 0.02) compared to normouricemia	[32]

UA: Uric acid; eGFR: Estimated glomerular filtration rate.

INTERVENTION STUDIES

Currently, no randomized, double-blind, controlled clinical trials of urate-lowering treatment on graft function and survival in renal allograft recipients are available. Table 2 shows the few published studies[11,34-37], all suffering from drawbacks such as low numerosity, lack of a placebo arm, single center, inconsistent reporting of adverse events and/or absence of blinding. In a study published in 2003, Perez-Ruiz *et al*[34] Studied 279 renal allograft recipient with hyperuricemia, 89 treated with allopurinol(mean dose 185 mg/d), and 190 with the uricosuric agent benziodarone(mean dose 73 mg/d); the immune-suppressive regimen included azathioprine in 49.1% of patients. Both drugs were effective in reducing serum UA, with similar withdrawal rate (11% for allopurinol and 8% for benziodarone). Major adverse events were rare, 3 in the allopurinol group (one case of pancytopenia, one hepatitis and one unexplained fever, all on high-dose treatment, 600 mg/d) and 2 in the benziodarone group (hypothyroidism). It must be remembered, however, that benziodarone was withdrawn from the market in many countries due to liver toxicity. More recently Numakura *et al*[11] studied 46 patients with post-transplant hyperuricemia treated with allopurinol (100-200 mg/d) compared

Table 2 Studies of uric-acid-lowering therapy in renal allograft recipients

Ref.	Study population	Average follow-up	Intervention/outcome(s)	Main study findings
Perez-Ruiz *et al*[34]	279 renal allograft recipients with hyperuricemia	38.6 ± 18.4 mo	Allopurinol, benziodarone/ serum UA levels	Both drugs effective in lowering serum UA; benziodarone safer in patients on azathioprine
Numakura *et al*[11]	121 renal allograft recipients with and without hyperuricemia	Up to 10 yr, mean not reported	Allopurinol/eGFR, graft survival	Hyperuricemia associated with reduced eGFR, but graft survival similar in normo and hyperuricemic patients
Osadchuck *et al*[35]	108 renal allograft recipients (54 patients treated *vs* 54 controls)	2 yr	Allopurinol/Serum UA levels, eGFR, graft survival	Reduced serum UA, preservation of eGFR in allopurinol treated patients; no differences in graft survival and blood pressure
Sofue *et al*[36]	93 renal allograft recipients (42 normouricemic, 51 hyperuricemic, 26 treated, 25 not treated)	1 yr	Febuxostat/serum UA levels, eGFR	Serum UA lower and eGFR stable in patients treated with febuxostat
Tojimbara *et al*[38]	23 renal allograft recipients with hyperuricemia	12 ± 2 mo	Febuxostat/serum UA, eGFR	Serum UA lower after treatment with febuxostat; eGFR stable

UA: Uric acid; eGFR: Estimated glomerular filtration rate.

to 75 normouricemic patients, followed up to 10 years. The former group had a lower eGFR, with a tendency for graft survival at 5 and 10 years to be reduced, with borderline statistical significancy. Rates of withdrawal from treatment or incidence of adverse events were not reported in this study. Osadchuk *et al*[35] in a retrospective case-control study, evaluated 54 hyperuricemic patients taking allopurinol because of gout, compared to 54 untreated controls matched for eGFR and time from transplant; mean baseline serum UA was 8.0 mg/dL in the allopurinol group and 6.8 mg/dL in the controls. At the end of the observation period (2 years) serum UA was reduced, and eGFR greater in the treatment group compared to controls, whereas no difference in graft survival was recorded. The dose of allopurinol used is not stated, and neither rate of withdrawal from treatment nor the incidence of adverse effects is reported. Sofue *et al*[36] studied 93 renal allograft recipients with stable renal function, 51 of them being hyperuricemic, 42 normouricemic. They treated 26 hyperuricemic patients with low-dose (10-20 mg/d) febuxostat, a novel xanthine-oxidase inhibitor associated with fewer adverse events than allopurinol[37]. After one year of treatment the majority of treated patients had achieved target serum UA levels and eGFR was stable. No serious adverse events were recorded and liver function tests were not altered by febuxostat. Finally, Tojimbara *et al*[37] assessed 22 hyperuricemic renal allograft recipients treated with low-dose febuxostat (10-20 mg/d). Despite the low dose administered, 73% of the patients achieved target serum UA levels (< 6.0 mg/dL). No serious adverse events were recorded, and only one patient withdrew from the study because of numbness in the arms. Immuno-suppressive drug levels were not affected by the co-administration of febuxostat.

CONCLUSION

Available evidence does not support widespread use of urate lowering therapies in asymptomatic hyperuricemic recipients of a renal allograft. At present, treatment should be limited to patients with gout, although patients with severe hyperuricemia (> 8.0 mg/dL) might benefit from serum UA lowering therapy; it is not known what serum UA target should be achieved, however, a recently published[39] long-term follow-up of a randomized, controlled clinical trial of allopurinol treatment in patients with CKD, has shown that nefro-protection can be attained by lowering serum UA just below its crystallization threshold (6.8 mg/dL). The therapeutic armamentarium is currently limited to xanthine-oxidase inhibitors, as uricosuric agents, with the possible exception of losartan, are mostly not indicated, or ineffective, in patients with CKD and/ or kidney transplant, uricase and its analogues are expensive, must be administered parenterally, and have important side effects; the discovery and isolation of urate transporters in the renal tubules, has led the way to the development of new hypouricemic drugs, currently under evaluation[40] but not immediately available for clinical use. The good news is that the data at hand seem to show that both allopurinol and febuxostat can be administered safely, at low doses, in renal transplant recipients, with the exception of those treated with azathioprine, the side-effects of which could be potentiated by xanthine-oxidase inhibition. In conclusion, randomized controlled trials of urate-lowering therapy are badly needed in this population of patients, to establish whether preservation of renal function and prolongation of graft survival can be achieved.

REFERENCES

1 **Jalal DI**, Chonchol M, Chen W, Targher G. Uric acid as a target of therapy in CKD. *Am J Kidney Dis* 2013; **61**: 134-146 [PMID: 23058478 DOI: 10.1053/j.ajkd.2012.07.021]

2 **Johnson RJ**, Nakagawa T, Jalal D, Sánchez-Lozada LG, Kang DH, Ritz E. Uric acid and chronic kidney disease: which is chasing which? *Nephrol Dial Transplant* 2013; **28**: 2221-2228 [PMID:

23543594 DOI: 10.1093/ndt/gft029]
3 **Bellomo G**. Uric acid and chronic kidney disease: A time to act? *World J Nephrol* 2013; **2**: 17-25 [PMID: 24175261 DOI: 10.5527/wjn.v2.i2.17]
4 **Feig DI**. Serum uric acid and the risk of hypertension and chronic kidney disease. *Curr Opin Rheumatol* 2014; **26**: 176-185 [PMID: 24419747 DOI: 10.1097/BOR.0000000000000033]
5 **Bose B**, Badve SV, Hiremath SS, Boudville N, Brown FG, Cass A, de Zoysa JR, Fassett RG, Faull R, Harris DC, Hawley CM, Kanellis J, Palmer SC, Perkovic V, Pascoe EM, Rangan GK, Walker RJ, Walters G, Johnson DW. Effects of uric acid-lowering therapy on renal outcomes: a systematic review and meta-analysis. *Nephrol Dial Transplant* 2014; **29**: 406-413 [PMID: 24042021 DOI: 10.1093/ndt/gft378]
6 **Stamp LK**, Chapman PT. Gout and organ transplantation. *Curr Rheumatol Rep* 2012; **14**: 165-172 [PMID: 22258500]
7 **Clive DM**. Renal transplant-associated hyperuricemia and gout. *J Am Soc Nephrol* 2000; **11**: 974-979 [PMID: 10770978]
8 **Kalantar E**, Khalili N, Hossieni MS, Rostami Z, Einollahi B. Hyperuricemia after renal transplantation. *Transplant Proc* 2011; **43**: 584-585 [PMID: 21440768 DOI: 10.1016/j.transproceed.2011.01.062]
9 **Malheiro J**, Almeida M, Fonseca I, Martins LS, Pedroso S, Dias L, Henriques AC, Cabrita A. Hyperuricemia in adult renal allograft recipients: prevalence and predictors. *Transplant Proc* 2012; **44**: 2369-2372 [PMID: 23026595 DOI: 10.1016/j.transproceed.2012.07.033]
10 **Kim KM**, Kim SS, Han DJ, Yang WS, Park JS, Park SK. Hyperuricemia in kidney transplant recipients with intact graft function. *Transplant Proc* 2010; **42**: 3562-3567 [PMID: 21094816]
11 **Numakura K**, Satoh S, Tsuchiya N, Saito M, Maita S, Obara T, Tsuruta H, Inoue T, Narita S, Horikawa Y, Kagaya H, Miura M, Habuchi T. Hyperuricemia at 1 year after renal transplantation, its prevalence, associated factors, and graft survival. *Transplantation* 2012; **94**: 145-151 [PMID: 22728291 DOI: 10.1097/TP.0b013e318254391b]
12 **Gerhardt U**, Grosse Hüttmann M, Hohage H. Influence of hyperglycemia and hyperuricemia on long-term transplant survival in kidney transplant recipients. *Clin Transplant* 1999; **13**: 375-379 [PMID: 10515217 DOI: 10.1034/j.1399-0012.1999.130502.x]
13 **Armstrong KA**, Johnson DW, Campbell SB, Isbel NM, Hawley CM. Does uric acid have a pathogenetic role in graft dysfunction and hypertension in renal transplant recipients? *Transplantation* 2005; **80**: 1565-1571 [PMID: 16371927 DOI: 10.1097/01.tp.0000183895.88572.13]
14 **Akgul A**, Bilgic A, Ibis A, Ozdemir FN, Arat Z, Haberal M. Is uric acid a predictive factor for graft dysfunction in renal transplant recipients? *Transplant Proc* 2007; **39**: 1023-1026 [PMID: 17524881 DOI: 10.1016/j.transproceed.2007.03.028]
15 **Saglam F**, Celik A, Sarioglu S, Cavdar C, Sifil A, Gulay H, Camsari T. Hyperuricemia influences chronic cyclosporine nephropathy. *Transplant Proc* 2008; **40**: 167-170 [PMID: 18261576 DOI: 10.1016/j.transproceed.2007.11.013]
16 **Akalin E**, Ganeshan SV, Winston J, Muntner P. Hyperuricemia is associated with the development of the composite outcomes of new cardiovascular events and chronic allograft nephropathy. *Transplantation* 2008; **86**: 652-658 [PMID: 18791445 DOI: 10.1097/TP.0b013e3181814f5b]
17 **Bandukwala F**, Huang M, Zaltzman JS, Nash MM, Prasad GV. Association of uric acid with inflammation, progressive renal allograft dysfunction and post-transplant cardiovascular risk. *Am J Cardiol* 2009; **103**: 867-871 [PMID: 19268747]
18 **Min SI**, Yun IJ, Kang JM, Park YJ, Min SK, Ahn C, Kim SJ, Ha J. Moderate-to-severe early-onset hyperuricaemia: a prognostic marker of long-term kidney transplant outcome. *Nephrol Dial Transplant* 2009; **24**: 2584-2590 [PMID: 19395726 DOI: 10.1093/ndt/gfp192]
19 **Karbowska A**, Boratynska M, Kusztal M, Klinger M. Hyperuricemia is a mediator of endothelial dysfunction and inflammation in renal allograft recipients. *Transplant Proc* 2009; **41**: 3052-3055 [PMID: 19857674 DOI: 10.1016/j.transproceed.2009.07.080]
20 **Meier-Kriesche HU**, Schold JD, Vanrenterghem Y, Halloran PF, Ekberg H. Uric acid levels have no significant effect on renal function in adult renal transplant recipients: evidence from the symphony study. *Clin J Am Soc Nephrol* 2009; **4**: 1655-1660 [PMID: 19713295 DOI: 10.2215/CJN.02700409]
21 **Haririan A**, Nogueira JM, Zandi-Nejad K, Aiyer R, Hurley H, Cooper M, Klassen DK, Weir MR. The independent association between serum uric acid and graft outcomes after kidney transplantation. *Transplantation* 2010; **89**: 573-579 [PMID: 19997058 DOI: 10.1097/TP.0b013e3181c73c18]
22 **Boratyńska M**, Karbowska A, Klinger M. The effect of hyperuricemia on endothelial biomarkers and renal function in kidney allograft recipients. *Transplant Proc* 2010; **42**: 4074-4077 [PMID: 21168631 DOI: 10.1016/j.transproceed.2010.09.069]
23 **Chung BH**, Kang SH, Hwang HS, Choi BS, Park CW, Kim YS, Kim JI, Moon IS, Yang CW. Clinical significance of early-onset hyperuricemia in renal transplant recipients. *Nephron Clin Pract* 2011; **117**: c276-c283 [PMID: 20847570]
24 **Kim KM**, Kim SS, Yun S, Lee MS, Han DJ, Yang WS, Park JS, Park SK. Uric acid contributes to glomerular filtration rate deterioration in renal transplantation. *Nephron Clin Pract* 2011; **118**: c136-c142 [PMID: 21150221 DOI: 10.1159/000320616]
25 **Wang W**, Li XB, Yin H, Yang XY, Liu H, Ren L, Hu XP, Wang Y, Zhang XD. Factors affecting the long-term renal allograft survival. *Chin Med J* (Engl) 2011; **124**: 1181-1184 [PMID: 21542992]
26 **Park JS**, Oh IH, Lee CH, Kim GH, Kang CM. The rate of decline of glomerular filtration rate is a predictor of long-term graft outcome after kidney transplantation. *Transplant Proc* 2013; **45**: 1438-1441 [PMID: 23726591 DOI: 10.1016/j.transproceed.2012.10.052]
27 **Choi JY**, Kwon OJ. The association between serum uric acid levels at 3 months after renal transplantation and the graft outcome in living donor renal transplantation. *Transplant Proc* 2013; **45**: 1548-1552 [PMID: 23726617 DOI: 10.1016/j.transproceed.2012.10.062]
28 **Dahle DO**, Jenssen T, Holdaas H, Leivestad T, Vårdal M, Mjøen G, Reisaeter AV, Toft I, Hartmann A. Uric acid has a J-shaped association with cardiovascular and all-cause mortality in kidney transplant recipients. *Clin Transplant* 2014; **28**: 134-140 [PMID: 24372653 DOI: 10.1111/ctr.12290]
29 **Hart A**, Jackson S, Kasiske BL, Mauer MS, Najafian B, Matas AJ, Spong R, Ibrahim HN. Uric acid and allograft loss from interstitial fibrosis/tubular atrophy: post hoc analysis from the angiotensin II blockade in chronic allograft nephropathy trial. *Transplantation* 2014; **97**: 1066-1071 [PMID: 24503762 DOI: 10.1097/01.TP.0000440952.29757.66]
30 **Weng SC**, Shu KH, Tarng DC, Cheng CH, Chen CH, Yu TM, Chuang YW, Huang ST, Wu MJ. Uric acid is highly associated with kidney allograft survival in a time-varying analysis. *Transplant Proc* 2014; **46**: 505-510 [PMID: 24656000 DOI: 10.1016/j.transproceed.2013.09.038]
31 **Boratyńska M**, Wakulenko A, Klinger M, Szyber P. Chronic allograft dysfunction in kidney transplant recipients: long-term single-center study. *Transplant Proc* 2014; **46**: 2673-2677 [PMID: 25380892 DOI: 10.1016/j.transproceed.2014.09.058]
32 **Weng SC**, Shu KH, Wu MJ, Cheng CH, Chen CH, Yu TM, Chuang YW, Huang ST, Tarng DC. Hyperuricemia predicts kidney disease progression after acute allograft dysfunction. *Transplant Proc* 2014; **46**: 499-504 [PMID: 24655999 DOI: 10.1016/j.transproceed.2013.09.040]
33 **Huang Y**, Li YL, Huang H, Wang L, Yuan WM, Li J. Effects of hyperuricemia on renal function of renal transplant recipients: a systematic review and meta-analysis of cohort studies. *PLoS One* 2012; **7**: e39457 [PMID: 22745759 DOI: 10.1371/journal.pone.0039457]
34 **Perez-Ruiz F**, Gomez-Ullate P, Amenabar JJ, Zarraga S, Calabozo M, Herrero-Beites AM, Nolla JM. Long-term efficacy of hyperuricaemia treatment in renal transplant patients. *Nephrol Dial Transplant* 2003; **18**: 603-606 [PMID: 12584286 DOI: 10.1093/ndt/18.3.603]
35 **Osadchuk L**, Bashir MH, Tangirala B, Marcus RJ, Nashar K, Hussain SM, Sureshkumar KK. Effect of allopurinol on slowing

allograft functional decline in kidney transplant recipients. *Exp Clin Transplant* 2014; **12**: 190-194 [PMID: 24907717]

36 **Sofue T**, Inui M, Hara T, Nishijima Y, Moriwaki K, Hayashida Y, Ueda N, Nishiyama A, Kakehi Y, Kohno M. Efficacy and safety of febuxostat in the treatment of hyperuricemia in stable kidney transplant recipients. *Drug Des Devel Ther* 2014; **8**: 245-253 [PMID: 24600205 DOI: 10.2147/DDDT.S56597]

37 **Tojimbara T**, Nakajima I, Yashima J, Fuchinoue S, Teraoka S. Efficacy and safety of febuxostat, a novel nonpurine selective inhibitor of xanthine oxidase for the treatment of hyperuricemia in kidney transplant recipients. *Transplant Proc* 2014; **46**: 511-513 [PMID: 24656001]

38 **Faruque LI**, Ehteshami-Afshar A, Wiebe N, Tjosvold L, Homik J, Tonelli M. A systematic review and meta-analysis on the safety and efficacy of febuxostat versus allopurinol in chronic gout. *Semin Arthritis Rheum* 2013; **43**: 367-375 [PMID: 24326033 DOI: 10.1016/j.semarthrit.2013.05.004]

39 **Goicoechea M**, Garcia de Vinuesa S, Verdalles U, Verde E, Macias N, Santos A, Pérez de Jose A, Cedeño S, Linares T, Luño J. Allopurinol and progression of CKD and cardiovascular events: long-term follow-up of a randomized clinical trial. *Am J Kidney Dis* 2015; **65**: 543-549 [PMID: 25595565 DOI: 10.1053/j.ajkd.2014.11.016]

40 **Diaz-Torné C**, Perez-Herrero N, Perez-Ruiz F. New medications in development for the treatment of hyperuricemia of gout. *Curr Opin Rheumatol* 2015; **27**: 164-169 [PMID: 25603039 DOI: 10.1097/BOR.0000000000000146]

P- Reviewer: Markic D, Mu JS **S- Editor**: Song XX **L- Editor**: A **E- Editor**: Yan JL

World Journal of Nephrology

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Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.5527/wjn.v4.i3.330

World J Nephrol 2015 July 6; 4(3): 330-344
ISSN 2220-6124 (online)

REVIEW

Pharmacokinetic and pharmacodynamic considerations of antimicrobial drug therapy in cancer patients with kidney dysfunction

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Author contributions: Keller F contributed to the pharmacokinetics and pharmacodynamics; Schröppel B contributed to the literature context and manuscript preparation; Ludwig U contributed to the clinical relevance and practical applicability.

Conflict-of-interest statement: There is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

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Received: November 27, 2014
Peer-review started: November 28, 2014
First decision: December 26, 2014
Revised: January 12, 2015
Accepted: May 5, 2015
Article in press: May 6, 2015
Published online: July 6, 2015

Abstract

Patients with cancer have a high inherent risk of infectious complications. In addition, the incidence of acute and chronic kidney dysfunction rises in this population. Anti-infective drugs often require dosing modifications based on an estimate of kidney function, usually the glomerular filtration rate (GFR). However, there is still no preferential GFR formula to be used, and in acute kidney injury there is always a considerable time delay between true kidney function and estimated GFR. In most cases, the anti-infective therapy should start with an immediate and high loading dose. Pharmacokinetic as well as pharmacodynamic principles must be applied for further dose adjustment. Anti-infective drugs with time-dependent action should be given with the target of high trough concentrations (*e.g.*, beta lactam antibiotics, penems, vancomycin, antiviral drugs). Anti-infective drugs with concentration-dependent action should be given with the target of high peak concentrations (*e.g.*, aminoglycosides, daptomycin, colistin, quinolones). Our group created a pharmacokinetic database, called NEPharm, hat serves as a reference to obtain reliable dosing regimens of anti-infective drugs in kidney dysfunction as well as renal replacement therapy. To avoid the risk of either too low or too infrequent peak concentrations, we prefer the eliminated fraction rule for dose adjustment calculations.

Key words: Anti-infective drugsl; Cancer; Kidney function; Pharmacodynamics; Pharmacokinetics; Dose adjustment; NEPharm

Core tip: Cancer patients are at an increased risk for both infection and kidney dysfunction. Infections need immediate treatment; during the further course, kidney function must be taken into account. Almost any drug can be adjusted to any kidney function in every patient. Observation of the pharmacokinetic principles allows avoiding adverse events.

Observation of the pharmacodynamic principles is needed to obtain anti-infective success. The target concentration for anti-infective drugs with a concentration-dependent effect is the high peak level. The target concentration for anti-infective drugs with a time-dependent effect is the high trough level. When in doubt, the peak should be the target.

Keller F, Schröppel B, Ludwig U. Pharmacokinetic and pharmacodynamic considerations of antimicrobial drug therapy in cancer patients with kidney dysfunction. *World J Nephrol* 2015; 4(3): 330-344 Available from: URL: http://www.wjgnet.com/2220-6124/full/v4/i3/330.htm DOI: http://dx.doi.org/10.5527/wjn.v4.i3.330

INTRODUCTION

The number of patients requiring anticancer therapy is rising due to the increase in life expectancy. Presently, there is almost no malignancy without an option for either curative or palliative, adjuvant or neo-adjuvant chemotherapy. Anticancer drugs bear not only the risk of infection and "febrile neutropenia"[1] but also the risk of nephrotoxicity[2].

Acute kidney injury (AKI) of any cause is a known risk factor for and a consequence of infectious complications. AKI can also be potentiated by the nephrotoxicity of the chemotherapeutics. In cancer patients, the incidence of AKI is estimated at 15%-45% per year[3]. The prevalence of chronic kidney disease (CKD) is reported at 15%-50% in cancer patients[4,5]. This high prevalence can be due to demographic trends but in contrast to previous speculations, CKD is not a risk factor for non-renal malignancies[3].

This review addresses the pharmacokinetics and pharmacodynamics (PK-PD) of anti-infective therapies in cancer patients with impaired kidney function.

Case report

The therapeutic dilemma might be illustrated by the case of a 73-year-old female with fever and leukopenia. The diagnosis of multiple myeloma had been made 18 mo before admission. As a third-line chemotherapy, she had received 4 cycles of bendamustine and prednisolone. Now she was referred from another hospital because of acute on chronic kidney failure requiring hemodialysis (HD). After persistent fever while on piperacillin-combactam and radiological evidence of pneumonia, she received 1000 mg meropenem every 12 h as rescue therapy. Since the half-life was assumed to increase from 1.0 to 9.7 h, the administration interval was prolonged from 8 to 12 h (Table 1). The renal failure dose of 500 mg twice daily as recommended by the manufacturer was considered to be under-dosed - in agreement with recent publications[6]. She remained dialysis-dependent but could be discharged home 3 wk later.

KIDNEY FUNCTION AND DRUG DOSE ADJUSTMENT

Anti-infective treatment is given with a therapeutic or a prophylactic indication. The preemptive treatment is distinguished from the induction therapy and the empirical differs from the sequential mode of therapy. For any mode of treatment, adjustment of anti-infective drug dose to the kidney function is recommended based on estimates of glomerular filtration rate (GFR) as well as pharmacokinetic and pharmacodynamic principles.

Kidney function

The kidney function can be measured by the GFR as this quantitates the primary and principal function of the nephron. It is an anachronism to use the endogenous creatinine clearance since urine collection errors are frequent[7]. This makes such estimates unreliable, resulting in under-dosing of anti-infective and anticancer drugs. For classifying the kidney dysfunction into one of the 5 stages of CKD, the standardized chronic kidney disease epidemiology collaboration (CKD-EPI) formula is currently preferred[8]. For drug dose adjustment, the GFR estimate easiest to access is the most appropriate[9]. Both, the modification of diet in renal disease (MDRD) or CKD-EPI equations estimate the GFR (eGFR) for a standard 1.73 m^2 body surface area (BSA). To estimate the BSA, we use the Mosteller formula[10].

$$GFR = eGFR \cdot \frac{BSA}{1.73}$$

$$BSA\ (m^2) = \frac{\sqrt{Height\ (cm) \cdot Weight\ (kg)}}{60}$$

The eGFR value is automatically calculated in most laboratories with the standardized MDRD and the CKD-EPI equations. Weight or body surface area are important determinants of the distribution volume and thus of the dose. Since oncologists are familiar with the use of BSA, the MDRD and CKD-EPI GFR might have advantages for dose adjustment calculations.

In the Cockcroft and Gault (C and G) formula the body weight is considered; it originally estimated the creatinine clearance. Like the other creatinine-based formulas, the C and G equation can also be used as an estimate of the GFR for drug dose adjustments[11]. Luzius Dettli proposed a coefficient-free version of the C and G equation[12] that was validated recently with the new calibrated serum creatinine measurements[13].

$$GFR = \frac{150 - Age\ (yr)}{Crea\ (mcmol/L)} \cdot Weight\ (kg)$$

Since the GFR is the independent and the serum creatinine is the dependent variable, there can be a time lag of 1 to 2 d behind the actual true kidney function and all creatinine-based GFR estimates in acute kidney injury (AKI). An interesting extension, therefore, is the so-called kinetic GFR for increasing and decreasing kidney function in patients with AKI[14]. The published

equation can be derived from the C and G equation and rearranged for readily available measurements of the initial serum creatinine ($Crea_0$) and differences (deltaX) between subsequent creatinine values ($Crea_{1,2\ldots}$).

$$kinetGFR = \frac{[150 - Age\ (yr)] \cdot Weight\ (kg)}{Crea_0\ (mcmol/L)} \cdot \left[1 - \frac{Crea_2 - Crea_1}{t_2 - t_1} \cdot \frac{24\ (h)}{182(mcmol/L)}\right]$$

This approach holds for changing creatinine and is based on creatinine production. It relates the increase in serum creatinine within a specified time interval to the maximum increase in creatinine within one day. Since creatinine production and renal excretion is constant at about 1000 mg/d and the creatinine distribution volume is 42 L, the maximum 24 h increase in serum creatinine is 182 μmol/L if GFR is zero (the original publication says 133 μmol/L). If AKI is progressing and the creatinine is increasing, the above 1 - deltaX term is < 1.0 whereas the 1 - deltaX term is > 1.0 for decreasing creatinine values and restitution of AKI. The kinetic GFR estimate makes the general GFR-based dose adjustment rules (see below) also applicable to AKI and the intensive care condition with renal replacement therapy[14].

Pharmacokinetics

The main pharmacokinetic parameters are clearance, volume and half-life. Malcolm Rowland claimed the primacy for the clearance term since elimination is driven by clearance not half-life[15]. Where clearance reflects a mechanistic model, however, the half-life reflects a mathematical approach. Friedrich Hartmut Dost argued that the clearance estimate depends on bioavailability and body weight - as does the volume as well - whereas half-life does not[16].

There is a close relationship between the three parameters of clearance (Cl), volume (Vd) and half-life ($T_{1/2}$) where the half-life is inversely proportional to the elimination rate constant (Ke).

$$Cl = Ke \cdot Vd$$

$$T_{1/2} = \frac{In\ (2)}{Ke} = \frac{0.693}{Ke} = 0.693 \cdot \frac{Vd}{Cl}$$

As discussed for antiviral drugs, the half-life is the pharmacokinetic parameter that most impacts drug action[17]. Since the half-life indicates how long an administration interval should be selected, and since the duration of drug action is correlated to the half-life, we consider the elimination half-life to be the most useful pharmacokinetic parameter for drug dosing[18]. In some cases the special half-life that represents the largest part of the area under the curve should be considered - Luzius Dettli coined it the "dominant half-life". Generally, the effect-indicative half-life at target concentrations should be used for dose calculations[18].

An increase and prolongation of the half-life was first reported by Kunin *et al*[19] for special drugs in patients with impaired kidney function. If the half-life is prolonged, drug accumulation kinetics will produce higher peak and higher trough concentrations with an increased risk for drug toxicity. According to the accumulation kinetics, the steady-state peak (C_{peak}) and the trough concentrations (C_{trough}) depend on the initial concentration after the first dose (C_0), on half-life ($T_{1/2}$) and administration interval (Tau).

$$C_{peak} = \frac{C_0}{1 - \exp(-\frac{0.693}{T_{1/2}} \cdot Tau)}$$

$$C_{trough} = \frac{C_0}{\exp(\frac{0.693}{T_{1/2}} \cdot Tau) - 1}$$

The relation between kidney function and half-life is as complex and hyperbolic as that between GFR and serum creatinine. It was a great advantage for drug dose adjustment that Luzius Dettli demonstrated the linear relationship between drug elimination and kidney function. This dependence was originally described as a linear function between the elimination rate constant and the creatinine clearance[20]. The modern approach describes this dependence as a linear function between drug clearance (Cl) and GFR.

$$Cl = a + b \cdot GFR = Cl_{nonren} + b \cdot GFR = Cl_{fail} + \frac{Cl_{norm} - Cl_{fail}}{GFR_{norm}} \cdot GFR$$

Based on this fundamental equation, the dose can be adjusted to the individual GFR in proportion to the decrease in drug clearance (Figure 1). The dose can also be adjusted in inverse proportion to the increase in half-life since in many published investigations, the inverse half-life, namely the elimination rate constant (Ke) has been related to the GFR. Based on the ideas of Luzius Dettli and for practical purposes, the fraction eliminated by the renal route (fren) has been proposed as the leading parameter for drug dose adjustment[21].

$$fren = \frac{A_{urine}}{D} = \frac{Cl_{ren}}{Cl_{tot}} = 1 - \frac{Cl_{fail}}{Cl_{norm}} = 1 - \frac{T_{1/2norm}}{T_{1/2fail}}$$

$$Cl = Cl_{norm} \cdot [1 - fren\ (1 - \frac{GFR}{GFR_{norm}})]$$

$$D = D_{norm} \cdot \frac{T_{1/2norm}}{T_{1/2}} = D_{norm} \cdot [1 - fren \cdot (1 - \frac{GFR}{GFR_{norm}})]$$

Since pharmacokinetics of anticancer drugs is rarely investigated in patients with CKD or AKI, it is an advantage that this fraction can be derived in volunteers with normal kidney function. However, kidney dysfunction also influences non-renal clearance, bioavailability and drug metabolism by the liver and intestines[22]. Therefore, the pharmacokinetics as determined in real patients with failing kidney function (CKD or AKI) should be the preferred source for drug dose adjustment calculations (*e.g.,* half-life estimates).

$$T_{1/2} = \frac{T_{1/2norm}}{1 - fren\ (1 - \frac{GFR}{GFR_{norm}})} = \frac{T_{1/2norm}}{1 - (1 - \frac{T_{1/2norm}}{T_{1/2fail}}) \cdot (1 - \frac{GFR}{GFR_{norm}})}$$

Table 1 Proposals for adjustment of an anti-infective drug dose to the estimated kidney function or to intermittent hemodialysis and continuous hemofiltration

Drug	Half life (h)		Loading dose	Normal kidney function (GFR = 100 mL/min)		Kidney impairment (GFR ≈ 30 mL/min)		Failure (GFR ⩽ 5 mL/min) and hemodialysis			Hemofiltration (2 L/h) and continuous dialysis	
								Off dialysis day Dfail		$D_{HD} = D_{fail} + D_{sup}$		
	Normal	Failure	D_{start} (mg)	Maintenancedose (mg)	Dose interval (h)	Maintenancedose (mg)	Dose interval (h)	Maintenancedose (mg)	Dose interval (h)	Post dialysis D_{HD} (mg)	Maintenancedose (mg)	Dose interval (h)
Abacavir (po)	1.5	2.1	600	600	12	600	12	600	12			
Aciclovir	2.5	25	750	750	8	500	12	500	24	750	750	24
Adefovir (po)	1.6	160	10	10	24	10	48	10	168	10		
Albendazole (po)	8	8	400	400	12	400	12	400	12			
Amantadine (iv)	13	600	200	200	8	200	72	200	168	200	200	72
(po)	20	610	100	100	12	100	72	100	168	100		
Amikacin	2	40	Norm/Failure 1500/750	1500	24	500	24	250	24	750	750	24
Amoxicillin (po)	1.2	12	1000	1000	8	1000	12	500	12	1000		
Amoxicillin + Clavulanic acid	1.2 + 1.2	12 + 4.3	500 + 125	500 + 125	8	500 + 125	12	500 + 125	12	500 + 125	500 + 125	12
			875 + 125	875 + 125	8	875 + 125	12	500 + 125	12			
Amphotericin B	24 (360)	35 (360)	70	70	24	70	24	50	24	50	50	24
Amphotericin B liposomal	24/92	24/160	200	200	24	200	48	200	24	200	200	24
Ampicillin	1	13	2000	2000	8	2000	12	1000	12	2000	2000	12
+ Sulbactam	+ 1	+ 6.6	+ 1000	+ 1000	8	+ 1000	12	+ 500	12	+ 1000	+ 1000	12
Amprenavir	8	8	1200	1200	12	1200	12	1200	12			
Anidulafungin	26	26	200	100	24	100	24	100	24	100	100	24
Artesunate	0.5			180								
Atazanavir (po)	9			300	24							
Atovaquone (po)	63	63	750	750	12							
Atovaquone +	63	63	250 + 100	250 + 100	24	250 + 100	24	250 + 100	24			
Proguanil (po)	14	23										
Azidothymidine	1	1.9 (52)	200	200	8	100	8	100	8	200	200	24
Azithromycin	39	40	1000	500	24	500	24	500	24	500	-	-
Brivudin (po)	14 (144)		125	125	24 for 7 d							
Caspofungin	10	10	70	50	24	50	24	50	24	50	50	24
Cefaclor (po)	0.7	3	1000	1000	8	1000	12	1000	12	1000		
Cefazolin	2.2	34	2000	2000	8	2000	12	500	12	1500	1000	12
Cefotaxime	1.2	7 (10)	2000	2000	8	2000	12	1000	12	2000	2000	12
Cefotiam	1	8	2000	2000	8	2000	12	1000	12	2000	2000	12
Ceftaroline fosamil	2.7	6	600	600	12	600	12	600	12	600	600	12
Ceftobiprol-medocaril	3.3	11	1000	1000	8	1000	12	500	12	1000	1000	12
Ceftazidime	2.1	25	2000	2000	8	2000	12	1000	24	2000	1000	12
Ceftriaxone	8	15	2000	2000	24	2000	24	2000	24	2000	2000	24
Cefuroxime (iv)	1.1	18	1500	1500	8	1500	12	750	24	1500	750	12
(po)			500	500	8	500	12	500	24			
Chinin = Quinine	13	15	600	600	12	600	12	600	12	600	600	12
Chloramphenicol	2.5	7	1000	1000	8	1000	8	1000	12	1000	1000	12
Chloroquine	48/212	300	250 mg/8 h	150	8	75	24					
Cidofovir	3.4	45	375 mg/168 h	375	336 h = 14 d	70	336 = 14 d	35	336 = 14 d	70	140	336 = 14 d
Ciprofloxacin (iv)	4.4	10	400	400	12	400	12	400	24	400	400	12
(po)			500	500								

Clarithromycin	6.8	17	500	500	12	500	12	500	24				
Clindamycin	3	3	900	600	6 - 8	600	6 - 8	600	6 - 8	600	600	6 - 8	
Colistin colistimethate Na	3 (9)	24 (11)	480 - 720 = 7 Mio IE	240 = 3 Mio IE	8	240 = 3 Mio IE	12	240 = 3 Mio IE	24	240 = 3 Mio IE	320 = 4 Mio IE	12	
Colistin (po)	3	16	160 mg = 2 Mio IE	2 Mio IE	12								
Co-trimoxazole	11/10	31/28	160/800	160/800	12	160/800	24	160/800	24	160/800	160/800	12	
Dalbavancin	336		1000	500	168								
Dapsone (po)	24	31	200	200	24	200	24	200	24	200			
Daptomycin	8	33	500	500	24	500	48	500	48	500	350	24	
				6-10 mg/kg		6-10 mg/kg		6-10 mg/kg		6-10 mg/kg			
Darunavir (po)	8			600	12								
Delavirdine	5.8			400	8								
Didanosine (po)	1.4	4.5	200	200	12	200	12	200	24				
Doripenem	1	8	1000	1000	8	1000	8	1000	12	1000	1000	8	
Doxycycline	15	23	200	100	24	100	24	100	24	100	100	24	
Efavirenz (po)	46.8	47	600	600	24	600	24	600	24				
Emtricitabine (po)	8.7	36	200	200	24	200	24	200	72				
Enfuvirtide	30		90		12								
Entecavir (po)	24 (138)	67 (384)	1.0	1.0	24	0.5	48	0.5	72	0.5			
Ertapenem	4.1	14.4	1000	1000	24	1000	24	1000	24	1000	1000	24	
Erythromycin	2.3	5	1000	1000	8	1000	12	1000	12	1000	1000	8	
Ethambutol	3.1	9.6	1600	1600	24	1200	24	1000	48	1600	1600	24	
			20 mg/kg										
Famciclovir (po)	2.2	14	250	250	12	250	12	250	24				
Flucloxacillin	0.8	3	2000	2000	8	2000	8	2000	8	2000	2000	8	
Fluconazole	25	110	800 or 400	800	24	400	24	400	48	400	800	24	
Flucytosine	4	150	2500	2500	6	2500	12	2500	48	2500	1250	24	
Fosamprenavir	19			700	12								
Foscarnet	4.5	100	6000	6000	12	3000	24	3000	72	4000	3000	24	
				4000	8								
Fosfomycin (iv)	1.5	20	5000	5000	8	5000	24	2500	24	5000	5000	12	
(po)			3000	single dose									
Ganciclovir	4.2	60	500	500	12	500	24	300	24	400	200	12	
			5 mg/kg KG										
Gentamicin	2	48	Norm/Fail	240	24	120	24	40	24	120	120	24	
			240/120					0	48	320 before HD			
Hydroxy-chloroquine	400			200	8								
Imipenem/	0.9/	3.3/	1000	1000	8	1000	12	500	12	1000	1000	12	
+ Cilastatin	0.9	13.8											
Indinavir (po)	1.8	2.1	800	800	8	800	8	800	8				
Isoniazid	1/3.3	5/12	300	300	24	300	24	300	24	300	300	24	
Itraconazole (po)	16	25	200	200	24	200	24	200	24	200			
Ketoconazole (po)	3	2	200	200	12	200	12	200	12	200			
Lamivudine (po)	6.2	21	150	150	12	150	24	100	24	150			
Levofloxacin	7.3	35	750	500	12	500	24	250	24	500	500	12	
Linezolid	4.9	6.9	600	600	12	600	12	600	12	600	600	12	
Lopinavir/Ritonavir	7/3.7	7/6.3	400 + 100	400 + 100	12	400 + 100	12	400 + 100	12				
Maraviroc (po)	36	36	300	300	12	300	12	300	12				

Mebendazole (po)	5		2 x 500	1000	8								
Mefloquine (po)	336	340	250	250	168	250	168						
Meropenem	1	9.7	1000	1000	8	1000	12	1000		1000		12	
Metronidazole (iv) (po)	10	11 (34)	500	500	8	500	12	500	500	500		12	
			400	400		400							
Micafungin	13	14	100	100	24	100	24	100					
Miconazole	24	24	1200	1200	24	1200	24	1200					
Moxifloxacin	12	15	400	400	24	400	24	400	400	400		24	
Nelfinavir (po)	4.5	4	750	750	8	750	8						
Nitrofurantoin (po)	1.0	1.2	100		8								
Nevirapine (po)	28	22	200/24	200	12	200	12						
Oritavancin	336		1200										
Oseltamivir (po)	7	(80)	75	30	24	75	72	75					
Paromomycin	2	40	500	500	24	500	48						
Penicillin G = Benzylpenicillin	0.5	10	10 mega	10 mega	8	10 mega	12	5 mega	12	5 mega	5 mega	8	
Penicillin V (po)	0.6	4.1	1 mega	1 mega	8	1 mega	8	1 mega	12				
Pentamidine (iv) (inhaled)	60	96	300	300	24	300	24	300	24				
			600		24								
			300		4 wk								
Piperacillin	1.1	4	4000	4000	8	4000	12	4000	12	4000	4000	12	
+ Sulbactam	1	8	500	500	8	500	12	500	12	500	500	12	
Piperacillin	1.1	4	4000	4000	8	4000	12	4000	12	4000	4000	12	
+ Tazobactam	1	8	500	500	8	500	12	500	12	500	500	12	
Posaconazole (po)	24	29	2 x 300	300	24	300	24	300	24				
Primaquine (po)	6.3	6.4	30	30	24	30	24	30	24				
Proguanil (po)	14	23		200	24								
Propicillin (po)	1		700 = 1 mega	700	8								
Prothionamide (po)	1.5		1000	1000	24	500	24	250	24	500	?	?	
Pyrazinamide (po)	9.1	19	2000	2000	24	2000	24	2000	48	2000	-	-	
Pyrimethamine	92	80	75	50	24	50	24	50	24				
Pyrvinium embonate	?	?	50	Single dosing									
Quinine	13	15	600	600	12	600	12	600	12	600	600	12	
Raltegravir (po)	5.5	2.5	400	400	12	400	12	400	12				
Ribavirin aerosol	44	26	6000	6000	12	6000	12	6000	12	6000	6000	12	
Ribavirin (po)	4/250	24/672	600	600	12	400	24	200	24	400	400	72	
(iv)			1000	1000	8	500	12	500	48	500	500	48	
Rifabutin (po)	25	37	600	600	24	600	24	300	24				
Rifabutin +	25	37	600	600	24	600	24	300	24				
Clarithromycin	6.8	17	300	300	24	300	24	300	24				
Rifampicin (iv)	4.5	4.5	600	600	24	600	24	600	24	600	600	24	
(po)			450	450	12	450	12	450	12				
Rifaximin (po)	intestine	unch	400	400	12	400	12	400	12				
Ritonavir (po)	3.7	6.3	600	600	12	600	12	600	12				
Roxithromycin	12	15	300	300	24	300	24	300	24	-	-	-	
Saquinavir (po)	7	13	2 x 500	1000	12	1000	12	600	12				
Stavudine (po)	1.5	6.0	40	40	12	40	12	40	12	40			
Sofosbuvir	1 (18)	(25)	400	400	24	400	24	300	24	400			
Streptomycin	2.6	100	1000	1000	24	500	48	250	72	250	500	24	

Teicoplanin	52	348	3 x (800/24)	1200	24	400	24	400	48	800	400	24
Telavancin	7.3	25	750	750	24	500	24	250	24	500	750	24
Telbivudine (po)	22		600	600	24							
Tenofovir (po)	14	28	245	245	24	245	24	245	48	245		
Terbinafine (po)	16	16	250	250	24	250	24	250	24			
Tetracycline (po)	8.9	83	500	500	8							
Tigecycline	40	47	100	50	12	50	12	50	12	50	50	12
Tipranavir (iv)	2.8	2.8	500	500	12							
+ Ritonavir (po)	3.7	6.3	+ 200	+ 200								
Tobramycin	2	48	Norm/Fail 240/120	240	24	120	24	40	24	120	120	24
Trimethoprim (iv) (po)	11	31	200	150	12	150	24	150	24	-	-	-
			100	100	24	100	24					
Trimethoprim +	11	31	160	160	12	160	24	160	24	160	160	12
Sulfamethoxazole	10	28	+ 800	+ 800	12	+ 800	24	+ 800	24	+ 800	+ 800	12
Trimethoprim +	11	31	400	400	8	320	12	400	24	400	400	12
Sulfamethoxazole (Pneumocystis)	10	28	+ 2000	+ 2000	8	+ 1600	12	+ 2000	24	+ 2000	+ 2000	12
Valacyclovir (po)	2.5	25	1000	1000	8	1000	12	500	24	1000		
Valganciclovir (po)	3.0	68	900	900	12	450	24	450	72	900		
Vancomycin	6	150	1000	1000	12	1000	24	500	72	1000	1000	24
Voriconazole	8	12	2 x 400/24	200	12	200	12	200	12	200	200	12
Zalcitabine (po)	1.8	11	0.75	0.75	8	0.75	12	0.75	24			
Zanamivir	2.5	13.7	10	10	12	10	12	10	12		10	24
Zidovudine	1	1.9 (52)	200	200	8	100	8	100	8	200	200	12

Drugs are listed in alphabetical order and the parameter values for the drug (or active metabolite) are taken from our NEPharm database. If the individual GFR is not exactly 100 mL/min, or 30 mL/min, or 5 mL/min, the dose could be estimated by interpolation between the stated proposals. GFR: Glomerular filtration rate.

Dose adjustment rules

According to the proportional dose adjustment rules as proposed by Luzius Dettli, either the dose (D) should be reduced or the interval (Tau) extended (Figure 2). When the dose is reduced (Dettli 1) the peak levels are lower than in normal conditions but the trough levels are higher. When the administration interval is extended (Dettli 2) the peak and the trough concentrations are kept constant but the dosing frequency will decrease.

$$\frac{D}{Tau} = \left(\frac{D}{Tau}\right)_{norm} \cdot \frac{T_{1/2norm}}{T_{1/2fail}}$$

The dosing alternative proposed by Calvin Kunin states: The loading dose is the normal dose ($D_{start} = D_{norm}$) and the maintenance dose is one half of the loading dose where the administration interval corresponds to one half-life[23]. The Kunin rule leads to normal peak levels but higher troughs, a larger area AUC and more frequent peaks than those obtained with the Dettli rule 2.

$$\frac{D}{Tau} = \frac{(1/2) \cdot D_{start}}{T_{1/2}} = \frac{(1/2) \cdot D_{norm}}{T_{1/2}}$$

The Kunin rule can be illustrated with the example of ampicillin. In kidney failure, the ampicillin dose is decreased from 2000 mg every 8 h to 1000 mg every 12 h, since the half-life increases from 1.0 to 13 h (Table 1). For a GFR of 30 mL/min, the ampicillin half-life can be estimated at 3.8 h, giving reason to extend the administration interval from 8 to 12 h but to not change the 2000 mg dose since the half-life is shorter than the administration interval.

A general dosing rule that combines the Kunin rule with the Dettli rule 2 has been mentioned by Luzius Dettli: the eliminated fraction rule (Dettli 3). With the Dettli

rule 3, the administration interval is selected according to the target trough concentration while the peak is kept constant (Figure 3).

$$D = D_{norm} \cdot \frac{1 - \exp\left(-0.693 \cdot \frac{Tau}{T_{1/2}}\right)}{1 - \exp\left(-0.693 \cdot \frac{Tau}{T_{1/2}}\right)_{norm}}$$

$$= D_{start} \cdot \left[1 - \exp\left(-0.693 \cdot \frac{Tau}{T_{1/2}}\right)\right]$$

$$= D_{start} \cdot \left[1 - \left(\frac{C_{trough}}{C_{peak}}\right)_{target}\right]$$

$$Tau = \frac{T_{1/2}}{0.693} \cdot \ln\left(\frac{C_{peak}}{C_{trough}}\right)_{target}$$

For the condition where peak as well as trough concentrations are constant and maintained as in the normal situation, the Dettli rule 3 corresponds to the Dettli rule 2 with a proportional extension of the administration interval. For the condition where the peak is constant but the trough should be no less than one half of the peak, the Dettli 3 rule corresponds to the Kunin rule.

Which rule should be applied cannot be decided by pharmacokinetic principles alone, but pharmacodynamic principles must be considered too. In addition, whenever possible, therapeutic drug monitoring should be utilized. In times where tandem mass spectrometry LC-MS/MS is possible, nearly every drug could be measured.

Therapeutic drug monitoring

Amikacin, gentamicin, tobramycin, teicoplanin and vancomycin, but recently also colistin, piperacillin, meropenem and linezolid are anti-infective drugs that routinely can be measured. When drug levels are measured for optimizing antimicrobial therapy, two important peculiarities must be observed. If impaired kidney function impacts pharmacokinetics, higher trough concentrations must be accepted to obtain efficient peak concentrations - this can be seen when the Dettli rule 1 or the Kunin rule are applied for dose adjustment (Figures 2 and 3). This was demonstrated by the use of aminoglycosides in HD patients where only troughs of at least 3 ng/mL are associated with peaks above 7 ng/mL and both peaks and troughs were significantly higher in those patients surviving than in those without anti-infective success[24,25].

In line with these statements, the target trough concentration for vancomycin has consistently been increased in the last 25 years. The area under the curve should be > 400 h x mg/L (= 24 h x Css; Css > 17 mg/L) to obtain an antimicrobial response with vancomycin[26]. The new targets are troughs of 15 ng/mL needed to guarantee peaks of 30 to 40 ng/mL[27]. The further increase in vancomycin dose and higher trough concentrations, however, might be associated with an increased risk of nephrotoxicity[28].

Counterintuitively, plasma binding does not have much impact on drug dosing since the absolute free drug concentration value (C_{free}) is unchanged when bound concentrations change[29].

$C_{free} = C - C_{bound} = (C - \Delta C_{bound}) - (C_{bound} - \Delta C_{bound}) = const$

If the binding decreases, only the total (C_{tot}) and the bound (C_{bound}) concentrations and not the free (C_{free}) concentration will decrease. Since the effect is supposed to depend on free concentrations, lower total concentrations do not need a change in dosage. However, plasma binding does have an effect on drug monitoring as far as total concentrations are measured ($C_{tot} = C_{initial} - \Delta C_{bound}$) and lower than normal concentrations must be the target when binding is less. This mainly applies to antibiotics with high plasma binding such as teicoplanin and ceftriaxone. And again, the decision as to which concentration should be the target can be made most rationally by considering pharmacodynamic criteria too.

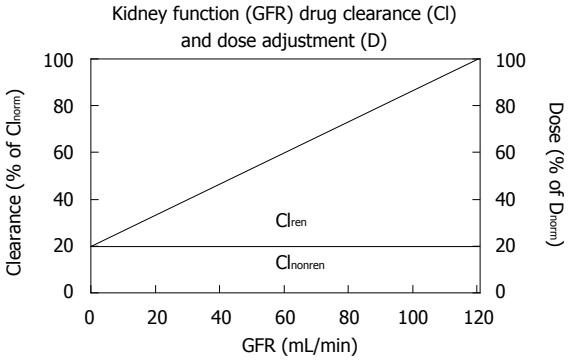

Figure 1 Linear correlation between drug clearance and the glomerular filtration rate as a measure of kidney function[20]. The dose can be adjusted in proportion to the reduced drug clearance, where $Cl = Cl_{ren} + Cl_{nonren}$. GFR: Glomerular filtration rate.

Pharmacodynamics

Pharmacokinetics is a necessary requirement for drug dose adjustment, but only the combined use of pharmacokinetics and pharmacodynamics is the sufficient condition for drug dose adjustment. Although some drug action might follow the dynamics of an irreversible effect, the most general concept of pharmacodynamics is based on the sigmoid Hill equation describing reversible effects. Even after mechanistic analysis of bacterial growth and killing dynamics, the Hill equation applies also to modeling the antimicrobial effect[30,31]. The actual effect (E) is a function of the maximum effect and of the concentration producing the half-maximum effect (CE_{50}). The Hill coefficient (H) gives a measure of the sigmoidicity of the effect concentration correlation.

$$E = \frac{E_{max}}{1 + \left(\frac{CE_{50}}{C}\right)^{H}}$$

From the above equation, the threshold concentration (CE_{05}) and the ceiling concentration (CE_{95}) can be derived[32]. The threshold concentration produces only 5% of the maximum effect and the ceiling concentration produces 95% of the maximum effect. The higher the Hill coefficient, the higher the threshold concentration is,

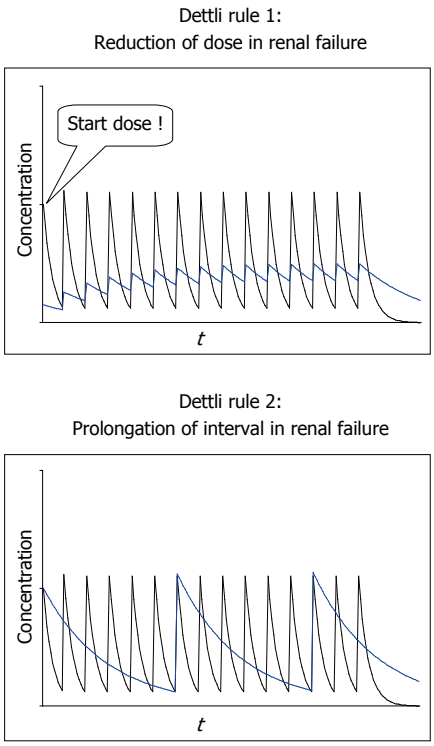

Figure 2 Dettli rules 1 and 2 for drug dose adjustment in kidney dysfunction. Dettli rule 1 leads to higher trough concentrations but lower peaks. To obtain an immediate antimicrobial effect, a loading dose must be given. With Dettli rules 1 and 2, the area under the curve AUC remains constant.

but the lower the ceiling concentration and the narrower the range of lower and upper target concentrations are (Figure 4).

$CE_{05} = CE_{50} \cdot 19^{-1/H}$

$CE_{95} = CE_{50} \cdot 19^{1/H}$

The ceiling concentration can be considered to be the upper limit of the target peak levels ($C_{peak} < CE_{95}$), whereas the threshold concentration marks the lower limit of effective trough levels ($C_{trough} > CE_{05}$). The distance between the ceiling and the threshold concentrations depends on H, not on CE_{50}, and the ceiling-to-threshold time $t_{ceiling\text{-}threshold}$ can be measured by multiples of the respective elimination half-life. For a drug with a short half-life and a high Hill coefficient, the therapeutic range of target concentrations can be very narrow (Figure 4).

$$CE_{05} = CE_{95} \cdot \exp\left(-\frac{\ln(2)}{T_{1/2}} \cdot t\right)$$

$$t_{ceiling\text{-}threshold} = T_{1/2} \cdot \frac{2}{H} \cdot \frac{\ln(19)}{\ln(2)} = T_{1/2} \cdot \frac{8.5}{H}$$

This conclusion might be illustrated with the beta lactam antibiotic ceftazidime where the half-life is 2.1 h and short in patients with normal kidney function (Table 1) but the Hill coefficient is 3.7 and high[33]. These values yield a short peak to trough or ceiling-to-threshold time $t_{ceiling\text{-}threshold}$ = 5 h, indicating that ceftazidime should be given at least every 6 h to maximize efficacy. In contrast, for gentamicin, the half-life is also 2 h (Table 1), but the Hill coefficient is 1.3 and low[33].

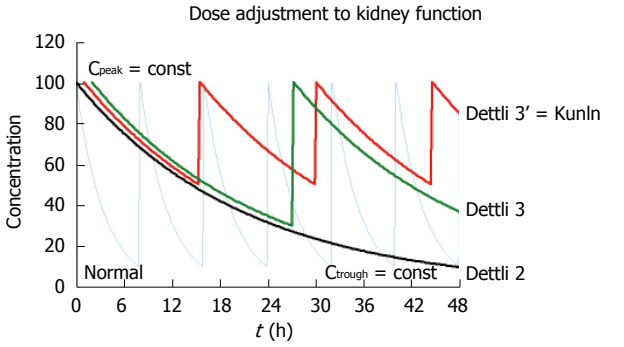

Figure 3 It is most practical to keep the peak concentration constant when the drug dose is adjusted to impaired kidney function[9]. With the eliminated fraction rule (Dettli 3), any dose and any interval can be estimated and selected. The Kunin rule is a special case of the Dettli rule 3 for the condition C_{trough} = 1/2 C_{peak}. With the Kunin rule and the Dettli rule 3, the area AUC is higher than under conditions with normal kidney function.

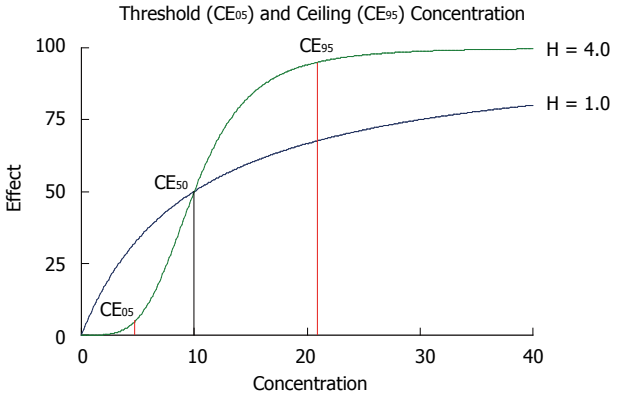

Figure 4 Pharmacodynamics. The threshold concentration CE_{05} produces 5% and the ceiling concentration CE_{95} produces 95% of the maximum effect. With a Hill coefficient of H = 1.0, the concentration is CE_{05} = 0.5 units and the CE_{95} = 190 units whereas for a higher Hill coefficient of H = 4.0, the threshold is high with CE_{05} = 6.0 units but the ceiling is low with CE_{95} = 21 units.

Thus, the estimated peak-to-trough time $t_{ceiling\text{-}threshold}$ is longer than 13 h: Here the administration interval could be extended to 12 h or more (Tau = $t_{ceiling\text{-}threshold}$).

The clinical progress in anti-infective dosing that has had the greatest impact has probably been achieved with the differentiation of drugs with time-dependent from drugs with concentration-dependent action[34,35]. Specific examples are the penicillins, cephalosporins, vancomycin, teicoplanin, the penems and the antiviral drugs with a time-dependent effect whereas gentamicin, amikacin, daptomycin, colistin, ciprofloxacin or levofloxacin possess a concentration-dependent activity.

It has been shown that anti-infective drugs with a time-dependent effect have a significantly higher Hill coefficient than those with concentration-dependent action[33]. This difference translates into practical consequences for the threshold and the ceiling concentration. A high Hill coefficient is associated with a relatively low ceiling concentration but simultaneously with a high threshold concentration (Figure 4). Thus, the time interval should be short between dosing of time-dependent

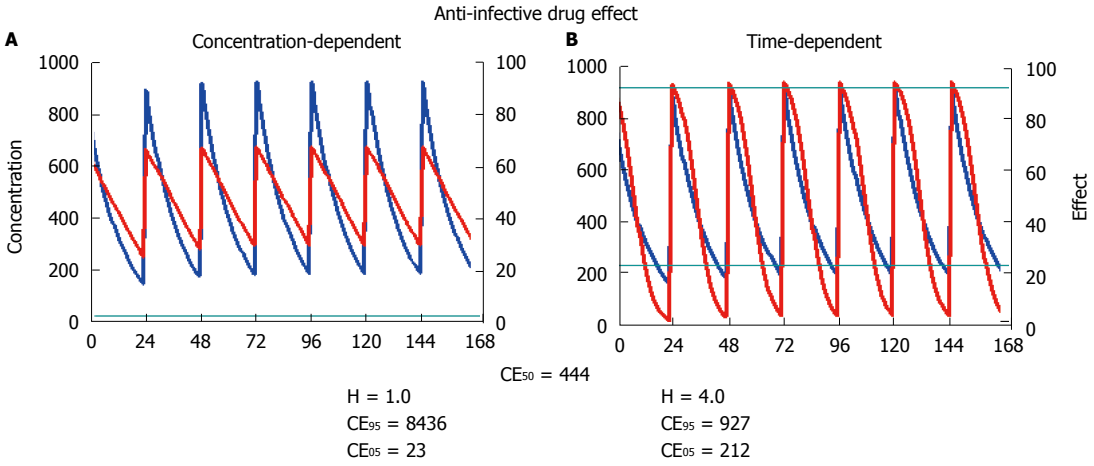

Figure 5 Pharmacodynamics of anti-infective drugs. The pharmacokinetics and the concentration curves are equal in both diagrams. Also the concentration producing the half-maximum effect is the same but the Hill coefficient is different. A: Concentration-dependent effect: With a Hill coefficient of H = 1.0, the calculated peak effect is only 60% and far from the ceiling effect CE_{95}. Thus, the concentration-dependent effect could be strengthened by increasing the dose; B: Time-dependent effect: With a Hill coefficient of H = 4.0, the calculated trough effect falls below the threshold concentration CE_{05} at the second part of the administration interval. Thus, the time-dependent effect could be strengthened by dosing more frequently.

anti-infective drugs and it makes no sense to increase the dose above the ceiling concentration. In contrast, a low Hill coefficient is associated with a high ceiling concentration and a low threshold concentration. Thus, it might increase the effect of concentration-dependent anti-infective drugs to give a single high bolus dose but it is not so critical to extend the administration interval - as proposed for aminoglycosides[36]. On a practical level, it might prove optimal to administer anti-infective drugs with time-dependent action more frequently, or even as a continuous infusion[37,38]. By contrast, anti-infective drugs with concentration-dependent action should be given with a bolus and a high maintenance dose to increase efficacy (Figure 5).

The usual measures of the antimicrobial effect such as the time over minimal inhibitory concentration MIC, or the AUC over MIC, or the peak over MIC can be unified by the following concept: A close correlation of the MIC and the concentration producing the half-maximum effect can be predicted. However, it has been shown[33] that for concentration-dependent antimicrobial action, the minimal inhibitory concentration could fall considerably below the concentration producing the half-maximum effect (MIC << CE_{50}). Consequently, it might be more reasonable to compare the bacteriological MIC with the pharmacodynamic parameter of a threshold concentration. Frequently the concentration target is stated as high as 4 times above the MIC. If this target corresponds to the CE_{50}, this translates into an average sized Hill coefficient of H = 2.1 since the following condition might hold true.

$C_{threshold} = MIC = CE_{05} = CE_{50} \cdot 19^{-1/H}$

In agreement with this equation, the Hill coefficient of meropenem is reported at H = 3.1 for the MIC of 1.0 mg/L and a CE_{50} at 2.6 mg/L[33].

Potency is also a significant measure of microbiology. The potency is inversely proportional to the concentration CE_{50} producing the half maximum effect. Therefore, resistance of the strain is just another word for a change in the CE_{50} and thus for reduced potency of the drug.

potency = $1/CE_{50}$

To overcome resistance, a higher dose might be necessary since a high concentration CE_{50} is required to produce the half-maximum effect. This concept allows a distinction to be made between relative resistance and absolute drug resistance. A pathogen with relative resistance can be made sensitive by increasing the dose[39-41]. Thus, it has been recommended to treat severe infections with resistant strains by increasing the standard meropenem dose to 3 x 2000 mg per day[42,43] or the daptomycin dose to > 8 mg/kg per day[44] with careful monitoring of side effects.

From the concept of potency and the interpretation of the Hill coefficient, it can be considered plausible that the time-dependent action and the concentration-dependent action are only the extreme positions of a continuum. Every drug can be considered both concentration-dependent and time-dependent - more or less, either the one or the other[31]. The antimicrobial drug effect needs the presence of leukocytes, and less bacterial killing is reported in neutropenia[31]. Therefore, these patients need a 1.5 to 2 times higher than usual dose of anti-infective drugs[45]. In addition, the increasing rate of drug resistance in febrile neutropenia also strongly supports the concept of high dosing[31,46].

Dose adjustment

Anticancer drugs and anti-infective drugs should be used differently. The adjustment of anticancer drugs must not only be based on the kidney function but also on the physical condition of a patient. Tumor patients are older and anticancer drugs have a considerable potential for toxicity. Therefore, anticancer chemotherapy must be adjusted to both kidney function and to the general medical condition (in cases with Karnofsky index < 40% or ECOG > 2 performance status). In contrast to

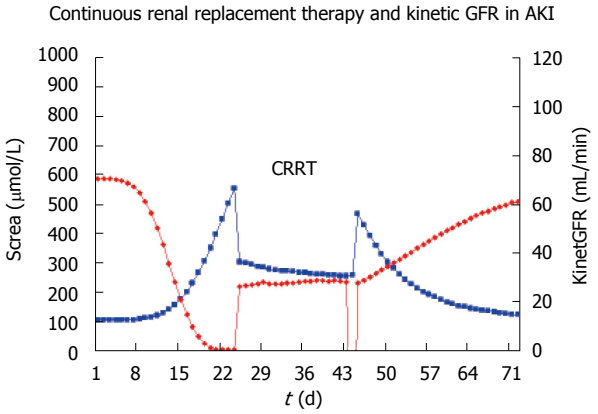

Figure 6 Serum creatinine (Screa) and estimated kinetic glomerular filtration rate in acute kidney injury. The kinetic GFR can also be estimated during continuous renal replacement therapy continuous hemofiltration (CRRT)[14]. GFR: Glomerular filtration rate; AKI: Acute kidney injury.

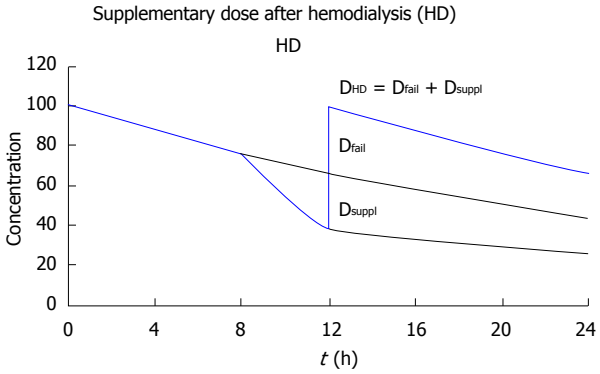

Figure 7 The dose after dialysis (D_{HD}) replaces both the dose adjusted for kidney failure (D_{fail}), and the supplementary dose (D_{suppl}) that compensates for the fraction (FR) removed during hemodialysis (HD).

anticancer drugs, however, the anti-infective therapy should be adjusted to kidney function alone, but a compromised or even poor general condition should not result in a reduced dose or selection of less active anti-infective therapy. An immediate and sufficiently high antimicrobial therapy is needed in the most vulnerable, that is, in elderly and immunocompromised cancer patients. Where the risk is low, oral dosing of anti-infective drugs is sufficient in febrile neutropenia[47]. In most cases, however, intravenous dosing might be preferable with sequential oral dosing only in responders.

Ehrlich[48] stated the principle of anti-infective therapy: "frapper vite et frapper fort" meaning "hit fast, hit hard".

For anti-infective drug therapy, the immediate and high loading dose is very important[49,50]. According to the "Tarragona strategy" the antibiotic regimen should be started fast and with a loading dose, whereas the dose adjustment follows the course and clinical condition[51]. It can be a deleterious mistake to adjust the dose to the impaired kidney function but to give no loading dose (Figure 2). The loading dose is usually the normal standard dose. However, many patients in the intensive care unit are over-hydrated and the distribution volume is much larger than under normal conditions[34]. The loading dose could well be adjusted to such volume changes by applying the BSA.

$$D_{start} = D_{norm} \cdot \frac{Vd}{Vd_{norm}} = D_{norm} \cdot \frac{Weight + V_{water}}{Weight_{norm}}$$

$$= D_{norm} \cdot \frac{BSA}{1.73\ m^2}$$

Thus, the required loading dose can be higher than the normal standard dose. In patients with sepsis, the gentamicin distribution volume was 0.35 L/kg *vs* 0.29 L/kg and significantly larger compared to intensive care patients without sepsis[52]. The need for a higher dose to initiate antimicrobial therapy can be stated as the rule when the immediate and high blood level is the target as with anti-infective therapy. The immediate start of treatment and an initially high concentration are also needed to avoid selection of resistant strains. Therefore, the antimicrobial treatment starts with a normal or even higher loading dose in the intensive care patients. Afterwards, the adjustment with a reduced maintenance dose is usually not needed before day 2 or 3 of anti-infective treatment[53].

A special problem occurs in the case of aminoglycosides: It is now standard practice to administer one single bolus dose per day instead of three divided doses[36]. Such a single high bolus dose will be associated with a 20-fold increase in the AUC if renal failure is present and the half-life increases from 2 to 40 h. For aminoglycosides, we propose administering only 50% of the standard high bolus loading dose to avoid excessive exposure in kidney failure or dialysis patients (Table 1). Following the loading dose, the maintenance dose can be estimated by one of the three Dettli rules, or the Kunin rule.

In addition to the case of over-hydration with an increase in distribution volume, the so-called augmented renal clearance has been brought into debate[54]. Augmented renal clearance is estimated from serum creatinine or endogenous creatinine clearance. If a patient is overhydrated, however, the serum creatinine is diluted, making creatinine clearance and creatinine-based GFR estimates falsely high. Since the clearance can be seen as the arithmetic product of elimination rate constant and distribution volume, the higher creatinine clearance in the patients with the systemic inflammatory response syndrome and sepsis could be explained by two mechanisms, augmented renal elimination and over-hydration. The consequences are different: augmented renal elimination needs a higher maintenance dose but over-hydration requires both an increase in the loading dose and a higher maintenance dose (= weight-based dosing as in pediatrics).

Renal replacement therapy

In the intensive care unit (ICU), three modalities are used as renal replacement therapy: Continuous hemofiltration (CRRT), sustained low efficiency daily dialysis (SLEDD) and intermittent HD. The hemofiltration is applied with variable modifications either of the surface area, of

the filter membrane, with predilution or post-dilution replacement fluid, and variable ultrafiltration rates that are used along with the corresponding flow rate of the substitution volume. Therefore, a global measure of the effect of hemofiltration on drug elimination will be very useful and the total creatinine clearance or the other creatinine-based measures of the GFR have been proposed for this purpose[9,55,56]. The recently introduced kinetic GFR applies also to patients with CRRT[14], and thus has clear advantages in the intensive care unit where the medical conditions can change rapidly (Figure 6).

$totalCL_{crea} = Filtration_{kedney} + Filtration_{CRRT}$

$totalCL_{crea} = eGFR = MDRD_{GFR} = CKD\text{ - }EPI_{GFR} = C\text{ and }G_{GFR}$

$totalCL_{crea} = kinetGFR$

There is a trend to underestimate drug elimination by CRRT and consequently under-dose antimicrobials in the ICU[57]. By using the total creatinine clearance, the creatinine-based GFR estimates or the kinetic GFR, the dose can be adjusted according to the rules of Dettli and Kunin also for patients on CRRT. As a rule and to avoid under-dosage, the normal standard dosage should be given and not be reduced if the total creatinine clearance is above 60 mL/min.

A combination of continuous and intermittent renal replacement is the SLEDD. The frequency of under-dosage is estimated with a median value of 70% whereas the risk of over-dosage was only 5% while on SLEDD[6,58]. If this kind of treatment is applied, the daily dose at least corresponds to the post HD dose (see below) but recommendations vary widely.

$D_{SLEDD} = D_{HD} \approx D_{start}$

More complex is the drug dosing when intermittent HD is performed (Figure 7). Off dialysis, the dose must be adjusted to the failing kidney function. For intermittent HD, we argue that it is better to give the dose not at the beginning but at the end or immediately after HD. With a pre-dialysis dose, no anti-infective effect will be maintained in the interval off dialysis[59].

If the drug is given after dialysis, the post-dialysis dose should replace first the amount eliminated during the interval off dialysis, that is, the dose for failing kidney function (D_{fail}). In addition to that, the effect of HD should be compensated by a supplementary dose (D_{suppl}) replacing the fraction eliminated on dialysis (FR).

$D_{HD} = D_{fail} + D_{suppl}$

$D_{suppl} = FR \cdot (D_{start} - D_{fail})$

$FR = 1 - \exp[(-0.693/T_{1/2on}) \cdot t_{on}]$

Thus, the dose after HD is higher than the adjusted maintenance dose[9]. In many cases the dose after HD is another loading dose (D_{start}). The post-dialysis dose (D_{HD}) can again be illustrated with the example of ampicillin: The fraction eliminated by dialysis is implicitly stated in NEPharm (40%) and the dose after dialysis is 2000 mg corresponding to the size of the normal loading dose (Table 1).

$D_{HD} \approx D_{start}$

In contrast to the usual post-dialysis dosing, it might be a good option to perform HD after drug administration for removal of high-dose anticancer therapy administered before dialysis. In analogy, the dosing immediately before dialysis has been also proposed for aminoglycosides[60]. With a pre-dialysis regimen, however, aminoglycosides must be given at a higher dose (gentamicin up to 400 mg) and HD should be performed on a daily basis in order to not miss the antimicrobial effect in the interval off dialysis.

CONCLUSION

The prevalence of CKD and incidence of AKI are high in patients with malignancies. This generally makes dose adjustment necessary, usually ending in a lower dose than normal. Since 1978, we have documented pharmacokinetic parameters in the NEPharm database from extracted PubMed citations[61-63]. With the parameters recorded in NEPharm and based on the above pharmacokinetic/pharmacodynamic considerations, we have made explicit dose proposals. These recommendations are used in our institution and subjected to continuous updates (Table 1).

Anti-infective therapy should start immediately without any delay and with a high dose. Dose adjustment follows on day 2 or later in the course of treatment[53]. A loading dose that takes into account the real volume especially in volume-expanded patients should be given. When in doubt, we propose that the peak level should be the target and the standard dose should be given with an extended administration interval when kidney function is impaired[9].

The anti-infective therapy should be optimized by therapeutic drug monitoring whenever possible (gentamicin, tobramycin, amikacin, vancomycin, teicoplanin, colistin, piperacillin, meropenem, linezolid). However, the adequate practical consequences should be drawn from the measured concentrations. In patients with impaired kidney function, higher trough concentrations result from the dose adjustment according to Dettli 1, Dettli 3 or Kunin. Only the Dettli rule 2 is associated with the same peak and trough concentrations as under normal conditions. On the other hand, the plasma binding of many drugs can decrease in kidney dysfunction. In this case, lower trough concentrations are acceptable (ceftriaxone, teicoplanin) since the absolute free concentration does not change when the bound fraction decreases but free concentrations produce the effect.

The modern distinction between time-dependent and concentration-dependent effects can be parameterized by the Hill coefficient. A high Hill coefficient (> 2.1) indicates time-dependent drug action, whereas a low Hill coefficient (< 2.1) indicates concentration-dependent action. Based on the Hill equation, the threshold concentration can be distinguished from the ceiling concentration. A high Hill coefficient determines that the ceiling concentration is low but the threshold concentration is relatively high (Figure 4). In contrast, a low Hill coefficient determines that the ceiling concentration is relatively high but the

threshold concentration is low. We suggest that the minimal inhibitory concentration from microbiology be correlated to the threshold concentration. The target concentration should not be less than the threshold concentration for time-dependent effects, but the target concentration could be as high as the ceiling concentration for concentration-dependent effects.

To decide between the pharmacokinetic dosing alternatives (Dettli 1-3), pharmacodynamic considerations can give an answer to whether the dose should be reduced or the interval extended in kidney dysfunction: (1) For time-dependent anti-infective action, more frequent dosing is more effective than maintaining the single high dose[35]: The target trough levels should be kept above the threshold concentration (Figure 5). The beta lactam antibiotics oxacillin or piperacillin are considered to exhibit a time-dependent action. Accordingly, it has been shown that continuous infusion produces a better antimicrobial response than intermittent dosing of the respective daily dose[37,38]; and (2) For concentration-dependent anti-infective action, however, the extension of the interval is less disadvantageous than reducing the single dose (Figure 5). The target peak levels should be close to the ceiling concentration and kept as high as possible[35]. The quinolone ciprofloxacin exhibits concentration-dependent action. Here, the high bolus dosing produced a more rapid bactericidal effect than the more frequent application of a lower dose[33,64]. Also for aminoglycosides, a high peak concentration is superior to more frequent dosing to induce bacterial killing[36,65].

For drugs with a high Hill coefficient, the area under the effect time curve may fall disproportionally less and result insufficient with a lower dose[61]. Therefore, we discourage proportional dose reduction, especially Dettli 1, if the Hill coefficient is unknown. The risk of selecting resistant strains is also less when the initial dose is high[31].

The time above MIC reflects effect duration. A pharmacodynamic measure for the duration of drug effect, the time of effect duration (TED), can be derived from the elimination half-life[18]. The intuitively most evident effect duration time is the effect bisection time (TED_{50}) that is correlated to the elimination half-life ($T_{1/2}$), the peak concentration (C_{peak}) and the Hill coefficient (H) along with the concentration (CE_{50}) producing the half-maximum effect[18].

$$TED_{50} = T_{1/2} \cdot \left(\frac{1.44}{H}\right) \cdot \ln\left[2 + \left(\frac{C_{peak}}{CE_{50}}\right)^{H}\right]$$

The longer the half-life and the higher the peak concentration - but the less the CE_{50} - the longer lasting the effect is. The half-life is 1.0 h (Table 1) and the Hill coefficient is stated at H = 3.1 for meropenem[33]. If the MIC of 6 mg/l[44] is equated to the threshold concentration (CE_{05} = MIC), the CE_{50} can be estimated at 37 mg/L. With a dose of 500 mg every 8 h and a peak concentration of 50 mg/L[44], the effect bisection time will be estimated at TED_{50} = 0.71 h. Doubling the dose, however, will more than double the effect bisection time TED_{50} to 1.5 h, thus extending the drug action while the pharmacokinetic half-life of 1.0 h is the same. However, the standard dose administered more frequently would not increase the effect bisection time.

The dose in patients with continuous renal replacement therapy can be derived from the creatinine-based GFR estimates or in case of changing kidney function, from the "kinetic GFR" (Figure 6). If this GFR estimate is above 60 mL/min, no dose adjustment is required. For intermittent HD a supplementary dose should be given after dialysis (Figure 7). The supplementary dose adds with the dose adjusted to renal failure to the post-HD dose that can be as high as the loading dose. This practice might be prudent also in cases where the drug fraction eliminated during HD is not known.

REFERENCES

1 **Nomura K**, Ishida Y. [Optimal administration of antimicrobial agents for febrile neutropenia in patients with hematological malignancies]. *Nihon Rinsho* 2010; **68**: 1403-1411 [PMID: 20662227]

2 **Perazella MA**. Onco-nephrology: renal toxicities of chemotherapeutic agents. *Clin J Am Soc Nephrol* 2012; **7**: 1713-1721 [PMID: 22879440 DOI: 10.2215/CJN.02780312]

3 **Lowrance WT**, Ordoñez J, Udaltsova N, Russo P, Go AS. CKD and the risk of incident cancer. *J Am Soc Nephrol* 2014; **25**: 2327-2334 [PMID: 24876115 DOI: 10.1681/ASN.2013060604]

4 **Launay-Vacher V**. Epidemiology of chronic kidney disease in cancer patients: lessons from the IRMA study group. *Semin Nephrol* 2010; **30**: 548-556 [PMID: 21146120 DOI: 10.1016/j.semnephrol.2010.09.003]

5 **Janus N**, Launay-Vacher V, Byloos E, Machiels JP, Duck L, Kerger J, Wynendaele W, Canon JL, Lybaert W, Nortier J, Deray G, Wildiers H. Cancer and renal insufficiency results of the BIRMA study. *Br J Cancer* 2010; **103**: 1815-1821 [PMID: 21063408 DOI: 10.1038/sj.bjc.6605979]

6 **Harris LE**, Reaves AB, Krauss AG, Griner J, Hudson JQ. Evaluation of antibiotic prescribing patterns in patients receiving sustained low-efficiency dialysis: opportunities for pharmacists. *Int J Pharm Pract* 2013; **21**: 55-61 [PMID: 23301534 DOI: 10.1111/j.2042-7174.2012.00226.x]

7 **Walser M**. Assessing renal function from creatinine measurements in adults with chronic renal failure. *Am J Kidney Dis* 1998; **32**: 23-31 [PMID: 9669420]

8 **Matsushita K**, Mahmoodi BK, Woodward M, Emberson JR, Jafar TH, Jee SH, Polkinghorne KR, Shankar A, Smith DH, Tonelli M, Warnock DG, Wen CP, Coresh J, Gansevoort RT, Hemmelgarn BR, Levey AS. Comparison of risk prediction using the CKD-EPI equation and the MDRD study equation for estimated glomerular filtration rate. *JAMA* 2012; **307**: 1941-1951 [PMID: 22570462 DOI: 10.1001/jama.2012.3954]

9 **Matzke GR**, Aronoff GR, Atkinson AJ, Bennett WM, Decker BS, Eckardt KU, Golper T, Grabe DW, Kasiske B, Keller F, Kielstein JT, Mehta R, Mueller BA, Pasko DA, Schaefer F, Sica DA, Inker LA, Umans JG, Murray P. Drug dosing consideration in patients with acute and chronic kidney disease-a clinical update from Kidney Disease: Improving Global Outcomes (KDIGO). *Kidney Int* 2011; **80**: 1122-1137 [PMID: 21918498 DOI: 10.1038/ki.2011.322]

10 **Mosteller RD**. Simplified calculation of body-surface area. *N Engl J Med* 1987; **317**: 1098 [PMID: 3657876 DOI: 10.1056/NEJM198710223171717]

11 **Park EJ**, Wu K, Mi Z, Dong T, Lawrence JP, Ko CW, Huang SM, Zhang L, Crentsil V, Zhang J, Xu NN. A systematic comparison of cockcroft-gault and modification of diet in renal disease equations for classification of kidney dysfunction and dosage adjustment. *Ann Pharmacother* 2012; **46**: 1174-1187 [PMID: 22932303 DOI:

10.1345/aph.1Q757]
12 **Keller F**, Hartmann B. An easy to calculate equation to estimate GFR based on inulin clearance. *Nephrol Dial Transplant* 2010; **25**: 1345-1346; author reply 1346-1347 [PMID: 20075434 DOI: 10.1093/ndt/gfp714]
13 **Tsinalis D**, Thiel GT. An easy to calculate equation to estimate GFR based on inulin clearance. *Nephrol Dial Transplant* 2009; **24**: 3055-3061 [PMID: 19395728 DOI: 10.1093/ndt/gfp193]
14 **Chen S**. Retooling the creatinine clearance equation to estimate kinetic GFR when the plasma creatinine is changing acutely. *J Am Soc Nephrol* 2013; **24**: 877-888 [PMID: 23704286 DOI: 10.1681/ASN.2012070653]
15 **Benet LZ**. Clearance (née Rowland) concepts: a downdate and an update. *J Pharmacokinet Pharmacodyn* 2010; **37**: 529-539 [PMID: 21113650 DOI: 10.1007/s10928-010-9187-8]
16 **Dost FH**. [Half value time and total clearance]. *Z Gesamte Inn Med* 1954; **9**: 546-551 [PMID: 13206142]
17 **Schiffer JT**, Swan DA, Corey L, Wald A. Rapid viral expansion and short drug half-life explain the incomplete effectiveness of current herpes simplex virus 2-directed antiviral agents. *Antimicrob Agents Chemother* 2013; **57**: 5820-5829 [PMID: 24018260 DOI: 10.1128/AAC.01114-13]
18 **Keller F**, Hartmann B, Czock D. Time of effect duration and administration interval for sitagliptin in patients with kidney failure. *Eur J Drug Metab Pharmacokinet* 2014; **39**: 77-85 [PMID: 24353117 DOI: 10.1007/s13318-013-0164-7]
19 **Kunin CM**, Rees SB, Merrill JP, Finland M. Persistence of antibiotics in blood of patients with acute renal failure. I. Tetracycline and chlortetracycline. *J Clin Invest* 1959; **38**: 1487-1497 [PMID: 14412754 DOI: 10.1172/JCI103927]
20 **Dettli LC**. Drug dosage in patients with renal disease. *Clin Pharmacol Ther* 1974; **16**: 274-280 [PMID: 4843318]
21 **Giusti DL**, Hayton WL. Dosage regimen adjustments in renal impairment. *Drug Intell Clin Pharm* 1973; **7**: 382-387
22 **Yeung CK**, Shen DD, Thummel KE, Himmelfarb J. Effects of chronic kidney disease and uremia on hepatic drug metabolism and transport. *Kidney Int* 2014; **85**: 522-528 [PMID: 24132209 DOI: 10.1038/ki.2013.399]
23 **Kunin CM**. A guide to use of antibiotics in patients with renal disease. A table of recommended doses and factors governing serum levels. *Ann Intern Med* 1967; **67**: 151-158 [PMID: 6028645 DOI: 10.7326/0003-4819-67-1-151]
24 **Keller F**, Wagner K, Borner K, Kemmerich B, Lode H, Offermann G, Distler A. Aminoglycoside dosage in hemodialysis patients. *J Clin Pharmacol* 1986; **26**: 690-695 [PMID: 3793962 DOI: 10.1002/j.1552-4604.1986.tb02974.x]
25 **Keller F**, Borner K, Schwarz A, Offermann G, Lode H. Therapeutic aminoglycoside monitoring in renal failure patients. *Ther Drug Monit* 1987; **9**: 148-153 [PMID: 3617152 DOI: 10.1097/00007691-198706000-00004]
26 **Moise-Broder PA**, Forrest A, Birmingham MC, Schentag JJ. Pharmacodynamics of vancomycin and other antimicrobials in patients with Staphylococcus aureus lower respiratory tract infections. *Clin Pharmacokinet* 2004; **43**: 925-942 [PMID: 15509186 DOI: 10.2165/00003088-200443130-00005]
27 **Davis SL**, Scheetz MH, Bosso JA, Goff DA, Rybak MJ. Adherence to the 2009 consensus guidelines for vancomycin dosing and monitoring practices: a cross-sectional survey of U.S. hospitals. *Pharmacotherapy* 2013; **33**: 1256-1263 [PMID: 23897602 DOI: 10.1002/phar.1327]
28 **Lodise TP**, Patel N, Lomaestro BM, Rodvold KA, Drusano GL. Relationship between initial vancomycin concentration-time profile and nephrotoxicity among hospitalized patients. *Clin Infect Dis* 2009; **49**: 507-514 [PMID: 19586413 DOI: 10.1086/600884]
29 **Benet LZ**, Hoener BA. Changes in plasma protein binding have little clinical relevance. *Clin Pharmacol Ther* 2002; **71**: 115-121 [PMID: 11907485]
30 **Czock D**, Markert C, Hartman B, Keller F. Pharmacokinetics and pharmacodynamics of antimicrobial drugs. *Expert Opin Drug Metab Toxicol* 2009; **5**: 475-487 [PMID: 19416084 DOI: 10.1517/17425250902913808]
31 **Ankomah P**, Levin BR. Exploring the collaboration between antibiotics and the immune response in the treatment of acute, self-limiting infections. *Proc Natl Acad Sci USA* 2014; **111**: 8331-8338 [PMID: 24843148 DOI: 10.1073/pnas.1400352111]
32 **Hartmann B**, Czock D, Keller F. Drug therapy in patients with chronic renal failure. *Dtsch Arztebl Int* 2010; **107**: 647-655; quiz 655-656 [PMID: 20959896 DOI: 10.3238/arztebl.2010.0647]
33 **Czock D**, Keller F. Mechanism-based pharmacokinetic-pharmacodynamic modeling of antimicrobial drug effects. *J Pharmacokinet Pharmacodyn* 2007; **34**: 727-751 [PMID: 17906920]
34 **Eyler RF**, Mueller BA. Antibiotic dosing in critically ill patients with acute kidney injury. *Nat Rev Nephrol* 2011; **7**: 226-235 [PMID: 21343897 DOI: 10.1038/nrneph.2011.12]
35 **Eyler RF**, Mueller BA. Antibiotic pharmacokinetic and pharmacodynamic considerations in patients with kidney disease. *Adv Chronic Kidney Dis* 2010; **17**: 392-403 [PMID: 20727509 DOI: 10.1053/j.ackd.2010.05.007]
36 **Kashuba AD**, Nafziger AN, Drusano GL, Bertino JS. Optimizing aminoglycoside therapy for nosocomial pneumonia caused by gram-negative bacteria. *Antimicrob Agents Chemother* 1999; **43**: 623-629 [PMID: 10049277]
37 **Hughes DW**, Frei CR, Maxwell PR, Green K, Patterson JE, Crawford GE, Lewis JS. Continuous versus intermittent infusion of oxacillin for treatment of infective endocarditis caused by methicillin-susceptible Staphylococcus aureus. *Antimicrob Agents Chemother* 2009; **53**: 2014-2019 [PMID: 19258261 DOI: 10.1128/AAC.01232-08]
38 **Lorente L**, Jiménez A, Martín MM, Iribarren JL, Jiménez JJ, Mora ML. Clinical cure of ventilator-associated pneumonia treated with piperacillin/tazobactam administered by continuous or intermittent infusion. *Int J Antimicrob Agents* 2009; **33**: 464-468 [PMID: 19150225 DOI: 10.1016/j.ijantimicag.2008.10.025]
39 **Gracia-Ahufinger I**, Gutiérrez-Aroca J, Cordero E, Vidal E, Cantisán S, del Castillo D, Martín-Gandul C, Rivero A, Torre-Cisneros J. Use of high-dose ganciclovir for the treatment of cytomegalovirus replication in solid organ transplant patients with ganciclovir resistance-inducing mutations. *Transplantation* 2013; **95**: 1015-1020 [PMID: 23407543 DOI: 10.1097/TP.0b013e31828555ac]
40 **Llor C**, Arranz J, Morros R, García-Sangenís A, Pera H, Llobera J, Guillén-Solà M, Carandell E, Ortega J, Hernández S, Miravitlles M. Efficacy of high doses of oral penicillin versus amoxicillin in the treatment of adults with non-severe pneumonia attended in the community: study protocol for a randomised controlled trial. *BMC Fam Pract* 2013; **14**: 50 [PMID: 23594463 DOI: 10.1186/1471-2296-14-50]
41 **Gould IM**, Miró JM, Rybak MJ. Daptomycin: the role of high-dose and combination therapy for Gram-positive infections. *Int J Antimicrob Agents* 2013; **42**: 202-210 [PMID: 23845504 DOI: 10.1016/j.ijantimicag.2013.05.005]
42 **Kiratisin P**, Keel RA, Nicolau DP. Pharmacodynamic profiling of doripenem, imipenem and meropenem against prevalent Gram-negative organisms in the Asia-Pacific region. *Int J Antimicrob Agents* 2013; **41**: 47-51 [PMID: 23127484 DOI: 10.1016/j.ijantimicag.2012.09.007]
43 **Binder L**, Schwörer H, Hoppe S, Streit F, Neumann S, Beckmann A, Wachter R, Oellerich M, Walson PD. Pharmacokinetics of meropenem in critically ill patients with severe infections. *Ther Drug Monit* 2013; **35**: 63-70 [PMID: 23318279 DOI: 10.1097/FTD.0b013e31827d496c]
44 **Kullar R**, Casapao AM, Davis SL, Levine DP, Zhao JJ, Crank CW, Segreti J, Sakoulas G, Cosgrove SE, Rybak MJ. A multicentre evaluation of the effectiveness and safety of high-dose daptomycin for the treatment of infective endocarditis. *J Antimicrob Chemother* 2013; **68**: 2921-2926 [PMID: 23928022 DOI: 10.1093/jac/dkt294]
45 **Drusano GL**, Louie A, Deziel M, Gumbo T. The crisis of resistance: identifying drug exposures to suppress amplification of resistant mutant subpopulations. *Clin Infect Dis* 2006; **42**: 525-532 [PMID: 16421797]
46 **Goulenok T**, Fantin B. Antimicrobial treatment of febrile neutropenia: pharmacokinetic-pharmacodynamic considerations. *Clin*

Pharmacokinet 2013; **52**: 869-883 [PMID: 23807657 DOI: 10.1007/s40262-013-0086-1]
47 **Kern WV**, Marchetti O, Drgona L, Akan H, Aoun M, Akova M, de Bock R, Paesmans M, Viscoli C, Calandra T. Oral antibiotics for fever in low-risk neutropenic patients with cancer: a double-blind, randomized, multicenter trial comparing single daily moxifloxacin with twice daily ciprofloxacin plus amoxicillin/clavulanic acid combination therapy--EORTC infectious diseases group trial XV. *J Clin Oncol* 2013; **31**: 1149-1156 [PMID: 23358983 DOI: 10.1200/JCO.2012.45.8109]
48 **Ehrlich P**. Chemotherapeutics: scientific principles, methods and results. *Lancet* 1913; **2**: 353-359
49 **Harbarth S**, Garbino J, Pugin J, Romand JA, Lew D, Pittet D. Inappropriate initial antimicrobial therapy and its effect on survival in a clinical trial of immunomodulating therapy for severe sepsis. *Am J Med* 2003; **115**: 529-535 [PMID: 14599631 DOI: 10.1016/j.amjmed.2003.07.005]
50 **Kumar A**, Ellis P, Arabi Y, Roberts D, Light B, Parrillo JE, Dodek P, Wood G, Kumar A, Simon D, Peters C, Ahsan M, Chateau D. Initiation of inappropriate antimicrobial therapy results in a fivefold reduction of survival in human septic shock. *Chest* 2009; **136**: 1237-1248 [PMID: 19696123 DOI: 10.1378/chest.09-0087]
51 **Sandiumenge A**, Diaz E, Bodí M, Rello J. Therapy of ventilator-associated pneumonia. A patient-based approach based on the ten rules of "The Tarragona Strategy". *Intensive Care Med* 2003; **29**: 876-883 [PMID: 12677369]
52 **Buijk SE**, Mouton JW, Gyssens IC, Verbrugh HA, Bruining HA. Experience with a once-daily dosing program of aminoglycosides in critically ill patients. *Intensive Care Med* 2002; **28**: 936-942 [PMID: 12122533 DOI: 10.1007/s00134-002-1313-7]
53 **Ulldemolins M**, Vaquer S, Llauradó-Serra M, Pontes C, Calvo G, Soy D, Martín-Loeches I. Beta-lactam dosing in critically ill patients with septic shock and continuous renal replacement therapy. *Crit Care* 2014; **18**: 227 [PMID: 25042938 DOI: 10.1186/cc13938]
54 **Claus BO**, Hoste EA, Colpaert K, Robays H, Decruyenaere J, De Waele JJ. Augmented renal clearance is a common finding with worse clinical outcome in critically ill patients receiving antimicrobial therapy. *J Crit Care* 2013; **28**: 695-700 [PMID: 23683557 DOI: 10.1016/j.jcrc.2013.03.003]
55 **Keller F**, Böhler J, Czock D, Zellner D, Mertz AK. Individualized drug dosage in patients treated with continuous hemofiltration. *Kidney Int Suppl* 1999; **(72)**: S29-S31 [PMID: 10560801]
56 **Bouman CS**. Antimicrobial dosing strategies in critically ill patients with acute kidney injury and high-dose continuous veno-venous hemofiltration. *Curr Opin Crit Care* 2008; **14**: 654-659 [PMID: 19023912]
57 **Seyler L**, Cotton F, Taccone FS, De Backer D, Macours P, Vincent JL, Jacobs F. Recommended β-lactam regimens are inadequate in septic patients treated with continuous renal replacement therapy. *Crit Care* 2011; **15**: R137 [PMID: 21649882 DOI: 10.1186/cc10257]
58 **Bogard KN**, Peterson NT, Plumb TJ, Erwin MW, Fuller PD, Olsen KM. Antibiotic dosing during sustained low-efficiency dialysis: special considerations in adult critically ill patients. *Crit Care Med* 2011; **39**: 560-570 [PMID: 21221000]
59 **Janus N**, Thariat J, Boulanger H, Deray G, Launay-Vacher V. Proposal for dosage adjustment and timing of chemotherapy in hemodialyzed patients. *Ann Oncol* 2010; **21**: 1395-1403 [PMID: 20118214 DOI: 10.1093/annonc/mdp598]
60 **Veinstein A**, Venisse N, Badin J, Pinsard M, Robert R, Dupuis A. Gentamicin in hemodialyzed critical care patients: early dialysis after administration of a high dose should be considered. *Antimicrob Agents Chemother* 2013; **57**: 977-982 [PMID: 23229487 DOI: 10.1128/AAC.01762-12]
61 **Aymanns C**, Keller F, Maus S, Hartmann B, Czock D. Review on pharmacokinetics and pharmacodynamics and the aging kidney. *Clin J Am Soc Nephrol* 2010; **5**: 314-327 [PMID: 20056753 DOI: 10.2215/CJN.03960609]
62 **Zellner D**, Schromm T, Frankewitsch T, Giehl M, Keller F. Structured data entry for reliable acquisition of pharmacokinetic data. *Methods Inf Med* 1996; **35**: 261-264 [PMID: 8952312]
63 **Keller F**, Frankewitsch T, Zellner D, Simon S, Czock D, Giehl M. Standardized structure and modular design of a pharmacokinetic database. *Comput Methods Programs Biomed* 1998; **55**: 107-115 [PMID: 9568383 DOI: 10.1016/S0169-2607(97)00061-8]
64 **Czock D**, Rasche FM. Dose adjustment of ciprofloxacin in renal failure: reduce the dose or prolong the administration interval? *Eur J Med Res* 2005; **10**: 145-148 [PMID: 15946909]
65 **Moore RD**, Lietman PS, Smith CR. Clinical response to aminoglycoside therapy: importance of the ratio of peak concentration to minimal inhibitory concentration. *J Infect Dis* 1987; **155**: 93-99 [PMID: 3540140 DOI: 10.1093/infdis/155.1.93]

P- Reviewer: Bellomo G, Friedman EA, Mahmoud KM, Yong D
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DOI: 10.5527/wjn.v4.i3.345

World J Nephrol 2015 July 6; 4(3): 345-353
ISSN 2220-6124 (online)

REVIEW

Estimating glomerular filtration rate in kidney transplantation: Still searching for the best marker

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Author contributions: Both authors contributed equally to this article.

Conflict-of-interest statement: The authors have read and understood BPG policy on declaration of interests and declare that they have no competing interests.

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Received: January 26, 2015
Peer-review started: January 28, 2015
First decision: March 6, 2015
Revised: May 6, 2015
Accepted: May 7, 2015
Article in press: May 8, 2015
Published online: July 6, 2015

Abstract

Kidney transplantation is the treatment of choice for end-stage renal disease. The evaluation of graft function is mandatory in the management of renal transplant recipients. Glomerular filtration rate (GFR), is generally considered the best index of graft function and also a predictor of graft and patient survival. However GFR measurement using inulin clearance, the gold standard for its measurement and exogenous markers such as radiolabeled isotopes (^{51}Cr EDTA, ^{99m}Tc DTPA or ^{125}I Iothalamate) and non-radioactive contrast agents (Iothalamate or Iohexol), is laborious as well as expensive, being rarely used in clinical practice. Therefore, endogenous markers, such as serum creatinine or cystatin C, are used to estimate kidney function, and equations using these markers adjusted to other variables, mainly demographic, are an attempt to improve accuracy in estimation of GFR (eGFR). Nevertheless, there is some concern about the inability of the available eGFR equations to accurately identify changes in GFR, in kidney transplant recipients. This article will review and discuss the performance and limitations of these endogenous markers and their equations as estimators of GFR in the kidney transplant recipients, and their ability in predicting significant clinical outcomes.

Key words: Glomerular filtration rate estimation; Creatinine; Cystatin C; Kidney transplantation; Clinical outcomes

Core tip: An accurate evaluation of allograft function is essential in the management of kidney transplant. Glomerular filtration rate (GFR), is generally considered the best index of graft function. Endogenous markers, such as serum creatinine or cystatin C, are used to estimate kidney function, and equations using these markers adjusted to other variables, are an attempt to improve accuracy in estimation of GFR. This article will review and discuss the performance and limitations of these endogenous markers and their equations as estimators of GFR in the kidney transplant recipients, and their ability in predicting clinical outcomes.

Santos J, Martins LS. Estimating glomerular filtration rate in

kidney transplantation: Still searching for the best marker. *World J Nephrol* 2015; 4(3): 345-353 Available from: URL: http://www.wjgnet.com/2220-6124/full/v4/i3/345.htm DOI: http://dx.doi.org/10.5527/wjn.v4.i3.345

INTRODUCTION

Kidney transplantation is the treatment of choice for end-stage renal disease. A successful kidney transplant improves the quality of life, reduces the mortality risk for most patients and is less costly when compared with maintenance dialysis[1-3]. The significant progress that has occurred over the last two decades in renal transplantation is mostly driven by improvements in short-term graft survival whereas long-term outcomes remained largely unchanged[4,5]. Nowadays, with the traditional short-term outcomes, namely the 1-year graft and patient survival rates in excess of 90% and 1-year acute rejection rate of less than 15%, the question arises if any further improvements are possible or even necessary[6]. However, these outstanding results have failed to anticipate long-term survival, so it becomes clear that identification of new, short-term end points capable of correlating with long-term graft outcome is necessary[7] ideally translating in longer graft maintenance.

Renal allograft function seems to be a tempting candidate as surrogate marker for research studies on transplantation[8], also for the assessment of new drugs[9,10], although its use as an outcome marker for graft loss is controversial[11,12]. In generally, the glomerular filtration rate (GFR), is considered to be the best index of overall kidney function[13,14], also an indicator of long-term graft survival[15], and an independent risk factor for cardiovascular mortality[16,17], the primary cause of death in kidney transplant recipients[17,18]. Of note, like in non-transplant chronic kidney disease, prevalence of complications related to loss of renal function such as hypertension, anemia and abnormal mineral metabolism increases significantly as the GFR declines[19]. Another important point is that the decline in GFR is also related with increased health care costs, and over the two years, transplantation was both more effective and less costly than dialysis[2,3]. Therefore, an accurate evaluation of renal allograft function is crucial in the clinical management of kidney transplant recipients.

Methods to measure GFR using exogenous markers, such as inulin clearance, the gold standard, and others such as radiolabeled isotopes (^{51}Cr EDTA, ^{99m}Tc DTPA or ^{125}I Iothalamate) and non-radioactive contrast agents (Iothalamate or Iohexol), are laborious as well as expensive, being rarely used in clinical practice. Therefore, endogenous markers, such as serum creatinine (SCr) or cystatin C (CyC), are used to estimate kidney function. Mathematical formulas employing these markers adjusted to other variables (mainly demographic) are an effort to ameliorate GFR estimation (eGFR) accuracy.

However, there is some concern about the accuracy of the available eGFR equations in kidney transplant recipients and guidelines still provide conflicting recommendations about GFR estimation methods in this population[20].

In this article, we aim to review the performance and limitations of these endogenous markers and their equations as estimators of GFR in the kidney transplant recipients, and their ability in predicting significant clinical outcomes.

ENDOGENOUS MARKERS

SCr

SCr concentration is the best known and most commonly used marker for estimation of GFR, since it was first described as a GFR marker in 1937[21], and SCr analysis is inexpensive and generally accessible. Creatinine is a breakdown product of creatinephosphate in muscle tissue, produced at a relatively constant rate, depending on the muscle mass, and filtered in the glomerulus but also actively secreted in the proximal tubule[22]. Tubular secretion contributes normally to 10% of renal Cr removal, but increases when GFR decreases[23], causing SCr to remain in the normal range until GFR drops below 60-70 mL/min. Some Cr is also incorporated from the diet. Ingestion of meat contributes substantially to the urinary Cr excretion, both as a result of expansion of the total creatine pool and as a result of gastrointestinal absorption of Cr[22]. Thus, multiple factors contribute to reduce the accuracy of SCr as an indicator of the GFR, including sex, age, race, muscle mass and dietary protein intake.

Particularly, in renal transplantation there are other determinants that may interfere with Cr metabolism such as corticosteroids, which have a direct catabolic effect[24] and cause a changed muscle mass ratio to total body weight[25]. Catabolic illnesses such as infection and acute rejection, and prolonged dialysis, can also be partly responsible[26].

Cr tubular secretion can be blocked by some drugs such as trimethoprim, commonly used in kidney transplantation[27]. Also, chronic rejection and acute tubular necrosis, can contribute, because tubular secretion of creatinine is reduced.

Because Cr secretion is not predictable, the GFR can decrease to nearly half the normal value before the SCr increases[13], with remarkable consequences in kidney transplant outcome, where subclinical progressive damage, such as calcineurin toxicity and rejection will not be early identified. Several studies in kidney transplantation demonstrated that the SCr and GFR were barely correlated[26,28].

In addition, SCr measurement by the most common method (Jaffé) is subject to interferences by chromogens such as bilirubin, glucose and uric acid, and the enzymatic method is prone to interference by bilirubin and some antibiotics. Considerable variations between SCr assays calibration may also cause inaccuracies in its determination[29]. An attempt to standardize measurement

has been recently introduced by adoption of a common calibration to isotope dilution mass spectrophotometry standard (IDMS) with substantial improvement and traceability of SCr measurements[30].

Nonetheless, SCr is recommended as a screening test for changes in allograft function[31], adjustments of immunosuppressive drugs[32], and it was shown that SCr by itself may be a predictor of long-term graft and patient survival[33].

Creatinine clearance

Creatinine clearance (CCr) as measured from 24-h urine collection is often used in clinical practice to calculate GFR, but it overestimates GFR due to the secretion of Cr by the renal tubules and the inherent limitations of SCr as a kidney marker. However, this calculation does not correct for tubular secretion, and overestimates GFR also in transplant populations[28,34,35], with additional errors in urine collection. Measurement of CCr using this method becomes more reliable after the administration of cimetidine, which inhibits tubular secretion[36], but still does not supply additional knowledge about renal function than other Cr-based methods[13,14].

Serum CyC

CyC is a 122-amino acid, 13-kDa protein that is a member of a family of competitive inhibitors of lysosomal cysteine proteinases. Its functions include involvement in extracellular proteolysis, immune modulation, and antibacterial and antiviral activities.

CyC has certain characteristics that make it an acceptable candidate as a kidney function marker, including a constant production rate, free glomerular filtration, complete reabsorption and catabolism by the proximal tubules with no reabsorption, and no tubular secretion[37].

Several clinical data demonstrated that serum CyC levels correlate better with GFR than does Cr alone, especially at higher levels of GFR, and it was also thought to be less influenced by certain demographic factors such as age, race, gender, or muscle mass compared with SCr[38,39]. However, some emerging new data have shown that serum CyC may be influence by these and other variables.

A recent study concluded that CyC was 9% lower in women and 6% higher in blacks for a given GFR[40]. In a cross-sectional study, Knight *et al*[41], found that older age, male gender, greater weight, greater height, current cigarette smoking, and higher serum C-reactive protein levels were independently associated with higher serum CyC levels after adjusting for CCr.

Moreover, in certain clinical settings, CyC level may be biased as a marker of kidney function, such as in patients with uncontrolled thyroid disease, rapid cell turnover, and those under steroid therapy[42], like kidney transplant recipients. Also, CyC is quite costly and unavailable in many transplant centers.

GFR ESTIMATION FROM SCR BASED EQUATIONS

To overcome some of the limitations of Cr as a marker for GFR, several formulas have been constructed to correct for the influences of weight, age, gender and/or race[26,43-46].

Some of these equations have been evaluated in renal transplant patients, and the most commonly used are the Modification of Diet in Renal Disease (MDRD) study[44], Cockcroft-Gault[43], and Nankivell[26] equations. The KDIGO position statement includes the proposal that Cr-based eGFR equations should be used to evaluate renal function in the everyday management of renal transplant recipients[14].

The Cockcroft-Gault equation was derived in 236 (96% male) hospitalized patients with a wide range of GFR values[43]. The MDRD equation, published in 1999 were derived in 1628 patients with chronic kidney disease (mean GFR, 40 mL/min per 1.73 m^2)[44], and this was simplified in 2000[47] and reexpressed in 2005, after standardization of the SCr assays to the reference method using IDMS[48,49]. The Nankivell equation is the only one that was derived from kidney transplant recipients[26], however some of these transplant patients were in an early post-transplant phase or with acute dysfunction, which has implications in prediction of GFR.

More recently, a new formula was published by the chronic kidney disease epidemiology collaboration (CKD-EPI)[50], to overcome the systematic underestimation of GFR and lack of precision of the MDRD formulas in patients with relatively well-preserved kidney function, but only 4% of the CKD-EPI derivation cohort consisted of organ transplant recipients.

PERFORMANCE OF CREATININE-BASED GFR ESTIMATION EQUATIONS IN KIDNEY TRANSPLANTATION

To certify graft function as a valid surrogate marker, we must know for certain that we use a solid measure of kidney function.

The eGFR equations were an alternative to estimate GFR in clinical context, as they allow us to overpass some of the limitations of the SCr[51].

To determine the performance of a given eGFR equation the K/DOQI guidelines[13] proposed a methodological approach according to simple and reproducible criteria: "BIAS", "PRECISION" and "ACCURACY". The absolute BIAS expresses the systematic deviation from the gold standard measurement of GFR, and was given by the mean difference between estimated GFR and gold standard clearance (true GFR). The relative BIAS, hereafter named percent BIAS, is expressed as the proportion of true GFR represented by the absolute bias, and was calculated as: absolute BIAS/true GFR × 100. PRECISION expresses the

variability or dispersion of predictions around the true GFR and corresponds to the standard deviation of the difference between the true and estimated GFR. The distribution of the differences between estimated and true GFR accounts for the ACCURACY of the GFR estimates (*e.g.*, 30% accuracy is the proportion of predicted GFR within ± 30% of the true GFR).

In several studies in kidney transplantation, the efficiency of MDRD, Cockcroft-Gault and Nankivell equations has been consistently reviewed[52], with a significant heterogeneity between studies, with low precision inducing limited accuracies, and this can be attributed to varied patient characteristics, differences in measure GFR methods and Cr assay calibration and, potentially, some inherent differences in this specific population of transplant recipients[52]. In the majority of these studies, all of these equations persistently testified progressive decrease in GFR overestimation and/or increase in GFR underestimation as graft function ameliorated [28,34,53].

The CKD-EPI equation[50] introduces a correction term to overcome the systematic underestimation of GFR of the MDRD formulas in patients with relatively well-preserved kidney function, as mentioned above. In a cohort of 207 stable Kidney transplant recipients[54] CKD-EPI shows improved estimation ability compared with MDRD equation, but still with suboptimal precision that limit the value of the CKD-EPI for monitoring changes in kidney function over time[54]. Other studies compare the performances of the MDRD and CKD-EPI equations in a large transplant patient's cohort[55,56] and the authors concluded that the latter equation does not offer a better GFR estimation in this population.

More recently, Shaffi *et al*[57], conducted a systematic evaluation of the development methods of all published Cr-based eGFR equations, and assess their performance in a large population (n = 3622) of solid-organ transplant recipients, including 53% kidney transplant recipients. They founded that the CKD-EPI[50] and IDMS-traceable 4-variable MDRD Study equations[48] were more accurate than the alternative equations, including those developed in populations including only transplant recipients, and as accurate as observed in non-transplanted populations. Nevertheless, we can't forget that these equations still misestimate true GFR by > 30% in 1 of 5 patients.

They also concluded that there was no difference between these two equations in the overall study population, but CKD-EPI equation showed better performance at higher GFRs compared with better performance of MDRD Study equation at lower GFRs, which is in agreement with the results of the systematic review performed by Earley *et al*[58]. This study[57] may have implications in clinical practice, support the use of these eGFR equations to routine access renal function in transplant patients as in other populations. Even though it was a good diagnostic test study design with a standardized reference test, the study population included few nonwhites and individuals with solid organ transplants other than liver and kidneys; therefore assessment of the equation performance in these subgroups is limited[57].

However we can't ignore that SCr levels are affected by factors besides GFR, and several studies suggest worse stage-based care in kidney transplant patients compared with native kidney diseases[59,60], so any eGFR equations based on SCr still have limitations.

PERFORMANCE OF CYSTATIN-BASED GFR ESTIMATION EQUATIONS IN KIDNEY TRANSPLANTATION

As with SCr, it is the CyC-based GFR, rather than the CyC itself, that is of greater clinical interest. Over the last decade, several serum CyC-based equations have been developed and proposed to estimate the GFR[61-67].

Only two of these equations (Rule *et al*[64] and Le Bricon *et al*[67]) were exclusively derived from a population of kidney transplant recipients.

Several studies in the renal transplant population, showed discordant results with some indicate advantage of CyC-based equations over Cr-based equations, whereas others showed no superiority of CyC over SCr[20,34,68]. One of the limitations of CyC-based eGFR formulas in this population is that the treatment with corticosteroids increases CyC levels by increasing the production of CyC[69]. Although the KDIGO recommendations on kidney transplantation comment the possible interest of using CyC to GFR estimation, they do not advocated its regular clinical use, due to the paucity of validation studies in this group of patients[20].

A recent systematic review[70], identified 10 studies, evaluating the accuracy of 14 different CyC-based eGFR equations in renal transplant recipients. The authors conclude that the Le Bricon equation[67] was the highest accurate, and the majority of the CyC-based equations exhibited 30% and 50% accuracy improvements compared with the Cr-based MDRD equation. However, as with the Cr equations, there was substantial variability between the studies. Much of this variability is consequence of different study populations, differences in the GFR reference standard measurement, and in variation in the calibrators for the CyC measurement, and this latter contributes to the greatest source of variation. Standardized reference material for CyC has already been developed[71], but none of the studies involved in this analysis[70], adopted this methodology.

In 2008 a new Cr- and CyC-based formula (CKD-EPI CyC equation) was developed[40], which besides serum CyC includes the variables of gender, age and race, and seems more accurate than the formulas based on Cr or CyC alone, but this formula requires further testing in various patients groups.

Recently, the CyC-based estimating equations were re-expressed for use with the standardized CyC reference material (ERM-DA47/IFCC)[72]. These and the equations with CyC in combination with SCr[40], improved in 2012 with lesser bias at GFR > 60 mL/min

(CKD-EPI Cr-CyC 2012)[73], were validate in a European cohort of renal transplants patients[74] but their accuracy needs to be evaluated in more studies with this population.

CREATININE-BASED AND CYC-BASED eGFR EQUATIONS AS PREDICTORS OF CLINICAL OUTCOMES

Although, it has been demonstrated that eGFR is a predictor of patient and transplant survival[75], disappointing results have been reported when several of Cr-based eGFR formulas where assessed against the most important outcome measures such as mortality and graft failure, with limited utility and no benefit over the use of SCr alone[76].

Another relevant problem in clinical practice is whether the eGFR equations were able to precisely predict variations in graft function over time. Several studies reported considerable variability of the Cr-based eGFR equations performance at different times post-transplant[28,77] with less accuracy within the first year of transplantation[78], indicating that those Cr-based equations must be worn with caution for GFR monitoring through time[79].

Nowadays there is an increasing interest in CyC-based equations as an outcome predictor in kidney transplantation. In general population, CyC-based eGFR equations are a stronger predictor of the risk of death and cardiovascular events, when compared with Cr[80,81], as well as the correlation of serum CyC with all-cause and cardiovascular mortality in chronic kidney disease (CKD)[82]. Recently, a meta-analysis of 11 general-population studies and 5 studies of cohorts with CKD[83], shows that the utilization of CyC alone or in combination with Cr reinforce the power of eGFR as a predictor of end-stage renal disease and death .

Whether this outcome prediction is true for transplant recipients needs to be confirmed. Although, some studies showed that CyC and or CyC-based equations predicted both patient mortality and graft outcome better than Cr-based eGFR equations[34,84], others founded that CyC and SCr were equally reliable predictors of graft outcome[85].

Interestingly, very recently, a study examined the extent to which the addition of serum CyC improves GFR estimation and mortality prediction, in comparison to various eGFR equations, in a population of 401 liver transplanted patients. In this work, the authors founded that CyC, by itself or as a part of an eGFR, was a significant predictor of mortality[86].

Another approach is a multimarker management, including combination of different markers of graft function, such as SCr, CyC, and kidney pathologic markers, such as proteinuria and/or albuminuria. Models that include Cr-based or CyC-based eGFR and albuminuria show better prediction to end-stage renal disease in general population[87,88], and CKD patients[89].

A clinical score constructed from a cross-validated French database of 2169 kidney transplant recipients, combining risk factors of graft loss, including SCr and proteinuria, demonstrated to be highly predictive of long-term kidney graft survival[90], and other study demonstrated that the combination of low-grade albuminuria and decreased eGFR was related with graft loss and mortality[91].

In a similar way, a recent small-sample single-center study[92] founded that predictors combining albuminuria and Cr- or CyC-based eGFR, performed better than those markers alone, to predict death censored graft loss, in kidney transplant recipients. Moreover, the best predictor of graft failure in this work was a product of CyC and the logarithm of albuminuria, and CyC-based predictors performed better than Cr-based predictors.

More recently, there has been some enthusiasm in new markers of kidney injury such as neutrophil gelatinase-associated lipocalin (NGAL) and kidney injury molecule-1 (KIM-1), and it potential in prediction of kidney function, not influenced by age, gender, race, body fat and muscle mass. Particularly, in kidney transplant recipients, it was demonstrated that urinary excretion of KIM-1, a proximal tubular protein, independently predicts graft failure[93]. However, more trials are required to validate these results in clinical setting.

PROTEINURIA

Proteinuria, although not a direct GFR marker, is also an important indicator of allograft dysfunction[94,95], associated with a reduced long-term graft survival[94,95] and increased patient mortality[94,96].

One of the limitations of proteinuria as an accurate marker of graft dysfunction is the native kidney excretion, and in that cases, should have a baseline value before transplantation, particularly in the setting of pre-existing glomerular disease. However, pretransplant proteinuria decreases or disappears after successful transplantation and *de novo* or increasing proteinuria is indicative of graft pathology[97].

Proteinuria can signal pathologic changes including recurrent or *de novo* glomerular disease, calcineurin inhibitor toxicity, alloantibody-mediated injury and chronic allograft nephropathy[98]. In this way, graft biopsy helps to determine the etiology of proteinuria[20,99] and to manage some treatable causes of graft injury. KDIGO guidelines[20] proposed monitoring of proteinuria as part of routine transplant follow-up.

CONCLUSION

An accurate evaluation of allograft function is crucial in the management of kidney transplant, and most importantly in predicting clinical outcomes. However any endogenous kidney function marker has limitations, and understandably, eGFR formulas derived from them will present similar barriers. Also, these prediction equations have inherent problems, namely the selected

populations used for their derivation, usually non-transplanted patients.

The Cr-based eGFR equations were the much widely used and recent studies, accessing the performance of MDRD study and CKD-EPI equations in kidney transplantation, support their use to routine access renal function in transplant patients as in other populations. But, we can't forget that Cr-based eGFR equations have never been demonstrated to improve the clinical recognition of changes in transplant function, compared to the use of Cr alone, and many transplant injuries occur without change in SCr level or eGFR.

In the last years, our attention is moving toward another markers and CyC seems to be a promising one. Although, some conflicting results, several studies in kidney transplants confirm the better performance of CyC-based equations over Cr-based equations in estimating GFR. The use of CyC alone or in combination with Cr reinforces the eGFR power as a predictor of end-stage kidney disease and death, in general and CKD population, but we need to confirm this outcome prediction in transplant recipients. However, like Cr, CyC is also influenced by non-GFR determinants, is more expensive than SCr and has suboptimal standardization, therefore its use is not widespread implemented.

Finally, a model combining different markers such as SCr, CyC, proteinuria and/or albuminuria can be useful in clinical practice, providing an improvement in outcome prediction. At moment, and regarding the kidney transplant management, we are still searching for the optimal combination and for the best marker.

REFERENCES

1 **Wolfe RA**, Ashby VB, Milford EL, Ojo AO, Ettenger RE, Agodoa LY, Held PJ, Port FK. Comparison of mortality in all patients on dialysis, patients on dialysis awaiting transplantation, and recipients of a first cadaveric transplant. *N Engl J Med* 1999; **341**: 1725-1730 [PMID: 10580071 DOI: 10.1056/NEJM199912023412303]

2 **Laupacis A**, Keown P, Pus N, Krueger H, Ferguson B, Wong C, Muirhead N. A study of the quality of life and cost-utility of renal transplantation. *Kidney Int* 1996; **50**: 235-242 [PMID: 8807593 DOI: 10.1038/ki.1996.307]

3 **Rocha MJ**, Ferreira S, Martins LS, Almeida M, Dias L, Pedroso S, Henriques AC, Almeida R, Cabrita A. Cost analysis of renal replacement therapy by transplant in a system of bundled payment of dialysis. *Clin Transplant* 2012; **26**: 529-531 [PMID: 22211715 DOI: 10.1111/j.1399-0012.2011.01571.x]

4 **Meier-Kriesche HU**, Schold JD, Kaplan B. Long-term renal allograft survival: have we made significant progress or is it time to rethink our analytic and therapeutic strategies? *Am J Transplant* 2004; **4**: 1289-1295 [PMID: 15268730 DOI: 10.1111/j.1600-6143.2004.00515.x]

5 **Chang SH**, Russ GR, Chadban SJ, Campbell SB, McDonald SP. Trends in kidney transplantation in Australia and New Zealand, 1993-2004. *Transplantation* 2007; **84**: 611-618 [PMID: 17876274 DOI: 10.1097/01.tp.0000280553.23898.ef]

6 **Lamb KE**, Lodhi S, Meier-Kriesche HU. Long-term renal allograft survival in the United States: a critical reappraisal. *Am J Transplant* 2011; **11**: 450-462 [PMID: 20973913 DOI: 10.1111/j.1600-6143.2010.03283.x]

7 **Mariat C**, Maillard N, Phayphet M, Thibaudin L, Laporte S, Alamartine E, Berthoux F. Estimated glomerular filtration rate as an end point in kidney transplant trial: where do we stand? *Nephrol Dial Transplant* 2008; **23**: 33-38 [PMID: 17967801 DOI: 10.1093/ndt/gfm708]

8 **Hariharan S**, McBride MA, Cohen EP. Evolution of endpoints for renal transplant outcome. *Am J Transplant* 2003; **3**: 933-941 [PMID: 12859527 DOI: 10.1034/j.1600-6143.2003.00176.x]

9 **Pascual M**, Theruvath T, Kawai T, Tolkoff-Rubin N, Cosimi AB. Strategies to improve long-term outcomes after renal transplantation. *N Engl J Med* 2002; **346**: 580-590 [PMID: 11856798 DOI: 10.1056/NEJMra011295]

10 **Vincenti F**, Larsen C, Durrbach A, Wekerle T, Nashan B, Blancho G, Lang P, Grinyo J, Halloran PF, Solez K, Hagerty D, Levy E, Zhou W, Natarajan K, Charpentier B. Costimulation blockade with belatacept in renal transplantation. *N Engl J Med* 2005; **353**: 770-781 [PMID: 16120857 DOI: 10.1056/NEJMoa050085]

11 **Hariharan S**, Kasiske B, Matas A, Cohen A, Harmon W, Rabb H. Surrogate markers for long-term renal allograft survival. *Am J Transplant* 2004; **4**: 1179-1183 [PMID: 15196079 DOI: 10.1111/j.1600-6143.2004.00484.x]

12 **Kaplan B**, Schold J, Meier-Kriesche HU. Poor predictive value of serum creatinine for renal allograft loss. *Am J Transplant* 2003; **3**: 1560-1565 [PMID: 14629286 DOI: 10.1046/j.1600-6135.2003.00275.x]

13 **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]

14 **Levey AS**, Eckardt KU, Tsukamoto Y, Levin A, Coresh J, Rossert J, De Zeeuw D, Hostetter TH, Lameire N, Eknoyan G. Definition and classification of chronic kidney disease: a position statement from Kidney Disease: Improving Global Outcomes (KDIGO). *Kidney Int* 2005; **67**: 2089-2100 [PMID: 15882252 DOI: 10.1111/j.1523-1755.2005.00365]

15 **First MR**. Renal function as a predictor of long-term graft survival in renal transplant patients. *Nephrol Dial Transplant* 2003; **18** Suppl 1: i3-i6 [PMID: 12738756 DOI: 10.1093/ndt/gfg1027]

16 **Fellström B**, Jardine AG, Soveri I, Cole E, Neumayer HH, Maes B, Gimpelewicz C, Holdaas H. Renal dysfunction is a strong and independent risk factor for mortality and cardiovascular complications in renal transplantation. *Am J Transplant* 2005; **5**: 1986-1991 [PMID: 15996249 DOI: 10.1111/j.1600-6143.2005.00983.x]

17 **Ojo AO**. Cardiovascular complications after renal transplantation and their prevention. *Transplantation* 2006; **82**: 603-611 [PMID: 16969281 DOI: 10.1097/01.tp.0000235527.81917.fe]

18 **Briggs JD**. Causes of death after renal transplantation. *Nephrol Dial Transplant* 2001; **16**: 1545-1549 [PMID: 11477152 DOI: 10.1093/ndt/16.8.1545]

19 **Karthikeyan V**, Karpinski J, Nair RC, Knoll G. The burden of chronic kidney disease in renal transplant recipients. *Am J Transplant* 2004; **4**: 262-269 [PMID: 14974949 DOI: 10.1046/j.1600-6143.2003.00315.x]

20 **Kidney Disease: Improving Global Outcomes (KDIGO) Transplant Work Group**. KDIGO clinical practice guideline for the care of kidney transplant recipients. *Am J Transplant* 2009; **9** Suppl 3: S1-S155 [PMID: 19845597 DOI: 10.1111/j.1600-6143.2009.02834.x]

21 **Popper H**, Mandel E. Filtrations-und reabsorptionsleitung in der Nierenpathologie. *Ergeb inn Med Kinderheilkd* 1937; **53**: 685-694 [DOI: 10.1007/978-3-642-90690-9_7]

22 **Perrone RD**, Madias NE, Levey AS. Serum creatinine as an index of renal function: new insights into old concepts. *Clin Chem* 1992; **38**: 1933-1953 [PMID: 1394976]

23 **Shemesh O**, Golbetz H, Kriss JP, Myers BD. Limitations of creatinine as a filtration marker in glomerulopathic patients. *Kidney Int* 1985; **28**: 830-838 [PMID: 2418254 DOI: 10.1038/ki.1985.205]

24 **Horber FF**, Scheidegger J, Frey FJ. Overestimation of renal function in glucocorticosteroid treated patients. *Eur J Clin Pharmacol* 1985; **28**: 537-541 [PMID: 4043198 DOI: 10.1007/bf00544064]

25 **El Haggan W**, Hurault de Ligny B, Partiu A, Sabatier JP, Lobbedez T, Levaltier B, Ryckelynck JP. The evolution of weight and body composition in renal transplant recipients: Two-year longitudinal study. *Transplant Proc* 2006; **38**: 3517-3519 [PMID: 17175319 DOI:

10.1016/j.transproceed.2006.10.121]
26 **Nankivell BJ**, Gruenewald SM, Allen RD, Chapman JR. Predicting glomerular filtration rate after kidney transplantation. *Transplantation* 1995; **59**: 1683-1689 [PMID: 7604438 DOI: 10.1097/00007890-199506270-00007]
27 **Berglund F**, Killander J, Pompeius R. Effect of trimethoprim-sulfamethoxazole on the renal excretion of creatinine in man. *J Urol* 1975; **114**: 802-808 [PMID: 1195454]
28 **Bosma RJ**, Doorenbos CR, Stegeman CA, van der Heide JJ, Navis G. Predictive performance of renal function equations in renal transplant recipients: an analysis of patient factors in bias. *Am J Transplant* 2005; **5**: 2193-2203 [PMID: 16095498 DOI: 10.1111/j.1600-6143.2005.00982.x]
29 **Hallan S**, Asberg A, Lindberg M, Johnsen H. Validation of the Modification of Diet in Renal Disease formula for estimating GFR with special emphasis on calibration of the serum creatinine assay. *Am J Kidney Dis* 2004; **44**: 84-93 [PMID: 15211442 DOI: 10.1053/j.ajkd.2004.03.027]
30 **Myers GL**, Miller WG, Coresh J, Fleming J, Greenberg N, Greene T, Hostetter T, Levey AS, Panteghini M, Welch M, Eckfeldt JH. Recommendations for improving serum creatinine measurement: a report from the Laboratory Working Group of the National Kidney Disease Education Program. *Clin Chem* 2006; **52**: 5-18 [PMID: 16332993 DOI: 10.1373/clinchem.2005.0525144]
31 **Kasiske BL**, Vazquez MA, Harmon WE, Brown RS, Danovitch GM, Gaston RS, Roth D, Scandling JD, Singer GG. Recommendations for the outpatient surveillance of renal transplant recipients. American Society of Transplantation. *J Am Soc Nephrol* 2000; **11** Suppl 15: S1-S86 [PMID: 11044969]
32 **Ojo A**. Assessing post-transplant renal function. *Medscape Transplant* 2004; **5**: 1-4
33 **Hariharan S**, McBride MA, Cherikh WS, Tolleris CB, Bresnahan BA, Johnson CP. Post-transplant renal function in the first year predicts long-term kidney transplant survival. *Kidney Int* 2002; **62**: 311-318 [PMID: 12081593 DOI: 10.1046/j.1523-1755.2002.00424.x]
34 **White C**, Akbari A, Hussain N, Dinh L, Filler G, Lepage N, Knoll GA. Estimating glomerular filtration rate in kidney transplantation: a comparison between serum creatinine and cystatin C-based methods. *J Am Soc Nephrol* 2005; **16**: 3763-3770 [PMID: 16236805 DOI: 10.1681/asn.2005050512]
35 **Mobb GE**, Veitch PS, Bell PR. Are serum creatinine levels adequate to identify the onset of chronic cyclosporine A nephrotoxicity? *Transplant Proc* 1990; **22**: 1708-1710 [PMID: 2389439]
36 **Kemperman FA**, Surachno J, Krediet RT, Arisz L. Cimetidine improves prediction of the glomerular filtration rate by the Cockcroft-Gault formula in renal transplant recipients. *Transplantation* 2002; **73**: 770-774 [PMID: 11907426 DOI: 10.1097/00007890-200203150-00020]
37 **Johnson D**. Evaluation of renal function: use of cystatin C measurement in evaluating kidney function. *Nephrology* (Carlton) 2005; **10** Suppl 4: S157-S167
38 **Hojs R**, Bevc S, Ekart R, Gorenjak M, Puklavec L. Serum cystatin C as an endogenous marker of renal function in patients with mild to moderate impairment of kidney function. *Nephrol Dial Transplant* 2006; **21**: 1855-1862 [PMID: 16524933 DOI: 10.1093/ndt/gfl073]
39 **Jonsson AS**, Flodin M, Hansson LO, Larsson A. Estimated glomerular filtration rate (eGFRCystC) from serum cystatin C shows strong agreement with iohexol clearance in patients with low GFR. *Scand J Clin Lab Invest* 2007; **67**: 801-809 [PMID: 17852801 DOI: 10.1080/00365510701397538]
40 **Stevens LA**, Coresh J, Schmid CH, Feldman HI, Froissart M, Kusek J, Rossert J, Van Lente F, Bruce RD, Zhang YL, Greene T, Levey AS. Estimating GFR using serum cystatin C alone and in combination with serum creatinine: a pooled analysis of 3,418 individuals with CKD. *Am J Kidney Dis* 2008; **51**: 395-406 [PMID: 18295055 DOI: 10.1053/j.ajkd.2007.11.018]
41 **Knight EL**, Verhave JC, Spiegelman D, Hillege HL, de Zeeuw D, Curhan GC, de Jong PE. Factors influencing serum cystatin C levels other than renal function and the impact on renal function measurement. *Kidney Int* 2004; **65**: 1416-1421 [PMID: 15086483 DOI: 10.1111/j.1523-1755.2004.00517.x]
42 **Thomas C**, Thomas L. Renal failure--measuring the glomerular filtration rate. *Dtsch Arztebl Int* 2009; **106**: 849-854 [PMID: 20062583 DOI: 10.3238/arztebl.2009.0849]
43 **Cockcroft DW**, Gault MH. Prediction of creatinine clearance from serum creatinine. *Nephron* 1976; **16**: 31-41 [PMID: 1244564 DOI: 10.1159/000180580]
44 **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]
45 **Rule AD**, Larson TS, Bergstralh EJ, Slezak JM, Jacobsen SJ, Cosio FG. Using serum creatinine to estimate glomerular filtration rate: accuracy in good health and in chronic kidney disease. *Ann Intern Med* 2004; **141**: 929-937 [PMID: 15611490 DOI: 10.7326/0003-4819-141-12-200412210-00009]
46 **Walser M**, Drew HH, Guldan JL. Prediction of glomerular filtration rate from serum creatinine concentration in advanced chronic renal failure. *Kidney Int* 1993; **44**: 1145-1148 [PMID: 8264148 DOI: 10.1038/ki.1993.361]
47 **Levey AS**, Greene T, Kusek J, Beck GJ; MDRD Study Group. A simplified equation to predict glomerular filtration rate from serum creatinine [abstract]. *J Am Soc Nephrol* 2000; **11**: AO828
48 **Levey AS**, Coresh J, Greene T, Marsh J, Stevens LA, Kusek J, Van Lente F. Expressing the MDRD study equation for estimating GFR with IDMS traceable (Gold Standard) serum creatinine values. [abstract]. *J Am Soc Nephrol* 2005; **16**: 69A
49 **Levey AS**, Coresh J, Greene T, Stevens LA, Zhang YL, Hendriksen S, Kusek JW, Van Lente F. Using standardized serum creatinine values in the modification of diet in renal disease study equation for estimating glomerular filtration rate. *Ann Intern Med* 2006; **145**: 247-254 [PMID: 16908915 DOI: 10.7326/0003-4819-145-4-200608150-00004]
50 **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]
51 **Stevens LA**, Coresh J, Greene T, Levey AS. Assessing kidney function--measured and estimated glomerular filtration rate. *N Engl J Med* 2006; **354**: 2473-2483 [PMID: 16760447 DOI: 10.1056/nejmra054415]
52 **White CA**, Huang D, Akbari A, Garland J, Knoll GA. Performance of creatinine-based estimates of GFR in kidney transplant recipients: a systematic review. *Am J Kidney Dis* 2008; **51**: 1005-1015 [PMID: 18455847 DOI: 10.1053/j.ajkd.2008.02.308]
53 **Raju DL**, Grover VK, Shoker A. Limitations of glomerular filtration rate equations in the renal transplant patient. *Clin Transplant* 2005; **19**: 259-268 [PMID: 15740565]
54 **White CA**, Akbari A, Doucette S, Fergusson D, Knoll GA. Estimating glomerular filtration rate in kidney transplantation: is the new chronic kidney disease epidemiology collaboration equation any better? *Clin Chem* 2010; **56**: 474-477 [PMID: 19959620 DOI: 10.1373/clinchem.2009.135111]
55 **Masson I**, Flamant M, Maillard N, Rule AD, Vrtovsnik F, Peraldi MN, Thibaudin L, Cavalier E, Vidal-Petiot E, Bonneau C, Moranne O, Alamartine E, Mariat C, Delanaye P. MDRD versus CKD-EPI equation to estimate glomerular filtration rate in kidney transplant recipients. *Transplantation* 2013; **95**: 1211-1217 [PMID: 23511243 DOI: 10.1097/tp.0b013e318288caa6]
56 **Buron F**, Hadj-Aissa A, Dubourg L, Morelon E, Steghens JP, Ducher M, Fauvel JP. Estimating glomerular filtration rate in kidney transplant recipients: performance over time of four creatinine-based formulas. *Transplantation* 2011; **92**: 1005-1011 [PMID: 22031008 DOI: 10.1097/TP.0b013e3182301602]
57 **Shaffi K**, Uhlig K, Perrone RD, Ruthazer R, Rule A, Lieske JC, Navis G, Poggio ED, Inker LA, Levey AS. Performance of creatinine-based GFR estimating equations in solid-organ transplant

recipients. *Am J Kidney Dis* 2014; **63**: 1007-1018 [PMID: 24703720 DOI: 10.1053/j.ajkd.2014.01.436]
58 **Earley A**, Miskulin D, Lamb EJ, Levey AS, Uhlig K. Estimating equations for glomerular filtration rate in the era of creatinine standardization: a systematic review. *Ann Intern Med* 2012; **156**: 785-95, W-270, W-271, W-272, W-273, W-274, W-275, W-276, W-277, W-278 [PMID: 22312131 DOI: 10.7326/0003-4819-156-6-201203200-00391]
59 **Pöge U**, Gerhardt T, Stoffel-Wagner B, Sauerbruch T, Woitas RP. Validation of the CKD-EPI formula in patients after renal transplantation. *Nephrol Dial Transplant* 2011; **26**: 4104-4108 [PMID: 21551088 DOI: 10.1093/ndt/gfr183]
60 **Akbari A**, Hussain N, Karpinski J, Knoll GA. Chronic kidney disease management: comparison between renal transplant recipients and nontransplant patients with chronic kidney disease. *Nephron Clin Pract* 2007; **107**: c7-13 [PMID: 17622770 DOI: 10.1159/000105138]
61 **Hoek FJ**, Kemperman FA, Krediet RT. A comparison between cystatin C, plasma creatinine and the Cockcroft and Gault formula for the estimation of glomerular filtration rate. *Nephrol Dial Transplant* 2003; **18**: 2024-2031 [PMID: 13679476 DOI: 10.1093/ndt/gfg349]
62 **Tan GD**, Lewis AV, James TJ, Altmann P, Taylor RP, Levy JC. Clinical usefulness of cystatin C for the estimation of glomerular filtration rate in type 1 diabetes: reproducibility and accuracy compared with standard measures and iohexol clearance. *Diabetes Care* 2002; **25**: 2004-2009 [PMID: 12401747 DOI: 10.2337/diacare.25.11.2004]
63 **Grubb A**, Nyman U, Björk J, Lindström V, Rippe B, Sterner G, Christensson A. Simple cystatin C-based prediction equations for glomerular filtration rate compared with the modification of diet in renal disease prediction equation for adults and the Schwartz and the Counahan-Barratt prediction equations for children. *Clin Chem* 2005; **51**: 1420-1431 [PMID: 15961546 DOI: 10.1373/clinchem.2005.051557]
64 **Rule AD**, Bergstralh EJ, Slezak JM, Bergert J, Larson TS. Glomerular filtration rate estimated by cystatin C among different clinical presentations. *Kidney Int* 2006; **69**: 399-405 [PMID: 16408133 DOI: 10.1038/sj.ki.5000073]
65 **Hojs R**, Bevc S, Ekart R, Gorenjak M, Puklavec L. Serum cystatin C-based equation compared to serum creatinine-based equations for estimation of glomerular filtration rate in patients with chronic kidney disease. *Clin Nephrol* 2008; **70**: 10-17 [PMID: 18793543 DOI: 10.5414/cnp70010]
66 **Larsson A**, Malm J, Grubb A, Hansson LO. Calculation of glomerular filtration rate expressed in mL/min from plasma cystatin C values in mg/L. *Scand J Clin Lab Invest* 2004; **64**: 25-30 [PMID: 15025426 DOI: 10.1080/00365510410003723]
67 **Le Bricon T**, Thervet E, Froissart M, Benlakehal M, Bousquet B, Legendre C, Erlich D. Plasma cystatin C is superior to 24-h creatinine clearance and plasma creatinine for estimation of glomerular filtration rate 3 months after kidney transplantation. *Clin Chem* 2000; **46**: 1206-1207 [PMID: 10926911]
68 **Zahran A**, Qureshi M, Shoker A. Comparison between creatinine and cystatin C-based GFR equations in renal transplantation. *Nephrol Dial Transplant* 2007; **22**: 2659-2668 [PMID: 17470455 DOI: 10.1093/ndt/gfm243]
69 **Pöge U**, Gerhardt T, Bökenkamp A, Stoffel-Wagner B, Klehr HU, Sauerbruch T, Woitas RP. Time course of low molecular weight proteins in the early kidney transplantation period--influence of corticosteroids. *Nephrol Dial Transplant* 2004; **19**: 2858-2863 [PMID: 15496561 DOI: 10.1093/ndt/gfh341]
70 **Harman G**, Akbari A, Hiremath S, White CA, Ramsay T, Kokolo MB, Craig J, Knoll GA. Accuracy of cystatin C-based estimates of glomerular filtration rate in kidney transplant recipients: a systematic review. *Nephrol Dial Transplant* 2013; **28**: 741-757 [PMID: 23275574 DOI: 10.1093/ndt/gfs498]
71 **Grubb A**, Blirup-Jensen S, Lindström V, Schmidt C, Althaus H, Zegers I. First certified reference material for cystatin C in human serum ERM-DA471/IFCC. *Clin Chem Lab Med* 2010; **48**: 1619-1621 [PMID: 21034257 DOI: 10.1515/CCLM.2010.318]
72 **Inker LA**, Eckfeldt J, Levey AS, Leiendecker-Foster C, Rynders G, Manzi J, Waheed S, Coresh J. Expressing the CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration) cystatin C equations for estimating GFR with standardized serum cystatin C values. *Am J Kidney Dis* 2011; **58**: 682-684 [PMID: 21855190 DOI: 10.1053/j.ajkd.2011.05.019]
73 **Inker LA**, Schmid CH, Tighiouart H, Eckfeldt JH, Feldman HI, Greene T, Kusek JW, Manzi J, Van Lente F, Zhang YL, Coresh J, Levey AS. Estimating glomerular filtration rate from serum creatinine and cystatin C. *N Engl J Med* 2012; **367**: 20-29 [PMID: 22762315 DOI: 10.1056/NEJMoa1114248]
74 **Masson I**, Maillard N, Tack I, Thibaudin L, Dubourg L, Delanaye P, Cavalier E, Bonneau C, Kamar N, Morelon E, Moranne O, Alamartine E, Mariat C. GFR estimation using standardized cystatin C in kidney transplant recipients. *Am J Kidney Dis* 2013; **61**: 279-284 [PMID: 23141866 DOI: 10.1053/j.ajkd.2012.09.010]
75 **Kasiske BL**, Israni AK, Snyder JJ, Skeans MA. The relationship between kidney function and long-term graft survival after kidney transplant. *Am J Kidney Dis* 2011; **57**: 466-475 [PMID: 21257243 DOI: 10.1053/j.ajkd.2010.10.054]
76 **He X**, Moore J, Shabir S, Little MA, Cockwell P, Ball S, Liu X, Johnston A, Borrows R. Comparison of the predictive performance of eGFR formulae for mortality and graft failure in renal transplant recipients. *Transplantation* 2009; **87**: 384-392 [PMID: 19202443 DOI: 10.1097/TP.0b013e31819004a1]
77 **Gaspari F**, Ferrari S, Stucchi N, Centemeri E, Carrara F, Pellegrino M, Gherardi G, Gotti E, Segoloni G, Salvadori M, Rigotti P, Valente U, Donati D, Sandrini S, Sparacino V, Remuzzi G, Perico N. Performance of different prediction equations for estimating renal function in kidney transplantation. *Am J Transplant* 2004; **4**: 1826-1835 [PMID: 15476483 DOI: 10.1111/j.1600-6143.2004.00579.x]
78 **Gera M**, Slezak JM, Rule AD, Larson TS, Stegall MD, Cosio FG. Assessment of changes in kidney allograft function using creatinine-based estimates of glomerular filtration rate. *Am J Transplant* 2007; **7**: 880-887 [PMID: 17391131 DOI: 10.1111/j.1600-6143.2006.01690.x]
79 **White CA**, Knoll GA, Poggio ED. Measuring vs estimating glomerular filtration rate in kidney transplantation. *Transplant Rev* (Orlando) 2010; **24**: 18-27 [PMID: 19942102 DOI: 10.1016/j.trre.2009.10.001]
80 **Shlipak MG**, Sarnak MJ, Katz R, Fried LF, Seliger SL, Newman AB, Siscovick DS, Stehman-Breen C. Cystatin C and the risk of death and cardiovascular events among elderly persons. *N Engl J Med* 2005; **352**: 2049-2060 [PMID: 15901858 DOI: 10.1056/nejmoa043161]
81 **Shlipak MG**, Wassel Fyr CL, Chertow GM, Harris TB, Kritchevsky SB, Tylavsky FA, Satterfield S, Cummings SR, Newman AB, Fried LF. Cystatin C and mortality risk in the elderly: the health, aging, and body composition study. *J Am Soc Nephrol* 2006; **17**: 254-261 [PMID: 16267155 DOI: 10.1681/asn.2005050545]
82 **Menon V**, Shlipak MG, Wang X, Coresh J, Greene T, Stevens L, Kusek JW, Beck GJ, Collins AJ, Levey AS, Sarnak MJ. Cystatin C as a risk factor for outcomes in chronic kidney disease. *Ann Intern Med* 2007; **147**: 19-27 [PMID: 17606957 DOI: 10.7326/0003-4819-147-1-200707030-00004]
83 **Shlipak MG**, Matsushita K, Ärnlöv J, Inker LA, Katz R, Polkinghorne KR, Rothenbacher D, Sarnak MJ, Astor BC, Coresh J, Levey AS, Gansevoort RT. Cystatin C versus creatinine in determining risk based on kidney function. *N Engl J Med* 2013; **369**: 932-943 [PMID: 24004120 DOI: 10.1056/NEJMoa1214234]
84 **Maillard N**, Mariat C, Bonneau C, Mehdi M, Thibaudin L, Laporte S, Alamartine E, Chamson A, Berthoux F. Cystatin C-based equations in renal transplantation: moving toward a better glomerular filtration rate prediction? *Transplantation* 2008; **85**: 1855-1858 [PMID: 18580481 DOI: 10.1097/TP.0b013e3181744225]
85 **Lezaic V**, Dajak M, Radivojevic D, Ristic S, Marinkovic J. Cystatin C and serum creatinine as predictors of kidney graft outcome. *Int Urol Nephrol* 2014; **46**: 1447-1454 [PMID: 24338493 DOI: 10.1007/s11255-013-0624-7]
86 **Allen AM**, Kim WR, Larson JJ, Colby C, Therneau TM, Rule AD.

Serum Cystatin C as an Indicator of Renal Function and Mortality in Liver Transplant Recipients. *Transplantation* 2015; Epub ahead of print [PMID: 25654627 DOI: 10.1097/tp.0000000000000552]

87 **Hallan SI**, Ritz E, Lydersen S, Romundstad S, Kvenild K, Orth SR. Combining GFR and albuminuria to classify CKD improves prediction of ESRD. *J Am Soc Nephrol* 2009; **20**: 1069-1077 [PMID: 19357254 DOI: 10.1681/ASN.2008070730]

88 **Peralta CA**, Shlipak MG, Judd S, Cushman M, McClellan W, Zakai NA, Safford MM, Zhang X, Muntner P, Warnock D. Detection of chronic kidney disease with creatinine, cystatin C, and urine albumin-to-creatinine ratio and association with progression to end-stage renal disease and mortality. *JAMA* 2011; **305**: 1545-1552 [PMID: 21482744 DOI: 10.1001/jama.2011.468]

89 **Tangri N**, Stevens LA, Griffith J, Tighiouart H, Djurdjev O, Naimark D, Levin A, Levey AS. A predictive model for progression of chronic kidney disease to kidney failure. *JAMA* 2011; **305**: 1553-1559 [PMID: 21482743 DOI: 10.1001/jama.2011.451]

90 **Foucher Y**, Daguin P, Akl A, Kessler M, Ladrière M, Legendre C, Kreis H, Rostaing L, Kamar N, Mourad G, Garrigue V, Bayle F, H de Ligny B, Büchler M, Meier C, Daurès JP, Soulillou JP, Giral M. A clinical scoring system highly predictive of long-term kidney graft survival. *Kidney Int* 2010; **78**: 1288-1294 [PMID: 20861817 DOI: 10.1038/ki.2010.232]

91 **Hernández D**, Pérez G, Marrero D, Porrini E, Rufino M, González-Posada JM, Delgado P, Torres A. Early association of low-grade albuminuria and allograft dysfunction predicts renal transplant outcomes. *Transplantation* 2012; **93**: 297-303 [PMID: 22228419 DOI: 10.1097/tp.0b013e31823ec0a7]

92 **Rodrigo E**, Ruiz JC, Fernández-Fresnedo G, Fernández MD, Piñera C, Palomar R, Monfá E, Gómez-Alamillo C, Arias M. Cystatin C and albuminuria as predictors of long-term allograft outcomes in kidney transplant recipients. *Clin Transplant* 2013; **27**: E177-E183 [PMID: 23373671 DOI: 10.1111/ctr.12082]

93 **van Timmeren MM**, Vaidya VS, van Ree RM, Oterdoom LH, de Vries AP, Gans RO, van Goor H, Stegeman CA, Bonventre JV, Bakker SJ. High urinary excretion of kidney injury molecule-1 is an independent predictor of graft loss in renal transplant recipients. *Transplantation* 2007; **84**: 1625-1630 [PMID: 18165774 DOI: 10.1097/01.tp.0000295982.78039.ef]

94 **Fernández-Fresnedo G**, Plaza JJ, Sánchez-Plumed J, Sanz-Guajardo A, Palomar-Fontanet R, Arias M. Proteinuria: a new marker of long-term graft and patient survival in kidney transplantation. *Nephrol Dial Transplant* 2004; **19** Suppl 3: iii47-iii51 [PMID: 15192136 DOI: 10.1093/ndt/gfh1015]

95 **Morath C**, Zeier M. When should post-transplantation proteinuria be attributed to the renal allograft rather than to the native kidney? *Nat Clin Pract Nephrol* 2007; **3**: 18-19 [PMID: 17183258 DOI: 10.1038/ncpneph0376]

96 **Halimi JM**, Matthias B, Al-Najjar A, Laouad I, Chatelet V, Marlière JF, Nivet H, Lebranchu Y. Respective predictive role of urinary albumin excretion and nonalbumin proteinuria on graft loss and death in renal transplant recipients. *Am J Transplant* 2007; **7**: 2775-2781 [PMID: 17949457 DOI: 10.1111/j.1600-6143.2007.02010.x]

97 **Schaub S**, Wilkins JA, Antonovici M, Krokhin O, Weiler T, Rush D, Nickerson P. Proteomic-based identification of cleaved urinary beta2-microglobulin as a potential marker for acute tubular injury in renal allografts. *Am J Transplant* 2005; **5**: 729-738 [PMID: 15760396 DOI: 10.1111/j.1600-6143.2005.00766.x]

98 **Rosenkranz AR**, Mayer G. Proteinuria in the transplanted patient. *Nephrol Dial Transplant* 2000; **15**: 1290-1292 [PMID: 10978380 DOI: 10.1093/ndt/15.9.1290]

99 **Shamseddin MK**, Knoll GA. Posttransplantation proteinuria: an approach to diagnosis and management. *Clin J Am Soc Nephrol* 2011; **6**: 1786-1793 [PMID: 21734095 DOI: 10.2215/CJN.01310211]

P- Reviewer: Duan SB, Nechifor G, Scarpioni R, Yorioka N
S- Editor: Ji FF **L- Editor**: A **E- Editor**: Yan JL

World Journal of Nephrology

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DOI: 10.5527/wjn.v4.i3.354

World J Nephrol 2015 July 6; 4(3): 354-362
ISSN 2220-6124 (online)

MINIREVIEWS

Modern approaches to incompatible kidney transplantation

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Author contributions: Wongsaroj P and Kahwaji J contributed equally to design, data acquisition, drafting, and final approval; Vo A and Jordan SC contributed to drafting, critical revisions and final approval.

Conflict-of-interest statement: The authors do not have any competing interests in relation to the submitted work.

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Received: October 20, 2014
Peer-review started: October 21, 2014
First decision: November 27, 2014
Revised: March 11, 2015
Accepted: April 1, 2015
Article in press: April 7, 2015
Published online: July 6, 2015

Abstract

The presence of human-leukocyte antigen (HLA)-antibodies and blood group incompatibility remain a large barrier to kidney transplantation leading to increased morbidity and mortality on the transplant waiting list. Over the last decade a number of new approaches were developed to overcome these barriers. Intravenous immunoglobulin (IVIG) remains the backbone of HLA desensitization therapy and has been shown in a prospective, randomized, placebo controlled trial to improve transplantation rates. Excellent outcomes with the addition of rituximab (anti-B cell) to IVIG based desensitization have been achieved. There is limited experience with bortezomib (anti-plasma cell) and eculizumab (complement inhibition) for desensitization. However, these agents may be good adjuncts for patients who are broadly sensitized with strong, complement-fixing HLA antibodies. Excellent short and long-term outcomes have been achieved in ABO incompatible transplantation with the combination of antibody removal, B cell depletion, and pre-transplant immunosuppression. Kidney paired donation has emerged as a reasonable alternative for programs who cannot provide desensitization or in conjunction with desensitization. Future therapies directed toward cytokines that alter B cell proliferation are under investigation.

Key words: Desensitization; Antibodies; Intravenous immunoglobulin; Rituximab; ABO incompatible; Eculizumab; Bortezomib

Core tip: Intravenous immunoglobulin (IVIG) remains the backbone of human-leukocyte antigen (HLA) desensitization therapy and excellent outcomes with the addition of rituximab (anti-B cell) have been achieved. Bortezomib (anti-plasma cell) and eculizumab (complement inhibition) may be good adjuncts for patients who are broadly sensitized with strong, complement-fixing HLA antibodies. Excellent outcomes have been achieved in ABO incompatible transplantation with the combination of antibody removal, B cell depletion, and pre-transplant immunosuppression. Kidney paired donation has emerged as a reasonable alternative for programs who cannot provide desensitization or in conjunction with

desensitization.

Wongsaroj P, Kahwaji J, Vo A, Jordan SC. Modern approaches to incompatible kidney transplantation. *World J Nephrol* 2015; 4(3): 354-362 Available from: URL: http://www.wjgnet.com/2220-6124/full/v4/i3/354.htm DOI: http://dx.doi.org/10.5527/wjn.v4.i3.354

INTRODUCTION

Kidney transplantation is the gold standard for treating end-stage kidney disease and remarkable strides have been made over the last thirty years. However, there are now over 100000 people awaiting kidney transplantation in the United States according to the Organ Procurement and Transplantation Network. A significant proportion of these patients are broadly human-leukocyte antigen (HLA) sensitized and will have to wait longer to find an acceptable match; some may never. There are also those on the wait list with living donors who are blood type incompatible (ABOi), but would otherwise be an acceptable match. The long wait times incurred lead to increased mortality on the kidney transplant list[1]. The ability to provide a blood type or HLA incompatible transplant decreases mortality and gives hope to those languishing on the wait list.

Desensitization therapies started to emerge in the 1980's. Donor specific blood transfusions were performed for HLA desensitization with limited success. There was more success with ABOi transplantation during this time period with techniques employing a combination of plasma exchange (PLEX) and splenectomy. HLA antibody desensitization with intravenous immunoglobulin (IVIG) was first reported in the mid-1990's and ushered in a new era of transplantation. New immunomodulatory therapies have since emerged that successfully allow HLA and blood type incompatible transplant. In this review, we will discuss the current approaches and future directions of desensitization therapies.

IVIG AND RITUXIMAB (ANTI-B CELL)

IVIG is a complex preparation derived from the gamma globulin fraction of pooled human plasma used to treat primary hypogammaglobulinemia, acquired antibody deficiency, and various autoimmune disorders. It modulates the auto- and allo-immune response *via* broad-acting mechanisms. These mechanisms include neutralization of circulating antibodies, inhibition of B and T cell proliferation *via* interactions with Fc receptors, alteration of cytokine production, and down-regulation of complement. It therefore has powerful immunomodulatory effects and is now widely used for desensitization and treatment of antibody-mediated rejection (ABMR).

The efficacy of high-dose IVIG (1-2 g/kg per dose) was initially described separately by Glotz *et al*[2] and Tyan *et al*[3]. IVIG was administered on a monthly basis to those awaiting either a living or deceased donor kidney transplant. An improvement in panel reactive antibodies (PRA) and transplant rates was observed. These early successes lead to the first randomized, multicenter, placebo-controlled trial for desensitization. The National Institute of Health Ig02 trial included a total of 101 highly sensitized patients with a PRA greater than 50%. Subjects were randomized to receive dialysis with IVIG (2 g/kg) monthly for 4 mo or dialysis with equivalent volume of placebo[4] (Figure 1). Patients receiving high-dose IVIG had a statistically significant reduction in PRA and an improved rate of transplantation with a shorter wait time (4.8 years *vs* 10.3 years). There was a higher rate of acute rejection observed in the IVIG group (53%) compared with the placebo group (10%). However, the 2-year graft survival rates were not significantly different. This approach was effective for both living and deceased donor transplants.

Another approach utilizes low-dose IVIG (100 mg/kg) plus PLEX (Figure 1). Montgomery *et al*[5] demonstrated the efficacy of this combined therapy to rescue three living donor kidney transplant recipients who experienced ABMR and to preemptively eliminate donor specific antibody (DSA) in four recipients scheduled for a living donor kidney transplant. Recently, Montgomery *et al*[1] reported a significant survival benefit of desensitization with the low-dose IVIG/PLEX regimen in 211 HLA sensitized patients compared to patients who remained on the waiting list for eight years[1]. This low-dose IVIG/PLEX regimen is primarily limited to living donor kidney transplantation due to rebound of HLA-antibody that is often seen within days following therapy.

Rituximab, a chimeric anti-CD20 (anti-B cell) monoclonal antibody, has emerged as an important drug for modification of B cell and antibody responses. It is approved for treatment of lymphoma and rheumatoid arthritis and has demonstrated a significant benefit in a number of autoimmune disorders[6]. Clinical data suggest that the beneficial effects of rituximab are likely related to modification of dysfunctional cellular immunity rather than simply a reduction in antibody. Rituximab binds to CD20 and marks the cell for destruction by antibody-dependent cell mediated cytotoxicity, complement-dependent cytotoxicity and cell-mediated apoptosis *via* CD20 cross-linking[7,8]. Rituximab depletes $CD20^+$ B-cells in the bone marrow, spleen and lymph nodes. It does not deplete plasma cells as they are CD20 negative. Rituximab may have some effect on plasmablasts that emerge primarily from the spleen. Data suggest that splenectomy is effective in treating ABMR because it removes DSA secreting plasmablasts that are the primary source of DSA production[9].

Over the past several years, rituximab has been studied and incorporated into desensitization protocols based on the synergistic effect with IVIG observed

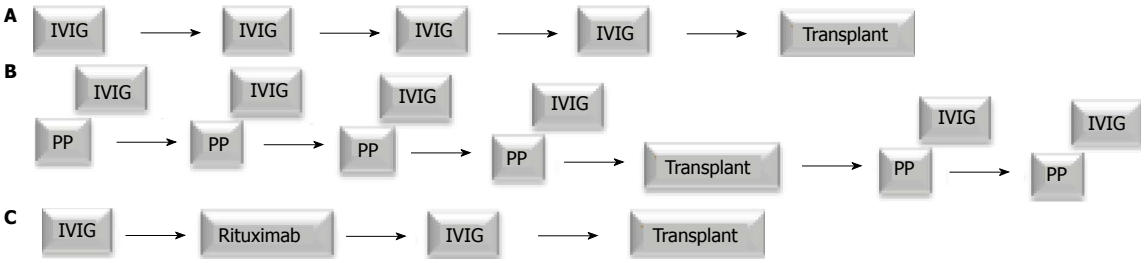

Figure 1 Desensitization protocols. A: The NIH Ig02 trial administered intravenous immunoglobulin (IVIG) in four monthly doses for patients awaiting a living or deceased donor transplant. This was followed by a living or deceased donor transplant once an acceptable crossmatch was achieved; B: Johns Hopkins University used a combination plasmapheresis (PP) with low-dose cytomegalovirus immune globulin following each PP session. The number and frequency of the PP sessions is dependent on the donor specific antibody titer. A living donor transplant occurs when an acceptable crossmatch is achieved. Additional sessions of PP/IVIG are administered after transplant; C: A modified protocol combining IVIG and rituximab was developed at Cedars-Sinai Medical Center. Two doses of IVIG are administered one month apart with one dose of rituximab given in between. A deceased or living donor transplant then takes place when an acceptable crossmatch is obtained.

in patients with autoimmune diseases. Our group evaluated the effect of adding two weekly doses of rituximab to a high-dose IVIG regimen in 20 highly sensitized patients. This protocol reduced PRA from an average 77% to 44%. There was an 80% rate of transplantation with excellent patient and allograft survival. Acute rejection occurred in 50% of patients who received a transplant. Most rejection episodes were diagnosed within the first month after transplantation and were reversible with treatment[10].

We subsequently reported a larger experience evaluating the efficacy of IVIG plus rituximab. Seventy-six highly sensitized patients with a positive cross-match who were treated with a desensitization regimen (IVIG 2 g/kg on day 1 and 30 and rituximab 1 g on day 15), had significant reductions in the T cell flow cytometry crossmatch, and were successfully transplanted. Thirty-one patients received a living donor and 45 patients received a deceased donor kidney transplant. Those awaiting a deceased donor were transplanted, on average, four months following desensitization. This was after waiting an average of 95 mo. There was a 37% rate of ABMR in this cohort, occurring mostly within the first month after transplant. ABMR was treated with pulse steroids, IVIG, and rituximab. PLEX was additionally administered for severe ABMR. The rejection episodes were reversible and did not translate to inferior outcomes. Patient and graft survival were 95% and 84%, respectively, at 24 mo[11].

These studies indicate that IVIG and rituximab offer a significant benefit in reduction of anti-HLA antibodies allowing improved rates of transplantation for highly sensitized patients. However, Marfo *et al*[12] found IVIG and rituximab to lack efficacy in a prospective cohort study that included highly sensitized kidney transplant candidates with a calculated PRA (cPRA) greater than 50%. The cPRA estimates the percentage of deceased donor offers that will be crossmatch incompatible for a candidate taking into account both class Ⅰ and class Ⅱ PRA. After a mean follow-up of 334 d, only two patients received a kidney transplant compared with 14 patients in the non-desensitized group (18% *vs* 52%). Desensitization did not lead to any significant reduction in patients' class Ⅰ and Ⅱ cPRA. There was also no change in the number of unacceptable antigens or their strength as measured by the mean fluorescence intensity (MFI). However, whole blood gene expression analyzed by microarrays demonstrated a down-regulation of immunoglobulin and B cell-associated transcripts after treatment[12].

More recently, our group conducted a double-blind randomized placebo-controlled trial comparing IVIG (2 g/kg, max 140 g administered at weeks 0 and 4) with rituximab (1 g administered at week 2) to IVIG (2 g/kg, max 140 g administered at weeks 0 and 4) with placebo (normal saline administered at week 2). Initially, 13 of 15 randomized patients received deceased donor transplants. The number of serious adverse events reported in the control group prohibited the completion of this trial and the study was un-blinded. There were six patients randomized to IVIG with rituximab and seven to IVIG with placebo. The data showed that all ABMR episodes occurred in the IVIG plus placebo group (N = 3, 43%) *vs* IVIG plus rituximab N = 0, 0%) (P = 0.06). The patients with ABMR episodes were treated with IVIG and rituximab with significantly improved renal function post-transplant at 6 and 12 mo. No transplant glomerulopathy was seen on protocol biopsies for the patients in IVIG plus rituximab group. It appeared that both protocols were effective in achieving an acceptable crossmatch allowing for transplantation. However, the combination of IVIG with rituximab was more effective at preventing DSA rebound, ABMR, and transplant glomerulopathy[13]. Desensitization using the combination of IVIG with rituximab was additionally shown to be cost-effective in a separate study[14].

BORTEZOMIB (ANTI-PLASMA CELL)

Bortezomib, a selective inhibitor of the 26S proteasome, was developed and approved by the United States Food and Drug Administration (FDA) for the treatment of multiple myeloma. Bortezomib inhibits antibody production from plasma cells, mediates apoptosis of this cell type and decreases the number of bone marrow-derived plasma cells. Therefore, it is expected to have

strong suppressive effects on humoral immunity and may represent a promising desensitization strategy.

Bortezomib has been used for the treatment of ABMR[15-18]. Nigos *et al*[19] performed a retrospective chart review of six kidney transplant patients with biopsy-proven ABMR. These six patients were treated with PLEX, IVIG (100 mg/kg after each PLEX and 300-400 mg/kg for 1-2 d after the last PLEX with a cumulative dose of 1 g/kg), steroids, and single-dose rituximab (375 mg/m^2) along with bortezomib (1.3 mg/m^2). Four out of the six patients had biopsy proven resolution of ABMR and stable allograft function over a median follow-up of 14 mo[19]. However, in a case series of four kidney transplant recipients with subacute ABMR and persistent DSA, Sberro-Soussan *et al*[16] found that bortezomib (1.3 mg/m^2) did not significantly decrease DSA MFI over a 5 mo follow-up. In this study, bortezomib was used as the sole therapeutic agent and only one dose was administered.

The potential effect of bortezomib on HLA antibody makes it an intriguing choice for desensitization. However, experiences with bortezomib as an alternative desensitizing agent are currently limited. Idica *et al*[20] reported the effect of bortezomib in thirteen highly sensitized patients. They found elimination of DSA in 10 of the patients and reduced MFI in the remaining three. Trivedi *et al*[17] reported a decrease in anti-HLA antibodies, both DSA and non-DSA, to less than 1000 MFI in nine of eleven patients treated with a combination of bortezomib and PLEX. The two patients without successful desensitization had strong HLA-antibodies with a peak MFI greater than 10000. Four patients had reappearance of anti-HLA antibodies after the initial reduction. However, all patients had stable graft function at a mean follow-up of 4 mo post-transplant[17]. Wahrmann used two cycles of bortezomib for pre-transplant desensitization in two highly sensitized kidney recipients[21]. Dexamethasone was added to the second cycle to enhance treatment efficacy. PRA decreased slightly from 87% to 80% in one patient and 37% to 13% in the second patient. However, both patients showed a greater than 50% reduction in the degree of complement fixing anti-HLA antibodies. Reghavan *et al*[22] reported a kidney transplant recipient with a weak binding DSA who successfully received a deceased-donor kidney transplant after using bortezomib in combination with rituximab. The patients cPRA was reduced from 57% to 31% and the DSA, became undetectable after transplant. The reduction in complement fixing antibodies is significant since they are mostly responsible the acute presentation of c4d positive ABMR and are difficult to modify. However, non-complement binding antibodies acting *via* antibody-dependent cell-mediated cytotoxicity are equally deleterious leading to chronic ABMR and transplant glomerulopathy[23].

In most studies, bortezomib shows promising outcomes for HLA desensitization. There is evidence, albeit limited, suggesting that bortezomib may be effective for altering complement fixing HLA-antibodies. These antibodies are difficult to modify with current therapies and are more deleterious to allografts. Proteasome inhibition alone may not provide durable modulation of HLA antibodies since there is no effect on precursor B cells or cytokines that promote antibody production. The main adverse effect of bortezomib is peripheral neuropathy that may occur in about 30% of treated patients. Severe events noted with bortezomib therapy include thrombocytopenia (28%) and neutropenia (11%). Given the limited experience and lack of long-term follow-up, bortezomib may be best utilized as an adjunct to other established therapies. Well-designed placebo-controlled studies are needed to further elucidate the role of bortezomib for HLA antibody desensitization.

ECULIZUMAB (COMPLEMENT INHIBITION)

Eculizumab is a humanized monoclonal antibody that binds to the complement factor C5 with high affinity, inhibiting its cleavage to C5a and C5b. This ultimately prevents the formation of the membrane attack complex. It is approved for the treatment of paroxysmal nocturnal hemoglobinuria and atypical hemolytic uremic syndrome (aHUS). It has been used primarily in transplantation to treat refractory ABMR and thrombotic microangiopathy. The binding of DSA to the donor endothelium initiates the classical pathway of the complement cascade. This, in turn, leads to the formation of the membrane attack complex (C5b-C9) and ultimately cell destruction. Eculizumab is administered as an adjunctive agent in desensitization to prevent complement dependent cytotoxicity mediated by antibody. Complement directed therapies do not have any depletive effects on HLA antibodies. Locke *et al*[24] presented a single case in which eculizumab was used combined with PLEX and IVIG to salvage an ongoing severe ABMR. Kidney allograft biopsies after treatment with eculizumab showed a dramatic reduction of the membrane attack complex without a significant change in C4d deposition or DSA. This is expected since C5 is located downstream from C4d in the complement activation cascade. Although the C5 epitope bound by eculizumab is located far from the C5a portion of C5, eculizumab can block C5 cleavage effectively. Eculizumab prevents the entry of the substrate molecule C5 into the C5 convertase, which means that C5 cleavage and the formation of C5a and C5b-9 are inhibited, resulting in blockade of the pro-inflammatory, pro-thrombotic and lytic functions of complement. The inhibition of complement activation at the level of C5 creates a functional C5 deficiency[25]. In the context of multiple different interventions, it was difficult to determine the impact of the anti-C5 antibody on the outcome. Burbach *et al*[26] reported the unsuccessful use of eculizumab in ABMR. However, this rejection was characterized by the absence of both C4d deposition in peritubular capillaries and complement binding DSA.

The role of eculizumab for HLA desensitization is not well defined. There is some evidence that eculizumab is effective in preventing ABMR in highly sensitized recipients. Stegall *et al*[27] reported significantly decreased incidence of early ABMR in 26 highly sensitized recipients with a positive crossmatch against their living donor. The incidence of ABMR was 7.7% (2/26) in the eculizumab group compared to 41.2% (21/51) in the control group. The two cases of ABMR in the eculizumab group occurred on post-transplant day seven and 14 in the setting of increased DSA and a biopsy that showed both C4d deposition and glomerular microthrombi. PLEX was instituted resulting in the resolution of the histologic features of ABMR in one week. The percentage of patients who developed high levels of DSA (MFI > 10000) in the first three months after transplant was similar in both groups. As expected, eculizumab did not have an impact on the presence and strength of DSA after transplant[27]. Long-term follow-up of eculizumab treated patients showed a much higher incidence of transplant glomerulopathy. Thus, C5 inhibition, alone, does not provide long-term protection from other forms of antibody-mediated injury. This raises the question of the need for concomitant B cell and antibody reduction therapies to prevent the development of transplant glomerulopathy.

There are some limitations to the use of eculizumab for desensitization. The duration of therapy after transplant has not been well established. Therefore, treatment may need to be continued indefinitely. Furthermore, it has no depletive effect on DSA and thus cannot alter the underlying immune disorder. Eculizumab only has effect against complement binding HLA antibodies. This can prevent acute ABMR but will likely be ineffective for the prevention of chronic ABMR and transplant glomerulopathy since this is mostly mediated by non-complement dependent pathways (antibody-dependent cell-mediated cytotoxicity). Finally, the cost of eculizumab is prohibitive in many settings and may ultimately limit its utility in kidney transplantation.

ABO INCOMPATIBLE TRANSPLANTATION

The development of protocols allowing for the transplantation of ABOi pairs has expanded the donor pool to recipients with a living donor who would otherwise be awaiting a deceased donor. ABOi transplantation has eased the pressure on the deceased donor waiting list and improved outcomes by transplanting recipients before they begin to experience the burden of chronic renal replacement therapy. Early experience in kidney transplantation showed that transplanting blood type incompatible donor/recipient pairs lead to hyperacute rejection and allograft thrombosis. Anti- A/B isoagglutinins bound to antigens on endothelial cells incite a cascade of events that leads to graft failure, often within minutes of graft reperfusion. Anti-A and B isoagglutinins are distinct from HLA antibodies in that they are natural antibodies that are likely produced in the bowel and peritoneum by precursor B1 cells in response to the presence of normal bacteria. Individuals are sensitized to non-self carbohydrate chains that exist on red blood cells and vascular endothelium. These chains are represented by the designation of blood types A, B, and AB. Those with blood type O do not express these chains and therefore develop antibodies to both A and B isoagglutinins.

There is now a wealth of experience with ABOi transplantation which dates back to the 1980s[28-30]. Early experience with ABOi transplantation occurred primarily in Japan where, at that time, there was limited availability of deceased donors. Through this early experience three essential components of successful ABOi transplantation were elucidated. They include antibody removal, B cell depletion, and pre-transplant immunosuppression. Many center specific protocols have been developed based on these principles[31] (Figure 2). PLEX was initially employed to remove anti-A/B antibodies prior to living donor transplantation, but this alone was not successful. It was the addition of B cell depletion by splenectomy that allowed early success[32]. However, the requirement of splenectomy for successful ABOi transplantation limited its wide spread acceptance given the added surgical risk and resultant life-long risk of infection. The development of rituximab eliminated the need for splenectomy allowing the process to be more palatable to both physicians and patients.

Outcomes in ABOi transplantation have markedly improved over the years. In Japan, higher rates of rejection and graft loss were seen prior to the introduction of tacrolimus and mycophenolate mofetil. Toki *et al*[33] analyzed the impact of ABMR on ABOi transplantation. In this study, 58 consecutive ABOi transplants were divided into two groups: those that developed ABMR within months and those that did not. Graft survival was statistically less at 3, 5, and 8 years after transplant (95% *vs* 49%) in the ABMR group. Multivariable analysis revealed the presence of HLA DSA and an anti A/B titer $\geqslant$ 1:32 to be predictive of ABMR while pre-transplant immunosuppression with mycophenolate mofetil was protective. Successful ABOi programs incorporate these elements into their protocol starting immunosuppression with mycophenolate mofetil with or without tacrolimus and steroids one to four weeks prior to transplant, achieving an anti-A/B titer of < 1:32 at the time of transplant, and screening for anti-HLA antibodies (Figure 2).

Isoagglutinin titers have a large impact on the incidence of rejection and graft outcomes after ABOi transplantation. However, recognition of titer rebound and post-transplant PLEX have improved outcomes dramatically. Won *et al*[34] explored the significance of isoagglutinin titers in a retrospective analysis of 95 patients receiving an ABOi allograft. The desensitization regimen consisted of pre-transplant immunosuppression with tacrolimus, mycophenolate mofetil, and steroids seven to ten days prior to transplant. Rituximab, fixed dose (200 mg or 500 mg), was administered two to 18 d prior to PLEX. The goal titer at the time of transplant was $\leqslant$ 1:4. Basiliximab was administered for induction at the

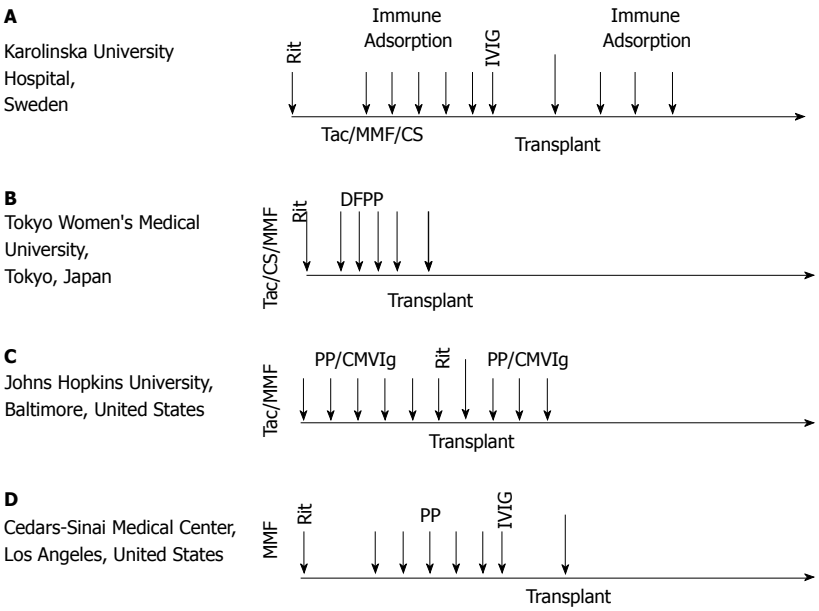

Figure 2 ABOi protocols. Successful ABOi transplantation has been achieved through various protocols around the world. These four protocols all contain the critical components of ABOi desensitization: antibody removal, pre-transplant immunosuppression and B cell depletion. Many protocols also use immune globulin. Rit: Rituximab; Tac: Tacrolimus; MMF: Mycophenolate mofetil; CS: Corticosteroid; DFPP: Double filtration plasmapheresis; PP: Plasmapheresis; CMVIg: Cytomegalovirus immune globulin.

time of transplant. Isoagglutinins rebounded and peaked two weeks after transplant. There were 34 patients (35.8%) that had a rebound in titer to $\geqslant$ 1:16. Titer rebound was associated with an initial pre-transplant titer $\geqslant$ 1:256, rituximab administration $\leqslant$ 7 d prior to initiation of PLEX, and blood type O. Titer rebound was treated with additional post-transplant PLEX. Only one episode of ABMR was reported with at titer of 1:32 at the time of rejection. The addition of immune globulin to the preconditioning regimen may also have the beneficial effect of limiting titer rebound and is used in many protocols around the world (Figure 2).

The use of rituximab facilitated the expansion of ABOi transplantation by obviating the need for splenectomy. Sonnenday *et al*[35] reported in 2004 an early case series of successful ABOi transplantation using a regimen consisting of PLEX, low-dose CMV immune globulin and rituximab. Many other groups have since reported good outcomes with rituximab making it a key ingredient in the current era of ABOi desensitization (Figure 2). More recently, there has been a trend toward minimizing the dose of rituximab in an effort to prevent infections and decrease costs. Some programs have successfully used doses as low as 200 mg[36]. Successful ABOi desensitization has also been described in the absence of both rituximab and splenectomy. Montgomery *et al*[37] reported a series of 24 patients who underwent ABOi transplantation without pre-transplant B cell depletion. Good short-term outcomes were achieved with 100% graft survival. There were three episodes of ABMR. Two were treated with additional PLEX and one with salvage splenectomy. B cell depletion, *via* splenectomy or rituximab, has long-term proven efficacy. A minimalist approach to ABOi transplantation should only be undertaken by experienced transplant centers.

Excellent short and long-term outcomes have been achieved following these principles. Genberg *et al*[38] reported no difference in rejection or allograft survival in ABOi recipients *vs* ABO compatible recipients at three years after transplantation using a protocol consisting of immunoadsorption (antibody removal), pre-transplant immunosuppression with mycophenolate mofetil, and B-cell depletion with rituximab (Figure 2). Analysis of variables associated with rejection revealed elevated anti-A/B titer prior to transplant, absence of pre-transplant immunosuppression, and presence of DSA.

KIDNEY PAIRED DONATION

The recent development of kidney paired donation programs has facilitated the transplant of hundreds of patients in the United States. These programs may be an option for kidney transplant recipients with an HLA or blood type incompatible living donor. Many paradigms exist and range from simple two way exchanges to long, so-called domino chains that have bridging donors (Figure 3). Programs are able to completely avoid unacceptable antigens and blood types removing the need for desensitization in patients with an easy to match phenotype. However, those who are very broadly sensitized with strong HLA antibodies remain a challenge.

A combination of desensitization therapies with kidney paired donation may result in donors with more favorable immunologic profiles. The group at Johns Hopkins recently reported on their experience combining desensitization with kidney paired donation[39]. Mathematical simulations have shown that this approach may improve the rates of transplantation with kidney paired donation

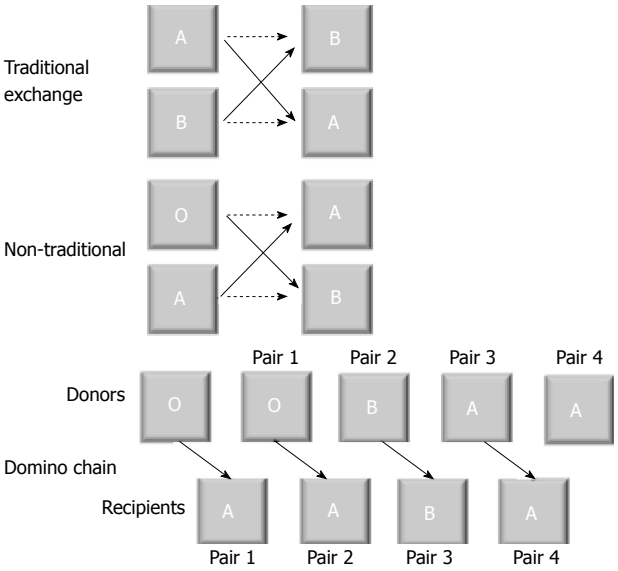

Figure 3 Paired kidney exchange paradigms. Traditional exchange: This exchange swaps two ABO incompatible pairs so that new donor recipient pairs are now ABO compatible. Non-traditional exchange: This exchange uses one human-leukocyte antigen (HLA) incompatible pair and one ABO incompatible pair and trades for two HLA compatible and ABO compatible transplants. Domino chain: This chain starts with a non-directed donor. The recipient of this kidney then has their donor available for another recipient. This occurs progressively until a donor cannot be matched. This donor can act as a bridge to a new chain or can donate to the deceased donor waiting list, thereby ending the chain.

programs. Yabu *et al*[40] recently reported the successful transplantation of five patients with a cPRA of 100% utilizing this approach. Desensitization consisted of IVIG and rituximab with the addition of bortezomib and PLEX in one case. Some centers have also adopted the approach of accepting an ABO incompatible donor in exchange for one that is HLA compatible.

Kidney paired donation provides a good alternative to desensitization in many circumstances; however it is not uniformly effective. A major limitation is that one must have a living donor available to present to the exchange. In addition, favorable donor characteristics including age and blood type must be taken into consideration and effect the likelihood of achieving a match. Patients who are very broadly sensitized with strong binding HLA antibody will be persistently difficult to match without the use of desensitization therapies.

FUTURE APPROACHES

The ongoing development of biologic agents particularly for the treatment of rheumatologic diseases may provide new avenues of exploration for desensitization. All of these agents modulate B cell activity. Epratuzumab targets CD22 on B cells and effectively modulates their activity. It has shown promise in patients with systemic lupus erythematosus (SLE)[41]. Belimumab, an antibody directed against B lymphocyte stimulator (BLyS) was recently approved for SLE and also has B cell modulator effects *via* inhibition of B cell proliferation[42]. Atacicept is currently under study in SLE and acts as a soluble receptor for the B cell proliferation cytokines BLyS and a proliferation-inducing ligand (APRIL) thereby neutralizing their activity. A decrease in total IgG levels has been demonstrated in early phase studies. Tocilizumab is a monoclonal antibody directed against the receptor for interleukin-6, a potent inflammatory cytokine. It is currently approved for rheumatoid arthritis and leads to reductions in IgG and inflammatory responses. It was shown in to modulate the development of DSA in a mouse model of allosensitization[43].

In summary, desensitization therapies with IVIG, rituximab and PLEX have greatly improved the access to and success of incompatible transplantation, both for HLA sensitized and ABOi patients. However, it is important to continue to pursue newer, potentially less toxic approaches that focus on B cells, plasma cells and inhibition of complement-activating antibodies. These basic therapies have also gained acceptance in treatment of ABMR and will likely become more important in transplant medicine as the impact of *de novo* DSA generation post-transplant is better understood.

REFERENCES

1 **Montgomery RA**, Lonze BE, King KE, Kraus ES, Kucirka LM, Locke JE, Warren DS, Simpkins CE, Dagher NN, Singer AL, Zachary AA, Segev DL. Desensitization in HLA-incompatible kidney recipients and survival. *N Engl J Med* 2011; **365**: 318-326 [PMID: 21793744 DOI: 10.1056/NEJMoa1012376]

2 **Glotz D**, Haymann JP, Sansonetti N, Francois A, Menoyo-Calonge V, Bariety J, Druet P. Suppression of HLA-specific alloantibodies by high-dose intravenous immunoglobulins (IVIg). A potential tool for transplantation of immunized patients. *Transplantation* 1993; **56**: 335-337 [PMID: 8356587]

3 **Tyan DB**, Li VA, Czer L, Trento A, Jordan SC. Intravenous immunoglobulin suppression of HLA alloantibody in highly sensitized transplant candidates and transplantation with a histoincompatible organ. *Transplantation* 1994; **57**: 553-562 [PMID: 8116041]

4 **Jordan SC**, Tyan D, Stablein D, McIntosh M, Rose S, Vo A, Toyoda M, Davis C, Shapiro R, Adey D, Milliner D, Graff R, Steiner R, Ciancio G, Sahney S, Light J. Evaluation of intravenous immunoglobulin as an agent to lower allosensitization and improve transplantation in highly sensitized adult patients with end-stage renal disease: report of the NIH IG02 trial. *J Am Soc Nephrol* 2004; **15**: 3256-3262 [PMID: 15579530]

5 **Montgomery RA**, Zachary AA, Racusen LC, Leffell MS, King KE, Burdick J, Maley WR, Ratner LE. Plasmapheresis and intravenous immune globulin provides effective rescue therapy for refractory humoral rejection and allows kidneys to be successfully transplanted into cross-match-positive recipients. *Transplantation* 2000; **70**: 887-895 [PMID: 11014642]

6 **Kahwaji J**, Tong C, Jordan SC, Vo AA. Rituximab: An emerging therapeutic agent for kidney transplantation. *Transpl Res Risk Manag* 2009; **1**: 15-29

7 **Jordan SC**, Pescovitz MD. Presensitization: the problem and its management. *Clin J Am Soc Nephrol* 2006; **1**: 421-432 [PMID: 17699241]

8 **Markasz L**, Vanherberghen B, Flaberg E, Otvös R, Stuber G, Gustafsson Jernberg A, Olah E, Skribek H, Szekely L. NK cell-mediated lysis is essential to kill Epstein-Barr virus transformed lymphoblastoid B cells when using rituximab. *Biomed Pharmacother* 2009; **63**: 413-420 [PMID: 18834693 DOI: 10.1016/j.biopha.2008.08.009]

9 **Orandi BJ**, Zachary AA, Dagher NN, Bagnasco SM, Garonzik-

Wang JM, Van Arendonk KJ, Gupta N, Lonze BE, Alachkar N, Kraus ES, Desai NM, Locke JE, Racusen LC, Segev DL, Montgomery RA. Eculizumab and splenectomy as salvage therapy for severe antibody-mediated rejection after HLA-incompatible kidney transplantation. *Transplantation* 2014; **98**: 857-863 [PMID: 25121475 DOI: 10.1097/TP.0000000000000298]

10 **Vo AA**, Lukovsky M, Toyoda M, Wang J, Reinsmoen NL, Lai CH, Peng A, Villicana R, Jordan SC. Rituximab and intravenous immune globulin for desensitization during renal transplantation. *N Engl J Med* 2008; **359**: 242-251 [PMID: 18635429 DOI: 10.1056/NEJMoa0707894]

11 **Vo AA**, Peng A, Toyoda M, Kahwaji J, Cao K, Lai CH, Reinsmoen NL, Villicana R, Jordan SC. Use of intravenous immune globulin and rituximab for desensitization of highly HLA-sensitized patients awaiting kidney transplantation. *Transplantation* 2010; **89**: 1095-1102 [PMID: 20110854 DOI: 10.1097/TP.0b013e3181d21e7f]

12 **Marfo K**, Ling M, Bao Y, Calder B, Ye B, Hayde N, Greenstein S, Chapochnick-Friedman J, Glicklich D, de Boccardo G, Kinkhabwala M, Akalin E. Lack of effect in desensitization with intravenous immunoglobulin and rituximab in highly sensitized patients. *Transplantation* 2012; **94**: 345-351 [PMID: 22820699]

13 **Vo AA**, Choi J, Cisneros K, Reinsmoen N, Haas M, Ge S, Toyoda M, Kahwaji J, Peng A, Villicana R, Jordan SC. Benefits of rituximab combined with intravenous immunoglobulin for desensitization in kidney transplant recipients. *Transplantation* 2014; **98**: 312-319 [PMID: 24770617]

14 **Vo AA**, Petrozzino J, Yeung K, Sinha A, Kahwaji J, Peng A, Villicana R, Mackowiak J, Jordan SC. Efficacy, outcomes, and cost-effectiveness of desensitization using IVIG and rituximab. *Transplantation* 2013; **95**: 852-858 [PMID: 23511212 DOI: 10.1097/TP.0b013e3182802f88]

15 **Everly MJ**, Everly JJ, Susskind B, Brailey P, Arend LJ, Alloway RR, Roy-Chaudhury P, Govil A, Mogilishetty G, Rike AH, Cardi M, Wadih G, Tevar A, Woodle ES. Bortezomib provides effective therapy for antibody- and cell-mediated acute rejection. *Transplantation* 2008; **86**: 1754-1761 [PMID: 19104417 DOI: 10.1097/TP.0b013e318190af83]

16 **Sberro-Soussan R**, Zuber J, Suberbielle-Boissel C, Candon S, Martinez F, Snanoudj R, Rabant M, Pallet N, Nochy D, Anglicheau D, Leruez M, Loupy A, Thervet E, Hermine O, Legendre C. Bortezomib as the sole post-renal transplantation desensitization agent does not decrease donor-specific anti-HLA antibodies. *Am J Transplant* 2010; **10**: 681-686 [PMID: 20121729 DOI: 10.1111/j.1600-6143.2009.02968.x]

17 **Trivedi HL**, Terasaki PI, Feroz A, Everly MJ, Vanikar AV, Shankar V, Trivedi VB, Kaneku H, Idica AK, Modi PR, Khemchandani SI, Dave SD. Abrogation of anti-HLA antibodies via proteasome inhibition. *Transplantation* 2009; **87**: 1555-1561 [PMID: 19461494 DOI: 10.1097/TP.0b013e3181a4b91b]

18 **Walsh RC**, Everly JJ, Brailey P, Rike AH, Arend LJ, Mogilishetty G, Govil A, Roy-Chaudhury P, Alloway RR, Woodle ES. Proteasome inhibitor-based primary therapy for antibody-mediated renal allograft rejection. *Transplantation* 2010; **89**: 277-284 [PMID: 20145517 DOI: 10.1097/TP.0b013e3181c6ff8d]

19 **Nigos JG**, Arora S, Nath P, Hussain SM, Marcus RJ, Ko TY, Sureshkumar KK. Treatment of antibody-mediated rejection in kidney transplant recipients: a single-center experience with a bortezomib-based regimen. *Exp Clin Transplant* 2012; **10**: 609-613 [PMID: 23216567]

20 **Idica A**, Kaneku H, Everly MJ, Trivedi HL, Feroz A, Vanikar AV, Shankar V, Trivedi VB, Modi PR, Khemchandani SI, Dave SD, Terasaki PI. Elimination of post-transplant donor-specific HLA antibodies with bortezomib. *Clin Transpl* 2008: 229-239 [PMID: 19708459]

21 **Wahrmann M**, Haidinger M, Körmöczi GF, Weichhart T, Säemann MD, Geyeregger R, Kikić Z, Prikoszovich T, Drach J, Böhmig GA. Effect of the proteasome inhibitor bortezomib on humoral immunity in two presensitized renal transplant candidates. *Transplantation* 2010; **89**: 1385-1390 [PMID: 20335829 DOI: 10.1097/TP.0b013e3181d9e1c0]

22 **Raghavan R**, Jeroudi A, Achkar K, Suki W, Gaber AO, Knight R, Land G, Dilioglou S, Patel S, Abdellatif A. Bortezomib in kidney transplant desensitization: a case report. *Clin Transpl* 2009: 339-342 [PMID: 20524295]

23 **Loupy A**, Hill GS, Jordan SC. The impact of donor-specific anti-HLA antibodies on late kidney allograft failure. *Nat Rev Nephrol* 2012; **8**: 348-357 [PMID: 22508180 DOI: 10.1038/nrneph.2012.81]

24 **Locke JE**, Magro CM, Singer AL, Segev DL, Haas M, Hillel AT, King KE, Kraus E, Lees LM, Melancon JK, Stewart ZA, Warren DS, Zachary AA, Montgomery RA. The use of antibody to complement protein C5 for salvage treatment of severe antibody-mediated rejection. *Am J Transplant* 2009; **9**: 231-235 [PMID: 18976298 DOI: 10.1111/j.1600-6143.2008.02451.x]

25 **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]

26 **Burbach M**, Suberbielle C, Brochériou I, Ridel C, Mesnard L, Dahan K, Rondeau E, Hertig A. Report of the inefficacy of eculizumab in two cases of severe antibody-mediated rejection of renal grafts. *Transplantation* 2014; **98**: 1056-1059 [PMID: 24839895]

27 **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]

28 **Tanabe K**. Japanese experience of ABO-incompatible living kidney transplantation. *Transplantation* 2007; **84**: S4-S7 [PMID: 18162988 DOI: 10.1097/01.tp.0000296008.08452.4c]

29 **Genberg H**, Kumlien G, Wennberg L, Berg U, Tydén G. ABO-incompatible kidney transplantation using antigen-specific immunoadsorption and rituximab: a 3-year follow-up. *Transplantation* 2008; **85**: 1745-1754 [PMID: 18580466 DOI: 10.1097/TP.0b013e3181726849]

30 **Montgomery RA**, Locke JE, King KE, Segev DL, Warren DS, Kraus ES, Cooper M, Simpkins CE, Singer AL, Stewart ZA, Melancon JK, Ratner L, Zachary AA, Haas M. ABO incompatible renal transplantation: a paradigm ready for broad implementation. *Transplantation* 2009; **87**: 1246-1255 [PMID: 19384174 DOI: 10.1097/TP.0b013e31819f2024]

31 **Kahwaji J**, Vo AA, Jordan SC. ABO blood group incompatibility: a diminishing barrier to successful kidney transplantation? *Expert Rev Clin Immunol* 2010; **6**: 893-900 [PMID: 20979554 DOI: 10.1586/eci.10.78]

32 **Alexandre GP**, Squifflet JP, De Bruyère M, Latinne D, Reding R, Gianello P, Carlier M, Pirson Y. Present experiences in a series of 26 ABO-incompatible living donor renal allografts. *Transplant Proc* 1987; **19**: 4538-4542 [PMID: 3321614]

33 **Toki D**, Ishida H, Setoguchi K, Shimizu T, Omoto K, Shirakawa H, Iida S, Horita S, Furusawa M, Ishizuka T, Yamaguchi Y, Tanabe K. Acute antibody-mediated rejection in living ABO-incompatible kidney transplantation: long-term impact and risk factors. *Am J Transplant* 2009; **9**: 567-577 [PMID: 19260836 DOI: 10.1111/j.1600-6143.2008.02538.x]

34 **Won D**, Choe W, Kim HJ, Kwon SW, Han DJ, Park SK. Significance of isoagglutinin titer in ABO-incompatible kidney transplantation. *J Clin Apher* 2014; **29**: 243-250 [PMID: 24375675 DOI: 10.1002/jca.21312]

35 **Sonnenday CJ**, Warren DS, Cooper M, Samaniego M, Haas M, King KE, Shirey RS, Simpkins CE, Montgomery RA. Plasmapheresis, CMV hyperimmune globulin, and anti-CD20 allow ABO-incompatible renal transplantation without splenectomy. *Am J Transplant* 2004; **4**: 1315-1322 [PMID: 15268734]

36 **Hatakeyama S**, Fujita T, Murakami R, Suzuki Y, Sugiyama N, Yamamoto H, Okamoto A, Imai A, Tobisawa Y, Yoneyama T, Mori K, Yoneyama T, Hashimoto Y, Koie T, Narumi S, Ohyama C. Outcome comparison of ABO-incompatible kidney transplantation with low-dose rituximab and ABO-compatible kidney transplantation: a single-center experience. *Transplant Proc* 2014; **46**: 445-448 [PMID: 24655984 DOI: 10.1016/j.transproceed.2013.09.036]

37 **Montgomery RA**, Locke JE. ABO-incompatible transplantation: less

may be more. *Transplantation* 2007; **84**: S8-S9 [PMID: 18162992 DOI: 10.1097/01.tp.0000296032.12974.bb]

38 **Genberg H**, Kumlien G, Wennberg L, Tydén G. Long-term results of ABO-incompatible kidney transplantation with antigen-specific immunoadsorption and rituximab. *Transplantation* 2007; **84**: S44-S47 [PMID: 18162990 DOI: 10.1097/01.tp.0000296031]

39 **Montgomery RA**, Lonze BE, Jackson AM. Using donor exchange paradigms with desensitization to enhance transplant rates among highly sensitized patients. *Curr Opin Organ Transplant* 2011; **16**: 439-443 [PMID: 21666478 DOI: 10.1097/MOT.0b013e32834897c1]

40 **Yabu JM**, Pando MJ, Busque S, Melcher ML. Desensitization combined with paired exchange leads to successful transplantation in highly sensitized kidney transplant recipients: strategy and report of five cases. *Transplant Proc* 2013; **45**: 82-87 [PMID: 23375278 DOI: 10.1016/j.transproceed.2012.08.007]

41 **Strand V**, Petri M, Kalunian K, Gordon C, Wallace DJ, Hobbs K, Kelley L, Kilgallen B, Wegener WA, Goldenberg DM. Epratuzumab for patients with moderate to severe flaring SLE: health-related quality of life outcomes and corticosteroid use in the randomized controlled ALLEVIATE trials and extension study SL0006. *Rheumatology* (Oxford) 2014; **53**: 502-511 [PMID: 24273022 DOI: 10.1093/rheumatology/ket378]

42 **Webber A**, Hirose R, Vincenti F. Novel strategies in immunosuppression: issues in perspective. *Transplantation* 2011; **91**: 1057-1064 [PMID: 21412186 DOI: 10.1097/TP.0b013e3182145306]

43 **Wu G**, Chai N, Kim I, Klein AS, Jordan SC. Monoclonal anti-interleukin-6 receptor antibody attenuates donor-specific antibody responses in a mouse model of allosensitization. *Transpl Immunol* 2013; **28**: 138-143 [PMID: 23562586 DOI: 10.1016/j.trim.2013.03.003]

P- Reviewer: Trimarchi H **S- Editor**: Song XX **L- Editor**: A
E- Editor: Yan JL

World Journal of Nephrology

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Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.5527/wjn.v4.i3.363

World J Nephrol 2015 July 6; 4(3): 363-366
ISSN 2220-6124 (online)

MINIREVIEWS

Epigenetics of epithelial Na^+ channel-dependent sodium uptake and blood pressure regulation

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Author contributions: The author solely contributed to this paper.

Supported by National Institutes of Health Grant 2R01 DK080236 06A1.

Conflict-of-interest statement: The author has declared that no conflict of interest exists.

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Received: November 1, 2014
Peer-review started: November 9, 2014
First decision: November 27, 2014
Revised: May 6, 2015
Accepted: May 16, 2015
Article in press: May 18, 2015
Published online: July 6, 2015

Abstract

The epithelial Na^+ channel (ENaC) consists of α, β, γ subunits. Its expression and function are regulated by aldosterone at multiple levels including transcription. ENaC plays a key role in Na^+ homeostasis and blood pressure. Mutations in ENaC subunit genes result in hypertension or hypotension, depending on the nature of the mutations. Transcription of αENaC is considered as the rate-limiting step in the formation of functional ENaC. As an aldosterone target gene, αENaC is activated upon aldosterone- mineralocorticoid receptor binding to the cis-elements in the αENaC promoter, which is packed into chromatin. However, how aldosterone alters chromatin structure to induce changes in transcription is poorly understood. Studies by others and us suggest that Dot1a-Af9 complex represses αENaC by directly binding and regulating targeted histone H3 K79 hypermethylation at the specific subregions of αENaC promoter. Aldosterone decreases Dot1a-Af9 formation by impairing expression of Dot1a and Af9 and by inducing Sgk1, which, in turn, phosphorylates Af9 at S435 to weaken Dot1a-Af9 interaction. MR attenuates Dot1a-Af9 effect by competing with Dot1a for binding Af9. Af17 relieves repression by interfering with Dot1a-Af9 interaction and promoting Dot1a nuclear export. *Af17*$^{-/-}$ mice exhibit defects in ENaC expression, renal Na^+ retention, and blood pressure control. This review gives a brief summary of these novel findings.

Key words: Gene transcription; Chromatin; Epithelial sodium channel; Histone; Blood pressure

Core tip: The epithelial Na^+ channel (ENaC) is a key player in sodium transport and blood pressure control. This minireview summarizes the epigenetic mechanisms governing the transcription of αENaC. The epigenetic control involves Dot1a-Af9-mediated repression through targeted hypermethylation of histone H3 K79. Aldosterone relieves the repression by decreasing Dot1a and Af9 mRNA levels and by weakening the protein-protein interaction between Dot1a and Af9 interaction *via*

Sgk1-catalyzed Af9 phosphorylation. Aldosterone-independent mechanism involves Af17 as a competitor of Af9 for binding Dot1a and stimulator of Dot1a nuclear export. *Af17*$^{-/-}$ mice exhibit decreased Na^+ reabsorption and lowered blood pressure, indicating the significance of this epigenetic control.

Zhang W. Epigenetics of epithelial Na^+ channel-dependent sodium uptake and blood pressure regulation. *World J Nephrol* 2015; 4(3): 363-366 Available from: URL: http://www.wjgnet.com/2220-6124/full/v4/i3/363.htm DOI: http://dx.doi.org/10.5527/wjn.v4.i3.363

EPITHELIAL SODIUM CHANNEL IS A KEY PLAYER IN NA^+ METABOLISM

Epithelial sodium channel (ENaC) or the amiloride-sensitive sodium channel contains α, β, and γ subunits[1]. Variations in the production of the subunits, its open probability and/or plasma membrane localization determine the rate of Na^+ entry. ENaC-dependent Na^+ entry occurs in the aldosterone-sensitive distal nephron (ASDN), which consists of the late distal convoluted tubule (DCT2), connecting tubule, and collecting duct[2]. The significance of ENaC in Na^+ metabolism and blood pressure regulation is illustrated by two human genetic diseases, Liddle's syndrome (Liddle syndrome or pseudoaldosteronism) and the autosomal recessive pseudohypoaldosteronism type 1[3].

The manifestations of Liddle's syndrome are similar to those caused by mineralocorticoid excess, including hypertension and, in some patients, hypokalemia and metabolic alkalosis. Moreover, plasma and urinary aldosterone levels are reduced, not increased as in primary aldosteronism. Presentation in most patients takes place at a young age, suggesting the possibility of a genetic disorder rather than an adrenal adenoma. Subsequent studies defined it as an autosomal dominant disorder in which excess loss of potassium and reabsorption of sodium take place in the ASDN. The therapy of the disease consists of a low sodium diet in conjunction with potassium-sparing diuretic medicines such as amiloride. The disorder is extremely rare. Less than 30 families or isolated occurrence have been described in the world as of 2008[4]. Patients with Liddle's syndrome have gain-of-function mutations in either βENaC or γENaC subunit, leading to increased ENaC function that produces inappropriately large Na^+ absorption by ASDN. All of these mutations impact a highly-conserved PPxY domain. Further analyses resulted in identification of the PPxY domain as the ENaC regulatory region. The mutations cause hyperactivity of the channel. Such hyperactivity probably results from changes in protein-protein interactions regulating the channel degradation through Nedd4 ubiquitin ligase[5,6]. Alternatively, the mutations may affect the clathrin-dependent endocytosis[7]. Autosomal recessive pseudohypoaldosteronism type 1 results from reduced ENaC function. The clinical features including aldosterone resistance, sodium wasting, hypovolemia, and hyperkalemia are presented in affected individuals in infancy. These features are similar to those in other forms of hypoaldosteronism in children, with exception of elevated, not reduced plasma aldosterone levels. The disorder results from loss-of-function mutations in any of the three genes encoding ENaC subunits.

Deletion of all three ENaC subunit genes induces perinatal lethality, associated with failure in lung fluid clearance, and/or an acute pseudohypoaldosteronism type 1 featured by metabolic acidosis and severe hyperkalemia[8].

EPIGENETIC CONTROL OF ENAC TRANSCRIPTION BY ALDOSTERONE-SENSITIVE DOT1A-AF9 COMPLEX

Chromatin has been well established to play a critical role in transcription regulation[9]. One of the mechanisms controlling chromatin structure is the post-translational modification of histone N-terminal tails such as acetylation and methylation. According to the histone code hypothesis[9], these histone tails are exposed, unstructured and accessible to various regulatory proteins that recognize a variety of modifications of specific amino acids in the histones or their combinations, giving rise to altered chromatin structures that control particular cellular processes.

Histone methylation can have distinct effects on gene activation, depending on its chromosomal location, the specifically targeted lysines, argines and combinations undergoing posttranslational modifications, and the enzyme (or protein complex) involved in the particular modifications[10]. Members of histone methyltransferase Dot1 family methylate histone H3 K79, which resides in the globular domain. They can modify H3 K79 with one, two, and three methyl groups, leading to mono-, di-, and tri-methylated K79[11]. These methylation events are referred as H3 m1, m2, and m3K79. Such complicity of the modifications may contribute to the functional diversity. In fact, Dot1 proteins have various functions, ranging from telomeric and HM silencing, cell cycle regulation, cell proliferation, meiotic checkpoint, DNA replication, apoptosis, leukemogenesis, to blood pressure control (reviewed in[12]).

Our previous work led to cloning of mouse Dot1-like (*Dot1l*) gene, which is featured by at least five isoforms (Dot1a-e). These isoforms are generated by alternative splicing. Among them, Dot1a is the most characterized variant[13]. The first clue of functional significance of Dot1a in renal physiology came from the observation that aldosterone downregulates Dot1a mRNA level in IMCD3 cells derived from mouse inner medullary collecting duct. Aldosterone regulates Dot1a

mRNA abundance in a time- and dose-dependent manner, resulting in a decrease in overall H3 K79 methylation[14]. Subsequent studies revealed that Dot1a represses α*ENaC* transcription. Chromatin immunoprecipitation (ChIP) coupled by real-time qPCR unearthed the repression associated with targeted H3 K79 hypermethylation at the specific subregions of α*ENaC* promoter. Dot1a is recruited to these subregions, most likely through Dot1a-binding partner ALL1-fused gene from chromosome 9 (*Af9*), a putative transcription factor. There are multiple independent lines of evidence in favor of this hypothesis. First, Dot1a interacts with Af9 in a variety of assays including yeast two-hybrid assays, mammalian two-hybrid assays, GST pulldown, co-immunoprecipitation, colocalization, and re-ChIP. Secondly, aldosterone reduces the levels of Af9 mRNA and protein; thirdly, Af9 overexpression induces hypermethylation of histone H3 K79 at particular subregions of the α*ENaC* promoter and decreases expression of the endogenous α*ENaC* mRNA and α*ENaC* promoter-luciferase reporters. In contrast, depletion of Af9 by specific RNAi causes the opposite results. Fourthly, ChIP assays unearth the association of Dot1a-Af9 protein complex in the corresponding subregions of α*ENaC* promoter[15,16], Finally, we identified the first Af9 cis-element (+78/+92) in the primary site for Dot1a-Af9 interaction and demonstrated Af9 binding to this element in electrophoretic mobility shift assay[17].

Dot1a-Af9-mediated repression can be relieved in an aldosterone-dependent and -independent manner through multiple mechanisms. Aldosterone impairs the formation of Dot1a-Af9 protein complex associated with the α*ENaC* promoter by (1) decreasing abundance of Dot1a and Af9; (2) attenuating the interaction between Dot1a and Af9 *via* Sgk1-catalyzed phosphorylation of Af9 at Ser 435; and (3) counterbalancing the repression through binding to mineralocorticoid receptor (MR) and facilitating its localization in the cell nucleus, where MR and Dot1a compete for binding Af9. Aldosterone-independent de-repression is achieved through the action of ALL1 fused gene from chromosome 17 (*Af17*). We first demonstrated that Af17 upregulates ENaC transcription and benzamil-sensitive Na^+ currents in 293T cells[18]. We showed that the same domain of Dot1a serves as the target for competitive binding by Af17 and Af9. Such competitive binding was mutually verified in a variety of assays. Functionally, Af17 and Af9 had antagonistic effects on expression and activity of ENaC. Af17 promoted decreased Dot1a nuclear expression, at least in part by facilitating its nuclear export, leading to a relief in repression of ENaC mediated by Dot1a-Af9 protein complex[18]. More importantly, whole-cell patch clamping analyses revealed that the alternation in ENaC transcription was translated to the corresponding changes in benzamil-sensitive Na^+ uptake[18]. In more physiologically relevant systems such as M1 and IMCD3 cells, we used equivalent short-circuit current and single-cell fluorescence imaging to examine ENaC activity. We confirmed similar mechanisms by which Dot1a and Af17 regulate ENaC expression and activity[19,20].

AF17$^{-/-}$ MICE HAVE INCREASED NA$^+$ EXCRETION AND DECREASED BLOOD PRESSURE

To demonstrate the functional significance of the epigenetic mechanisms involving Dot1a-Af9-Af17 in regulating Na^+ metabolism and blood pressure control, we created the first *Af17*$^{-/-}$ mice, characterized *Af17* expression pattern during development, and found that *Af17* is not required for hematopoiesis and embryogenesis. Deletion of Af17 has little effect on long-term survival[21], despite increased H3 m2K79 and reduced ENaC function[22]. The impaired ENaC function is a result of downregulated ENaC mRNA and protein levels, lowered channel open probability, decreased active channel numbers, and attenuated effective activity[22]. The abnormalities in sodium handling and blood pressure (BP) were completely corrected when *Af17*$^{-/-}$ mice were treated with a low Na^+ diet, a high K^+ diet, or aldosterone infusion, all of which bolster plasma aldosterone to high levels. These studies establish *Af17* as a potential player for tight regulation of sodium and BP and a potential target for developing new therapeutic strategies in fighting abnormal BP[22].

REFERENCES

1 **Canessa CM**, Schild L, Buell G, Thorens B, Gautschi I, Horisberger JD, Rossier BC. Amiloride-sensitive epithelial Na+ channel is made of three homologous subunits. *Nature* 1994; **367**: 463-467 [PMID: 8107805 DOI: 10.1038/367463a0]

2 **Nesterov V**, Dahlmann A, Krueger B, Bertog M, Loffing J, Korbmacher C. Aldosterone-dependent and -independent regulation of the epithelial sodium channel (ENaC) in mouse distal nephron. *Am J Physiol Renal Physiol* 2012; **303**: F1289-F1299 [PMID: 22933298 DOI: 10.1152/ajprenal.00247.2012]

3 **Pradervand S**, Barker PM, Wang Q, Ernst SA, Beermann F, Grubb BR, Burnier M, Schmidt A, Bindels RJ, Gatzy JT, Rossier BC, Hummler E. Salt restriction induces pseudohypoaldosteronism type 1 in mice expressing low levels of the beta-subunit of the amiloride-sensitive epithelial sodium channel. *Proc Natl Acad Sci USA* 1999; **96**: 1732-1737 [PMID: 9990093 DOI: 10.1073/pnas.96.4.1732]

4 **Rossier BC**, Schild L. Epithelial sodium channel: mendelian versus essential hypertension. *Hypertension* 2008; **52**: 595-600 [PMID: 18711011 DOI: 10.1161/HYPERTENSIONAHA.107.097147]

5 **Staub O**, Dho S, Henry P, Correa J, Ishikawa T, McGlade J, Rotin D. WW domains of Nedd4 bind to the proline-rich PY motifs in the epithelial Na+ channel deleted in Liddle's syndrome. *EMBO J* 1996; **15**: 2371-2380 [PMID: 8665844]

6 **Abriel H**, Loffing J, Rebhun JF, Pratt JH, Schild L, Horisberger JD, Rotin D, Staub O. Defective regulation of the epithelial Na+ channel by Nedd4 in Liddle's syndrome. *J Clin Invest* 1999; **103**: 667-673 [PMID: 10074483 DOI: 10.1172/JCI5713]

7 **Shimkets RA**, Lifton RP, Canessa CM. The activity of the epithelial sodium channel is regulated by clathrin-mediated endocytosis. *J Biol Chem* 1997; **272**: 25537-25541 [PMID: 9325269 DOI: 10.1074/

jbc.272.41.25537]
8 **Hummler E**, Vallon V. Lessons from mouse mutants of epithelial sodium channel and its regulatory proteins. *J Am Soc Nephrol* 2005; **16**: 3160-3166 [PMID: 16192419 DOI: 10.1681/ASN.2005040450]
9 **Strahl BD**, Allis CD. The language of covalent histone modifications. *Nature* 2000; **403**: 41-45 [PMID: 10638745 DOI: 10.1038/47412]
10 **Jenuwein T**, Allis CD. Translating the histone code. *Science* 2001; **293**: 1074-1080 [PMID: 11498575 DOI: 10.1126/science.1063127]
11 **van Leeuwen F**, Gafken PR, Gottschling DE. Dot1p modulates silencing in yeast by methylation of the nucleosome core. *Cell* 2002; **109**: 745-756 [PMID: 12086673 DOI: 10.1016/S0092-8674(02)00759-6]
12 **Nguyen AT**, Zhang Y. The diverse functions of Dot1 and H3K79 methylation. *Genes Dev* 2011; **25**: 1345-1358 [PMID: 21724828 DOI: 10.1101/gad.2057811]
13 **Zhang W**, Hayashizaki Y, Kone BC. Structure and regulation of the mDot1 gene, a mouse histone H3 methyltransferase. *Biochem J* 2004; **377**: 641-651 [PMID: 14572310 DOI: 10.1042/BJ20030839]
14 **Zhang W**, Xia X, Jalal DI, Kuncewicz T, Xu W, Lesage GD, Kone BC. Aldosterone-sensitive repression of ENaCalpha transcription by a histone H3 lysine-79 methyltransferase. *Am J Physiol Cell Physiol* 2006; **290**: C936-C946 [PMID: 16236820 DOI: 10.1152/ajpcell.00431.2005]
15 **Zhang W**, Xia X, Reisenauer MR, Rieg T, Lang F, Kuhl D, Vallon V, Kone BC. Aldosterone-induced Sgk1 relieves Dot1a-Af9-mediated transcriptional repression of epithelial Na+ channel alpha. *J Clin Invest* 2007; **117**: 773-783 [PMID: 17332896 DOI: 10.1172/JCI29850]
16 **Zhang W**, Xia X, Reisenauer MR, Hemenway CS, Kone BC. Dot1a-AF9 complex mediates histone H3 Lys-79 hypermethylation and repression of ENaCalpha in an aldosterone-sensitive manner. *J Biol Chem* 2006; **281**: 18059-18068 [PMID: 16636056 DOI: 10.1074/jbc.M601903200]
17 **Zhang W**, Yu Z, Wu H, Chen L, Kong Q, Kone BC. An Af9 cis-element directly targets Dot1a to mediate transcriptional repression of the αENaC gene. *Am J Physiol Renal Physiol* 2013; **304**: F367-F375 [PMID: 23152297 DOI: 10.1152/ajprenal.00537.2011]
18 **Reisenauer MR**, Anderson M, Huang L, Zhang Z, Zhou Q, Kone BC, Morris AP, Lesage GD, Dryer SE, Zhang W. AF17 competes with AF9 for binding to Dot1a to up-regulate transcription of epithelial Na+ channel alpha. *J Biol Chem* 2009; **284**: 35659-35669 [PMID: 19864429 DOI: 10.1074/jbc.M109.038448]
19 **Wu H**, Chen L, Zhou Q, Zhang W. AF17 facilitates Dot1a nuclear export and upregulates ENaC-mediated Na+ transport in renal collecting duct cells. *PLoS One* 2011; **6**: e27429 [PMID: 22087315 DOI: 10.1371/journal.pone.0027429]
20 **Reisenauer MR**, Wang SW, Xia Y, Zhang W. Dot1a contains three nuclear localization signals and regulates the epithelial Na+ channel (ENaC) at multiple levels. *Am J Physiol Renal Physiol* 2010; **299**: F63-F76 [PMID: 20427473 DOI: 10.1152/ajprenal.00105.2010]
21 **Zhang Z**, Huang L, Reisenauer MR, Wu H, Chen L, Zhang Y, Xia Y, Zhang W. Widely expressed Af17 is likely not required for embryogenesis, hematopoiesis, and animal survival. *Genesis* 2010; **48**: 693-706 [PMID: 21170927 DOI: 10.1002/dvg.20679]
22 **Chen L**, Wu H, Pochynyuk OM, Reisenauer MR, Zhang Z, Huang L, Zaika OL, Mamenko M, Zhang W, Zhou Q, Liu M, Xia Y, Zhang W. Af17 deficiency increases sodium excretion and decreases blood pressure. *J Am Soc Nephrol* 2011; **22**: 1076-1086 [PMID: 21546577 DOI: 10.1681/ASN.2010121270]

P- Reviewer: Sanlioglu AD, Wang QE **S- Editor**: Gong XM
L- Editor: A **E- Editor**: Yan JL

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DOI: 10.5527/wjn.v4.i3.367

World J Nephrol 2015 July 6; 4(3): 367-373
ISSN 2220-6124 (online)

MINIREVIEWS

How tubular epithelial cells dictate the rate of renal fibrogenesis

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Conflict-of-interest statement: I declare, as the corresponding author, that neither I nor Kevin Louis have any conflict of interest with regard to the manuscript.

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Received: February 10, 2015
Peer-review started: February 11, 2015
First decision: March 20, 2015
Revised: May 6, 2015
Accepted: May 16, 2015
Article in press: May 18, 2015
Published online: July 6, 2015

Abstract

The main threat to a kidney injury, whatever its cause and regardless of whether it is acute or chronic, is the initiation of a process of renal fibrogenesis, since fibrosis can auto-perpetuate and is of high prognostic significance in individual patients. In the clinic, a decrease in glomerular filtration rate correlates better with tubulointerstitial damage than with glomerular injury. Accumulation of the extracellular matrix should not be isolated from other significant cellular changes occurring in the kidney, such as infiltration by inflammatory cells, proliferation of myofibroblasts, obliteration of peritubular capillaries and atrophy of tubules. The aim of this review is to focus on tubular epithelial cells (TEC), which, necessarily involved in the repair process, eventually contribute to accelerating fibrogenesis. In the context of injury, TEC rapidly exhibit phenotypic and functional changes that recall their mesenchymal origin, and produce several growth factors known to activate myofibroblasts. Because they are high-demanding energy cells, TEC will subsequently suffer from the local hypoxia that progressively arises in a microenvironment where the matrix increases and capillaries become rarified. The combination of hypoxia and metabolic acidosis may induce a vicious cycle of sustained inflammation, at the center of which TEC dictate the rate of renal fibrogenesis.

Key words: Epithelium; Fibroblasts; Acute kidney injury; Chronic kidney diseases; Fibrosis

Core tip: In this review, we explain why and how tubular epithelial cells should be regarded not only as victims in the context of chronic kidney disease, but also as actors playing an ambiguous role. In particular, we report on studies which demonstrated that they can actively contribute to fibrogenesis itself, either directly, because their function has been reprogrammed in a way reminiscent of their mesenchymal origin, or from a distance, by influencing endothelial and myofibroblast functions. Last, they are seen as potential targets for new drugs aiming at controlling fibrosis.

Louis K, Hertig A. How tubular epithelial cells dictate the rate of renal fibrogenesis. *World J Nephrol* 2015; 4(3): 367-373 Available from: URL: http://www.wjgnet.com/2220-6124/full/v4/i3/367.htm DOI: http://dx.doi.org/10.5527/wjn.v4.i3.367

INTRODUCTION

In the clinic, decrease in glomerular filtration rate correlates better with tubulointerstitial damage than with glomerular injury[1]. Myofibroblasts are the main source of extracellular matrix in fibrotic organs, but the view that they merely result from the proliferation of resident interstitial fibroblasts at the onset of an injury is considered simplistic. Bone marrow derived stem cells, vascular smooth muscle cells, epithelial cells, endothelial cells and, more recently, pericytes, have all been suggested as significant sources of myofibroblasts[2-4]. If anything, this three-ring circus reflects a real shift in the paradigm of the cell differentiation and fate process. In contrast to the established idea that cells are terminally differentiated, a more dynamic and plastic vision of how cells behave and react to environmental constraints has emerged[5]. With respect to epithelial cells, a switch to a mesenchymal phenotype (stricto sensu, essentially a cell program that produces the extracellular matrix) makes sense since they are mesenchymal in origin: during embryogenesis the entire nephron, apart from the collecting duct, is derived from the mesenchymal to epithelial transition of the metanephric blastema[6]. The concept of the reverse phenomenon, epithelial to mesenchymal transition (EMT), is well known to embryologists since primary epiblasts acquire mesenchymal proteins in order to disperse, and to oncologists because, at the invasive front of carcinomas, transformed epithelial cells may also acquire migratory properties and metastasize. In 1995, Strutz *et al*[7] extended the concept of EMT to the field of fibrogenesis and its occurrence in adult solid organs, and discussed the possibility that tubular epithelial cells (TEC) might also acquire migratory properties and eventually create *de novo* myofibroblasts[7]. It was proposed that TEC, properly stimulated, would convert and progress from the tubular structure to the interstitium. This major new idea was corroborated by one experimental study[3], but contradicted by other studies[2,8,9]. Overall, the concept of EMT has focused on the TEC phenotype as a potential contributor to fibrogenesis. Rather than suggesting epithelial cells are the main source of myofibroblasts, we use the term "epithelial phenotypic changes" (EPC) to refer to *in situ* EMT[10,11]. Analyzing sequential surveillance biopsies performed in kidney recipients, we and others have demonstrated that EPC are detectable in TEC[12] and are associated with accelerated fibrogenesis and poor graft outcome[10], results confirmed elsewhere. How the external microenvironment influences the phenotype of TEC is an area of intense research, although it is safe to say that the members of the Smad family play a major role. The balance between pro-fibrotic Smads (Smad 2/3) and anti-fibrotic Smads (Smad 1 and Smad 7) is controlled both inside the cells, for example by micro RNAs, and outside, where growth factors such as transforming growth factor β (TGFβ), bone morphogenetic protein 7 (BMP7), hepatocyte growth factor (HGF), their trap proteins [connective tissue growth factor (CTGF), kielin/chordin-like protein (KCP)[13]], and their cognate membrane receptors, all regulate the transient phenotype of "bistable" TEC. Excising *ALK3*, the gene encoding the receptor for BMP7, specifically in TEC, is sufficient to induce a worsening of renal fibrosis in mice subjected to different models of renal injury[14,15]. This demonstrates that TEC exert some control on the process of fibrogenesis. The aim of this review is to provide an update on why EMT is detrimental and contributes *in situ* to renal fibrogenesis. Schematically, EMT reprograms TEC in a way that allows them to produce aberrant amounts of extracellular matrix, activate myofibroblasts from a distance, and eventually impair tissue oxygenation by decreasing the secretion of vascular endothelial growth factor (VEGF) by the epithelium. Table 1 indicates the main molecules produced by TEC and involved in renal fibrogenesis.

TUBULAR EPITHELIAL CELLS AS ABERRANT PRODUCERS OF EXTRACELLULAR MATRIX

The continuous decline in renal function is closely associated with the progressive accumulation of ECM proteins such as collagens and fibronectin. Excessive matrix is scattered between tubular structures, and also around tubules in what pathologists term "tubular atrophy". Beneath the circular ECM that surrounds it, the epithelium often appears flattened, yet Nadasdy *et al*[16] have observed a high cell proliferation rate in those atrophic tubules, *i.e.,* higher than in normal tubules or damaged but non-atrophic tubules[17,18], which suggests that cells are actively engaged in damage repair. In non-atrophic tubules located in non-fibrotic areas, EPC may be detected by immunohistochemistry, using antibodies targeting cytoskeletal proteins typical of myofibroblasts rather than epithelial cells. For example, vimentin, alpha-smooth muscle actin, and even fibroblast-specific protein 1, may be aberrantly expressed in cortical tubules, at the expense of epithelial proteins such as cytokeratins, cadherins, or ZO-1, which are lost. Importantly, this cytoskeletal switch occurs at the same time as increased production of two proteins that help to assemble ECM components: (1) heat shock protein 47 (HSP47), a collagen-specific molecular chaperone which helps to synthesize, process and secrete procollagen from the endoplasmic reticulum, and then acts in the folding and assembly of procollagen

Table 1 Major molecules produced by tubular epithelial cells and involved in renal fibrogenesis

	Role in renal fibrosis	Ref.
Transforming growth factor beta pathway		
TGFβ	Pro-fibrotic agent *via* EMT, activation of myofibroblasts.	[8,15,25-27,30]
CTGF	Trap ligand for TGFβ (promotes its action)	[21,28-31]
BMP7	Anti-Fibrotic agent. Counteracts TGFβ	[14,15]
KCP	Trap ligand for BMP7 (promotes its action)	[13]
Hypoxia pathway		
HIF	Promotes fibrosis through the induction of TGFβ, CTGF, PDGF, and PAI-1. Promotes endothelial survival through the induction of VEGF.	[34-36,41-42]
VEGF	Promotes endothelial fenestration, and survival.	[38-40,42,43]
PAI-1	Pro-fibrotic agent. Inhibitis plasmin formation.	[32,33]
Ph		
Acidotic pH	Induces EMT, enhances angiotensin 2 and endothelin secretion.	[44,50,52-53]

TGFβ: Transforming growth factor β; CTGF: Connective tissue growth factor; BMP7: Bone morphogenetic protein 7; KCP: Kielin/chordin-like protein; HIF: Hypoxia inducible factor; VEGF: Vascular endothelial growth factor; PAI-1: Type 1 plasminogen activator inhibitor.

molecules[19]; and (2) prolyl 4-hydroxylase (P4H), which stabilizes collagen triple helix molecules[20]. We have reported on the *de novo* expression of HSP47 in proximal TEC from human renal allografts, which strongly suggests collagen synthesis[21]. Alpha and beta chains of P4H were similarly found in the tubular cells of most biopsy samples (but not in normal kidneys)[17]. ECM proteins, in particular collagens and laminins, were indeed shown to be synthesized by TEC: Rastaldi *et al*[17], using *in situ* hybridization, were the first to demonstrate that, in a number of human diseases affecting the native kidneys, TEC produce detectable amounts of collagens even before they lose cytokeratins[17]. Of note, the fact that TEC are able to produce ECM is not surprising, since TEC must build their own basement membrane. Nevertheless, manufacturing significant amounts of ECM and modifying the cytoskeleton in the same way as mesenchymal cells, attests to a cell reprogramming which precisely mirrors mesenchymal function (and as such would help to "contain" the injured area). One last point should be highlighted: cell matrix interactions also regulate the epithelial phenotype, hence qualitative changes in the matrix also matter. For instance, the deposition of fibrillar collagen types Ⅰ and Ⅲ (but not type Ⅳ) might further divert TEC from a normal (epithelial) differentiation, thus creating a vicious circle[22,23].

Importantly, the intensity of EPC was found to be predictive of a more rapid progression of interstitial fibrosis and tubular atrophy in renal grafts undergoing sequential biopsies taken for immunological surveillance, and of a poorer allograft function in the long run[10,21]. To what extent TEC contribute to net fibrogenesis by the direct production of ECM is, however, unknown. EPCs may still serve as biomarkers to identify patients who have a high propensity for renal fibrosis, although the anti-fibrotic intervention required for these patients has yet to be developed. We have used two robust markers of EPC which resemble EMT, namely, the *de novo* expression of vimentin, and the translocation of beta-catenin into the cytoplasm, in the decision tree of the Certitem study, a prospective, multicenter trial performed in France. In this study, patients were stratified depending on the presence of EPC on a graft biopsy sample taken at three months' post-transplant, and then randomized either to a conventional immunosuppressive regimen to prevent graft rejection, or to discontinue cyclosporine A and replace it with a mammalian target of rapamycin (mTOR) inhibitor[24]. This strategy was chosen because at the time the trial was designed, calcineurin inhibitors were regarded as the main cause of graft fibrogenesis. The main results of the Certitem trial are that the conversion from cyclosporine to everolimus at 3 mo (a timepoint at which interstitial fibrosis was not present or was very mild) failed to protect EPC^+ grafts from fibrogenesis, since conversion to everolimus increased both clinical and infra-clinical graft rejection episodes. Any benefit that could have been expected from cyclosporine withdrawal was thus masked by inflammatory lesions. However, the predictive value of EPC was good, especially for patients who had a pristine kidney at three months' post-transplant, and this study may serve as a proof of concept that the epithelial phenotype can be used in everyday practice. Should an anti-fibrotic agent enter our materia medica in the future, these markers would undoubtedly be helpful.

TUBULAR EPITHELIAL CELLS SECRETE PRO-INFLAMMATORY AND PRO-FIBROTIC AGENTS

TEC placed under cellular stress may produce various cytokines and chemokines promoting the recruitment of leucocytes. Interstitial inflammation is frequently present in fibrotic areas, such that pathologists often

disregard this kind of inflammation. For obvious reasons, it is difficult to measure the respective contribution of each cell type in this production (a complex crosstalk probably exists between epithelial and inflammatory cells, and potentially between endothelial cells as well). Among factors that sustain the growth and the activation of fibroblasts, TGFβ is a powerful cytokine. TGFβ signals through its cognate receptor, ALK5, and induces Smad 2/3 phosphorylation. By doing so, TGFβ contributes to multiple tubular phenotypic changes in epithelial cells, including EMT and death by apoptosis, but conversely promotes activation and proliferation in fibroblasts[25]. Bechtel *et al*[26] elegantly demonstrated that durably exposed to TGFβ, fibroblasts will undergo epigenetic changes that will auto-perpetuate their proliferation. TEC were repeatedly found to be a source of TGFβ themselves, and thereby contribute to fibrosis progression[27]. CTGF is also an important molecule, since it can act as a positive trap for TGFβ (*i.e.,* facilitating its binding to ALK5) and as a negative trap for BMP7 (preventing its binding to ALK3)[28]. Of note, TGFβ increases the transcription of CTGF, and this positive feedback loop amplifies the process. In renal allografts, we have detected that TEC also produce CTGF, and that, unlike Banff acute or chronic scores, the intensity of CTGF staining in TEC correlates well with graft dysfunction and proteinuria at the time of allograft biopsy[21]. In observations made by others, tubular cells were also found to produce CTGF in diabetes mellitus nephropathy[29], IgA nephropathy[30] and renal allografts[31]. Type 1 plasminogen activator inhibitor (PAI-1) is another important target gene of TGFβ: by controlling the production of plasmin, PAI-1 regulates the activation of matrix proteases and of TGFβ itself, and is involved in inflammatory pathways[32]. Many studies have demonstrated that it may be secreted by renal epithelial cells during pathology[33].

The capacity for activated TEC to produce pro-fibrotic and pro-inflammatory agents directly can be enhanced by various circumstances, including renal hypoxia.

TUBULOINTERSTITIAL INJURY, INTRARENAL HYPOXIA AND FIBROSIS

Although renal blood flow represents 20% of the cardiac output, the kidney is physiologically at risk of hypoxia because of the presence of a complex arterio-venous oxygen shunt[34]. Hypoxia is instantly sensed by and in cells by the oxygen-dependent hypoxia inducible factor (HIF) pathway. HIF proteins (HIF-1 in epithelial cells, and HIF-2, also known as EPAS-1, in endothelial cells and fibroblasts) are heterodimeric transcription factors, composed of an α subunit and a common β subunit[35,36]. These two units only assemble under hypoxic conditions, because otherwise oxygen causes the ubiquitination of HIF-α through a complex system involving prolyl hydroxylases (PHDs) and Von-Hippel-Lindau (VHL) proteins. In the absence of oxygen, HIF-α heterodimerizes with HIF-1β, and the complex enters the nucleus to promote the expression of target genes. Of note, many growth factor stimulating fibroblasts, such as TGFβ, CTGF and PDGF, are also induced by HIF[37,38]. In addition, glycolytic enzymes which facilitate anaerobic production of ATP, and angiogenic factors including VEGF, are among HIF-target genes. In turn, VEGF promotes endothelial functions and survival. VEGF is constitutively and selectively expressed in podocytes and TEC in normal kidneys, whereas expression of the VEGF receptor (KDR/VEGFR2) is largely restricted to adjacent peritubular capillaries[39]. Transcription and translation of VEGF-A in TEC is up-regulated by hypoxia, and VEGF expression correlates with expansion or regression of peritubular capillaries[40]. To what extent is the epithelial secretion of VEGF important in the context of a renal injury? It has been found that the conditional knockout of VHL in tubular cells (artificially increasing HIF-α even in the absence of hypoxia) resulted in the enhancement of VEGF and PDGF-B expression, an increase in endothelial cell proliferation and an attenuation of the tubulointerstitial damage following ischemia/reperfusion injury[41]. Accordingly, the specific ablation of VEGF-A in tubules leads to a specific dropout of peritubular capillaries, and reflects the importance of an intimate tubulo-vascular crosstalk to maintain peritubular microvascularization. Conversely, inhibitors of PHD (and thus upregulation of HIF and hence of VEGF) were recently shown to exert a protective role in a model of diabetic nephropathy where carbonyl and oxidative stress are particularly high.

A loss of VEGF expression by TEC has been documented in progressive renal diseases[40,42]. This data is counterintuitive since interstitial fibrosis could theoretically alter oxygen supply. By increasing the distance between capillaries and TEC, accumulation of ECM probably impairs oxygen diffusion. However, tissue oxygenation is decreased early in chronic renal failure and this precedes the accumulation of ECM, suggesting causality the other way around, *i.e.,* a primary endothelial defect is probably there in the first place[35,37]. It could be speculated that the cell reprogramming that induces EPC also includes the decrease in secretion of VEGF, an important epithelial function. This would in turn promote capillary loss and, eventually, hypoxia[43]. Under hypoxia, TEC may either undergo apoptosis or survive with a mesenchymal phenotype[35].

TUBULAR CELL METABOLISM, RENAL TISSUE ACIDOSIS AND FIBROSIS

Proximal tubular cells, the predominant cell type in the interstitium, are notable in that they have a high

level of energy consumption because of multiple functions such as fluid and electrolyte homeostasis, active solute secretion and hormonal production[44]. They depend solely on aerobic oxidative metabolism[45] and, like cardiomyocytes, they use fatty acid oxidation (FAO) to produce energy. An abnormal accumulation of lipids was recently identified in epithelial cells in both mouse and human kidneys presenting fibrotic lesions, suggesting that β-oxidation is altered because of hypoxia. This accumulation might also alter epithelial functions and phenotype, and even lead to apoptosis[46].

In homeostasis, 80% of renal oxygen consumption is used for the tubular sodium reabsorption driven by Na-K-ATPase, which creates a negative membrane potential and a Na^+ gradient. Na^+-dependent co-transporters and counter-transporters use the energy of this gradient to promote the uptake of $HCO3^-$ and the secretion of H^+ which both ensure the systemic acid base balance[44,45]. Proximal TEC respond to acidosis by an increased bicarbonate reabsorption and transport into the blood and an increased extraction and catabolism of plasma glutamine, which allows for increased ammoniagenesis[44]. But the significant plasticity of intercalated cells eventually prevents acidosis in the collecting duct. They may alternatively secrete protons or bicarbonates, a phenotypic switch which is not due to EMT, but to a process of trans-differentiation[47]. However, how acidosis is sensed by cells from the collecting ducts remains unelucidated. Despite the fact that "systemic" metabolic acidosis usually appears at a late stage of chronic kidney disease[48], acid retention occurs earlier in the renal tissue. Thus, mice subjected to a 2/3 nephrectomy have H^+ retention, but without alteration of the renal function. Intrarenal acidosis, or even dietary H^+, can activate the renin angiotensin system, and increase intrarenal angiotensin 2 activity[49]. An oral alkali diet preserves GFR better than angiotensin 2 receptors or endothelin antagonists in experimental models of moderate chronic kidney disease in mice. In these models, H^+ renal retention is present but not sufficient to induce a metabolic acidosis in plasma[50,51]. Thus, a dysfunction of TEC metabolism, in particular of acid base regulation, probably contributes to renal fibrogenesis and reduction of GFR. Clinical studies are ongoing to determine whether an alkali diet or an increased fruit consumption (*i.e.,* a basic as opposed to acid dietary regimen) will affect the deterioration of GFR in patients with chronic kidney disease[52,53].

CONCLUSION

Preventing the progression of chronic kidney disease is still a major goal of modern medicine. It requires interventions that target and ideally reverse renal fibrogenesis. Of all the renal cell populations, whether resident and injured, or infiltrating and exacerbating injury, TEC are under closest scrutiny since they play a pivotal role in the process. They contribute directly to fibrogenesis by secreting aberrant amounts of extracellular matrix, and indirectly through the production of pro-fibrotic factors, which will act in a paracrine way and stimulate myofibroblasts and inflammatory cells. Progressively isolated by the surrounding matrix, and placed in a microenvironment where hypoxia and oxidative stress increase, they can no longer perform a protective function, including the promotion of endothelial cell survival and sufficient secretion of acid, in the absence of which fibrosis and inflammation increases. This circle is vicious on many levels, but also offers points of therapeutic intervention for the future.

ACKNOWLEDGMENTS

Alexandre Hertig is the recipient of a Contrat d'Interface Hospitalier with INSERM (2014-2017).

REFERENCES

1 **Mackensen-Haen S**, Bader R, Grund KE, Bohle A. Correlations between renal cortical interstitial fibrosis, atrophy of the proximal tubules and impairment of the glomerular filtration rate. *Clin Nephrol* 1981; **15**: 167-171 [PMID: 7237863]

2 **Humphreys BD**, Lin SL, Kobayashi A, Hudson TE, Nowlin BT, Bonventre JV, Valerius MT, McMahon AP, Duffield JS. Fate tracing reveals the pericyte and not epithelial origin of myofibroblasts in kidney fibrosis. *Am J Pathol* 2010; **176**: 85-97 [PMID: 20008127 DOI: 10.2353/ajpath.2010.090517]

3 **Iwano M**, Plieth D, Danoff TM, Xue C, Okada H, Neilson EG. Evidence that fibroblasts derive from epithelium during tissue fibrosis. *J Clin Invest* 2002; **110**: 341-350 [PMID: 12163453 DOI: 10.1172/JCI0215518]

4 **LeBleu VS**, Taduri G, O'Connell J, Teng Y, Cooke VG, Woda C, Sugimoto H, Kalluri R. Origin and function of myofibroblasts in kidney fibrosis. *Nat Med* 2013; **19**: 1047-1053 [PMID: 23817022 DOI: 10.1038/nm.3218]

5 **Neilson EG**. Plasticity, nuclear diapause, and a requiem for the terminal differentiation of epithelia. *J Am Soc Nephrol* 2007; **18**: 1995-1998 [PMID: 17568015 DOI: 10.1681/ASN.2007040457]

6 **Galichon P**, Hertig A. Epithelial to mesenchymal transition as a biomarker in renal fibrosis: are we ready for the bedside? *Fibrogenesis Tissue Repair* 2011; **4**: 11 [PMID: 21470408 DOI: 10.1186/1755-1536-4-11]

7 **Strutz F**, Okada H, Lo CW, Danoff T, Carone RL, Tomaszewski JE, Neilson EG. Identification and characterization of a fibroblast marker: FSP1. *J Cell Biol* 1995; **130**: 393-405 [PMID: 7615639 DOI: 10.1083/jcb.130.2.393]

8 **Koesters R**, Kaissling B, Lehir M, Picard N, Theilig F, Gebhardt R, Glick AB, Hähnel B, Hosser H, Gröne HJ, Kriz W. Tubular overexpression of transforming growth factor-beta1 induces autophagy and fibrosis but not mesenchymal transition of renal epithelial cells. *Am J Pathol* 2010; **177**: 632-643 [PMID: 20616344 DOI: 10.2353/ajpath.2010.091012]

9 **Kriz W**, Kaissling B, Le Hir M. Epithelial-mesenchymal transition (EMT) in kidney fibrosis: fact or fantasy? *J Clin Invest* 2011; **121**: 468-474 [PMID: 21370523 DOI: 10.1172/JCI44595]

10 **Hertig A**, Anglicheau D, Verine J, Pallet N, Touzot M, Ancel PY, Mesnard L, Brousse N, Baugey E, Glotz D, Legendre C, Rondeau E, Xu-Dubois YC. Early epithelial phenotypic changes predict graft fibrosis. *J Am Soc Nephrol* 2008; **19**: 1584-1591 [PMID: 18434568 DOI: 10.1681/ASN.2007101160]

11 **Galichon P**, Vittoz N, Xu-Dubois YC, Cornaire E, Vandermeersch S, Mesnard L, Hertig A, Rondeau E. Epithelial phenotypic

changes detect cyclosporine in vivo nephrotoxicity at a reversible stage. *Transplantation* 2011; **92**: 993-998 [PMID: 21909056 DOI: 10.1097/TP.0b013e31822fa495]

12 **Hertig A**, Verine J, Mougenot B, Jouanneau C, Ouali N, Sebe P, Glotz D, Ancel PY, Rondeau E, Xu-Dubois YC. Risk factors for early epithelial to mesenchymal transition in renal grafts. *Am J Transplant* 2006; **6**: 2937-2946 [PMID: 17061992 DOI: 10.1111/j.1600-6143.2006.01559.x]

13 **Lin J**, Patel SR, Cheng X, Cho EA, Levitan I, Ullenbruch M, Phan SH, Park JM, Dressler GR. Kielin/chordin-like protein, a novel enhancer of BMP signaling, attenuates renal fibrotic disease. *Nat Med* 2005; **11**: 387-393 [PMID: 15793581 DOI: 10.1038/nm1217]

14 **Sugimoto H**, LeBleu VS, Bosukonda D, Keck P, Taduri G, Bechtel W, Okada H, Carlson W, Bey P, Rusckowski M, Tampe B, Tampe D, Kanasaki K, Zeisberg M, Kalluri R. Activin-like kinase 3 is important for kidney regeneration and reversal of fibrosis. *Nat Med* 2012; **18**: 396-404 [PMID: 22306733 DOI: 10.1038/nm.2629]

15 **Zeisberg M**, Hanai J, Sugimoto H, Mammoto T, Charytan D, Strutz F, Kalluri R. BMP-7 counteracts TGF-beta1-induced epithelial-to-mesenchymal transition and reverses chronic renal injury. *Nat Med* 2003; **9**: 964-968 [PMID: 12808448]

16 **Nadasdy T**, Laszik Z, Blick KE, Johnson DL, Silva FG. Tubular atrophy in the end-stage kidney: a lectin and immunohistochemical study. *Hum Pathol* 1994; **25**: 22-28 [PMID: 7906246 DOI: 10.1016/0046-8177(94)90166-X]

17 **Rastaldi MP**, Ferrario F, Giardino L, Dell'Antonio G, Grillo C, Grillo P, Strutz F, Müller GA, Colasanti G, D'Amico G. Epithelial-mesenchymal transition of tubular epithelial cells in human renal biopsies. *Kidney Int* 2002; **62**: 137-146 [PMID: 12081572 DOI: 10.1046/j.1523-1755.2002.00430.x]

18 **Risdon RA**, Sloper JC, De Wardener HE. Relationship between renal function and histological changes found in renal-biopsy specimens from patients with persistent glomerular nephritis. *Lancet* 1968; **2**: 363-366 [PMID: 4173786 DOI: 10.1016/S0140-6736(68)90589-8]

19 **Nagata K**. Hsp47: a collagen-specific molecular chaperone. *Trends Biochem Sci* 1996; **21**: 22-26 [PMID: 8848834 DOI: 10.1016/S0968-0004(06)80023-X]

20 **Kivirikko KI**, Myllylä R, Pihlajaniemi T. Protein hydroxylation: prolyl 4-hydroxylase, an enzyme with four cosubstrates and a multifunctional subunit. *FASEB J* 1989; **3**: 1609-1617 [PMID: 2537773]

21 **Xu-Dubois YC**, Baugey E, Peltier J, Colombat M, Ouali N, Jouanneau C, Rondeau E, Hertig A. Epithelial phenotypic changes are associated with a tubular active fibrogenic process in human renal grafts. *Hum Pathol* 2013; **44**: 1251-1261 [PMID: 23332931 DOI: 10.1016/j.humpath.2012.10.010]

22 **Zeisberg M**, Bonner G, Maeshima Y, Colorado P, Müller GA, Strutz F, Kalluri R. Renal fibrosis: collagen composition and assembly regulates epithelial-mesenchymal transdifferentiation. *Am J Pathol* 2001; **159**: 1313-1321 [PMID: 11583959 DOI: 10.1016/S0002-9440(10)62518-7]

23 **Becker GJ**, Hewitson TD. The role of tubulointerstitial injury in chronic renal failure. *Curr Opin Nephrol Hypertens* 2000; **9**: 133-138 [PMID: 10757217 DOI: 10.1097/00041552-200003000-00006]

24 **Rostaing L**, Hertig A, Albano L, Anglicheau D, Durrbach A, Vuiblet V, Moulin B, Merville P, Hazzan M, Lang P, Touchard G, Hurault deLigny B, Quéré S, Di Giambattista F, Dubois YC, Rondeau E. Fibrosis progression according to epithelial-mesenchymal transition profile: a randomized trial of everolimus versus CsA. *Am J Transplant* 2015; **15**: 1303-1312 [PMID: 25808994]

25 **Böttinger EP**, Bitzer M. TGF-beta signaling in renal disease. *J Am Soc Nephrol* 2002; **13**: 2600-2610 [PMID: 12239251 DOI: 10.1097/01.ASN.0000033611.79556.AE]

26 **Bechtel W**, McGoohan S, Zeisberg EM, Müller GA, Kalbacher H, Salant DJ, Müller CA, Kalluri R, Zeisberg M. Methylation determines fibroblast activation and fibrogenesis in the kidney. *Nat Med* 2010; **16**: 544-550 [PMID: 20418885]

27 **Loeffler I**, Wolf G. Transforming growth factor-β and the progression of renal disease. *Nephrol Dial Transplant* 2014; **29** Suppl 1: i37-i45 [PMID: 24030832 DOI: 10.1093/ndt/gft267]

28 **Abreu JG**, Ketpura NI, Reversade B, De Robertis EM. Connective-tissue growth factor (CTGF) modulates cell signalling by BMP and TGF-beta. *Nat Cell Biol* 2002; **4**: 599-604 [PMID: 12134160 DOI: 10.1038/ncb826]

29 **Burns WC**, Twigg SM, Forbes JM, Pete J, Tikellis C, Thallas-Bonke V, Thomas MC, Cooper ME, Kantharidis P. Connective tissue growth factor plays an important role in advanced glycation end product-induced tubular epithelial-to-mesenchymal transition: implications for diabetic renal disease. *J Am Soc Nephrol* 2006; **17**: 2484-2494 [PMID: 16914537]

30 **Nonaka Takahashi S**, Fujita T, Takahashi T, Wada Y, Fuke Y, Satomura A, Matsumoto K. TGF-beta1 and CTGF mRNAs are correlated with urinary protein level in IgA nephropathy. *J Nephrol* 2008; **21**: 53-63 [PMID: 18264937]

31 **Cheng O**, Thuillier R, Sampson E, Schultz G, Ruiz P, Zhang X, Yuen PS, Mannon RB. Connective tissue growth factor is a biomarker and mediator of kidney allograft fibrosis. *Am J Transplant* 2006; **6**: 2292-2306 [PMID: 16889607 DOI: 10.1111/j.1600-6143.2006.01493.x]

32 **Eddy AA**, Fogo AB. Plasminogen activator inhibitor-1 in chronic kidney disease: evidence and mechanisms of action. *J Am Soc Nephrol* 2006; **17**: 2999-3012 [PMID: 17035608 DOI: 10.1681/ASN.2006050503]

33 **Xu Y**, Hagege J, Mougenot B, Sraer JD, Rønne E, Rondeau E. Different expression of the plasminogen activation system in renal thrombotic microangiopathy and the normal human kidney. *Kidney Int* 1996; **50**: 2011-2019 [PMID: 8943484]

34 **Evans RG**, Gardiner BS, Smith DW, O'Connor PM. Intrarenal oxygenation: unique challenges and the biophysical basis of homeostasis. *Am J Physiol Renal Physiol* 2008; **295**: F1259-F1270 [PMID: 18550645 DOI: 10.1152/ajprenal.90230.2008]

35 **Nangaku M**. Chronic hypoxia and tubulointerstitial injury: a final common pathway to end-stage renal failure. *J Am Soc Nephrol* 2006; **17**: 17-25 [PMID: 16291837 DOI: 10.1681/ASN.2005070757]

36 **Rosenberger C**, Mandriota S, Jürgensen JS, Wiesener MS, Hörstrup JH, Frei U, Ratcliffe PJ, Maxwell PH, Bachmann S, Eckardt KU. Expression of hypoxia-inducible factor-1alpha and -2alpha in hypoxic and ischemic rat kidneys. *J Am Soc Nephrol* 2002; **13**: 1721-1732 [PMID: 12089367 DOI: 10.1097/01.ASN.0000017223.49823.2A]

37 **Manotham K**, Tanaka T, Matsumoto M, Ohse T, Inagi R, Miyata T, Kurokawa K, Fujita T, Ingelfinger JR, Nangaku M. Transdifferentiation of cultured tubular cells induced by hypoxia. *Kidney Int* 2004; **65**: 871-880 [PMID: 14871406 DOI: 10.1111/j.1523-1755.2004.00461.x]

38 **Choi YJ**, Chakraborty S, Nguyen V, Nguyen C, Kim BK, Shim SI, Suki WN, Truong LD. Peritubular capillary loss is associated with chronic tubulointerstitial injury in human kidney: altered expression of vascular endothelial growth factor. *Hum Pathol* 2000; **31**: 1491-1497 [PMID: 11150374 DOI: 10.1053/hupa.2000.20373]

39 **Kang DH**, Kanellis J, Hugo C, Truong L, Anderson S, Kerjaschki D, Schreiner GF, Johnson RJ. Role of the microvascular endothelium in progressive renal disease. *J Am Soc Nephrol* 2002; **13**: 806-816 [PMID: 11856789]

40 **Simon M**, Gröne HJ, Jöhren O, Kullmer J, Plate KH, Risau W, Fuchs E. Expression of vascular endothelial growth factor and its receptors in human renal ontogenesis and in adult kidney. *Am J Physiol* 1995; **268**: F240-F250 [PMID: 7864162]

41 **Theilig F**, Enke AK, Scolari B, Polzin D, Bachmann S, Koesters R. Tubular deficiency of von Hippel-Lindau attenuates renal disease progression in anti-GBM glomerulonephritis. *Am J Pathol* 2011; **179**: 2177-2188 [PMID: 21925138 DOI: 10.1016/j.ajpath.2011.07.012]

42 **Yuan HT**, Li XZ, Pitera JE, Long DA, Woolf AS. Peritubular capillary loss after mouse acute nephrotoxicity correlates with down-regulation of vascular endothelial growth factor-A and hypoxia-inducible factor-1 alpha. *Am J Pathol* 2003; **163**: 2289-2301 [PMID: 14633603 DOI: 10.1016/S0002-9440(10)63586-9]

43 **Dimke H**, Sparks MA, Thomson BR, Frische S, Coffman TM, Quaggin SE. Tubulovascular cross-talk by vascular endothelial growth factor a maintains peritubular microvasculature in kidney. *J Am Soc Nephrol* 2015; **26**: 1027-1038 [PMID: 25385849 DOI:

10.1681/ASN.2014010060]
44 **Curthoys NP**, Moe OW. Proximal tubule function and response to acidosis. *Clin J Am Soc Nephrol* 2014; **9**: 1627-1638 [PMID: 23908456 DOI: 10.2215/CJN.10391012]
45 **Epstein FH**. Oxygen and renal metabolism. *Kidney Int* 1997; **51**: 381-385 [PMID: 9027710 DOI: 10.1038/ki.1997.50]
46 **Kang HM**, Ahn SH, Choi P, Ko YA, Han SH, Chinga F, Park AS, Tao J, Sharma K, Pullman J, Bottinger EP, Goldberg IJ, Susztak K. Defective fatty acid oxidation in renal tubular epithelial cells has a key role in kidney fibrosis development. *Nat Med* 2015; **21**: 37-46 [PMID: 25419705 DOI: 10.1038/nm.3762]
47 **Hagége J**, Gabe M, Richet G. Scanning of the apical pole of distal tubular cells under differing acid-base conditions. *Kidney Int* 1974; **5**: 137-146 [PMID: 4205572 DOI: 10.1038/ki.1974.18]
48 **Hsu CY**, Chertow GM. Elevations of serum phosphorus and potassium in mild to moderate chronic renal insufficiency. *Nephrol Dial Transplant* 2002; **17**: 1419-1425 [PMID: 12147789 DOI: 10.1093/ndt/17.8.1419]
49 **Wesson DE**, Jo CH, Simoni J. Angiotensin II receptors mediate increased distal nephron acidification caused by acid retention. *Kidney Int* 2012; **82**: 1184-1194 [PMID: 22832514 DOI: 10.1038/ki.2012.267]
50 **Wesson DE**, Simoni J. Acid retention during kidney failure induces endothelin and aldosterone production which lead to progressive GFR decline, a situation ameliorated by alkali diet. *Kidney Int* 2010; **78**: 1128-1135 [PMID: 20861823 DOI: 10.1038/ki.2010.348]
51 **Manotham K**, Tanaka T, Matsumoto M, Ohse T, Miyata T, Inagi R, Kurokawa K, Fujita T, Nangaku M. Evidence of tubular hypoxia in the early phase in the remnant kidney model. *J Am Soc Nephrol* 2004; **15**: 1277-1288 [PMID: 15100368 DOI: 10.1097/01.ASN.0000125614.35046.10]
52 **Goraya N**, Simoni J, Jo CH, Wesson DE. Treatment of metabolic acidosis in patients with stage 3 chronic kidney disease with fruits and vegetables or oral bicarbonate reduces urine angiotensinogen and preserves glomerular filtration rate. *Kidney Int* 2014; **86**: 1031-1038 [PMID: 24694986 DOI: 10.1038/ki.2014.83]
53 **Goraya N**, Simoni J, Jo CH, Wesson DE. A comparison of treating metabolic acidosis in CKD stage 4 hypertensive kidney disease with fruits and vegetables or sodium bicarbonate. *Clin J Am Soc Nephrol* 2013; **8**: 371-381 [PMID: 23393104 DOI: 10.2215/CJN.02430312]

P- Reviewer: Eirini G, Sakhaee K, Trimarchi H, Taheri S
S- Editor: Ji FF **L- Editor**: A **E- Editor**: Yan JL

World Journal of Nephrology

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DOI: 10.5527/wjn.v4.i3.374

World J Nephrol 2015 July 6; 4(3): 374-378
ISSN 2220-6124 (online)

MINIREVIEWS

Early renal failure as a cardiovascular disease: Focus on lipoprotein(a) and prothrombotic state

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Author contributions: Catena C and Sechi LA planned the article outline, retrived articles from the literature, wrote the manuscript; Colussi G, Nait F, Pezzutto F and Marinis F retrieved articles from the literature and critically reviewed the manuscript.

Supported by A European Cooperation in the field of Scientific and Technical Research (COST-BM1301) grant (to Catena C); and by a generous contribution of the PierSilverio Nassimbeni Foundation (to Catena C and Sechi LA).

Conflict-of-interest statement: The authors have no conflict of interest to declare.

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Received: January 20, 2015
Peer-review started: January 20, 2015
First decision: February 7, 2015
Revised: April 2, 2015
Accepted: April 27, 2015
Article in press: April 29, 2015
Published online: July 6, 2015

Abstract

Patients with renal failure are at increased risk of cardiovascular events even at the earliest stages of disease. In addition to many classic cardiovascular risk factors, many conditions that are commonly identified as emerging risk factors might contribute to occurrence of cardiovascular disease. Changes in circulating levels of many of these emerging risk factors have been demonstrated in patients with early stages of renal failure caused by different types of renal disease and have been associated with detection of cardiovascular complications. However, for most of these factors evidence of benefits of correction on cardiovascular outcome is missing. In this article, we comment on the role of lipoprotein(a) and prothrombotic factors as potential contributors to cardiovascular events in patients with early renal failure.

Key words: Early renal failure; Cardiovascular disease; Risk factors; Lipoprotein(a); Prothrombotic state

Core tip: Cardiovascular disease is the leading cause of morbidity and mortality in patients with chronic renal failure and even patients with moderate impairment of renal function have an increased risk to develop cardiovascular events. Traditional cardiovascular risk factors have a leading role in the pathophysiology of accelerated atherosclerosis of patients with renal failure, but emerging non-traditional factors might also be involved. Evidence of a possible contribution of lipoprotein(a) and prothrombotic state to cardiovascular outcomes of patients with early renal failure is discussed in this editorial.

Catena C, Colussi G, Nait F, Pezzutto F, Martinis F, Sechi LA. Early renal failure as a cardiovascular disease: Focus on lipoprotein(a) and prothrombotic state. *World J Nephrol*

2015; 4(3): 374-378 Available from: URL: http://www.wjgnet.com/2220-6124/full/v4/i3/374.htm DOI: http://dx.doi.org/10.5527/wjn.v4.i3.374

INTRODUCTION

Strong epidemiological evidence indicates that subjects with impaired renal function have shorter life-expectancy than subjects with normal renal function[1]. In subjects with renal failure, cardiovascular events are the leading cause of death and disability[2,3] and many mechanisms can contribute to increased cardiovascular risk. These mechanisms include, on one hand, conditions that are specific to renal failure, such as reduced hemoglobin and acid-base and electrolyte disturbances and, on the other hand, increased prevalence of classic traditional risk factors for atherosclerosis, such as age, diabetes, hypertension, and dyslipidemia[4]. In addition to classic cardiovascular risk factors, other conditions that are commonly identified as "emerging" risk factors[5], have been called into play because they can contribute to cardiovascular events occurring in subjects with relatively low cardiovascular risk as estimated by the current charts[6,7]. Many of these non-traditional emerging risk factors have been reported to be significantly increased in patients with end-stage renal failure, strongly suggesting a contribution to occurrence of cardiovascular events in these patients[8,9] (Table 1). It is clear, however, as also previously stated, that subjects with severely impaired renal function have a myriad of additional conditions that contribute to cardiovascular risk and can hide the relevance of some emerging risk factors. Therefore, the weight of these factors on cardiovascular outcomes of renal patients would be more appropriately examined in subjects at the initial stage of renal disease. Noteworthy, some conditions that increase the cardiovascular risk of end-stage renal patients have also been detected in the earliest stages of renal failure and could possibly contribute to cardiovascular outcomes also in patients with mild impairment of renal function[10]. Here we comment on the evidence supporting the view that early renal failure is a condition associated with high cardiovascular risk and focus on the possible contribution of lipoprotein(a) [Lp(a)] and prothrombotic state to the cardiovascular outcomes of these patients.

EARLY RENAL FAILURE AND CARDIOVASCULAR DISEASE

Evidence obtained in clinical studies demonstrates that the cardiovascular outcome is worse in subjects with initial impairment of renal function as compared to subjects with normal renal function. In a cross-sectional investigation of patients with primary hypertension and different degree of renal function impairment, prevalence of coronary heart, cerebrovascular, and peripheral artery disease was significantly higher in patients with glomerular filtration rate (GFR) comprised from 30 to 89 mL/min per 1.72 m^2 than in patients with a GFR of 90 mL/min per 1.72 m^2 or more[11]. In a first prospective cohort study with 10-year follow-up, incidence of myocardial and cerebral infarction in patients with GFR between 20 and 50 mL/min per 1.72 m^2 was three-times higher than in general population[12]. Patients who had cardiovascular events in this study also had elevated plasma levels of Lp(a), fibrinogen, and homocysteine. A subanalysis of the Hypertension Optimal Treatment study was conducted in hypertensive individuals to estimate the risk of patients with plasma creatinine of 1.7 mg/dL or more to have cardiovascular events or death over a 3.8-year period[13]. In this analysis, incidence of myocardial infarction and stroke resulted significantly greater in patients with high plasma creatinine levels. Moreover, increased plasma creatinine was associated with a risk of cardiovascular events that was higher than that attributed to other risk factors, including diabetes and previous myocardial infarction. In a post-hoc analysis of the Antihypertensive and Lipid Lowering Treatment to Prevent Heart Attack Trial, frequency of coronary events and end-stage renal failure was estimated in 3 groups of patients with hypertension who were stratified according to GFR in a 6-year follow-up[14]. Patients with a GFR < 60 mL/min per 1.72 m^2 had a six-fold higher probability to have a cardiovascular event than to require dialysis, clearly showing that patients with early renal disease are more likely to have cardiovascular disease than evolve to uremia. Conclusive evidence of a graded association between decreasing GFR and increasing rate of cardiovascular events, however, was reached after publication of two milestone studies that came out in 2004. First, in the Valsartan in Acute Myocardial Infarction Trial (VALIANT) 14527 subjects with myocardial infarction and decreased left ventricular ejection fraction were followed for 2 years[15]. In patients with a GFR lower than 81 mL/min per 1.72 m^2 relative risk of death and non-fatal cardiovascular events increased by 10% for each 10 mL/min per 1.72 m^2 reduction in GFR. Second, in a longitudinal prospective study, Go *et al*[16] followed more than one million adults who had been included in a health care system to examine the possible association of GFR, as estimated by the MDRD formula, with the risk of death, cardiovascular events and hospitalization. After a follow-up of 2.8 years, mortality progressively increased as the baseline GFR fell below 60 [hazard ratio (HR): 1.2], 45 (HR: 1.8), and 30 (HR: 3.2) mL/min per 1.72 m^2. Similarly, the HR for nonfatal cardiovascular events was progressively higher with decreasing GFR. Thus, these two important studies together with previous observations obtained in more limited investigation definitely demonstrate that even mild impairment of renal function increases significantly the risk of cardiovascular morbidity and mortality[17].

Table 1 Classic traditional and emerging non-traditional cardiovascular risk factors in chronic renal failure

Classic traditional cardiovascular risk factors	Emerging non-traditional cardiovascular risk factors
Older age	Proteinuria
Male sex	Left ventricular hypertrophy
Arterial hypertension	Anemia
Diabetes mellitus	Electrolyte abnormalities
Smoking	Acid-base imbalance
Increased LDL-cholesterol	Abnormal calcium/ phosphate metabolism
Decreased HDL-cholesterol	Extracellular fluid overload
Family history of cardiovascular events	Lipoprotein(a) and apolipoprotein(a) isoforms
Physical inactivity	Prothrombotic state
	Homocysteine
	Insulin resistance
	Oxidative stress
	Endothelial dysfunction
	Arterial stiffening

LDL: Low-density lipoprotein; HDL: High-density lipoprotein.

EARLY RENAL FAILURE AND EMERGING NON-TRADITIONAL CARDIOVASCULAR RISK FACTORS

As stated above, a variety of emerging non-traditional cardiovascular risk factors might contribute to increase the cardiovascular risk of patients in the early stages of renal failure (Table 1). Most of these conditions meet three of the requisites needed to define a risk factor, that is biological plausibility as to why it may cause cardiovascular disease, demonstration of a dose-response relationship with decreasing renal function, and demonstration of an independent association with cardiovascular disease and renal failure in observational studies. However, so far none of them has met the fourth and most important requisite, that is demonstration in controlled clinical trials that correction of the risk factor is beneficial for cardiovascular outcomes.

It is well known that some lipoproteins are fundamental to the atherosclerotic process and can increase the impact of renal failure on cardiovascular outcomes. Lp(a) is a heterogeneous low-density lipoprotein that incorporates the highly polymorphic apolipoprotein(a) [apo(a)][18]. The *apo(a)* gene is the major gene controlling Lp(a) concentrations[19] that vary over a broad range and are inversely related to the size of apo(a)[20]. In 160 hypertensive patients with early impairment of renal function (GFR 30-89 mL/min per 1.72 m^2) Lp(a) levels were significantly higher than those of 257 hypertensive patients with normal renal function (GFR 90 mL/min per 1.72 m^2 or more) and a significant inverse and independent relationship between Lp(a) levels and GFR was reported[21]. In a further study, it was shown that elevated serum Lp(a) concentrations are not related to the size polymorphism of apo(a) in patients with early renal failure, indicating that in these patients Lp(a) increase can not be ascribed to variations at the *apo(a)* gene locus[22] and strongly suggesting that Lp(a) increase is secondary to impairment of renal function. In this view, one possibility is that the kidney might have a catabolic function on Lp(a) as suggested by detection of degraded apo(a) fragments in urine in an amont that is correlated with GFR[23]. Furthermore, elevated Lp(a) levels and decreased GFR were significantly associated with increased prevalence of cardiovascular events[24], suggesting a contribution of this lipoprotein to cardiovascular outcomes in patients with early renal failure. The association of GFR with Lp(a) levels was investigated in the 7675 participants of the Third National Health and Nutrition Survey (NHANES)[25]. In this population study, a moderate impairment of GFR was associated with greater Lp(a) levels although this association was more prominent in non-Hispanic blacks and Mexican Americans than non-Hispanic whites. Despite this association of elevated Lp(a) levels with early decrease of GFR, other studies demonstrated that this lipoprotein does not contribute to progression of chronic kidney disease[26]. The mechanisms through which Lp(a) promotes atherosclerosis in patients with or without renal failure are not clearly understood. Proposed mechanisms include and increased Lp(a)-associated cholesterol capture in the arterial intima, inflammatory cell recruitment, and carrying of proinflammatory oxidized phospholipids[27].

In addition to the proatherogenic properties, prothrombotic effects of Lp(a) due to its structural homology with plasminogen might explain the contribution of this lipoprotein to cardiovascular events[24]. Also, elevated Lp(a) levels have been found to be frequently associated with hyperhomocysteinemia in patients with pre-dialysis renal failure[28]. Although an inverse relationship of Lp(a) levels with dietary alcohol[29] and omega-3 polyunsaturated acid[30] consumption has been reported and levels of Lp(a) were slightly decreased by use of nicotinic acid[31] and mipomersen[32], impact of either dietary or pharmacologic interventions on Lp(a) levels is minimally relevant. Thus, Lp(a) levels are inversely related with renal function and might contribute to cardiovascular outcomes in patients with early impairment of renal function, but lack of treatments that effectively decrease its levels limits this evidence.

Because a prothrombotic state has been demonstrated in patients with end-stage renal disease, research on emerging risk factors potentially contributing to cardiovascular disease in early renal failure has focused on the hemostatic system[33]. Assessment of the state of activation of the coagulation cascade can be obtained by measurement in plasma of prothrombin fragment 1 + 2 (F1 + 2) that is released when activated factor X converts prothrombin to thrombin, fibrin D-dimer, a breakdown fragment of fibrin, and fibrinogen. In 425 hypertensive patients, 172 of whom had GFR from 30 to 89 mL/min per 1.72 m^2, we measured hemostatic variables and assessed prevalence of cardiovascular events. After adjustment for confounders, GFR was significantly and inversely correlated with plasma

levels of F1 + 2, D-dimer, and fibrinogen, and for the latter two variables correlation was independent of demographic and anthropometric variables, blood pressure levels, plasma lipids, and urinary protein excretion[34]. This observation indicated that and activated hemostatic cascade can be detected also in subjects with mild-to-moderate renal failure, possibly leading to a prothrombotic state and increased incidence of atherothrombotic vascular complications. In these patients with early renal failure and activated coagulation system, prevalence of coronary heart disease, cerebrovascular disease, and peripheral arteriopathy was significantly higher than in patients with GFR of 90 mL/min per 1.72 m^2 or more and cardiovascular disease was independently predicted by both plasma D-dimer and fibrinogen levels. Consistently, in 50 patients with stage 2-3 renal failure plasma fibrinogen was significantly increased possibly contributing to the high cardiovascular morbidity of these patients[35]. In the 3758 patients with GFR of 20 to 70 mL/min per 1.72 m^2 of the Chronic Renal Insufficiency Cohort Study, a prothrombotic state was associated with increased prevalence of peripheral artery disease[36]. In a prospective study of 4029 men aged 60-79 years who were followed for an average period of 6 years, mild-to-moderate renal failure was associated with increased plasma levels of hemostatic markers and caused significantly increased cardiovascular mortality[37].

Thus, it is clear that changes in coagulation parameters suggesting a prothrombotic state occur early in the course of renal disease and could contribute to increase the cardiovascular risk[38]. Similar to Lp(a), in the case of hemostatic variables there is no evidence supporting possible benefits on the cardiovascular outcomes of these patients of treatments that may correct the prothrombotic state.

CONCLUSION

Cardiovascular disease is the leading cause of morbidity and mortality in patients with chronic renal failure and even patients in the early stages of renal disease have much greater risk to have cardiovascular disease than to require substitutive treatment with either dialysis or transplantation. It is clear that traditional cardiovascular risk factors play a major role in the pathophysiology of accelerated atherosclerosis typical of renal failure patients, but emerging non-traditional factors might be involved and contribute to cardiovascular disease. For some of these factors, contribution to cardiovascular outcomes might be relevant from the earliest stages of renal failure. However, conclusive evidence should be obtained from intervention trials with correction of these factors and this is currently missing. Therefore, evidence on whether these and other, as yet unidentified, factors contribute to cardiovascular morbidity and mortality in patients with early renal failure is not conclusive and is the subject of ongoing investigation.

REFERENCES

1 **Keith DS**, Nichols GA, Gullion CM, Brown JB, Smith DH. Longitudinal follow-up and outcomes among a population with chronic kidney disease in a large managed care organization. *Arch Intern Med* 2004; **164**: 659-663 [PMID: 15037495 DOI: 10.1001/archinte.164.6.659]

2 **Foley RN**, Parfrey PS, Sarnak MJ. Epidemiology of cardiovascular disease in chronic renal disease. *J Am Soc Nephrol* 1998; **9**: S16-S23 [PMID: 11443763 DOI: 10.1053/ajkd.1998.v32.pm9820470]

3 **Parfrey PS**, Foley RN. The clinical epidemiology of cardiac disease in chronic renal failure. *J Am Soc Nephrol* 1999; **10**: 1606-1615 [PMID: 10405218]

4 **Sarnak MJ**, Levey AS, Schoolwerth AC, Coresh J, Culleton B, Hamm LL, McCullough PA, Kasiske BL, Kelepouris E, Klag MJ, Parfrey P, Pfeffer M, Raij L, Spinosa DJ, Wilson PW. Kidney disease as a risk factor for development of cardiovascular disease: a statement from the American Heart Association Councils on Kidney in Cardiovascular Disease, High Blood Pressure Research, Clinical Cardiology, and Epidemiology and Prevention. *Circulation* 2003; **108**: 2154-2169 [PMID: 14581387 DOI: 10.1161/01.CIR.0000095676.90936.80]

5 **Catena C**, Novello M, Lapenna R, Baroselli S, Colussi G, Nadalini E, Favret G, Cavarape A, Soardo G, Sechi LA. New risk factors for atherosclerosis in hypertension: focus on the prothrombotic state and lipoprotein(a). *J Hypertens* 2005; **23**: 1617-1631 [PMID: 16093903 DOI: 10.1097/01.hjh.0000178835.33976.e7]

6 **Conroy RM**, Pyörälä K, Fitzgerald AP, Sans S, Menotti A, De Backer G, De Bacquer D, Ducimetière P, Jousilahti P, Keil U, Njølstad I, Oganov RG, Thomsen T, Tunstall-Pedoe H, Tverdal A, Wedel H, Whincup P, Wilhelmsen L, Graham IM. Estimation of ten-year risk of fatal cardiovascular disease in Europe: the SCORE project. *Eur Heart J* 2003; **24**: 987-1003 [PMID: 12788299 DOI: 10.1016/S0195-668X(03)00114-3]

7 **Mancia G**, De Backer G, Dominiczak A, Cifkova R, Fagard R, Germano G, Grassi G, Heagerty AM, Kjeldsen SE, Laurent S, Narkiewicz K, Ruilope L, Rynkiewicz A, Schmieder RE, Boudier HA, Zanchetti A, Vahanian A, Camm J, De Caterina R, Dean V, Dickstein K, Filippatos G, Funck-Brentano C, Hellemans I, Kristensen SD, McGregor K, Sechtem U, Silber S, Tendera M, Widimsky P, Zamorano JL, Erdine S, Kiowski W, Agabiti-Rosei E, Ambrosioni E, Lindholm LH, Viigimaa M, Adamopoulos S, Agabiti-Rosei E, Ambrosioni E, Bertomeu V, Clement D, Erdine S, Farsang C, Gaita D, Lip G, Mallion JM, Manolis AJ, Nilsson PM, O'Brien E, Ponikowski P, Redon J, Ruschitzka F, Tamargo J, van Zwieten P, Waeber B, Williams B. 2007 Guidelines for the Management of Arterial Hypertension: The Task Force for the Management of Arterial Hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC). *J Hypertens* 2007; **25**: 1105-1187 [PMID: 17563527 DOI: 10.1097/HJH.0b013e3281fc975a]

8 **Zoccali C**, Mallamaci F, Tripepi G. Inflammatory proteins as predictors of cardiovascular disease in patients with end-stage renal disease. *Nephrol Dial Transplant* 2004; **19** Suppl 5: V67-V72 [PMID: 15284363 DOI: 10.1093/ndt/gfh1059]

9 **Sechi LA**, Catena C, Zingaro L, Melis A, De Marchi S. Abnormalities of glucose metabolism in patients with early renal failure. *Diabetes* 2002; **51**: 1226-1232 [PMID: 11916949 DOI: 10.2337/diabetes.51.4.1226]

10 **Sarnak MJ**, Levey AS. Cardiovascular disease and chronic renal disease: a new paradigm. *Am J Kidney Dis* 2000; **35**: S117-S131 [PMID: 10766010 DOI: 10.1016/S0272-6386(00)70239-3]

11 **Sechi LA**, Zingaro L, Catena C, De Marchi S. Increased fibrinogen levels and hemostatic abnormalities in patients with arteriolar nephrosclerosis: association with cardiovascular events. *Thromb Haemost* 2000; **84**: 565-570 [PMID: 11057851]

12 **Jungers P**, Massy ZA, Nguyen Khoa T, Fumeron C, Labrunie M, Lacour B, Descamps-Latscha B, Man NK. Incidence and risk factors of atherosclerotic cardiovascular accidents in predialysis chronic renal failure patients: a prospective study. *Nephrol Dial*

Transplant 1997; **12**: 2597-2602 [PMID: 9430858 DOI: 10.1093/ndt/12.12.2597]
13 **Ruilope LM**, Salvetti A, Jamerson K, Hansson L, Warnold I, Wedel H, Zanchetti A. Renal function and intensive lowering of blood pressure in hypertensive participants of the hypertension optimal treatment (HOT) study. *J Am Soc Nephrol* 2001; **12**: 218-225 [PMID: 11158211]
14 **Rahman M**, Pressel S, Davis BR, Nwachuku C, Wright JT, Whelton PK, Barzilay J, Batuman V, Eckfeldt JH, Farber MA, Franklin S, Henriquez M, Kopyt N, Louis GT, Saklayen M, Stanford C, Walworth C, Ward H, Wiegmann T. Cardiovascular outcomes in high-risk hypertensive patients stratified by baseline glomerular filtration rate. *Ann Intern Med* 2006; **144**: 172-180 [PMID: 16461961 DOI: 10.7326/0003-4819-144-3-200602070-00005]
15 **Anavekar NS**, McMurray JJ, Velazquez EJ, Solomon SD, Kober L, Rouleau JL, White HD, Nordlander R, Maggioni A, Dickstein K, Zelenkofske S, Leimberger JD, Califf RM, Pfeffer MA. Relation between renal dysfunction and cardiovascular outcomes after myocardial infarction. *N Engl J Med* 2004; **351**: 1285-1295 [PMID: 15385655 DOI: 10.1056/NEJMoa041365]
16 **Go AS**, Chertow GM, Fan D, McCulloch CE, Hsu CY. Chronic kidney disease and the risks of death, cardiovascular events, and hospitalization. *N Engl J Med* 2004; **351**: 1296-1305 [PMID: 15385656 DOI: 10.1056/NEJMoa041031]
17 **Daly C**. Is early chronic kidney disease an important risk factor for cardiovascular disease? A background paper prepared for the UK Consensus Conference on early chronic kidney disease. *Nephrol Dial Transplant* 2007; **22** Suppl 9: ix19-ix25 [PMID: 17998227]
18 **Utermann G**. The mysteries of lipoprotein(a). *Science* 1989; **246**: 904-910 [PMID: 2530631 DOI: 10.1126/science.2530631]
19 **Boerwinkle E**, Leffert CC, Lin J, Lackner C, Chiesa G, Hobbs HH. Apolipoprotein(a) gene accounts for greater than 90% of the variation in plasma lipoprotein(a) concentrations. *J Clin Invest* 1992; **90**: 52-60 [PMID: 1386087 DOI: 10.1172/JCI115855]
20 **Utermann G**, Menzel HJ, Kraft HG, Duba HC, Kemmler HG, Seitz C. Lp(a) glycoprotein phenotypes. Inheritance and relation to Lp(a)-lipoprotein concentrations in plasma. *J Clin Invest* 1987; **80**: 458-465 [PMID: 2956279 DOI: 10.1172/JCI113093]
21 **Sechi LA**, Zingaro L, De Carli S, Sechi G, Catena C, Falleti E, Dell' Anna E, Bartoli E. Increased serum lipoprotein(a) levels in patients with early renal failure. *Ann Intern Med* 1998; **129**: 457-461 [PMID: 9735083 DOI: 10.7326/0003-4819-129-6-199809150-00006]
22 **Sechi LA**, Zingaro L, Catena C, Perin A, De Marchi S, Bartoli E. Lipoprotein(a) and apolipoprotein(a) isoforms and proteinuria in patients with moderate renal failure. *Kidney Int* 1999; **56**: 1049-1057 [PMID: 10469373 DOI: 10.1046/j.1523-1755.1999.00621.x]
23 **Oida K**, Takai H, Maeda H, Takahashi S, Shimada A, Suzuki J, Tamai T, Nakai T, Miyabo S. Apolipoprotein(a) is present in urine and its excretion is decreased in patients with renal failure. *Clin Chem* 1992; **38**: 2244-2248 [PMID: 1424118]
24 **Sechi LA**, Catena C, Casaccio D, Zingaro L. Lipoprotein (a), haemostatic variables and cardiovascular damage in hypertensive patients. *J Hypertens* 2000; **18**: 709-716 [PMID: 10872555 DOI: 10.1097/00004872-200018060-00008]
25 **Kovesdy CP**, Astor BC, Longenecker JC, Coresh J. Association of kidney function with serum lipoprotein(a) level: the third National Health and Nutrition Examination Survey (1991-1994). *Am J Kidney Dis* 2002; **40**: 899-908 [PMID: 12407633 DOI: 10.1053/ajkd.2002.36319]
26 **Rahman M**, Yang W, Akkina S, Alper A, Anderson AH, Appel LJ, He J, Raj DS, Schelling J, Strauss L, Teal V, Rader DJ. Relation of serum lipids and lipoproteins with progression of CKD: The CRIC study. *Clin J Am Soc Nephrol* 2014; **9**: 1190-1198 [PMID: 24832097 DOI: 10.2215/CJN.09320913]
27 **Jacobson TA**. Lipoprotein(a), cardiovascular disease, and contemporary management. *Mayo Clin Proc* 2013; **88**: 1294-1311 [PMID: 24182706 DOI: 10.1016/j.mayocp.2013.09.003]
28 **Parsons DS**, Reaveley DA, Pavitt DV, Brown EA. Relationship of renal function to homocysteine and lipoprotein(a) levels: the frequency of the combination of both risk factors in chronic renal impairment. *Am J Kidney Dis* 2002; **40**: 916-923 [PMID: 12407635 DOI: 10.1053/ajkd.2002.36321]
29 **Catena C**, Novello M, Dotto L, De Marchi S, Sechi LA. Serum lipoprotein(a) concentrations and alcohol consumption in hypertension: possible relevance for cardiovascular damage. *J Hypertens* 2003; **21**: 281-288 [PMID: 12569257 DOI: 10.1097/00004872-200302000-00018]
30 **Colussi G**, Catena C, Baroselli S, Nadalini E, Lapenna R, Chiuch A, Sechi LA. Omega-3 fatty acids: from biochemistry to their clinical use in the prevention of cardiovascular disease. *Recent Pat Cardiovasc Drug Discov* 2007; **2**: 13-21 [PMID: 18221098]
31 **Seed M**, O'Connor B, Perombelon N, O'Donnell M, Reaveley D, Knight BL. The effect of nicotinic acid and acipimox on lipoprotein(a) concentration and turnover. *Atherosclerosis* 1993; **101**: 61-68 [PMID: 8216503 DOI: 10.1016/0021-9150(93)90102-Z]
32 **Gouni-Berthold I**, Berthold HK. Lipoprotein(a): current perspectives. *Curr Vasc Pharmacol* 2011; **9**: 682-692 [PMID: 21529331 DOI: 10.2174/157016111797484071]
33 **Vaziri ND**, Gonzales EC, Wang J, Said S. Blood coagulation, fibrinolytic, and inhibitory proteins in end-stage renal disease: effect of hemodialysis. *Am J Kidney Dis* 1994; **23**: 828-835 [PMID: 8203365 DOI: 10.1016/S0272-6386(12)80136-3]
34 **Catena C**, Zingaro L, Casaccio D, Sechi LA. Abnormalities of coagulation in hypertensive patients with reduced creatinine clearance. *Am J Med* 2000; **109**: 556-561 [PMID: 11063957]
35 **Lai S**, Dimko M, Galani A, Coppola B, Innico G, Frassetti N, Mazzei ED, Mariotti A. Early markers of cardiovascular risk in chronic kidney disease. *Ren Fail* 2015; **37**: 254-261 [PMID: 25394527 DOI: 10.3109/0886022X.2014.982489]
36 **Chen J**, Mohler ER, Xie D, Shlipak MG, Townsend RR, Appel LJ, Raj DS, Ojo AO, Schreiber MJ, Strauss LF, Zhang X, Wang X, He J, Hamm LL. Risk factors for peripheral arterial disease among patients with chronic kidney disease. *Am J Cardiol* 2012; **110**: 136-141 [PMID: 22465315 DOI: 10.1016/j.amjcard.2012.02.061]
37 **Wannamethee SG**, Shaper AG, Lowe GD, Lennon L, Rumley A, Whincup PH. Renal function and cardiovascular mortality in elderly men: the role of inflammatory, procoagulant, and endothelial biomarkers. *Eur Heart J* 2006; **27**: 2975-2981 [PMID: 17132648 DOI: 10.1093/eurheartj/ehl402]
38 **Jalal DI**, Chonchol M, Targher G. Disorders of hemostasis associated with chronic kidney disease. *Semin Thromb Hemost* 2010; **36**: 34-40 [PMID: 20391294 DOI: 10.1055/s-0030-1248722]

P- Reviewer: Bellomo G, Nemcsik J, Taheri S **S- Editor**: Gong XM
L- Editor: A **E- Editor**: Yan JL

World Journal of Nephrology

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Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.5527/wjn.v4.i3.379

World J Nephrol 2015 July 6; 4(3): 379-387
ISSN 2220-6124 (online)

MINIREVIEWS

Erectile dysfunction in chronic kidney disease: From pathophysiology to management

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Author contributions: Papadopoulou E and Doumas M conceived the idea of the manuscript; Papadopoulou E, Varouktsi A, Lazaridis A, and Boutari C performed the systematic search of the literature and drafted different parts of the manuscript; Doumas M overviewed the final draft; all authors approved the final version of the manuscript.

Conflict-of-interest statement: The authors declare that there are no conflicts of interest.

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Received: November 20, 2014
Peer-review started: November 22, 2014
First decision: December 12, 2014
Revised: April 3, 2015
Accepted: May 27, 2015
Article in press: May 28, 2015
Published online: July 6, 2015

Abstract

Chronic kidney disease (CKD) is encountered in millions of people worldwide, with continuously rising incidence during the past decades, affecting their quality of life despite the increase of life expectancy in these patients. Disturbance of sexual function is common among men with CKD, as both conditions share common pathophysiological causes, such as vascular or hormonal abnormalities and are both affected by similar coexisting comorbid conditions such as cardiovascular disease, hypertension and diabetes mellitus. The estimated prevalence of erectile dysfunction reaches 70% in end stage renal disease patients. Nevertheless, sexual dysfunction remains under-recognized and under-treated in a high proportion of these patients, a fact which should raise awareness among clinicians. A multifactorial approach in management and treatment is undoubtedly required in order to improve patients' quality of life and cardiovascular outcomes.

Key words: Chronic kidney disease; Erectile dysfunction; Management; Quality of life; Hypertension; Diabetes mellitus; Phosphodiesterase-5 inhibitors

Core tip: Erectile dysfunction is highly prevalent among patients with chronic kidney disease in rates that reach even 70%, especially in those suffering from end stage renal disease. The rates of patients suffering from sexual dysfunction tend to be higher when additional risk factors, such as coronary artery disease, diabetes mellitus, hypertension or prescription of antihypertensive drugs, coexist. Integrated management of these patients through lifestyle measures, hormonal replacement, and use of drugs such as phosphodiesterase-5 inhibitors, is essential in order to improve sexual function among these patients, thereby maintaining a satisfactory quality of life.

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Doumas M. Erectile dysfunction in chronic kidney disease: From pathophysiology to management. *World J Nephrol* 2015; 4(3): 379-387 Available from: URL: http://www.wjgnet.com/2220-6124/full/v4/i3/379.htm DOI: http://dx.doi.org/10.5527/wjn.v4.i3.379

INTRODUCTION

Sexual dysfunction is highly prevalent in patients with chronic kidney disease (CKD) especially those receiving dialysis. Almost 70% of men with CKD report erectile dysfunction (ED) and these estimates are higher than in the general population[1]. The cause is generally multifactorial with psychological, neurological, endocrinological, vascular and iatrogenic factors acting in concert to increase the likelihood of ED[2]. A number of recent studies suggest an association between endothelial dysfunction and ED[3].

This review aims to analyze the pathophysiology of ED in patients with CKD, to present its prevalence rates in various stages of CKD, to highlight comorbid conditions and common risk factors, which both diseases share and, eventually, to discuss possible therapeutic options, which might improve sexual function of CKD patients.

We performed a systemic search of the literature using the PubMed, OVID, EMBASE and Cochrane Central Register databases from their inception to July 2014. The studies addressing the association between ED and CKD were identified by using the following terms in various combinations: CKD, erectile dysfunction, impotence, renal failure, end stage renal disease, kidney transplantation, phosphodiesterase (PDE)-5 inhibitors. In addition, we reviewed the reference lists of the identified original papers, the studies citing identified papers and review papers relevant to this topic.

Data were extracted by four independent members of our team (Papadopoulou E, Varouktsi A, Lazaridis A, Boutari C) and were discussed with the senior author of our paper (Doumas M). The following criteria were required for a study to be included in our review: observational studies with at least 20 participants, detailed description of a proper estimation of renal function and erectile function.

PATHOPHYSIOLOGY

Erectile dysfunction is defined as the persistent inability to attain and/or maintain penile erection sufficient for sexual intercourse[4]. The erectile process is a complex neurovascular event. In response to sexual stimulation, cavernous nerve terminals and endothelial cells release nitric oxide (NO) which is believed to be the main vasoactive mediator of penile erection. NO promotes penile vasodilation and blood flow, by activating soluble guanyl cyclase to produce 3, 5-cyclic guanosine monophosphate resulting in an enzymatic cascade that reduces intracellular calcium and induces relaxation of cavernosal smooth muscle[5].

Appropriate hormonal environment permits a successful erection with testosterone playing a primary role[6]. Disturbances in neurovascular control, abnormal hormone levels or psychological factors are responsible for the vast majority of ED that is broadly classified as psychogenic (generalized, situational), organic (vasculogenic, neurogenic, anatomic, endocrinologic) or mixed[7].

Psychological factors include primary lack of sexual arousal, chronic disorder of sexual intimacy, depression and performance anxiety. Physiological factors are a more common aetiology and neurological disorders such as Parkinson's disease, stroke, tumour, multiple sclerosis and spinal cord injury are noted to be associated with ED. In addition hormonal disorders such as hypogonadism, hyperprolactinemia and both hyperthyroid and hypothyroid states are known to result in ED. Arterial insufficiency associated with diabetes, hypertension, dyslipidemia, cigarette smoking, blunt perineal or pelvic trauma and pelvic irradiation, tend to be the most common cause of ED[8].

In men with chronic renal disease a combination of testicular failure and secondary disturbances in the pituitary-gonadal axis can be detected in the early stages of CKD and progressively worsen as the renal disease progresses[9]. Some authors have reported that successful kidney transplantation may improve sexual function with reference to the previous situation of haemodialysis[10]. Impaired spermatogenesis and testicular damage with decreased volume of ejaculate, either low or complete azoospermia, low percentage of motility and infertility were reported[11].

Histological changes in the testes revealed decreased spermatogenic activity especially in the later stages of spermatogenesis which are hormonally dependent[12]. Testicular biopsy is often performed to demonstrate reduced spermatogenesis[13]. Leydig and Sertoli cells show absence of hypertrophy or hyperplasia and the reduced levels of total and free testosterone presented in CKD, suggest a Leydig cell dysfunction[14].

Hypogonadism (low testosterone) defined as total testosterone below 300 ng/mL is a prevalent condition in men with CKD especially in those undergoing dialysis and can contribute to decreased libido, ED, oligospermia infertility and anaemia[15]. On the other hand total plasma estrogen concentration is often elevated. The plasma concentration of the pituitary gonadotropin luteinizing hormone (LH) is elevated probably as a result of the decreased release of testosterone from the Leydig cells and the consequent loss of normal negative feedback. In addition the metabolic clearance rate of LH is reduced and it is not corrected by dialysis[16].

In uremic subjects disturbances in LH secretion has been observed but it is not known whether this is the result of a change in GnRH release from the hypothalamus or a change in the responsiveness of the pituitary. However kidney transplantation seems to restore the secretory pattern of LH. Follicle-stimulating hormone (FSH) secretion is also elevated in men with CKD. A peptide

called inhibin produced by the Sertoli cells has a negative feedback on the release of FSH. Uremic patients with severe damage in seminiferous tubules and Sertoli cells tend to have higher plasma FSH concentrations as less inhibin is secreted[9].

Prolactin levels also appear substantially elevated in men with CKD, with a prevalence of hyperprolactinemia from 30%-65%, as a consequence of both reduced renal clearance and increased production. Again these abnormalities seem to resolve after kidney transplantation. Evidence indicates that hyperprolactinemia is associated with infertility, loss of libido, testosterone deficiency and increased risk of cardiovascular events and mortality in CKD. Bromocriptine treatment reduces prolactin levels with no significant side effects[14].

According to the "artery size hypothesis" atherosclerosis is more likely to develop first in the smaller arteries than in the larger ones. Since penile arteries are significantly smaller (1-2 mm diameter) than coronary arteries (3-4 mm), symptoms of ED occur several years before coronary artery disease (CAD) symptoms. ED is also found to be a stronger predictor of CAD than any of the traditional risk factors such as family history, hypertension, dyslipidemia and can be considered as a marker of ischemic heart disease in both CKD and non CKD patients[17,18].

NO is the primary neurotransmitter of penile erection. In chronic renal failure NO bioavailability is reduced. The expression of NO-synthase (NOS) has been shown to be alterned thus leading to a disturbance in sexual function[12]. Possible causes of NO deficiency are substrate limitation (L-arginine), as a result of disturbances in the renal biosynthesis of this amino acid and increased levels of circulating endogenous inhibitors of NOS especially asymmetric dimethylarginine (ADMA). Elevated levels of ADMA has emerged as an independent risk factor in end stage renal disease and reducing ADMA concentration might be a therapeutic goal[19].

Uremic polyneuropathy is an important contributor of ED. Patients undergoing haemodialysis are reported to have an abnormal response to Valsalva manoeuvre, impaired nocturnal penile tumescence and bulbocavernous reflex as evidence of autonomic and peripheral neuropathy, all correlated to sexual dysfunction[20].

CKD is associated with higher anxiety, higher distress, high depression and especially dialysis patients report interpersonal difficulties, lower employment, reduced social activity and low quality of life (QoL)[21]. Changes in body shape and image (catheter, fistula) also contribute to lack of desire and sexual dysfunction. The presence of higher depressive symptoms which are highly prevalent in patients undergoing haemodialysis are independently associated with sexual dysfunction and probably common factors are responsible for both[22].

Treatment of hypertension has also been associated with sexual dysfunction. B-blockers could cause ED by decreasing testosterone levels and potentiating α1-adrenergic activity in the penis. Patients taking thiazide diuretics report difficulty in gaining and maintaining an erection and difficulty with ejaculation. Spironolactone can cause gynecomastia, decreased libido and ED while drugs such as calcium antagonists, angiotensin converting enzyme (ACE) inhibitors and angiotensin II receptor blockers, are associated with lower incidence of these side effects. Other drugs commonly involved in the development of ED are cimetidine, tricyclic antidepressants and metoclopramide[9,23,24].

Available data point towards a detrimental effect of CKD on spermatogenesis[9]. Moreover, zinc deficiency caused by reduced dietary intake, malabsorption and possible loss during haemodialysis, has been implicated in the pathophysiology of reduced sperm motility in CKD patients[9,23,25].

PREVALENCE

Sexual dysfunction is a common feature in patients with CKD despite the fact that is often underestimated by clinicians. Existing comorbidities such as diabetes mellitus, hypertension, atherosclerosis, and certain medications (medications *e.g.,* antidepressants diuretics, beta-blockers and other antihypertensive drugs) as well as pathophysiological conditions such as peripheral vascular disease, peripheral neuropathy and uremia are associated with a decrease in erectile function of male patients[26].

Since 1997, Rosen *et al*[27] have developed the International Index of Erectile Dysfunction (IIEF), a questionnaire which includes all aspects of male sexual functions (erectile function, orgasmic function, sexual desire, intercourse satisfaction, overall satisfaction) and can evaluate as objectively as possible sexual function in male patients. Variable prevalence rates have been reported, mainly due to the study populations' diversity and the variety of sexual dysfunction assessment instruments. Bellinghieri *et al*[11] reported a direct correlation between IIEF and GFR, an inverse correlation between testosterone and cholesterol and an increased number of diabetic patients with ED in stage 3 of chronic renal failure.

The prevalence of erectile dysfunction is strongly age-dependent. The prevalence rises sharply with age. In particular, in the Massachusetts Male Aging Study (MMAS), erectile dysfunction is found in 8% in patients aged in their 40 s and rises up to 80% in patients over 70 years of age[28]. Messina *et al*[29] reported that men under 50 years old with CKD have a higher prevalence of ED than men over age 50 years, while in the MMAS the level of impotence and the prevalence of erectile dysfunction was positively associated with the subjects' age[30].

During the last decades the prevalence of end stage renal disease has significantly increased worldwide and due to the progress in renal replacement therapy. People with end stage renal disease (ESRD) appear to have a reduction in the QoL, which is associated with several factors such as age, therapy complications, psychological factors, and co-existing diseases[31]. Mesquita *et al*[32] reported that the prevalence of ED

was 76.5%, with 72.3% in stage 3 CKD, 81.5% in stage 4 and 87.5% in stage 5 CKD.

A study of 174 male HD patients (controls: 1133 healthy males) revealed that the prevalence of ED in men older than 40 years was higher than 80%, significantly higher than that described in control groups of the same age[33]. Espinoza *et al*[34] reported an ED prevalence of 48.9% in kidney transplant recipients in a study conducted among men with kidney transplantations. Rosas *et al*[26] reported that the prevalence of ED was 82% for all HD patients in a cross sectional study of 302 subjects treated with haemodialysis. ED was present in 90% of older HD patients (> 50 years) whereas its prevalence in younger subjects (< 50 years) was 63%[26].

A large systematic review and meta-analysis of observational studies (50 studies, 8343 patients) reported that the prevalence of any level of erectile dysfunction is approximately 70% (21 studies, 4389 patients) with no difference in prevalence rates among hemodialysis and peritoneal dialysis patients. However, in kidney transplant recipients the prevalence was lower (59% *vs* 75%)[35].

EFFECT OF COMORBIDITIES

Erectile and kidney dysfunction share common risk factors and are associated with diseases involving endothelial impairment such as diabetes mellitus, hypertension, dyslipidemia, coronary artery disease, smoking and obesity[32].

Cigarette smoking is an established modifiable risk factor of arteriosclerosis. As a result of the MMAS, Feldman *et al*[28] reported that current smokers presented an adjusted odds ratio of 1.97 for incident ED compared to non-smokers (P = 0.03). Other studies also report a higher prevalence of ED in the groups of smokers compared to non-smokers and a higher percentage of smoking in men with severe/complete ED but these results did not appear to be significant in multivariate analysis[36].

As far as obesity is concerned, the nine-year follow-up prospective study from the MMAS revealed that it had an independent effect on ED, as a BMI $\geqslant$ 28 kg/m^2 predicted incident ED (OR: 1.96, P = 0.01). Similar results were observed in the Rancho-Bernando Study, where the age-adjusted BMI appeared to be significantly higher in men with severe ED or men who were sexually inactive (P < 0.05)[28,37].

Dyslipidemia is associated with an increased risk of erectile dysfunction due to its effect on endothelium and smooth muscle cells of the corpus cavernosum. The prospective study of MMAS failed to indicate a link between serum lipids and prediction of ED[28]. On the other hand, in the Rancho-Bernando Study elevated serum cholesterol levels and triglyceride levels were associated with more severe ED, as men without ED had lower cholesterol levels compared to men with moderate ED (P < 0.05), and men with no sexual activity or severe/complete ED had higher triglyceride levels than men without ED (P < 0.05)[37]. In a prospective study among 2869 men, Ponholzer *et al*[38] reported that hyperlipidemia was independently and significantly correlated to the presence of erectile dysfunction with an OR of 2.29 (P = 0.04). Hyperlipidemia is common among men with ED at rates that may reach 40%[39]. There have been reports that lipid-lowering therapy and use of statins may have a negative impact on erectile function; nevertheless statins are not generally accepted as a cause of ED and through their pleiotropic effects, statins may increase vascular NO activity and improve endothelial function[40].

Diabetes mellitus is considered to be a risk factor for ED due to vasculopathy and autonomic neuropathy and, additionally, one of the most frequent causes of CKD. Several studies have shown that it is highly prevalent and independently associated with erectile dysfunction in general population. Giuliano *et al*[41] reported a prevalence of ED of 71% among patients with DM and noted a trend of association between decreased mean IIEF-5 score and an increased duration of type 1-diabetes, lack of glycemic control and existence of complications. In a study of arterial risk factors (diabetes, hypertension, hyperlipidemia and smoking) among 440 impotent men, diabetes was the only risk factor that in isolation significantly reduced the penile blood-pressure index[42]. In another large prospective study including 2869 men diabetes was associated with a three times higher risk for ED[38]. Diabetic nephropathy is one of the most common causes of CKD and highly prevalent among end stage renal disease patients. Diabetes mellitus is significantly associated and considered to be an independent risk factor for erectile dysfunction in these patients[29,33,43]. No association between ED and cause of CKD has been proven. Nevertheless, in a study including 119 men with CRF in hemodialysis program, the highest prevalence of ED was among men whose kidney disease was due to diabetes[36]. Rosas *et al*[26] found that men with diabetes had twice the odds of suffering from erectile dysfunction compared to non-diabetic men in a cohort study among 302 subjects in hemodialysis, whereas Mesquita *et al*[32] reported that among 81 patients with CKD, diabetic patients were 4 times more likely to have impaired erectile function compared to non-diabetic subjects (P = 0.048).

In the general population, hypertension is considered to be a risk factor for erectile dysfunction due to its contribution in the atherosclerotic process and the endothelial dysfunction in penile vessels. In a multicenter prospective study conducted in Spain among 2130 men with primary hypertension, erectile dysfunction was reported in 45.8% of them[44]. In a smaller study, which included 634 Greek patients with essential hypertension, erectile dysfunction was prevalent in 35.2% of them compared to a rate of 14.1% found in the normotensives subjects (P < 0.01) and was associated with the severity of hypertension[23]. Despite the fact that a

strong association between hypertension and the emerge of erectile dysfunction has been well established in several other studies[38,41,45], data occasionally remain uncertain if hypertension is an independent risk factor for[28,42]. Although the frequency of hypertension is high among patients with CKD in rates reaching even 70%-95%, its association with sexual dysfunction among these patients is not always statistically significant[29,32,36,43]. Erectile dysfunction observed in hypertensive patients may be associated with the disease itself or it may be caused by the antihypertensive therapy being administered to these patients. In CKD patients, the drug therapy prescribed is often multifactorial including many types of old-antihypertensive drugs such as central acting antihypertensive drugs, diuretics (*e.g.,* thiazide diuretics and spironolactone), and beta-blockers, especially nonselective ones, which have shown a major influence on erectile function. New-generation agents, which include calcium-channel blockers, nebivolol and renin-angiotensin system inhibitors seem to have less deteriorating effects in sexual activity[6,46]. Indeed, Giuliano *et al*[41] reported greater IIEF-5 scores in patients receiving ACE inhibitors or angiotensin II receptor blockers compared to other antihypertensive treatment, while Rosas *et al*[26] revealed a significant association between ACE inhibitors and a decreased prevalence of erectile dysfunction in hemodialysis patients (OR: 0.42). As far as CKD patients are concerned, other widely used drugs, which are traditionally associated with increased rates of erectile dysfunction such as antidepressants, H_2 antagonists and benzodiazepines, should be taken into account as their use may impair sexual function[46].

Atherosclerosis and vascular calcification are very common in the CKD population, thus contributing to higher rates of cardiovascular disease and mortality among these patients. These findings tend to be present even among young adults undergoing hemodialysis compared to healthy subjects of the same age[47]. In the uremic subjects with ESRD, cardiovascular disease (CVD) is responsible for 40%-50% of all deaths and CVD mortality rates in those patients are approximately 15 times higher than the general population[21]. In addition to typical cardiovascular disease risk factors, end stage renal disease patients have impaired calcium and phosphorus homeostasis, deficiency of calcification inhibitors and receive high doses of vitamin D treatment, factors which have shown to promote the vascular calcification process[48]. In the general population, it has been reported that erectile dysfunction is associated with silent coronary disease as patients experiencing sexual dysfunction symptoms tend to have higher coronary artery calcification scores even in the absence of angina symptoms[49,50], a finding which is additionally observed among hemodialysis patients with severe ED, who also tend to present greater coronary artery calcium scores[3]. In a retrospective cohort study of 12825 ED patients, it was reported that there was a two-fold increase in the risk of acute myocardial infarction among men with ED[51]. Due to the apparent link between erectile dysfunction and other vascular abnormalities, CKD patients suffering from erectile dysfunction are in high risk of CVD. Therefore, clinicians should perform a careful evaluation in order to assess coexisting comorbid conditions and modify possible risk factors.

Cardiovascular risk and its association with sexual activity should be evaluated in all men with indications or confirmed cardiovascular disease. According to the Second Princeton Consensus Conference algorithm, patients are classified as low, intermediate or high cardiac risk depending on their sexual activity and their management depends on which category they are integrated. Although the Princeton-Ⅱ algorithm is based on acknowledged cardiovascular risk factors such as hypertension, diabetes mellitus or history of myocardial infarction or angina symptoms, CKD is not included as a condition increasing the cardiovascular risk in men with erectile dysfunction. Considering the increased risk of cardiovascular disease among patients suffering from renal failure and sexual dysfunction, it becomes obvious that these patients should be likewise evaluated and managed[52].

MANAGEMENT OF ED

The improvement of sexual function in CKD patients through a multifactorial approach is associated with an increase in patients' QoL and improved cardiovascular outcomes.

Lifestyle and general measures

Treatment of erectile dysfunction should start with an assessment of general status, evaluation of possible covariates and adoption of lifestyle measures, such as quit smoking, decrease of alcohol consumption and regular physical activity. As far as dialysis patients are concerned, clinicians should focus on optimization of dialysis delivery and adequate nutritional intake of these patients. The medication profile of each patient should be reviewed, considering that many drugs such as diuretics, beta-blockers, antidepressants, and H_2-antagonists are related to erectile dysfunction. Moreover, drugs inducing hyperprolactinaemia such as metoclopramide, haloperidol, phenothiazine, chlorpromazine, and methyldopa should be taken into account[53].

"Curable" causes of erectile dysfunction

Psychogenic ED: Psychotherapy and psychoeducational interventions such as rational, emotive therapy, sex group therapy and sexual counseling should be recommended when depression and other psychogenic causes of erectile dysfunction are suspected or in cases, in which it is indicated[53].

Hormonal-endocrine approach: Therapy with recombinant human Erythropoietin (rHuEPO) has shown to improve many aspects of functional health, such as exercise tolerance, sexual function, and QoL

of patients with CKD[54]. This improvement is likely to be associated with the correction of anemia, induced by the introduction of rHuEPO. In addition, some studies have shown that rHuEPO therapy is associated with alterations on endocrine function, affecting the pituitary-gonadal feedback mechanism. There is evidence supporting that it is associated with reduced prolactin, FSH and LH levels and increased plasma testosterone levels[55,56], although some small studies support that the prolactin levels suppression remains controversial among rHuEPO recipients[57]. Testosterone deficiency is a recognizable contributing factor in the development of anemia in CKD patients. Testosterone replacement therapy may increase blood count, QOL and sexual function[56].

Testosterone replacement therapy has been associated with multiple benefits in men with late onset hypogonadism. However, its effectiveness in men with CKD remains controversial considering that the improvement noted in libido, sexual desire, mood and energy is more profound than in erectile dysfunction individually[58]. Testosterone treatment may be also beneficial in increasing muscle mass and strength and in enhancing erythropoiesis[56]. Derivatives of testosterone can be delivered as injectable, oral, buccal, transdermal and subdermal preparations. Potential side effects such as cardiovascular adverse events, prostate cancer or exacerbation of sleep apnea should be identified and carefully assessed by clinicians[56].

An additional potential therapeutic option affecting endocrine disorders in CKD patients is dopaminergic agonists such as bromocryptine, which normalize prolactin levels, elevate plasma testosterone levels and improve libido and potency[59]. It has been reported that oral zinc supplements improve testosterone levels but its effect on sexual function remains conflicting. Subsidiary administration of oral vitamin E has shown that it may decrease prolactin and plasma testosterone levels[56].

First line therapy-oral pharmacotherapy

Since their introduction in 1998, phosphodiesterase-5 inhibitors are considered first-line agents for erectile dysfunction treatment in the general population. Sildenafil which is the agent most widely used is metabolized mainly in the liver and excreted approximately 80% in the feces and 13% in the urine; therefore, its pharmacokinetics are not significantly different in mild to moderate renal disease compared to healthy men, although its bioavailability may be increased in patients with creatinine clearance < 30 mL/min[60]. In several RCTs for treatment of sexual dysfunction in patients with CKD, treatment with sildenafil and vardenafil is associated with improvement in the overall score of IIEF-5, an increase of the score of all individual IIEF-5 tool domains (erection frequency, erection quality, penetration ability, maintenance frequency of penetration, maintenance of erection after penetration and erection confidence) compared to placebo and an increase of the overall satisfaction score of the IIEF-15 sexual assessment tool. For the use of other agents such as tadalafil or mirodenafil in CKD patients data is limited[61]. Sildenafil citrate is also considered an important first-line therapeutic option among kidney transplant recipients with sexual dysfunction as it has no effect on renal function or immunosuppressive drug levels[62]. The frequency of adverse events in CKD patients is similar to the general population, with headaches, flushing, dyspepsia, myalgia, and back pain, nasal congestion being most commonly reported, while more serious adverse events such nonarteritic anterior ischemic optic neuropathy or cardiovascular events are extremely rare. Due to the possible emerge of hypotension these agents are contraindicated in patients receiving nitrates. In addition, PDE-5 inhibitors should not be administered with PDE-3 inhibitors, such as cilostazol which is used for the management of peripheral artery disease.

Other therapeutic options beyond PDE-5 inhibitors

Vacuum constriction devices are an alternative therapeutic option. They provide negative pressure to the penis, resulting in increased blood flow and thus, causing erection. However, the satisfaction rates among patients remain variable.

Intraurethral or intracavernosal delivery of alprostadil (prostaglandin E_1) individually or in combination with other drugs such as papaverine or phentolamine can be used as a second-line therapy in case of non-response to oral drugs. A penile prosthesis is another therapeutic option in case of previous therapeutic failure and is preferred by some patients as it provides more permanent results. Nevertheless, it is recommended to be delayed after renal transplantation, as a percentage of ESRD patients may improve their sexual function afterwards[53].

Erectile dysfunction in post-transplant patients

Kidney transplantation is considered to be the most effective therapeutic option for patients suffering from CKD. The majority of kidney transplantations are carried out in middle age, where sexual function and fertility remain important[62]. Several studies suggest that erectile dysfunction remains highly prevalent, reaching 50% after kidney transplantation[34,63]. Sexual function post-operatively may be limited by graft malfunction, preexisting comorbid conditions of diabetes mellitus, hypertension, smoking and dyslipidemia, duration of dialysis before transplantation, effects of immunosuppressive or hypertension therapy and is associated with the original cause of kidney insufficiency[10,64]. The influence of haemodialysis duration before kidney transplantation observed by Rebollo *et al*[10] may be owing to the longer duration of peripheral vascular disease, and thus, prolonged vascular damage and hormonal changes in dialysis patients. With regard to immunosuppressive treatment, Malavaud *et al*[63] reported no statistically significant

association between cyclosporine therapy and erectile dysfunction, while the study of Rebollo *et al*[10] showed no association between ED and use of tacrolimus as immunosuppressant. The combination of cyclosporine and prednisone may have a more beneficial effect than azathioprine in gonadal function after kidney transplantation[65]. Renal transplantation usually results in normalization of hormonal profiles of kidney transplant recipients, reducing high levels of prolactin and LH and elevating plasma testosterone[65-68]. Despite these alterations, recovery of sexual function is not present in all patients, as erectile dysfunction may be affected by various factors and thus, can be highly prevalent in patients with renal insufficiency even after kidney transplantation.

CONCLUSION

Sexual dysfunction and chronic renal failure share common pathophysiological pathways and are affected by similar comorbid conditions. Erectile dysfunction tends to be more frequent in patients with CKD. Its incidence is strongly associated with age and stage of renal failure. Despite the advances in therapeutic options, especially the emerge of PDE-5 inhibitors, and the potential relief they may offer, erectile dysfunction still remains highly prevalent and further studies are needed.

REFERENCES

1 **Vecchio M**, Palmer SC, Tonelli M, Johnson DW, Strippoli GF. Depression and sexual dysfunction in chronic kidney disease: a narrative review of the evidence in areas of significant unmet need. *Nephrol Dial Transplant* 2012; **27**: 3420-3428 [PMID: 22942174 DOI: 10.1093/ndt/gfs135]

2 **Bortolotti A**, Parazzini F, Colli E, Landoni M. The epidemiology of erectile dysfunction and its risk factors. *Int J Androl* 1997; **20**: 323-334 [PMID: 9568524 DOI: 10.1046/j.1365-2605.1998.00081.x]

3 **Inci K**, Hazirolan T, Aki FT, Oruc O, Tombul T, Tasar C, Erkan I, Bakkaloglu M, Turgan C, Ergen A. Coronary artery calcifications in hemodialysis patients and their correlation with the prevalence of erectile dysfunction. *Transplant Proc* 2008; **40**: 77-80 [PMID: 18261550 DOI: 10.1016/j.tranproceed.2007.11.046]

4 NIH Consensus Conference. Impotence. NIH Consensus Development Panel on Impotence. *JAMA* 1993; **270**: 83-90 [PMID: 8510302 DOI: 10.1001/jama.1993.03510010089036]

5 **Burnett AL**. The role of nitric oxide in erectile dysfunction: implications for medical therapy. *J Clin Hypertens* (Greenwich) 2006; **8**: 53-62 [PMID: 17170606 DOI: 10.1111/j.1524-6175.2006.06026.x]

6 **Manolis A**, Doumas M. Hypertension and sexual dysfunction. *Arch Med Sci* 2009; **5**: S337-S350

7 **Dean RC**, Lue TF. Physiology of penile erection and pathophysiology of erectile dysfunction. *Urol Clin North Am* 2005; **32**: 379-395, v [PMID: 16291031 DOI: 10.1016/j.ucl.2005.08.007]

8 **Solomon H**, Man JW, Jackson G. Erectile dysfunction and the cardiovascular patient: endothelial dysfunction is the common deno minator. *Heart* 2003; **89**: 251-253 [PMID: 12591819 DOI: 10.1136/heart.89.3.251]

9 **Palmer BF**. Sexual dysfunction in uremia. *J Am Soc Nephrol* 1999; **10**: 1381-1388 [PMID: 10361878]

10 **Rebollo P**, Ortega F, Valdés C, Fernández-Vega F, Ortega T, García-Mendoza M, Gómez E. Factors associated with erectile dysfunction in male kidney transplant recipients. *Int J Impot Res* 2003; **15**: 433-438 [PMID: 14671663 DOI: 10.1038/sj.ijir.3901056]

11 **Bellinghieri G**, Santoro D, Mallamace A, Savica V. Sexual dysfunction in chronic renal failure. *J Nephrol* 2008; **21** Suppl 13: S113-S117 [PMID: 18446743]

12 **Palmer BF**. Outcomes associated with hypogonadism in men with chronic kidney disease. *Adv Chronic Kidney Dis* 2004; **11**: 342-347 [PMID: 15492970 DOI: 10.1053/j.ackd.2004.07.008]

13 **Dohle GR**, Elzanaty S, van Casteren NJ. Testicular biopsy: clinical practice and interpretation. *Asian J Androl* 2012; **14**: 88-93 [PMID: 22157985 DOI: 10.1038/aja.2011.57]

14 **de Vries CP**, Gooren LJ, Oe PL. Haemodialysis and testicular function. *Int J Androl* 1984; **7**: 97-103 [PMID: 6539303 DOI: 10.1111/j.1365-2605.1984.tb00765.x]

15 **Carrero JJ**, Bárány P, Yilmaz MI, Qureshi AR, Sonmez A, Heimbürger O, Ozgurtas T, Yenicesu M, Lindholm B, Stenvinkel P. Testosterone deficiency is a cause of anaemia and reduced responsiveness to erythropoiesis-stimulating agents in men with chronic kidney disease. *Nephrol Dial Transplant* 2012; **27**: 709-715 [PMID: 21617198 DOI: 10.1093/ndt/gfr288]

16 **Prem AR**, Punekar SV, Kalpana M, Kelkar AR, Acharya VN. Male reproductive function in uraemia: efficacy of haemodialysis and renal transplantation. *Br J Urol* 1996; **78**: 635-638 [PMID: 8944524 DOI: 10.1046/j.1464-410X.1996.14624.x]

17 **Manolis A**, Doumas M. Sexual dysfunction: the 'prima ballerina' of hypertension-related quality-of-life complications. *J Hypertens* 2008; **26**: 2074-2084 [PMID: 18854743 DOI: 10.1097/HJH.0b013e32830dd0c6]

18 **Steele TE**, Wuerth D, Finkelstein S, Juergensen D, Juergensen P, Kliger AS, Finkelstein FO. Sexual experience of the chronic peritoneal dialysis patient. *J Am Soc Nephrol* 1996; **7**: 1165-1168 [PMID: 8866408]

19 **Baylis C**. Arginine, arginine analogs and nitric oxide production in chronic kidney disease. *Nat Clin Pract Nephrol* 2006; **2**: 209-220 [PMID: 16932427 DOI: 10.1038/ncpneph0143]

20 **Kersh ES**, Kronfield SJ, Unger A, Popper RW, Cantor S, Cohn K. Autonomic insufficiency in uremia as a cause of hemodialysis-induced hypotension. *N Engl J Med* 1974; **290**: 650-653 [PMID: 4813726 DOI: 10.1056/NEJM197403212901203]

21 **National Kidney Foundation**. K/DOQI clinical practice guidelines for chronic kidney disease: evaluation, classification, and stratification. *Am J Kidney Dis* 2002; **39**: S1-S266 [PMID: 11904577]

22 **Peng YS**, Chiang CK, Hung KY, Chiang SS, Lu CS, Yang CS, Wu KD, Yang CC, Lin RP, Chang CJ, Tsai TJ, Chen WY. The association of higher depressive symptoms and sexual dysfunction in male haemodialysis patients. *Nephrol Dial Transplant* 2007; **22**: 857-861 [PMID: 17121784 DOI: 10.1093/ndt/gfl666]

23 **Doumas M**, Tsakiris A, Douma S, Grigorakis A, Papadopoulos A, Hounta A, Tsiodras S, Dimitriou D, Giamarellou H. Factors affecting the increased prevalence of erectile dysfunction in Greek hypertensive compared with normotensive subjects. *J Androl* 2006; **27**: 469-477 [PMID: 16339456 DOI: 10.2164/jandrol.04191]

24 **Chang SW**, Fine R, Siegel D, Chesney M, Black D, Hulley SB. The impact of diuretic therapy on reported sexual function. *Arch Intern Med* 1991; **151**: 2402-2408 [PMID: 1746997 DOI: 10.1001/archinte.1991.00400120048008]

25 **Chia SE**, Ong CN, Chua LH, Ho LM, Tay SK. Comparison of zinc concentrations in blood and seminal plasma and the various sperm parameters between fertile and infertile men. *J Androl* 2000; **21**: 53-57 [PMID: 10670519]

26 **Rosas SE**, Joffe M, Franklin E, Strom BL, Kotzker W, Brensinger C, Grossman E, Glasser D, Feldman HI. Prevalence and determinants of erectile dysfunction in hemodialysis patients. *Kidney Int* 2001; **59**: 2259-2266 [PMID: 11380829 DOI: 10.1046/j.1523-1755.2001.00742.x]

27 **Rosen RC**, Riley A, Wagner G, Osterloh IH, Kirkpatrick J, Mishra A. The international index of erectile function (IIEF): a multidimensional scale for assessment of erectile dysfunction. *Urology* 1997; **49**: 822-830 [PMID: 9187685 DOI: 10.1016/S0090-4295(97)00238-0]

28 **Feldman HA**, Johannes CB, Derby CA, Kleinman KP, Mohr BA, Araujo AB, McKinlay JB. Erectile dysfunction and coronary risk factors: prospective results from the Massachusetts male aging study. *Prev Med* 2000; **30**: 328-338 [PMID: 10731462 DOI: 10.1006/

pmed.2000.0643]
29 **Messina LE**, Claro JA, Nardozza A, Andrade E, Ortiz V, Srougi M. Erectile dysfunction in patients with chronic renal failure. *Int Braz J Urol* 2007; **33**: 673-678 [PMID: 17980064 DOI: 10.1590/S1677-55382007000500008]
30 **Feldman HA**, Goldstein I, Hatzichristou DG, Krane RJ, McKinlay JB. Construction of a surrogate variable for impotence in the Massachusetts Male Aging Study. *J Clin Epidemiol* 1994; **47**: 457-467 [PMID: 7730871 DOI: 10.1016/0895-4356(94)90292-5]
31 **Türk S**, Guney I, Altintepe L, Tonbul Z, Yildiz A, Yeksan M. Quality of life in male hemodialysis patients. Role of erectile dysfunction. *Nephron Clin Pract* 2004; **96**: c21-c27 [PMID: 14752250 DOI: 10.1159/000075568]
32 **Mesquita JF**, Ramos TF, Mesquita FP, Bastos Netto JM, Bastos MG, Figueiredo AA. Prevalence of erectile dysfunction in chronic renal disease patients on conservative treatment. *Clinics* (Sao Paulo) 2012; **67**: 181-183 [PMID: 22358245 DOI: 10.6061/clinics/2012(02)15]
33 **Naya Y**, Soh J, Ochiai A, Mizutani Y, Ushijima S, Kamoi K, Ukimura O, Kawauchi A, Fujito A, Ono T, Iwamoto N, Aoki T, Imada N, Marumo K, Murai M, Miki T. Significant decrease of the International Index of Erectile Function in male renal failure patients treated with hemodialysis. *Int J Impot Res* 2002; **14**: 172-177 [PMID: 12058244 DOI: 10.1038/sj.ijir.3900854]
34 **Espinoza R**, Gracida C, Cancino J, Ibarra A. Prevalence of erectile dysfunction in kidney transplant recipients. *Transplant Proc* 2006; **38**: 916-917 [PMID: 16647509 DOI: 10.1016/j.transproceed.2006.02.045]
35 **Navaneethan SD**, Vecchio M, Johnson DW, Saglimbene V, Graziano G, Pellegrini F, Lucisano G, Craig JC, Ruospo M, Gentile G, Manfreda VM, Querques M, Stroumza P, Torok M, Celia E, Gelfman R, Ferrari JN, Bednarek-Skublewska A, Dulawa J, Bonifati C, Hegbrant J, Wollheim C, Jannini EA, Strippoli GF. Prevalence and correlates of self-reported sexual dysfunction in CKD: a meta-analysis of observational studies. *Am J Kidney Dis* 2010; **56**: 670-685 [PMID: 20801572 DOI: 10.1053/j.ajkd.2010.06.016]
36 **Cerqueira J**, Moraes M, Glina S. Erectile dysfunction: prevalence and associated variables in patients with chronic renal failure. *Int J Impot Res* 2002; **14**: 65-71 [PMID: 11979319 DOI: 10.1038/sj.ijir.3900811]
37 **Fung MM**, Bettencourt R, Barrett-Connor E. Heart disease risk factors predict erectile dysfunction 25 years later: the Rancho Bernardo Study. *J Am Coll Cardiol* 2004; **43**: 1405-1411 [PMID: 15093875 DOI: 10.1016/j.jacc.2003.11.041]
38 **Ponholzer A**, Temml C, Mock K, Marszalek M, Obermayr R, Madersbacher S. Prevalence and risk factors for erectile dysfunction in 2869 men using a validated questionnaire. *Eur Urol* 2005; **47**: 80-85; discussion 85-86 [PMID: 15582253 DOI: 10.1016/j.eururo.2004.08.017]
39 **Seftel AD**, Sun P, Swindle R. The prevalence of hypertension, hyperlipidemia, diabetes mellitus and depression in men with erectile dysfunction. *J Urol* 2004; **171**: 2341-2345 [PMID: 15126817 DOI: 10.1097/01.ju.0000125198.32936.38]
40 **Vrentzos GE**, Paraskevas KI, Mikhailidis DP. Dyslipidemia as a risk factor for erectile dysfunction. *Curr Med Chem* 2007; **14**: 1765-1770 [PMID: 17627514 DOI: 10.2174/092986707781058931]
41 **Giuliano FA**, Leriche A, Jaudinot EO, de Gendre AS. Prevalence of erectile dysfunction among 7689 patients with diabetes or hypertension, or both. *Urology* 2004; **64**: 1196-1201 [PMID: 15596196 DOI: 10.1016/j.urology.2004.08.059]
42 **Virag R**, Bouilly P, Frydman D. Is impotence an arterial disorder? A study of arterial risk factors in 440 impotent men. *Lancet* 1985; **1**: 181-184 [PMID: 2857264 DOI: 10.1016/S0140-6736(85)92023-9]
43 **Neto AF**, de Freitas Rodrigues MA, Saraiva Fittipaldi JA, Moreira ED. The epidemiology of erectile dysfunction and its correlates in men with chronic renal failure on hemodialysis in Londrina, southern Brazil. *Int J Impot Res* 2002; **14** Suppl 2: S19-S26 [PMID: 12161764 DOI: 10.1038/sj.ijir.3900894]
44 **Aranda P**, Ruilope LM, Calvo C, Luque M, Coca A, Gil de Miguel A. Erectile dysfunction in essential arterial hypertension and effects of sildenafil: results of a Spanish national study. *Am J Hypertens* 2004; **17**: 139-145 [PMID: 14751656 DOI: 10.1016/j.amjhyper.2003.09.006]
45 **Johannes CB**, Araujo AB, Feldman HA, Derby CA, Kleinman KP, McKinlay JB. Incidence of erectile dysfunction in men 40 to 69 years old: longitudinal results from the Massachusetts male aging study. *J Urol* 2000; **163**: 460-463 [PMID: 10647654 DOI: 10.1016/S0022-5347(05)67900-1]
46 **Derby CA**, Barbour MM, Hume AL, McKinlay JB. Drug therapy and prevalence of erectile dysfunction in the Massachusetts Male Aging Study cohort. *Pharmacotherapy* 2001; **21**: 676-683 [PMID: 11401181 DOI: 10.1592/phco.21.7.676.34571]
47 **Goodman WG**, Goldin J, Kuizon BD, Yoon C, Gales B, Sider D, Wang Y, Chung J, Emerick A, Greaser L, Elashoff RM, Salusky IB. Coronary-artery calcification in young adults with end-stage renal disease who are undergoing dialysis. *N Engl J Med* 2000; **342**: 1478-1483 [PMID: 10816185 DOI: 10.1056/NEJM200005183422003]
48 **Vo TM**, Disthabanchong S. Are there ways to attenuate arterial calcification and improve cardiovascular outcomes in chronic kidney disease? *World J Cardiol* 2014; **6**: 216-226 [PMID: 24944752]
49 **Jackson G**, Padley S. Erectile dysfunction and silent coronary artery disease: abnormal computed tomography coronary angiogram in the presence of normal exercise ECGs. *Int J Clin Pract* 2008; **62**: 973-976 [PMID: 18479289 DOI: 10.1111/j.1742-1241.2008.01788.x]
50 **Chiurlia E**, D'Amico R, Ratti C, Granata AR, Romagnoli R, Modena MG. Subclinical coronary artery atherosclerosis in patients with erectile dysfunction. *J Am Coll Cardiol* 2005; **46**: 1503-1506 [PMID: 16226175 DOI: 10.1016/j.jacc.2005.06.068]
51 **Blumentals WA**, Gomez-Caminero A, Joo S, Vannappagari V. Should erectile dysfunction be considered as a marker for acute myocardial infarction? Results from a retrospective cohort study. *Int J Impot Res* 2004; **16**: 350-353 [PMID: 14985780 DOI: 10.1038/sj.ijir.3901174]
52 **Hatzimouratidis K**, Amar E, Eardley I, Giuliano F, Hatzichristou D, Montorsi F, Vardi Y, Wespes E. Guidelines on male sexual dysfunction: erectile dysfunction and premature ejaculation. *Eur Urol* 2010; **57**: 804-814 [PMID: 20189712 DOI: 10.1016/j.eururo.2010.02.020]
53 **Ayub W**, Fletcher S. End-stage renal disease and erectile dysfunction. Is there any hope? *Nephrol Dial Transplant* 2000; **15**: 1525-1528 [PMID: 11007818]
54 **Beusterien KM**, Nissenson AR, Port FK, Kelly M, Steinwald B, Ware JE. The effects of recombinant human erythropoietin on functional health and well-being in chronic dialysis patients. *J Am Soc Nephrol* 1996; **7**: 763-773 [PMID: 8738812]
55 **Kokot F**, Wiecek A, Schmidt-Gayk H, Marcinkowski W, Gilge U, Heidland A, Rudka R, Trembecki J. Function of endocrine organs in hemodialyzed patients of long-term erythropoietin therapy. *Artif Organs* 1995; **19**: 428-435 [PMID: 7625922 DOI: 10.1111/j.1525-1594.1995.tb02354.x]
56 **Iglesias P**, Carrero JJ, Díez JJ. Gonadal dysfunction in men with chronic kidney disease: clinical features, prognostic implications and therapeutic options. *J Nephrol* 2012; **25**: 31-42 [PMID: 21748720 DOI: 10.5301/JN.2011.8481]
57 **Lawrence IG**, Price DE, Howlett TA, Harris KP, Feehally J, Walls J. Erythropoietin and sexual dysfunction. *Nephrol Dial Transplant* 1997; **12**: 741-747 [PMID: 9141005 DOI: 10.1093/ndt/12.4.741]
58 **Anantharaman P**, Schmidt RJ. Sexual function in chronic kidney disease. *Adv Chronic Kidney Dis* 2007; **14**: 119-125 [PMID: 17395114 DOI: 10.1053/j.ackd.2007.01.002]
59 **Vircburger MI**, Prelević GM, Perić LA, Knezević J, Djukanović L. Testosterone levels after bromocriptine treatment in patients undergoing long-term hemodialysis. *J Androl* 1985; **6**: 113-116 [PMID: 3921506]
60 **Grossman EB**, Swan SK, Muirhead GJ, Gaffney M, Chung M, DeRiesthal H, Chow D, Raij L. The pharmacokinetics and hemodynamics of sildenafil citrate in male hemodialysis patients. *Kidney Int* 2004; **66**: 367-374 [PMID: 15200445 DOI: 10.1111/j.1523-1755.2004.00739.x]

61 **Vecchio M**, Navaneethan SD, Johnson DW, Lucisano G, Graziano G, Querques M, Saglimbene V, Ruospo M, Bonifati C, Jannini EA, Strippoli GF. Treatment options for sexual dysfunction in patients with chronic kidney disease: a systematic review of randomized controlled trials. *Clin J Am Soc Nephrol* 2010; **5**: 985-995 [PMID: 20498250 DOI: 10.2215/CJN.09081209]

62 **Lasaponara F**, Paradiso M, Milan MG, Morabito F, Sedigh O, Graziano ME, Abbona A, Piccoli GB, Rossetti M, Mezza E, Ferrando U. Erectile dysfunction after kidney transplantation: our 22 years of experience. *Transplant Proc* 2004; **36**: 502-504 [PMID: 15110572 DOI: 10.1016/j.transproceed.2004.02.014]

63 **Malavaud B**, Rostaing L, Rischmann P, Sarramon JP, Durand D. High prevalence of erectile dysfunction after renal transplantation. *Transplantation* 2000; **69**: 2121-2124 [PMID: 10852609 DOI: 10.1097/00007890-200005270-00027]

64 **Barroso LV**, Miranda EP, Cruz NI, Medeiros MA, Araújo AC, Mota Filho FH, Medeiros FC. Analysis of sexual function in kidney transplanted men. *Transplant Proc* 2008; **40**: 3489-3491 [PMID: 19100420 DOI: 10.1016/j.transproceed.2008.07.141]

65 **Burgos FJ**, Pascual J, Gomez V, Orofino L, Liaño F, Ortuño J. Effect of kidney transplantation and cyclosporine treatment on male sexual performance and hormonal profile: a prospective study. *Transplant Proc* 1997; **29**: 227-228 [PMID: 9122973 DOI: 10.1016/S0041-1345(96)00072-3]

66 **Chu SH**, Tay SK, Chiang YJ, Chuang CK, Chen HW, Chen CS, Chou CC, Huang CC. Male sexual performance and hormonal studies in uremic patients and renal transplant recipients. *Transplant Proc* 1998; **30**: 3062-3063 [PMID: 9838349 DOI: 10.1016/S0041-1345(98)00930-0]

67 **Akbari F**, Alavi M, Esteghamati A, Mehrsai A, Djaladat H, Zohrevand R, Pourmand G. Effect of renal transplantation on sperm quality and sex hormone levels. *BJU Int* 2003; **92**: 281-283 [PMID: 12887484 DOI: 10.1046/j.1464-410X.2003.04323.x]

68 **Tsujimura A**, Matsumiya K, Tsuboniwa N, Yamanaka M, Miura H, Kitamura M, Kishikawa H, Nishimura K, Ichikawa Y, Nagano S, Kokado Y, Takahara S, Okuyama A. Effect of renal transplantation on sexual function. *Arch Androl* 2002; **48**: 467-474 [PMID: 12425764 DOI: 10.1080/01485010290099381]

P- Reviewer: Azadzoi K, Barzilay JI, Kadoglu A **S- Editor**: Gong XM
L- Editor: A **E- Editor**: Yan JL

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DOI: 10.5527/wjn.v4.i3.388

World J Nephrol 2015 July 6; 4(3): 388-395
ISSN 2220-6124 (online)

MINIREVIEWS

Epidemiology, clinical characteristics, and management of chronic kidney disease in human immunodeficiency virus-infected patients

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Conflict-of-interest statment: None declared.

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Received: June 24, 2014
Peer-review started: June 25, 2014
First decision: September 16, 2014
Revised: October 10, 2014
Accepted: April 8, 2015
Article in press: April 9, 2015
Published online: July 6, 2015

Abstract

Antiretroviral therapy has markedly reduced acquired immune deficiency syndrome-related deaths and opportunistic infectious diseases. This has resulted in prolonged survival of individuals infected with the human immunodeficiency virus (HIV). However, this improvement in survival has been accompanied by an increase in the incidence of chronic kidney disease (CKD) and end-stage renal disease. CKD is now epidemic among HIV-infected populations in both Western and Eastern countries. Risk factors associated with CKD in HIV-infected populations include aging, hypertension, diabetes mellitus, co-infection with hepatitis C virus, a low CD4 cell count, and a high HIV viral load. Clinical experience has shown that HIV-infected individuals often have one or more concurrent risk factors for CKD. The cumulative effect of multiple risk factors on the development of CKD should be noted in this population. Glomerular disease directly related to HIV infection, so-called HIV-associated nephropathy, remains an important cause of CKD among a limited HIV population of African descent, but is less likely to be common among other urban HIV populations. The impact of exposure to nephrotoxic antiretroviral agents on the development of kidney disease is both an old and a new concern. In particular, the association of tenofovir with kidney tubular injury has been an area of great interest. The findings regarding tenofovir's adverse effect on long-term kidney function vary among studies. The early identification and treatment of CKD is recommended for reducing the burden of patients requiring dialysis in HIV-infected populations. Periodic monitoring of urinary concentrations of albumin, protein, and tubular injury markers such as low-molecular-weight proteins may be useful for the early diagnosis of patients at risk for incident CKD. This review focuses on recent epidemiology, clinical characteristics, and management of CKD in a contemporary HIV-infected population.

Key words: Antiretroviral therapy; Tenofovir; Human immunodeficiency virus-associated nephropathy; Albuminuria; Renal tubular biomarkers; Cystatin C; Diabetes mellitus; Hypertension

Core tip: Kidneys are affected by the human immunodeficiency virus (HIV) and its associated therapies. As HIV subjects now have longevity while they receive combination anti-retroviral therapy (cART), kidney disease has been prominent among the current HIV subjects on cART. HIV subjects often have several coexisting risk factors of kidney disease, including diabetes and hypertension. Measurements of albuminuria, proteinuria, urinary low-molecular weight proteins, and serum cystatin C are necessary for early detection of kidney disease. Collaborative discussions between HIV experts and nephrologists are warranted to achieve the good treatment of chronic kidney disease in HIV patients.

Ando M, Yanagisawa N. Epidemiology, clinical characteristics, and management of chronic kidney disease in human immunodeficiency virus-infected patients. *World J Nephrol* 2015; 4(3): 388-395 Available from: URL: http://www.wjgnet.com/2220-6124/full/v4/i3/388.htm DOI: http://dx.doi.org/10.5527/wjn.v4.i3.388

INTRODUCTION

Although combination anti-retroviral therapy (cART) has contributed to the longevity in individuals affected by human immunodeficiency virus (HIV), the life-span extension is followed by the emergence of chronic kidney disease (CKD), leading to their high morbidity and mortality[1-9]. Now, nephrologists are faced with several problems related to CKD among HIV populations, including how to find out subclinical kidney insults, to identify incipient stage of kidney illness, and to collaborate with HIV healthcare staff to offer how to treat CKD. The frequency of CKD is increasing in HIV patients living in Asian countries[10,11] likewise in Western countries[12,13]. Generally speaking, as the early identification of kidney disease gives a chance to exert treatments that inhibit progression of kidney dysfunction[14-16], it could be most crucial to find out HIV patients at high risk of incident CKD as the first step in weakening the frequency of CKD in this population[17-19]. The 2012 KDIGO guidelines elaborated the identification and prognosis of CKD by combining albuminuria with estimated glomerular filtration rate (eGFR)[20,21]. The review attempted to summarize recent advances in the study on CKD in the current HIV individuals.

PREVALENCE OF CKD: PROTEINURIA, ALBUMINURIA, AND A LOSS IN RENAL FUNCTION IN HIV INDIVIDUALS

A simple and reliable biomarker of renal insult is persistent urinary excretion of protein or albumin. Whereas 7.2%-13.7% of HIV-infected subjects manifest proteinuria on a urine dipstick test[7,9,10,22-26], 8.7%-17.8% of those subjects have albuminuria, based on the urinary excretion of albumin[10,27,28]. The frequency of a persistent loss in renal function less than 60 mL/min per 1.73 m^2 varies between 3.5% and 9.7% in different HIV populations[9-12,26]. When both of the existence of urinary protein and a decline in glomerular function were considered, the frequency of CKD stages 1 to 5 ranged 15% to 24%[2,9,10,12,26]. Difference of the CKD prevalence across various countries has not been studied yet. Table 1 demonstrates the frequency of kidney disease in Japan, China, Europe, and the United States, as previously reported.

Numerous reports have shown that albuminuria seems to be one of independent risk factors of a poor prognosis among HIV-infected individuals[27,28]. A quite recent paper has shown that low-grade proteinuria is highly prevalent in a large HIV-infected white cohort on cART[29]. It is therefore reasonable to assume that the KDIGO classification would be more practical for the identification of CKD and for estimating prognosis in HIV-infected individuals than the conventional KDOQI staging. However, the measurement of albuminuria is expensive, with public health care insurance systems in most countries limiting the application to follow-up for diabetic nephropathy. Therefore, a total of 1447 HIV-infected Japanese (1351 males, 96 females; mean age, 44.4 ± 11.5 years) were classified using the 2012 KDIGO guidelines for estimating CKD risk: a combination of eGFR and dipstick proteinuria, as a convenient alternative to albuminuria[30]. Proteinuria was classified into 3 grades: [A1] $\leqslant$ +/-, [A2] 1+ to 2+, and [A3] 3+ $\leqslant$ eGFR was classified into 6 grades: [Gade 1] $\geqslant$ 90, [Grade 2] 60-89, [Grade 3a] 45-59, [Grade 3b] 30-44, [Grade 4] 15-29, and [Grade 5] < 15 mL/min per 1.73 m^2, using colored heat map zones. It was shown that the prevalence rates of individuals in the green, yellow, orange, and red zones were 85.9%, 11.0%, 2.1%, and 1.0%, respectively. The prevalence of individuals at high and very high risk for a poor prognosis in the KDIGO classification was nearly halved, compared with the risk for CKD $\geqslant$ stage 3 in the KDOQI system (3.1% *vs* 6.6%) (Figure 1).

GLOMERULAR AND TUBULAR DISEASES IN HIV-INFECTED PATIENTS

Glomerular and tubular diseases that are often identified in HIV-infected patients are summarized in Table 2. The traditional problems of HIV-associated nephropathy (HIVAN), HIVIC, and TMA are still crucial because of the delay in HIV diagnosis or the non-response to ART even in the contemporary cART years[31]. Patients at the earlier stage of HIVAN may manifest almost normal kidney glomerular function, albuminuria, or subclinical proteinuria. Their renal function often remains constant over some years after the start of cART[32,33]. HIV-

GFR grade	eGFR (mL/min per 1.73 m^2)	A1	A2	A3
G1	⩾ 90	G1A1 518 (35.8%)	G1A2 25 (1.7%)	G1A3 0 (0.0%)
G2	60-89	G2A1 725 (50.1%)	G2A2 79 (5.5%)	G2A3 4 (0.3%)
G3a	45-59	G3aA1 55 (3.8%)	G3aA2 21 (1.5%)	G3aA3 3 (0.2%)
G3b	30-44	G3bA1 5 (0.3%)	G3bA2 5 (0.3%)	G3bA3 1 (0.1%)
G4	15-29	G4A1 2 (0.1%)	G4A2 3 (0.2%)	G4A3 1 (0.1%)
G5	< 15	G5A1 0 (0.0%)	G5A2 0 (0.0%)	G5A3 0 (0.0%)

Figure 1 Distribution of human immunodeficiency virus-infected individuals determined by the KDIGO 2012 classification. The percentage of HIV-infected individuals in each category is expressed in each color box. The cohort includes 1447 HIV-infected patients. The prevalence of individuals in the green, yellow, orange, and red zone was 85.9%, 11.0%, 2.1%, and 1.0%, respectively. KDIGO: Kidney Disease: Outcomes Quality Initiative; CKD: Chronic kidney disease; eGFR: Estimated glomerular filtration rate; A1: No proteinuria (dipstick - or +/-); A2: Mild proteinuria (dipstick 1+ or 2+); A3: Heavy proteinuria (dipstick ⩾ 3+); HIV: Human immunodeficiency virus.

Table 1 Comparisons of prevalence of chronic kidney disease in human immunodeficiency virus-infected patients across previous studies

	Prevalence (%)	Countries	Ref.
Proteinuria			
	7.20	United States	[7]
	9.50	Japan	[11]
	13.70	China	[10]
Albuminuria			
	8.70	Norway	[25]
	11.00	United States	[26]
	17.80	Japan	[11]
CKD stages 1-5			
	15.40	Japan	[11]
	15.50	United States	[23]
	16.80	China	[10]
	23.70	United States	[13]
CKD stages ⩾ 3			
	3.50	EuroSIDA	[12]
	5.60	China	[10]
	5.90	United States	[23]
	9.70	Japan	[11]
	9.70	United States	[13]

CKD: Chronic kidney disease; EuroSIDA: European study of patients with HIV-1 infection including 93 centers across Europe.

infected individuals with African pedigree have been considered being at higher risk of HIVAN arising from podocyte proliferation and tubular dilatation with atrophy and flattening of the tubular epithelial cells[34,35].

RISK FACTORS OF CKD IN HIV SUBJECTS

Known risk factors of CKD in HIV patients are shown in Table 3. Epidemiologic investigations showed that variates associated with CKD in HIV-infected patients include traditional risks including elder age, hypertension, and DM[9-12,22-26]. This has been confirmed by a report from a prospective study with a 6-year median follow-up including a large white HIV cohort receiving antiretroviral treatment[36]. Lipids levels, decreased CD4 cell counts, and elevated HIV RNA load are perhaps specific risks for HIV-infected subjects[10,11,23-25]. Moreover, HCV infection contributes to renal insults in HIV people[11,25,31]. Nearly 30% of subjects with HIV are affected with HCV[37]. Liangpunsakul *et al*[38] performed a study to see the association between non-diabetic patients concurrently having HCV and albuminuria, based on a database from the NHANES Ⅲ. Adjusted for known variates, they demonstrated that HCV co-infection was independently involved in microalbuminuria in individuals without diabetes mellitus. Furthermore, Tsui *et al*[39] showed a significant relationship between albuminuria and HCV seropositivity in people who were classified by age.

ART AND CKD

Some antiretroviral agents are related to kidney disease, hyperlipidemia, diabetes mellitus, and hypertension which may intensify the risk of incidence of CKD[40]. Whereas HIVAN was the major renal involvement before the era of ART, comorbidities and adverse renal effects of various drugs for ART now complicate the landscape of kidney disease in HIV[41]. Drug-induced decrease in kidney function was shown in some NRTIs, TDF, and protease inhibitors (PIs). In those PIs, indinavir is predisposed to generate crystalline stones and it has been changed by PIs with safer agents with integrase inhibition. In addition, atazanavir (ATV) is likely associated with acute interstitial nephritis[42,43] and sub-acute or chronic renal insufficiency due to granulomatous interstitial nephritis characterized by the coexistence of crystalline deposition[44-46]. TDF is secreted from proximal renal tubules, and may be associated with its tubular damage representing mitochondrial dysfunction[47,48]. Although studies of the Gallant *et al*[49] did not show that tenofovir was responsible for renal failure, HIV-infected groups on TDF at the Johns Hopkins Clinical Cohort had a significant decrease in creatinine clearance for 3 years, as compared to patients not having tenofovir[50]. Nevertheless, another study using the same cohort

Table 2 Glomerular or tubular diseases in human immunodeficiency virus-infected patients

Diseases	Clinical characteristics
HIV-specific glomerular disease	
HIVAN	Detectable viral load, a high amount of proteinuria, albuminuria, RPGN
HIVIC	Proteinuria and/or hematuria, variable manifestation including AKI
TMA	AKI, proteinuria, hematuria with microangiopathic hemolytic anemia and thrombocytopenia
HIV-non-specific glomerular disease	
HCV-related MPGN/ cryoglobulinemia	Proteinuria and/or hematuria, nephritic syndrome, a decrease in serum complements
Diabetec nephropathy	Proteinuria (microalbuminuria to nephrotic syndrome), a decrease in GFR
Glomerular sclerosis	Older patients, hypertension, no or low amount of proteinuria, coexistence of atherosclerotic diseases
Membranous glomerulopathy	Nephrotic syndrome; idiopathic and secondary causes associated with HBV or cancers
Minimal change disease	Nephrotic syndrome, use of NSAIDs
IgA nephropathy	Hematuria and/or proteinuria with or without renal failure
Post-infectious glomerulonephritis	Hematuria and/or proteinuria with or without renal failure
ART-associated tubular injury	
Acute tubular necrosis	Use of TDF
Cristal nephropathy	Use of IDV and ATV
Acute or chronic interstitial nephritis	Use of ATV

HIVAN: HIV-associated nephropathy; HIVIC: HIV-associated immune complex kidney disease; TMA: Thrombotic microangiopathy; HCV: Hepatitis C virus; HBV: Hepatitis B virus; AKI: Acute kidney injury; GFR: Glomerular filtration rate; NSAID: Non-steroidal anti-inflammatory drug; ART: Antiretroviral therapy; TDF: Tenofovir disoproxil fumarate; IDV: Indinavir; ATV: Atazanavir.

showed that kidney function did not significantly change between HIV-infected subjects on cART with or without the regimen including tenofovir[51]. These differences between the two studies may be derived from the difference in the cumulative time for cART. The latter included only cART-naïve subjects, while the former included both cART-naïve and -experienced subjects. These disparate results on the TDF's nephrotoxicity remain conflicting, but a recent meta-analysis showed that the relevance of the adverse impact of TDF is mild, which may imply that restriction of "TDF use without regular monitoring of renal function" is not basically necessary[52]. Table 3 shows the known factors related to CKD in HIV-infected individuals.

HOW TO IDENTIFY HIV-INFECTED INDIVIDUALS AT HIGH RISK OF CKD

Measurement of albuminuria and proteinuria

The early diagnosis of renal illness in HIV patients is critical for preventing progression of prevalent renal injury and adding suitable treatment promptly. To help

Table 3 Traditional and human immunodeficiency virus-related factors associated with chronic kidney disease

Variables	Ref.
Black race	[34,35]
Older age	[9-12,22-26]
Low CD4 cell count	[10,11,23-25]
High HIV-RNA viral load	[10,11,23-25]
Diabetes mellitus	[9-12,22-26]
Hypertension	[9-12,22-26]
Hepatitis C virus coinfection	[11,25,31,37]
Proteinuria	[3,27,28,30]
Albuminuria	[3,29,55]
eGFR < 90 mL/min per 1.73 m^2	[10,12,23-25]
Elevation of urinary tubular markers	[56-64]
Use of TDF or ATV	[40-52]

eGFR: Estimated glomerular filtration rate; TDF: Tenofovir disoproxil fumarate; ATV: Atazanavir.

HIV experts with the identification of kidney disease, the IDSA guidelines suggest to conduct urinalysis and the evaluation of glomerular function at the diagnosis of HIV infection[3]. Although a dipstick test is a simple measure to use, it is unable to identify subclinical levels of urinary albumin. A comparison of a dipstick test and urinary protein concentration corrected for creatinine (PCR) in HIV-infected patients showed that the dipstick test could not detect individuals with mild or moderate proteinuria[53]. Therefore, the screening of proteinuria should be done according to PCR than dipstick test[54]. Ando *et al*[55] have found that a moderate to mild level of ACR (30 mg/g > ACR $\geqslant$ 10 mg/g) is an indicator of the incidence of CKD, likely emphasizing that the measurement of the ACR may be of higher relevance than that of the PCR for the detection of new CKD among HIV individuals.

Urinary low-molecular weight proteins for detection of tubular damage

The measurement of urinary biomarkers for identifying early tubular damage in HIV subjects, especially receiving cART has special importance. Some researchers measured urinary low-molecular weight proteins to examine whether patients on cART may have kidney tubular injury in the absence of renal dysfunction[56-62]. Approximately a quarter of HIV-infected patients on cART could have prevalent kidney tubular injury in the absence of renal dysfunction, probably resulting in a near future decrease in glomerular function and a higher emergence of urinary protein[63]. Also, Shlipak *et al*[64] indicated that novel urine biomarkers for tubular injury including KIM-1 and interleukin-18 identify risk for ensuing decrease in renal function in HIV-infected women in the Women's Interagency HIV Study cohort. Measuring urinary low-molecular-weight proteins could be helpful to the early detection of subjects, particular those who take tenofovir, who have high risk of definite CKD. In addition, Peralta *et al*[65] showed that some urinary indices of tubular damage are relevant to mortality in the Women's Interagency HIV Study.

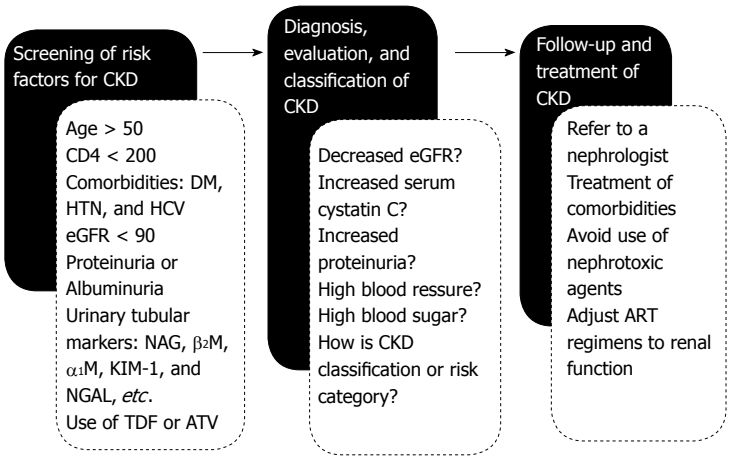

Figure 2 Flow chart for management of chronic kidney disease in human immunodeficiency virus-infected patients. This algorithm includes a clinical flow from screening of risk factors to identification, evaluation and follow-up care of patients for prevalent or incident CKD. CKD: Chronic kidney disease; HTN: Hypertension; DM: Diabetes mellitus; eGFR: Estimated glomerular filtration rate; NAG: N-acetyl-D-glucosaminidase; M: Microglobulin; ART: Antiretroviral therapy; ATV: Atazanavir; TDF: Tenofovir disoproxil fumarate.

Comprehensive assessment of risk factors

HIV-infected subjects usually possess several co-existing risks associated with renal illness, but the clinical impact of them on the emergence of chronic renal disease has remained unknown. A clinical model of predicting the incidence of CKD has been constructed. This model including age, CD4 cell count, diabetes, proteinuria, and a loss in glomerular function less than 90 mL/min per 1.73 m^2 were related to the incident CKD and predicted the development of CKD[66]. In addition, Scherzer *et al*[67] developed a point-based score to discriminate an HIV patient's risk of CKD over 5 years. Figure 2 shows a screening algorithm of the detection and practical management for patients infected with HIV.

CLINICAL SIGNIFICANCE OF CYSTATIN C FOR HIV-INFECTED PATIENTS

Cystatin C is an index for early glomerular dysfunction and may be a potential marker of chronic inflammation. Accordingly, cystatin C is something more than a marker of renal function. In fact, its elevation portends the incidence of heart and vessel diseases and all-cause mortality in the older people[68]. Moreover, it may be associated with a high likelihood of developing cancers[69,70]. However, serum cystatin C concentration are sometimes affected by non-renal factors including age, sex, race, and others[71]. The serum cystatin C level among HIV-infected patients could be greater in those with HIV infection than those without[72], as the serum cystatin C concentration is influenced by prevalent inflammatory diseases and the HIV viral replication[73]. Validation would be needed to confirm the utility of serum cystatin C level for assessing kidney function in HIV individuals.

MAHAGEMENT OF HIV-INFECTED INDIVIDUALS WITH CKD

A careful examination of the medical history and cumulative ART exposure is important for the past and further investigation of HIV individuals with CKD. The cART has beneficial effects on HIV-related diseases, such as HIVAN and HIVIC, but has adverse effects due to the long-term cumulative exposure. In addition to the metabolic changes of glucose and lipids induced by ART, some antiretroviral drugs may directly affect kidney function. Therefore, the detection of patients at high risk of CKD by the periodic measurements of ACR, PCR, and tubular biomarkers is most crucial with special reference to renal protection.

Further examination includes the follow-up of glomerular function, the test of urinary sediments, the ultrasonography of kidneys, and the pathological assessment of biopsied kidney tissues. Renal biopsy study is required for differentiating HIVAN from other glomerular nephtitis including diabetic nephropathy and HCV-related glomerulonephropathies, however, the risk of biopsy-related complications should be fully considered.

Adverse effects of cART on kidney are likely based on the overdosing of medications[74], and thus drug dosages have to be correctly altered according to eGFR. The major treatments for CKD may involve the strict control of high blood pressure, serum sugar and lipids. ART initiation in those having HIVAN is advocated, being independent on the control of $CD4^+$ cell count and HIV infection[75,76]. Prednisolone and ACE inhibitors could be useful for caring HIVAN[77,78].

KEY MESSAGE

The periodic examination of proteinuria or albuminuria combined with eGFR, serum cystatin C, and markers for renal tubular damage may enable the early detection of CKD in HIV-infected subjects.

ACKNOWLEDGMENTS

We thank all doctors in the Department of Infectious Diseases in Komagome General Hospital for their invaluable help with writing this review.

REFERENCES

1 **Lohse N**, Hansen AB, Pedersen G, Kronborg G, Gerstoft J,

Sørensen HT, Vaeth M, Obel N. Survival of persons with and without HIV infection in Denmark, 1995-2005. *Ann Intern Med* 2007; **146**: 87-95 [PMID: 17227932 DOI: 10.7326/0003-4819-146-2-200701160-00003]

2 **Mocroft A**, Ledergerber B, Katlama C, Kirk O, Reiss P, d'Arminio Monforte A, Knysz B, Dietrich M, Phillips AN, Lundgren JD. Decline in the AIDS and death rates in the EuroSIDA study: an observational study. *Lancet* 2003; **362**: 22-29 [PMID: 12853195 DOI: 10.1016/S0140-6736(03)13802-0]

3 **Lucas GM**, Ross MJ, Stock PG, Shlipak MG, Wyatt CM, Gupta SK, Atta MG, Wools-Kaloustian KK, Pham PA, Bruggeman LA, Lennox JL, Ray PE, Kalayjian RC. Clinical practice guideline for the management of chronic kidney disease in patients infected with HIV: 2014 update by the HIV Medicine Association of the Infectious Diseases Society of America. *Clin Infect Dis* 2014; **59**: e96-e138 [PMID: 25234519 DOI: 10.1093/cid/ciu617]

4 **Eggers PW**, Kimmel PL. Is there an epidemic of HIV Infection in the US ESRD program? *J Am Soc Nephrol* 2004; **15**: 2477-2485 [PMID: 15339998 DOI: 10.1097/01.ASN.0000138546.53152]

5 **Schwartz EJ**, Szczech LA, Ross MJ, Klotman ME, Winston JA, Klotman PE. Highly active antiretroviral therapy and the epidemic of HIV+ end-stage renal disease. *J Am Soc Nephrol* 2005; **16**: 2412-2420 [PMID: 15987747 DOI: 10.1681/ASN.2005040340]

6 **Lucas GM**, Mehta SH, Atta MG, Kirk GD, Galai N, Vlahov D, Moore RD. End-stage renal disease and chronic kidney disease in a cohort of African-American HIV-infected and at-risk HIV-seronegative participants followed between 1988 and 2004. *AIDS* 2007; **21**: 2435-2443 [PMID: 18025880]

7 **Gardner LI**, Holmberg SD, Williamson JM, Szczech LA, Carpenter CC, Rompalo AM, Schuman P, Klein RS. Development of proteinuria or elevated serum creatinine and mortality in HIV-infected women. *J Acquir Immune Defic Syndr* 2003; **32**: 203-209 [PMID: 12571531]

8 **Palella FJ**, Baker RK, Moorman AC, Chmiel JS, Wood KC, Brooks JT, Holmberg SD. Mortality in the highly active antiretroviral therapy era: changing causes of death and disease in the HIV outpatient study. *J Acquir Immune Defic Syndr* 2006; **43**: 27-34 [PMID: 16878047 DOI: 10.1097/01.qai.0000233310.90484.16]

9 **Ryom L**, Kirk O, Lundgren JD, Reiss P, Pedersen C, De Wit S, Buzunova S, Gasiorowski J, Gatell JM, Mocroft A. Advanced chronic kidney disease, end-stage renal disease and renal death among HIV-positive individuals in Europe. *HIV Med* 2013; **14**: 503-508 [PMID: 23590641 DOI: 10.1111/hiv.12038]

10 **Cheung CY**, Wong KM, Lee MP, Liu YL, Kwok H, Chung R, Chau KF, Li CK, Li CS. Prevalence of chronic kidney disease in Chinese HIV-infected patients. *Nephrol Dial Transplant* 2007; **22**: 3186-3190 [PMID: 17575315 DOI: 10.1093/ndt/gfm350]

11 **Yanagisawa N**, Ando M, Ajisawa A, Imamura A, Suganuma A, Tsuchiya K, Nitta K. Clinical characteristics of kidney disease in Japanese HIV-infected patients. *Nephron Clin Pract* 2011; **118**: c285-c291 [PMID: 21212692 DOI: 10.1159/000322278]

12 **Mocroft A**, Kirk O, Gatell J, Reiss P, Gargalianos P, Zilmer K, Beniowski M, Viard JP, Staszewski S, Lundgren JD. Chronic renal failure among HIV-1-infected patients. *AIDS* 2007; **21**: 1119-1127 [PMID: 17502722 DOI: 10.1097/QAD.0b013e3280f774ee]

13 **Fernando SK**, Finkelstein FO, Moore BA, Weissman S. Prevalence of chronic kidney disease in an urban HIV infected population. *Am J Med Sci* 2008; **335**: 89-94 [PMID: 18277114 DOI: 10.1097/MAJ.0b013e31812e6b34]

14 **Taal MW**, Brenner BM. Predicting initiation and progression of chronic kidney disease: Developing renal risk scores. *Kidney Int* 2006; **70**: 1694-1705 [PMID: 16969387 DOI: 10.1038/sj.ki.5001794]

15 **Yamagata K**, Iseki K, Nitta K, Imai H, Iino Y, Matsuo S, Makino H, Hishida A. Chronic kidney disease perspectives in Japan and the importance of urinalysis screening. *Clin Exp Nephrol* 2008; **12**: 1-8 [PMID: 18175065 DOI: 10.1007/s10157-007-0010-9]

16 **Ripley E**. Complementary effects of angiotensin-converting enzyme inhibitors and angiotensin receptor blockers in slowing the progression of chronic kidney disease. *Am Heart J* 2009; **157**: S7-S16 [PMID: 19450722 DOI: 10.1016/j.ahj.2009.04.008]

17 **Gansevoort RT**, Bakker SJ, de Jong PE. Early detection of progressive chronic kidney disease: is it feasible? *J Am Soc Nephrol* 2006; **17**: 1218-1220 [PMID: 16611711 DOI: 10.1681/ASN.2006030247]

18 **Chen N**, Hsu CC, Yamagata K, Langham R. Challenging chronic kidney disease: experience from chronic kidney disease prevention programs in Shanghai, Japan, Taiwan and Australia. *Nephrology (Carlton)* 2010; **15** Suppl 2: 31-36 [PMID: 20586945 DOI: 10.1111/j.1440-1797.2010.01309.x]

19 **Peralta CA**, Shlipak MG, Judd S, Cushman M, McClellan W, Zakai NA, Safford MM, Zhang X, Muntner P, Warnock D. Detection of chronic kidney disease with creatinine, cystatin C, and urine albumin-to-creatinine ratio and association with progression to end-stage renal disease and mortality. *JAMA* 2011; **305**: 1545-1552 [PMID: 21482744 DOI: 10.1001/jama.2011.468]

20 **Levey AS**, de Jong PE, Coresh J, El Nahas M, Astor BC, Matsushita K, Gansevoort RT, Kasiske BL, Eckardt KU. The definition, classification, and prognosis of chronic kidney disease: a KDIGO Controversies Conference report. *Kidney Int* 2011; **80**: 17-28 [PMID: 21150873 DOI: 10.1038/ki.2010.483]

21 **Levin A**, Stevens PE. Summary of KDIGO 2012 CKD Guideline: behind the scenes, need for guidance, and a framework for moving forward. *Kidney Int* 2014; **85**: 49-61 [PMID: 24284513 DOI: 10.1038/ki.2013.444]

22 **Szczech LA**, Hoover DR, Feldman JG, Cohen MH, Gange SJ, Goozé L, Rubin NR, Young MA, Cai X, Shi Q, Gao W, Anastos K. Association between renal disease and outcomes among HIV-infected women receiving or not receiving antiretroviral therapy. *Clin Infect Dis* 2004; **39**: 1199-1206 [PMID: 15486845 DOI: 10.1086/424013]

23 **Szczech LA**, Gange SJ, van der Horst C, Bartlett JA, Young M, Cohen MH, Anastos K, Klassen PS, Svetkey LP. Predictors of proteinuria and renal failure among women with HIV infection. *Kidney Int* 2002; **61**: 195-202 [PMID: 11786101 DOI: 10.1046/j.1523-1755.2002.00094.x]

24 **Gupta SK**, Mamlin BW, Johnson CS, Dollins MD, Topf JM, Dubé MP. Prevalence of proteinuria and the development of chronic kidney disease in HIV-infected patients. *Clin Nephrol* 2004; **61**: 1-6 [PMID: 14964451 DOI: 10.5414/CNP61001]

25 **Fulop T**, Olivier J, Meador RS, Hall J, Islam N, Mena L, Henderson H, Schmidt DW. Screening for chronic kidney disease in the ambulatory HIV population. *Clin Nephrol* 2010; **73**: 190-196 [PMID: 20178717 DOI: 10.5414/CNP73190]

26 **Wyatt CM**, Winston JA, Malvestutto CD, Fishbein DA, Barash I, Cohen AJ, Klotman ME, Klotman PE. Chronic kidney disease in HIV infection: an urban epidemic. *AIDS* 2007; **21**: 2101-2103 [PMID: 17885301 DOI: 10.1097/QAD.0b013e3282ef1bb4]

27 **Wyatt CM**, Hoover DR, Shi Q, Seaberg E, Wei C, Tien PC, Karim R, Lazar J, Young MA, Cohen MH, Klotman PE, Anastos K. Microalbuminuria is associated with all-cause and AIDS mortality in women with HIV infection. *J Acquir Immune Defic Syndr* 2010; **55**: 73-77 [PMID: 20098331 DOI: 10.1097/QAI.0b013e3181cc1070]

28 **Choi AI**, Li Y, Deeks SG, Grunfeld C, Volberding PA, Shlipak MG. Association between kidney function and albuminuria with cardiovascular events in HIV-infected persons. *Circulation* 2010; **121**: 651-658 [PMID: 20100969 DOI: 10.1161/CIRCULATIONAHA.109.898585]

29 **Gravemann S**, Brinkkoetter PT, Vehreschild JJ, Franke B, Ehren K, Bünemann E, Orbach H, Weiß V, Hellmich M, Benzing T, Fätkenheuer G. Low-grade proteinuria is highly prevalent in HIV-positive patients on antiretroviral treatment. *AIDS* 2014; **28**: 1783-1789 [PMID: 24841129 DOI: 10.1097/QAD.0000000000000324]

30 **Yanagisawa N**, Muramatsu T, Yamamoto Y, Tsuchiya K, Nitta K, Ajisawa A, Fukutake K, Ando M. Classification of human immunodeficiency virus-infected patients with chronic kidney disease using a combination of proteinuria and estimated glomerular filtration rate. *Clin Exp Nephrol* 2014; **18**: 600-605 [PMID: 23955325 DOI: 10.1007/s10157-013-0853-1]

31 **Ross MJ**, Klotman PE. Recent progress in HIV-associated nephropathy. *J Am Soc Nephrol* 2002; **13**: 2997-3004 [PMID: 12444220 DOI: 10.1097/01.ASN.0000040750.40907.99]

32 **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]

33 **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]

34 **Freedman BI**, Soucie JM, Stone SM, Pegram S. Familial clustering of end-stage renal disease in blacks with HIV-associated nephropathy. *Am J Kidney Dis* 1999; **34**: 254-258 [PMID: 10430971 DOI: 10.1016/S0272-6386(99)70352-5]

35 **Szczech LA**, Gupta SK, Habash R, Guasch A, Kalayjian 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]

36 **Ryom L**, Mocroft A, Kirk O, Ross M, Reiss P, Fux CA, Morlat P, Moranne O, Smith C, El-Sadr W, Law M, Lundgren JD. Predictors of advanced chronic kidney disease and end-stage renal disease in HIV-positive persons. *AIDS* 2014; **28**: 187-199 [PMID: 24361680 DOI: 10.1097/QAD.0000000000000042]

37 **Vallet-Pichard A**, Pol S. Natural history and predictors of severity of chronic hepatitis C virus (HCV) and human immunodeficiency virus (HIV) co-infection. *J Hepatol* 2006; **44**: S28-S34 [PMID: 16343684 DOI: 10.1016/j.jhep.2005.11.008]

38 **Liangpunsakul S**, Chalasani N. Relationship between hepatitis C and microalbuminuria: results from the NHANES III. *Kidney Int* 2005; **67**: 285-290 [PMID: 15610253 DOI: 10.1111/j.1523-1755.2005.00080.x]

39 **Tsui JI**, Vittinghoff E, Shlipak MG, O'Hare AM. Relationship between hepatitis C and chronic kidney disease: results from the Third National Health and Nutrition Examination Survey. *J Am Soc Nephrol* 2006; **17**: 1168-1174 [PMID: 16524948 DOI: 10.1681/ASN.2005091006]

40 **Daugas E**, Rougier JP, Hill G. HAART-related nephropathies in HIV-infected patients. *Kidney Int* 2005; **67**: 393-403 [PMID: 15673287 DOI: 10.1111/j.1523-1755.2005.67096.x]

41 **Kumar N**, Perazella MA. Differentiating HIV-associated nephropathy from antiretroviral drug-induced nephropathy: a clinical challenge. *Curr HIV/AIDS Rep* 2014; **11**: 202-211 [PMID: 24924830 DOI: 10.1007/s11904-014-0209-9]

42 **Brewster UC**, Perazella MA. Acute interstitial nephritis associated with atazanavir, a new protease inhibitor. *Am J Kidney Dis* 2004; **44**: e81-e84 [PMID: 15492941 DOI: 10.1053/j.ajkd.2004.07.017]

43 **Izzedine H**, M'rad MB, Bardier A, Daudon M, Salmon D. Atazanavir crystal nephropathy. *AIDS* 2007; **21**: 2357-2358 [PMID: 18090291 DOI: 10.1097/QAD.0b013e3282f17503]

44 **Viglietti D**, Verine J, De Castro N, Scemla A, Daudon M, Glotz D, Pillebout E. Chronic interstitial nephritis in an HIV type-1-infected patient receiving ritonavir-boosted atazanavir. *Antivir Ther* 2011; **16**: 119-121 [PMID: 21311116 DOI: 10.3851/IMP1700]

45 **Kanzaki G**, Tsuboi N, Miyazaki Y, Yokoo T, Utsunomiya Y, Hosoya T. Diffuse tubulointerstitial nephritis accompanied by renal crystal formation in an HIV-infected patient undergoing highly active antiretroviral therapy. *Intern Med* 2012; **51**: 1543-1548 [PMID: 22728488 DOI: 10.2169/internalmedicine.51.7093]

46 **Schmid S**, Opravil M, Moddel M, Huber M, Pfammatter R, Keusch G, Ambuhl P, Wuthrich RP, Moch H, Varga Z. Acute interstitial nephritis of HIV-positive patients under atazanavir and tenofovir therapy in a retrospective analysis of kidney biopsies. *Virchows Arch* 2007; **450**: 665-670 [PMID: 17464512 DOI: 10.1007/s00428-007-0418-3]

47 **Izzedine H**, Harris M, Perazella MA. The nephrotoxic effects of HAART. *Nat Rev Nephrol* 2009; **5**: 563-573 [PMID: 19776778 DOI: 10.1038/nrneph.2009.142]

48 **Izzedine H**, Hulot JS, Vittecoq D, Gallant JE, Staszewski S, Launay-Vacher V, Cheng A, Deray G. Long-term renal safety of tenofovir disoproxil fumarate in antiretroviral-naive HIV-1-infected patients. Data from a double-blind randomized active-controlled multicentre study. *Nephrol Dial Transplant* 2005; **20**: 743-746 [PMID: 15741212 DOI: 10.1093/ndt/gfh658]

49 **Gallant JE**, Parish MA, Keruly JC, Moore RD. Changes in renal function associated with tenofovir disoproxil fumarate treatment, compared with nucleoside reverse-transcriptase inhibitor treatment. *Clin Infect Dis* 2005; **40**: 1194-1198 [PMID: 15791522 DOI: 10.1086/428840]

50 **Gallant JE**, Moore RD. Renal function with use of a tenofovir-containing initial antiretroviral regimen. *AIDS* 2009; **23**: 1971-1975 [PMID: 19696652 DOI: 10.1097/QAD.0b013e32832c96e9]

51 **Zimmermann AE**, Pizzoferrato T, Bedford J, Morris A, Hoffman R, Braden G. Tenofovir-associated acute and chronic kidney disease: a case of multiple drug interactions. *Clin Infect Dis* 2006; **42**: 283-290 [PMID: 16355343 DOI: 10.1086/499048]

52 **Cooper RD**, Wiebe N, Smith N, Keiser P, Naicker S, Tonelli M. Systematic review and meta-analysis: renal safety of tenofovir disoproxil fumarate in HIV-infected patients. *Clin Infect Dis* 2010; **51**: 496-505 [PMID: 20673002 DOI: 10.1086/655681]

53 **Siedner MJ**, Atta MG, Lucas GM, Perazella MA, Fine DM. Poor validity of urine dipstick as a screening tool for proteinuria in HIV-positive patients. *J Acquir Immune Defic Syndr* 2008; **47**: 261-263 [PMID: 18223364 DOI: 10.1097/QAI.0b013e31815ac4ad]

54 **Levey AS**, Coresh J, Balk E, Kausz AT, Levin A, Steffes MW, Hogg RJ, Perrone RD, Lau J, Eknoyan G. National Kidney Foundation practice guidelines for chronic kidney disease: evaluation, classification, and stratification. *Ann Intern Med* 2003; **139**: 137-147 [PMID: 12859163 DOI: 10.7326/0003-4819-139-2-200307150-00013]

55 **Ando M**, Yanagisawa N, Ajisawa A, Tsuchiya K, Nitta K. Urinary albumin excretion within the normal range is an independent risk for near-term development of kidney disease in HIV-infected patients. *Nephrol Dial Transplant* 2011; **26**: 3923-3929 [PMID: 21427068 DOI: 10.1093/ndt/gfr129]

56 **Kabanda A**, Vandercam B, Bernard A, Lauwerys R, van Ypersele de Strihou C. Low molecular weight proteinuria in human immunodeficiency virus-infected patients. *Am J Kidney Dis* 1996; **27**: 803-808 [PMID: 8651244 DOI: 10.1016/S0272-6386(96)90517-X]

57 **Gatanaga H**, Tachikawa N, Kikuchi Y, Teruya K, Genka I, Honda M, Tanuma J, Yazaki H, Ueda A, Kimura S, Oka S. Urinary beta2-microglobulin as a possible sensitive marker for renal injury caused by tenofovir disoproxil fumarate. *AIDS Res Hum Retroviruses* 2006; **22**: 744-748 [PMID: 16910829 DOI: 10.1089/aid.2006.22.744]

58 **Kinai E**, Hanabusa H. Progressive renal tubular dysfunction associated with long-term use of tenofovir DF. *AIDS Res Hum Retroviruses* 2009; **25**: 387-394 [PMID: 19361280 DOI: 10.1089/aid.2008.0202]

59 **Labarga P**, Barreiro P, Martin-Carbonero L, Rodriguez-Novoa S, Solera C, Medrano J, Rivas P, Albalater M, Blanco F, Moreno V, Vispo E, Soriano V. Kidney tubular abnormalities in the absence of impaired glomerular function in HIV patients treated with tenofovir. *AIDS* 2009; **23**: 689-696 [PMID: 19262355 DOI: 10.1097/QAD.0b013e3283262a64]

60 **Hall AM**, Edwards SG, Lapsley M, Connolly JO, Chetty K, O'Farrell S, Unwin RJ, Williams IG. Subclinical tubular injury in HIV-infected individuals on antiretroviral therapy: a cross-sectional analysis. *Am J Kidney Dis* 2009; **54**: 1034-1042 [PMID: 19783343 DOI: 10.1053/j.ajkd.2009.07.012]

61 **Jaafar A**, Séronie-Vivien S, Malard L, Massip P, Chatelut E, Tack I. Urinary cystatin C can improve the renal safety follow-up of tenofovir-treated patients. *AIDS* 2009; **23**: 257-259 [PMID: 19098496 DOI: 10.1097/QAD.0b013e328314e382]

62 **Paragas N**, Nickolas TL, Wyatt C, Forster CS, Sise M, Morgello S, Jagla B, Buchen C, Stella P, Sanna-Cherchi S, Carnevali ML, Mattei S, Bovino A, Argentiero L, Magnano A, Devarajan P, Schmidt-Ott KM, Allegri L, Klotman P, D'Agati V, Gharavi AG, Barasch J. Urinary NGAL marks cystic disease in HIV-associated

nephropathy. *J Am Soc Nephrol* 2009; **20**: 1687-1692 [PMID: 19628667 DOI: 10.1681/ASN.2009010065]

63 **Ando M**, Yanagisawa N, Ajisawa A, Tsuchiya K, Nitta K. Kidney tubular damage in the absence of glomerular defects in HIV-infected patients on highly active antiretroviral therapy. *Nephrol Dial Transplant* 2011; **26**: 3224-3229 [PMID: 21372250 DOI: 10.1093/ndt/gfr020]

64 **Shlipak MG**, Scherzer R, Abraham A, Tien PC, Grunfeld C, Peralta CA, Devarajan P, Bennett M, Butch AW, Anastos K, Cohen MH, Nowicki M, Sharma A, Young MA, Sarnak MJ, Parikh CR. Urinary markers of kidney injury and kidney function decline in HIV-infected women. *J Acquir Immune Defic Syndr* 2012; **61**: 565-573 [PMID: 23023103 DOI: 10.1097/QAI.0b013e3182737706]

65 **Peralta C**, Scherzer R, Grunfeld C, Abraham A, Tien P, Devarajan P, Bennett M, Butch A, Anastos K, Cohen M, Nowicki M, Sharma A, Young M, Sarnak M, Parikh C, Shlipak M. Urinary biomarkers of kidney injury are associated with all-cause mortality in the Women's Interagency HIV Study (WIHS). *HIV Med* 2014; **15**: 291-300 [PMID: 24313986 DOI: 10.1111/hiv.12113]

66 **Ando M**, Yanagisawa N, Ajisawa A, Tsuchiya K, Nitta K. A simple model for predicting incidence of chronic kidney disease in HIV-infected patients. *Clin Exp Nephrol* 2011; **15**: 242-247 [PMID: 21246239 DOI: 10.1007/s10157-010-0393-x]

67 **Scherzer R**, Gandhi M, Estrella MM, Tien PC, Deeks SG, Grunfeld C, Peralta CA, Shlipak MG. A chronic kidney disease risk score to determine tenofovir safety in a prospective cohort of HIV-positive male veterans. *AIDS* 2014; **28**: 1289-1295 [PMID: 24922479 DOI: 10.1097/QAD.0000000000000258]

68 **Shlipak MG**, Wassel Fyr CL, Chertow GM, Harris TB, Kritchevsky SB, Tylavsky FA, Satterfield S, Cummings SR, Newman AB, Fried LF. Cystatin C and mortality risk in the elderly: the health, aging, and body composition study. *J Am Soc Nephrol* 2006; **17**: 254-261 [PMID: 16267155 DOI: 10.1681/ASN.2005050545]

69 **Yanagisawa N**, Ando M, Tsuchiya K, Nitta K. HIV-infected men with elevated level of serum cystatin C have a high likelihood of developing cancers. *J Antivir Antiretrovir* 2012; **4**: 38-42 [DOI: 10.4172/jaa.1000044]

70 **Yanagisawa N**, Ando M, Tsuchiya K, Nitta K. Impact of cystatin C elevation and albuminuria on probability of adverse outcomes in HIV-infected men receiving HAART. *Clin Nephrol* 2013; **79**: 362-369 [PMID: 23253904 DOI: 10.5414/CN107668]

71 **Köttgen A**, Selvin E, Stevens LA, Levey AS, Van Lente F, Coresh J. Serum cystatin C in the United States: the Third National Health and Nutrition Examination Survey (NHANES III). *Am J Kidney Dis* 2008; **51**: 385-394 [PMID: 18295054 DOI: 10.1053/j.ajkd.2007.11.019]

72 **Odden MC**, Scherzer R, Bacchetti P, Szczech LA, Sidney S, Grunfeld C, Shlipak MG. Cystatin C level as a marker of kidney function in human immunodeficiency virus infection: the FRAM study. *Arch Intern Med* 2007; **167**: 2213-2219 [PMID: 17998494 DOI: 10.1001/archinte.167.20.2213]

73 **Knight EL**, Verhave JC, Spiegelman D, Hillege HL, de Zeeuw D, Curhan GC, de Jong PE. Factors influencing serum cystatin C levels other than renal function and the impact on renal function measurement. *Kidney Int* 2004; **65**: 1416-1421 [PMID: 15086483 DOI: 10.1111/j.1523-1755.2004.00517.x]

74 **Choi AI**, Rodriguez RA, Bacchetti P, Volberding PA, Havlir D, Bertenthal D, Bostrom A, O'Hare AM. Low rates of antiretroviral therapy among HIV-infected patients with chronic kidney disease. *Clin Infect Dis* 2007; **45**: 1633-1639 [PMID: 18190326 DOI: 10.1086/523729]

75 **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]

76 **Hammer SM**, Eron JJ, Reiss P, Schooley RT, Thompson MA, Walmsley S, Cahn P, Fischl MA, Gatell JM, Hirsch MS, Jacobsen DM, Montaner JS, Richman DD, Yeni PG, Volberding PA. Antiretroviral treatment of adult HIV infection: 2008 recommendations of the International AIDS Society-USA panel. *JAMA* 2008; **300**: 555-570 [PMID: 18677028 DOI: 10.1001/jama.300.5.555]

77 **Hammer SM**, Eron JJ, Reiss P, Schooley RT, Thompson MA, Walmsley S, Cahn P, Fischl MA, Gatell JM, Hirsch MS, Jacobsen DM, Montaner JS, Richman DD, Yeni PG, Volberding PA. Antiretroviral treatment of adult HIV infection: 2008 recommendations of the International AIDS Society-USA panel. *JAMA* 2008; **300**: 555-570 [PMID: 18677028 DOI: 10.1001/jama.300.5.555]

78 **Wei A**, Burns GC, Williams BA, Mohammed NB, Visintainer P, Sivak SL. Long-term renal survival in HIV-associated nephropathy with angiotensin-converting enzyme inhibition. *Kidney Int* 2003; **64**: 1462-1471 [PMID: 12969167 DOI: 10.1046/j.1523-1755.2003.00230.x]

P- Reviewer: Bellomo G, Bhimma R, Kusztal M, Tanaka H
S- Editor: Tian YL **L- Editor**: A **E- Editor**: Yan JL

World Journal of Nephrology

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Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.5527/wjn.v4.i3.396

World J Nephrol 2015 July 6; 4(3): 396-405
ISSN 2220-6124 (online)

ORIGINAL ARTICLE

Retrospective Study

Urethral complications after tension-free vaginal tape procedures: A surgical management case series

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Author contributions: Larsson PG and Jarlshammar B performed the surgical management of all cases; Sergouniotis F performed the follow-up urethrocystoscopy; all authors contributed to the drafting and approval of the manuscript.

Ethics approval statement: No ethical approval was required for this case-report series after contacting the Gothenburg Regional Ethical Committee.

Informed consent statement: All study participants provided informed verbal consent prior to the study enrollment, which was obtained at the visits for postoperative follow-up urethrocystoscopy following the tape removal. The patients encouraged us to report the complications, to prevent others from suffering.

Conflict-of-interest statement: All the authors declare no conflict of interest.

Data sharing statement: No additional data are available.

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Received: December 18, 2014
Peer-review started: December 18, 2014
First decision: January 20, 2015
Revised: February 25, 2015
Accepted: April 10, 2015
Article in press: April 14, 2015
Published online: July 6, 2015

Abstract

AIM: To analyze the clinical features, diagnostic modalities, and the surgical management of urethral complications after tension-free vaginal tape procedures.

METHODS: This study encompasses a retrospective review of nine patients presented with urethral complications after midurethral sling procedures. The patients underwent the procedures during a period from 1999 to 2012 in three different regional hospitals in the southwest part of Sweden. The time from sling placement to diagnosis, the risk factors, clinical features, diagnosis, surgical management, and functional outcome are presented. The presenting symptoms were described as either early onset (< 12 mo) or late onset (> 12 mo) according to when they were first reported.

RESULTS: Eight cases of urethral erosion and one case of bladder-neck erosion were detected. The mean interval for diagnoses of the erosions ranged from 3 mo to 11 years. The most common presenting symptoms included *de novo* urgency with or without incontinence (7/9 patients), urinary retention/voiding dysfunction (4/9 patients), urethritis (4/9 patients), relapse of stress-incontinence (3/9 patients), recurrent urinary tract infections (5/9 patients), and hematuria (1/9 patient). In most cases, voiding dysfunction and urethritis occurred early after the operation. The surgical management applied in most cases was transurethral resection of the intraurethral part of the mesh. The removal of the intraurethral mesh resulted in improvement or complete cure of urgency symptoms

in 5/7 patients with urgency. Four patients were reoperated with a new stress-incontinence surgery, one with laparoscopic Burch, and three with retropubic tension-free vaginal tape procedures.

CONCLUSION: Urethral complications should be suspected in the case of *de novo* urgency and relapse of stress-incontinence. Transurethral excision of the intraurethral mesh is the recommended treatment.

Key words: Bladder neck erosion; Complications; Intraurethral mesh; Stress incontinence; Tension-free vaginal tape; Urethral erosion

Core tip: We present eight cases of urethral erosion and one of bladder neck erosion after tension-free vaginal tape procedures. The mean interval for complication diagnoses ranged from 3 mo to 11 years. The clinical profile of the complications included *de novo* urgency, voiding dysfunction, urethritis, relapse of stress incontinence, recurrent urinary tract infections, and hematuria. It is important to consider urethral complications in the postoperative follow-up if these symptoms occur. A control urethrocystoscopy is important for the diagnosis. The transurethral excision of the intraurethral part of the mesh is recommended as the treatment of choice.

Sergouniotis F, Jarlshammar B, Larsson PG. Urethral complications after tension-free vaginal tape procedures: A surgical management case series. *World J Nephrol* 2015; 4(3): 396-405 Available from: URL: http://www.wjgnet.com/2220-6124/full/v4/i3/396.htm DOI: http://dx.doi.org/10.5527/wjn.v4.i3.396

INTRODUCTION

Stress urinary incontinence (SUI) is a significant and common problem in women. SUI is defined as an involuntary leakage of urine on effort, straining, or coughing[1]. Some of the potential causes of SUI include childbirth, older age, obesity, chronic bronchitis, and chronic constipation[2]. Although relatively mild, symptoms often have a negative effect on the patient quality of life in terms of physical and social well-being.

In 1996, Petros and Ulmsten introduced a new, minimally invasive sling procedure for the treatment of SUI. The new procedure applied a tension-free vaginal tape (TVT) under the mid-urethra. The midurethral sling reinforced the weakened pubourethral ligaments and recreated the "hammock" support of the lax anterior vaginal wall and endopelvic fascia[3-5]. The advantage of the TVT procedure is that it is minimally invasive and can be performed under local or regional anesthesia as outpatient surgery.

The TVT procedure has undergone numerous modifications and improvements since its initial introduction. In 2001, De lorme *et al*[6] described a surgical approach where the polypropylene suburethral sling was placed between the two obturator foramina. The goal was to maintain the same position of the sling under the mid-urethra while reducing the risk of complications associated with the blind passage in the retropubic space, such as bladder, bowel, and iliac vessel injury. The procedure is an "outside-in" technique. The technique involves a blind percutaneous introduction of a curved trocar lateral to the vagina, around the inferior ischiopubic ramus, through the obturator foramen and into the anterior vaginal wall at the midurethral level.

A later modification of the Delorme technique was described by de Leval[7] in 2003. De Leval designed a trans-obturator inside-out procedure (TVT-O), a technically more convenient method than the outside-in technique. The surgeon does not need to use an index finger to guide the needle coming from the outside. Furthermore, a "wing-guide" is used during the dissection of the paraurethral tunnel in order to protect the urinary tract[5].

In 2006, a third generation of midurethral slings was introduced with the development of single incision mini-slings. The new method applied a shorter polypropylene mesh with a single suburethral incision. Because the technique avoided the need for blind passage through the retropubic or obturator spaces, it aimed to reduce the complications and increase the safety of the procedure[8-11]. An additional benefit of the procedure was that it could also be performed under local anesthesia.

Since 1996 when it was first introduced, the TVT technique has become the gold standard of minimally invasive surgery in the treatment of stress incontinence. The efficacy of the TVT procedure has been comprehensively documented in the literature. However, TVT-associated complications and their management are less well understood[12-15].

Perioperative perforation of the bladder is a common complication associated with retropubic TVT sling procedures. In a Swedish study that evaluated the results of over 700 patients treated with TVT, the frequency rate of bladder perforations was 1.7%[16]. Urethral erosion of the mesh is a rare complication after a sling operation and may present with various symptoms. The complication occurs when the sling placed outside the urinary tract gradually erodes into the urethra[17]. The first described case of urethral erosion after a TVT operation was published in 2001[18]. Since then, there have been a limited number of reports and case series published on this unusual complication[12,19-26].

The objective of this study was to evaluate the clinical features, physical findings, and diagnostic procedures of postoperative urethral complications. Furthermore, the effectiveness of our management approach in controlling the condition, as well as the outcomes after treatment, were addressed.

MATERIALS AND METHODS

Our first case of urethral complication was noted in 2006.

We had examined a woman *via* urethrocystoscopy due to postoperative urinary tract discomfort, including dysuria and frequency and voiding dysfunctions. The urethrocystoscopy revealed an intraurethral section of the displaced mesh. Following the initial case, all women with dysuria, frequency and voiding dysfunctions, and *de novo* urgency after TVT-procedures underwent urethrocystoscopy.

Herein, we performed an analysis of women with urethral complications after synthetic midurethral sling procedures. The patients' medical records were reviewed retrospectively. The time between sling placement to diagnosis, risk factors, presenting symptoms, diagnostic procedures, surgical management, and postoperative outcomes were recorded. A control urethrocystoscopy was performed on the patients with remaining symptoms.

Five different surgeons from three different regional hospitals in the southwest part of Sweden had performed the sling procedures over a 13-year period (1999-2012) in women diagnosed with intraurethral displaced tape. The presenting symptoms were described as either early onset (< 12 mo postoperatively) or late onset (> 12 mo postoperatively), based on when the symptoms were first reported.

The manufacturers' standard recommendations were followed when placing the mesh, so that the mesh laid tension-free under the urethra. All the surgeons were very experienced and had performed more than 200 TVT procedures. Vaginal sonography was applied as part of the routine postoperative control.

RESULTS

In the period from 1999-2012, nine cases of intraurethrally displaced mesh after midurethral sling procedures were identified. In all nine cases, the mesh was surgically removed at our clinic.

Case 1

A 46-year-old woman was operated upon with TVT-O for genuine SUI in May 2006. The patient was 2-para, had a hormone intrauterine device (IUD; Mirena®) and a body mass index (BMI) of 23.0. The patient also had a medical history of chronic cough, appendectomy, and her mother was operated on with Burch-plastic. During the procedure, the band was doubled with an Allis clamp in a little loop in order to avoid tension of the sling.

Postoperatively, the patient developed urinary retention and had to use intermittent catheterization. The patient complained of pain in the urethra and was treated for urinary tract infection (UTI). An urethrocystoscopy performed 19 d after the surgery showed swelling over the bladder neck; hence, a suprapubic catheter was applied. Two weeks later, the patient had spontaneous voiding, and the suprapubic catheter was removed.

Five months after the surgery, the patient complained of *de novo* urgency, voiding dysfunction, urethral pain, and dyspareunia. An urethrocystoscopy showed an erosion of the sling directly across the urethra. A second surgery, 5.5 mo after the primary TVT operation, was performed using a transvaginal urethroplasty, and the tape was removed to restore the urethra. A suprapubic catheter was applied for three weeks.

At the three-month short-term follow-up, the patient had improved voiding, no urgency, fewer frequency symptoms, and little vaginal pain. Over the long-term, the patient had a relapse of SUI and *de novo* urge incontinence. The former was first treated with two paraurethral silicon injections and then with laparoscopic Burch colposuspension. The latter was treated with anticholinergics.

Case 2

A 55-year-old postmenopausal woman underwent a TVT-O for mixed incontinence. The patient was 2-para, obese with a BMI of 40.7, and on medication for hypertension and type Ⅱ diabetes. The TVT-O procedure was performed without complications. Perioperatively, it was noticed that she had a short urethra. The mesh was placed approximately 1 cm from meatus.

During the first postoperative month, the patient experienced worsening of her urgency symptoms, together with urethral pain. The patient was treated with antibiotics for urethritis and had two urethrocystoscopy procedures without any signs of erosion. Due to voiding difficulties, the patient received urethral dilatations with a slight reduction in her symptoms, and she was scheduled for further dilatations. After urethrocystoscopy, 3 mo postoperatively, it was noticed that the patient had urethral erosion.

The patient was reoperated upon 3 mo and 9 d after the sling application. The sling was cut outside the urethra, excised transurethrally, and the defect in the urethra was closed. A suprapubic catheter was applied for three weeks.

During the short-term follow-up, the patient developed a local vaginal infection and UTI; however, the urgency symptoms improved. Over the long-term follow-up, the patient had a worsening of her urgency and slight relapse of SUI. The patient was treated with anticholinergics for her urgency. During this time, the patient underwent an operation for disc herniation in the lower spine and had a gastric bypass. The patient was later offered polyacrylamide injection treatment for the stress component of her incontinence but felt that she needed no further operative treatment.

Case 3

A 60-year-old woman, 3-para, with a normal BMI of 24.1, ulcerative colitis, and cardiac arrhythmia underwent a TVT-O in 2005 for genuine SUI with no complication. The cardiac arrhythmia was later treated with a pacemaker. The patient had voiding dysfunction shortly after the TVT-O operation and used intermittent catheters for 1 mo.

Twenty months after the TVT-O procedure, the patient developed *de novo* urge incontinence and local

vaginal pain, and was prescribed antibiotics for her UTI. An urethrocystoscopy showed a small part of the mesh in the urethra. A new operation was performed two years postoperatively, with transurethral excision of the intraurethral part of the mesh. No urethroplasty was needed. A suprapubic catheter was placed for 10 d, and antibiotics were given for two weeks.

In the long-term follow-up, the patient had no urgency symptoms, no vaginal pain, and no SUI.

Case 4

A 43-year-old woman, 2-para, with a normal BMI of 26.1 and no previous surgery, underwent a mini-sling TVT-Secur operation for genuine SUI in 2009. After the application of the sling, the perioperative urethrocystoscopy was normal.

A short time after the operation, the patient developed *de novo* urgency with leakage, urethral pain, local vaginal pain, and dyspareunia, but her SUI had been cured. An urethrocystoscopy was performed 2 mo postoperatively. The procedure showed a small erosion of two loops of the TVT mesh in the urethra at the 5 o'clock position. The patient was treated conservatively and received antibiotics for urethritis.

The mesh continued to erode into the urethra, and the next urethrocystoscopy, conducted 6 mo postoperatively, showed progression of the erosion and visible passing of the sling right through the urethra between the 3 o'clock and 9 o'clock positions. The patient was followed conservatively for 1.5 years, but the sling did not progress further through the urethra. A transurethral excision of the intraurethral part of the mesh was performed 20 mo postoperatively. The patient was then treated with antibiotics for 1 mo.

Three months after the operation the patient had no urethral pain, less dyspareunia and vaginal pain, and improved urgency symptoms. However, the patient's SUI relapsed. Therefore, a TVT-retropubic procedure was performed two years after the first sling placement. This dissection was somewhat more difficult due to the presence of the scar tissue.

In the long-term follow-up, the patient had no stress incontinence, slight vaginal pain, and began anticholinergic treatment.

Case 5

A 38-year-old woman underwent a retropubic TVT for genuine SUI in 1999. The patient was 5-para, with a BMI of 36.2, had been sterilized with a laparoscopic procedure, and then re-sterilized with a new laparoscopy because of an ectopic pregnancy. The TVT procedure was uncomplicated, but the patient did have a voiding dysfunction and irritative urinary symptoms after the operation. An urethrocystoscopy 3 mo postoperatively was normal.

In 2004, five years after the first operation, the patient had a relapse of her SUI, and a TVT-O operation was performed in 2005. No preoperative urethrocystoscopy was done at this procedure. With the exception of mild voiding problems, the patient was satisfied. The patient had also undergone a vaginal hysterectomy after the second sling procedure.

In 2010, the patient experienced a macroscopic hematuria. An urethrocystoscopy was performed 11 years and 1 mo after the first TVT. This procedure revealed a 1 cm horizontal erosion of the white TVT-classic mesh into the urethra. This tape used in 1999 was undyed, whereas the tape used in 2005 was dyed blue. A transurethral extirpation of the intraurethral part of the mesh was performed 12 years and 3 mo after the first TVT. The patient was treated with antibiotics for three weeks postoperatively.

In the long-term follow-up, the patient had no voiding problems, little urgency, and experienced improvement of her incontinence after all procedures.

Case 6

In 2011, a 47-year-old woman was operated upon with the MiniArc™ single-incision sling system for genuine SUI. The patient was 4-para, obese with a BMI of 35.3, had asthma, fibromyalgia, and irritable bowel syndrome. She was previously sterilized laparoscopically. The preoperative cystoscopy was normal, and the MiniArc™ procedure was uncomplicated. Postoperatively, the patient had voiding difficulties and did not experience any improvement in her incontinence.

In a follow-up, 8 mo after the operation, the patient had a worsening of her SUI and was treated with antibiotics because of a UTI. The urethrocystoscopy showed that the mesh had eroded 0.5 cm into the urethra. A reoperation was performed 10 mo after placement of the sling. Transurethrally, the intraurethral mesh was cut on both sides, a catheter was placed, and local antibiotics were applied in the urethra. The patient was treated with oral antibiotics postoperatively.

At the three-month follow-up, the patient had unchanged SUI and recurrent UTIs. However, the urethrocystoscopy was normal. The patient later had a successful retropubic TVT. The postoperative follow-up was uncomplicated with the exception of a UTI.

Case 7

A 44-year-old woman was operated upon with retropubic TVT for genuine SUI in 2002. The patient was 3-para, had a BMI of 29.7, and an appendectomy in her medical history. There were no preoperative complications. In the long-term, the patient complained of recurrent UTIs, *de novo* urgency without incontinence, nocturia, and minor voiding dysfunctions. An urethrocystoscopy, nine years after the primary operation, showed one part of the mesh eroded into the urethra at the right side near the bladder neck.

A reoperation was conducted 1 mo later, and the intraurethral mesh was cut and removed. The excision was performed transurethrally, and urethroplasty was not needed. Antibiotics were given for prophylaxis.

Three months after the reoperation, the patient had a slight relapse of her SUI. The urgency symptoms and

Table 1 Background factors for patients with urethral erosion after tension-free vaginal tape procedure

Case	Age (yr)	Parity	BMI	Menopause	Previous operations	Postoperative medical history	Primary operation
Case 1	46	2	23.0	Hormone IUD (Mirena®)	Appendectomy	No	TVT-O, Gynecare®
Case 2	55	2	40.7	Yes	No	Gastric by-pass	TVT-O, Gynecare®
Case 3	60	3	24.1	Yes	No	Pacemaker	TVT-O, Gynecare®
Case 4	43	2	26.1	No	No	No	TVT-Secure, Gynecare®
Case 5	38	5	36.2	No	Laparoscopic sterilization, Re-sterilization because of ectopic pregnancy	Hysteroscopy, vaginal hysterectomy	TVT-retropubic, Gynecare®
Case 6	48	4	35.3	No	Laparoscopic sterilization	No	MinArcTM, AMS®
Case 7	44	3	29.7	No	Appendectomy	No	TVT-retropubic, Gynecare®
Case 8	40	1	21.3	No	No	Abdominal total hysterectomy and bilateral salpingo-oophorectomy for ovarian mass, re-laparotomy because of ileus	TVT-retropubic, Gynecare®
Case 9	66	2	26.0	Yes	Abdominal hysterectomy, pelvic organ prolapse procedure	Laparoscopic operation of adhesions	TVT-retropubic, Gynecare®

BMI: Body mass index; IUD: Intrauterine device; TVT: Tension-free vaginal tape; TVT-O: Trans-obturator inside-out procedure.

voiding problems did improve, and the patient no longer had the nocturia. The urethrocystoscopy was normal at 3 mo. However, a year later during the follow-up urethrocystoscopy, a small section of threads from the mesh was noticed at the 3 o'clock position. As the patient was symptom free, it was handled conservatively, and an urethrocystoscopy was planned for the following year.

Case 8

A 40-year-old woman underwent a retropubic TVT for genuine SUI in 2004. The patient was 1-para, healthy, and had a BMI of 21.3. There were no perioperative complications, and the patient was asymptomatic for 6.5 years. She had also undergone an abdominal salpingo-oophorectomy and total hysterectomy due to a large ovarian cyst. Five days later, the patient was reoperated upon because of an adherent ileus of the small intestine.

Seven years and 8 mo after the sling placement, the patient had an examination due to a relapse of her SUI and minor *de novo* urgency. An urethrocystoscopy showed erosion of the tape into the urethra. A transurethral procedure was performed seven years and 9 mo postoperatively. Local antibiotics were applied in the urethra along with systemic antibiotic prophylaxis.

In the follow-up 5 mo later, the symptoms of SUI with minor urgency remained. The urethrocystoscopy was normal, and there was a slight detrusor contraction while filling the bladder. However, there was no need for anticholinergics. The patient was operated upon with a new retropubic TVT, and at the one-year follow-up she had no SUI or urgency.

Case 9

A 66-year-old woman, 2-para, with a BMI of 26.0, and a medical history of hypertension and gastritis underwent a TVT-retropubic procedure for SUI in 2010. She was operated upon at a perimenopausal age for an abdominal hysterectomy because of a fast-growing, but benign myoma. The patient had also previously undergone a vaginal operation for pelvic organ prolapse in the form of a rectocele and enterocele.

The patient developed SUI after her pelvic organ prolapse operation. Therefore, the previously mentioned TVT retropubic procedure was performed in 2010. The mesh was doubled with Allis forceps, and no leakage was observed. The perioperative urethrocystoscopy was normal.

Five months after the operation, the patient was examined for severe urgency symptoms. However, her SUI had been cured. The urethrocystoscopy showed that the mesh had eroded to the left side of the bladder neck.

A reoperation was performed 8 mo after the TVT procedure. The mesh had eroded from the bladder neck into the bladder making it difficult to cut the mesh transurethrally. Using a suprapubic trocar (laparoscopic trocar) from the abdomen to the bladder, the mesh was excised using laparoscopic scissors under the guidance of a cystoscope. The patient had a suprapubic catheter for 1 d and bladder catheter for 2 d. Antibiotics were given for one week.

In the long-term, the patient had an improvement in her urgency and no incontinence symptoms. An urethrocystoscopy performed 5 mo postoperatively was normal.

The patient had to undergo later a laparoscopic lysis of pelvic adhesions due to a chronic dyspareunia. These adhesions were caused by the previous hysterectomy.

Patient characteristics

Pertinent patient characteristics are presented in Table 1. The mean patient age at the primary operation was 48.9 years (range: 38-66 years). Six of the patients were premenopausal, and three were postmenopausal. The median BMI was 29.2 (range: 21.3-40.7), and three patients had a BMI > 30. Only one patient had a medical history of a pelvic organ prolapse operation

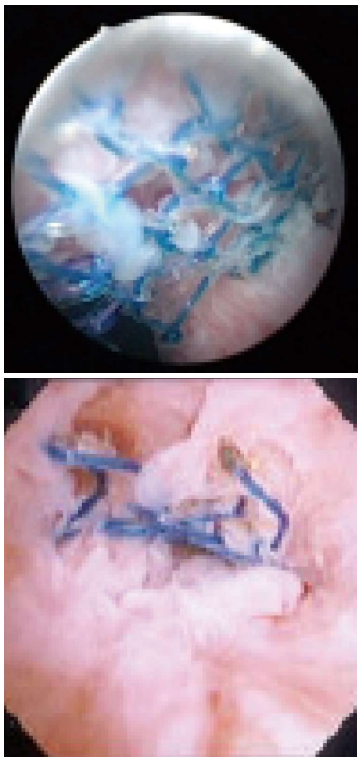

Figure 1 Mesh visible in the urethra.

before the primary incontinence procedure. Two patients had medical records of chronic asthma, and three patients had physically demanding work. Four of the patients had undergone other surgical procedures after the primary TVT operation. There were no obvious common predisposing factors for urethral erosion.

Primary procedure characteristics

Eight of the patients were operated upon for genuine SUI and one for mixed incontinence. The type of primary operation varied from TVT-retropubic to TVT-obturator and mini-slings (Table 1). Therefore, no obvious correlations with the types of operations were found. We do not have data on the number of different TVT operations performed since 1999 in the three hospitals. Therefore, the frequency of the urethral erosion by the mesh cannot be calculated.

In two cases, the sling was doubled preoperatively to make the sling tension-free. Finally, postoperatively, four of the nine patients used intermittent catheterization because of voiding difficulties.

Complication characteristics

Eight of the presented cases had urethral erosions, and one had erosion of the mesh in the bladder neck. The mean interval for diagnosing the erosions was 42 mo (range: 3-133 mo). Four patients were diagnosed within the first year after the operation and five later, up to 11 years after the primary procedure (Table 2). The presenting symptoms were divided into an early-onset (< 12 mo postoperatively) and late-onset (> 12 mo) group according to when they were first reported. It is not clear if all symptoms were directly related to the described complications, but they might be a useful indicator of suspected erosion. Seven patients had clear clinical symptoms less than 12 mo after their operation. The list of early-onset symptoms for each patient is listed in Table 2. Four patients had new symptoms reported after 12 mo postoperatively.

Overall, the majority of patients (77.7%) had some grade of *de novo* urgency with or without urge incontinence and recurrent UTIs (55.5%). The most common early-onset symptoms included urinary retention and voiding dysfunctions, urethritis, and vaginal pain. The late-onset symptoms were more often recurrent UTIs and recurrence of SUI.

Surgical treatment

The median interval between the diagnosis and surgical treatment was 1.5 mo (range: 10 d to 3 mo) for seven cases of urethral erosion. There were two cases with an interval of 13 mo and 18 mo, which were first treated conservatively.

The first case required transvaginal excision of the intraurethral mesh followed by transvaginal urethroplasty. This was a rather complicated and time-consuming operation, which led us to change our approach to transurethral resection of the intraurethral part of the mesh.

Best method for removing the intraurethral mesh based on our experience

The best position for identifying and removing the intraurethral mesh was to have the patient in the lithotomy position under general or regional anesthesia. Using a regular cystoscope [Karl Storz Gmbh, Tuttlingen, Germany; Charrière (Ch) 22, 0° or 30° optics, with a deflecting mechanism with Albarran lever, and a working channel with two ports] the intraurethral mesh was identified (Figure 1), and a 5 Ch "open-end" ureteral catheter was inserted into the urethra. A 0-0 monofilament thread was then introduced through a loop in the central part of the mesh, and a good portion of the thread was pushed through the mesh into the bladder. Then, the cystoscope was retracted, and the thread position in the urethra was secured by grasping it at the meatus. The ureteral catheter was removed from the cystoscope, which was reintroduced into the bladder, and the distal part of the thread was located and grasped with cystoscopic pincers and extracted through the urethra. This created a thread loop through the mesh allowing the application of tension on the mesh while cutting.

To avoid cutting the loop by mistake, it is recommended to use a monofilament thread that is a different color than the mesh. It can be technically challenging to insert the monofilament thread into one of the loops of the TVT mesh. The procedure is easier when using a child cystoscope (Ch 10, one port channel, with an optic of 0°). The small cystoscope is more easily handled in the urethra and does not require a

Table 2 Clinical symptoms of urethral erosion and outcomes after the intraurethral mesh was removed

Case	Interval for diagnosis	Early-onset symptoms (< 12 mo)	Late-onset symptoms (> 12 mo)	Interval for surgical management	Outcome after surgical management	Further treatment
Case 1	5 mo	Urinary retention/voiding dysfunction, UTI, urethral pain (urethritis), *de novo* urgency, dyspareunia	_	5 mo	Improved urgency, voiding dysfunction resolved, relapse of SUI	Anticholinergic, incontinence pad, electric stimulation, two macroplasty procedures, laparoscopic Burch
Case 2	3 mo	Urethral pain (urethritis), worsening of urgency	_	3 mo	Worsening of urge incontinence, relapse of SUI, recurrent urethritis	Anticholinergic, local antibiotics into the urethra, no further SUI treatments
Case 3	1 yr 8 mo	Urinary retention/voiding dysfunction	*De novo* urgency, recurrent UTI, vaginal pain	1 yr 11 mo	Urgency resolved, vaginal pain resolved	No further SUI treatments
Case 4	2 mo	*De novo* urgency, urethral pain (urethritis), vaginal pain, dyspareunia	_	1 yr 8 mo	Improved urgency, urethral pain resolved, relapse of SUI	Anticholinergic, retropubic TVT
Case 5	11 yr 1 mo	Urinary retention/voiding dysfunction, UTI, voiding pain (urethritis)	Relapse of SUI, hematuria after TVT-O	12 yr 2 mo	Recurrent minor SUI (patient already had second incontinence operation), improved urgency, urethral pain, voiding dysfunction resolved	No further SUI treatments
Case 6	8 mo	Urinary retention/voiding dysfunction, direct relapse of SUI, UTI	_	10 mo	Same as before the surgical management	Retropubic TVT
Case 7	9 yr 1 mo	_	Recurrent UTI, *de novo* urgency, nocturia	9 yr 2 mo	Slight relapse of SUI, improved urgency and voiding dysfunction	Local estrogens, no further SUI treatments
Case 8	7 yr 8 mo	_	Relapse of SUI, minor *de novo* urgency, nocturia	7 yr 9 mo	Same as before surgical management	Retropubic TVT
Case 9	4 mo	*De novo* urgency	_	7 mo	Improved urgency, cured SUI, no relapse after the surgical management	No further SUI treatments

SUI: Stress urinary incontinence; TVT: Tension-free vaginal tape; TVT-O: Trans-obturator inside-out procedure; UTI: Urinary tract infection.

ureteral catheter to pass the monofilament thread through the instrument and the mesh. After placing the thread, the procedure is continued as described above with a normal cystoscope. From our experience, the monofilament thread of 0-0 size is the most convenient to use.

The next step was to carefully dilate the urethra to Hegar pin number 10. A nephroscope (Storz S27092 AMA, 0° optic and an operating sheath 27093BN, Ch 28 with a working channel of 5 mm) (Figure 2) has been the most convenient instrument to use when cutting the mesh. The regular cystoscope was not the best choice, as the small scissors used through a cystoscope were too weak to cut the mesh and easily broke. With a video technique, it was possible for the assistant to manipulate the urethra with one finger in the vagina. Through the working channel of the nephroscope, laparoscopic scissors (Storz, Metzenbaum scissor 5 mm, 34210 MW or Hak scissor, 34210 EH) were used to cut the mesh. It was easy to cut the mesh at the first side, and with the monofilament suture, it was possible to keep the mesh tensioned while cutting the other side. Cutting of the mesh at the mucosal level did not require suturing.

As much as possible of the visible mesh should be cut. If some small part remains, it will probably disappear. Only one case had small remains of the mesh at the control cystoscopy performed a couple of months after surgery. This patient will be followed-up with a new urethrocystoscopy after one year.

Preoperative antibiotics and intraurethral antibiotics were given as prophylaxis. Additionally, we have postoperatively left a suprapubic catheter in place for 7 d. During this time, oral antibiotics were given.

Under the ideal circumstances, the procedure took 30 min or less.

Outcomes

Four patients had relapses of their SUI after the intraurethral mesh was removed (Table 2). For five patients, no further SUI treatment was necessary after the removal of the intraurethral tape. Five out

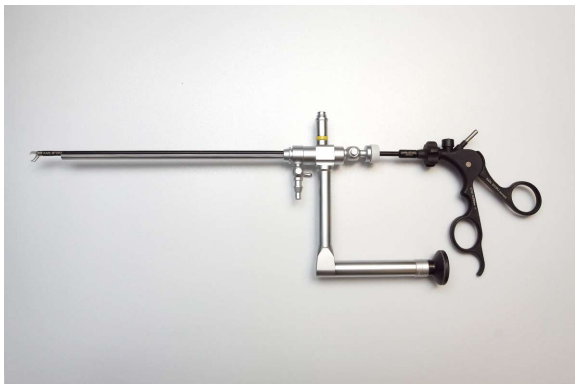

Figure 2 Operating nephroscope.

of seven patients patients with urgency experienced improvements or cures of their symptoms after the removal of the intraurethral mesh. Only three out of seven patients with *de novo* urgency were in need of anticholinergics after the last follow-up. Three patients also experienced improvements or cures of their voiding difficulties.

DISCUSSION

Risk factors

The exact pathophysiology behind erosion of the sling materials is not fully understood[3,17]. Various factors may predispose a patient to erosion. Factors associated with the pelvic anatomy include urogenital atrophy, poorly estrogenized tissue, previous pelvic radiation, previous vaginal surgery, or concomitant procedure and local infection, as well as a high body weight[13,17,27,28]. Our series had only one patient with a previous pelvic procedure, so these risk factors could not be verified.

Predisposing factors associated with the surgical technique include excessive tensioning of the sling and placement of the mesh too close to the urethra, which is the most feasible explanation, but it is not possible to extract this information from the medical records. When operating with mini-slings, it has been suggested to put the sling closer to the urethra than with traditional slings. This could explain our two cases of urethral complication after mini-sling procedures. Other possible explanations are inadequate vaginal tissue coverage and improper dissecting near the urethra, which might damage the urethral tissue and its vascularity[3,13,17,27]. Two of our cases had the sling doubled under the urethra perioperatively to ensure a tension-free application of the mesh. Four of our cases suffered from tight placement of the sling under the urethra; two patients had to use intermittent catheterization and two experienced voiding difficulties postoperatively.

The surgical manuals on retropubic TVT procedures recommend a rigid catheter guide inserted into the indwelling catheter for contralateral displacement of the bladder to minimize the risk of perforation. The urethra is then pulled towards the TVT needle, potentially increasing the risk of perforating the edge of the urethra. This might explain cases of urethral perforations and erosions in retropubic TVTs, but not in the other methods, as there was no displacement of the urethra during the operation.

More than one million TVT procedures have been performed worldwide since 1996 when the method was introduced[3,19]. In Sweden, about 4000-5000 women are operated on annually for SUI, most of whom receive the retropubic technique[16].

Urethral complications might be more common than reported, but that does not mean that the TVT procedures should be restricted. Urethral injury is still a rare complication, while the TVT procedures have a high success rate and a great improvement in the quality of life of the operated patients.

Symptoms

Urethral erosion may present with various symptoms. These include postoperative urinary retention, voiding dysfunction, hematuria, urethral or pelvic pain, recurrent UTIs, relapse of SUI, and *de novo* urgency[15].

There are only sporadic case reports referring to the clinical profile of erosions after sling procedures. As it seems in our study, the clinical profile of urethral complications might vary depending on the early or late onset of the symptoms after the operation. In the early period, we should expect voiding difficulties and urethritis symptoms, whereas in the later stage, it is more common with the relapse of SUI. *De novo* urgency (with or without leakage) and recurrent UTIs are also common symptoms that might appear early or late in the postoperative process.

In a review of 376 women with adverse events after suburethral sling procedures, Petri *et al*[28] analyzed the most common complications. *De novo* urgency with or without leakage was presented in 54% of the cases, voiding dysfunction in 48%, vaginal erosion in 19%, and urethral and vaginal pain in 14%. A total of 17 (4.5%) cases of urethral and bladder base perforation were found in their study, and most often the complications had occurred peroperatively. However, urethral and bladder base perforations might be associated with severe morbidity, and even lead to urethravaginal fistulae if undetected. Our study describes similar clinical symptoms secondary to urethral erosions.

A majority (77.7%) of our cases had *de novo* urgency. Postoperative *de novo* urgency has been reported in 10% of TVT procedures. If urethrocystoscopy is implemented postoperatively, the number of undiagnosed urethral erosions could considerably be reduced.

Treatment

There has been a variety of approaches to the surgical management of urethral complications after

sling procedures. The transvaginal excision of the intraurethral part of the sling with urethroplasty was the first approach to be used[18]. We used this method for our first case, but the transurethral excision of the mesh has become the preferred method for the majority of the patients in our series.

Another alternative is the conservative approach. In case 3, only one small loop of the mesh was seen in the urethra during the first cystoscopy. Cystoscopies were performed every 6 mo as a follow-up, and the mesh migrated directly across the urethra, but did not progress further after that.

We tried conservative treatment after receiving a report from colleagues who found a part of the mesh in the urethra but did not remove it. Therefore, we believe that urethral erosion might be a much common complication, at least in Sweden, where gynecologists perform TVT procedures, but do not routinely carry out urethrocystoscopy.

We also introduced preoperative urethrocystoscopy to all pubovaginal sling procedures. Including urethrocystoscopy in the preoperative investigation had many advantages. First, filling the bladder with 300 mL of saline solution without any detrusor contraction made cystometry unnecessary. Second, conducting a preoperative pad test with 300 mL in the bladder, with the patient exercising for 1 min and coughing ten times, would be much faster, if it is done at the same time as the urethrocystoscopy. Third, it would make urethrocystoscopy a standard and familiar procedure for the gynecologist.

Urethral complications after sling procedures might be more common than previously thought. One of the reasons is that the urethrocystoscopy is not included routinely in the postoperative follow-up of patients with residual or new symptoms from the urinary or vaginal tracts. This makes it difficult to identify complications because of the wide variation in the clinical profile and the timing of the presenting symptoms. It is important to suspect urethral complications if symptoms, such as urgency, voiding dysfunction, recurrent UTIs, or relapse of SUI occur after sling procedures.

We recommend the transurethral approach for the excision of the intraurethral mesh as the treatment of choice for urethral erosion.

ACKNOWLEDGEMENTS

We are grateful to all the patients who participated in the study.

COMMENTS

Background

Introduction of the tension-free vaginal tape (TVT) operations in 1996 to treat stress incontinence changed the surgical management of the condition. Instead of being an abdominal operation with one week of hospitalized postoperative care, TVT became a minimal invasive outpatient surgery, with same-day discharge. This meant many more women could be operated upon with this minimally invasive technique improving the patient's quality of life tremendously. However, even the minimally invasive procedures have associated complications with a unique set of symptoms.

Research frontiers

Mesh erosions are very serious complications, especially in prolapse surgery, with a high patient morbidity rate. In TVT operations, very few complications have been reported, mostly referring to perforation of the bladder. Urethral complications have been reported very sporadically.

Innovations and breakthroughs

The authors have performed routine urethrocystoscopy on women with some voiding difficulties after a TVT operation. Nine cases of urethral mesh erosions were identified. The initially used method required removal of the tape with an intravaginal approach, a rather long and complicated operation. The authors made a necessary modification with an intraurethral removal of the tape using a nephroscope. This device is normally used in kidney operations and not in vaginal procedures.

Applications

As much as 10% of reported cases of *de novo* urgency occur after a TVT operation. Many of these women might have urethral erosion that can be easily operated upon, if identified during urethrocystoscopy.

Terminology

Stress incontinence is leakage of urine during coughing, laughing, and running. Urge incontinence is leakage after a strong feeling of need to void. *De novo* urge is a symptom of urgency that develops after the stress incontinence procedure. TVT procedures are minimally invasive operations with placing of a small tape under the urethra. Cystoscopy is a diagnostic procedure where a small instrument with a camera is introduced into the bladder and identifies the inside. Urethrocystoscopy is the same procedure that also includes examining of the urethra. It is not always performed during a routine cystoscopy.

Peer-review

This article is very interesting for individuals involved in the treatment of stress incontinence. Authors present their experience in the treatment of urethral erosion after tension-free vaginal tape procedures. The diagnostic and therapeutic approach is clearly explained. Also, an unusual and rare but very interesting operation of transurethral excision of the intraurethral part of the mesh was performed.

REFERENCES

1 **Abrams P**, Cardozo L, Fall M, Griffiths D, Rosier P, Ulmsten U, van Kerrebroeck P, Victor A, Wein A. The standardisation of terminology of lower urinary tract function: report from the Standardisation Sub-committee of the International Continence Society. *Am J Obstet Gynecol* 2002; **187**: 116-126 [PMID: 12114899 DOI: 10.1067/mob.2002.125704]

2 **Altman D**, Falconer C, Zetterström, J. Urogynekologi. Lund: Studentlitteratur. Print, 2010

3 **Brophy MM**, Klutke JJ, Klutke CG. A review of the tension-free vaginal tape procedure: outcomes, complications, and theories. *Curr Urol Rep* 2001; **2**: 364-369 [PMID: 12084243 DOI: 10.1007/s11934-996-0021-1]

4 **Ulmsten U**, Henriksson L, Johnson P, Varhos G. An ambulatory surgical procedure under local anesthesia for treatment of female urinary incontinence. *Int Urogynecol J Pelvic Floor Dysfunct* 1996; **7**: 81-85; discussion 85-86 [PMID: 8798092 DOI: 10.1007/BF01902378]

5 **Long CY**, Hsu CS, Wu MP, Liu CM, Wang TN, Tsai EM. Comparison of tension-free vaginal tape and transobturator tape procedure for the treatment of stress urinary incontinence. *Curr Opin Obstet Gynecol* 2009; **21**: 342-347 [PMID: 19528799 DOI: 10.1097/GCO.0b013e32832e07bf]

6 **Delorme E**. [Transobturator urethral suspension: mini-invasive procedure in the treatment of stress urinary incontinence in women]. *Prog Urol* 2001; **11**: 1306-1313 [PMID: 11859672]

7 **de Leval J**. Novel surgical technique for the treatment of female stress urinary incontinence: transobturator vaginal tape inside-out. *Eur Urol* 2003; **44**: 724-730 [PMID: 14644127 DOI: 10.1016/j.eururo.2003.09.003]

8 **Walsh CA**. TVT-Secur mini-sling for stress urinary incontinence: a review of outcomes at 12 months. *BJU Int* 2011; **108**: 652-657

[PMID: 21756280]
9 **Abdel-Fattah M**, Ford JA, Lim CP, Madhuvrata P. Single-incision mini-slings versus standard midurethral slings in surgical management of female stress urinary incontinence: a meta-analysis of effectiveness and complications. *Eur Urol* 2011; **60**: 468-480 [PMID: 21621321 DOI: 10.1016/j.eururo.2011.05.003]
10 **Oliveira R**, Silva C, Dinis P, Cruz F. Suburethral single incision slings in the treatment of female stress urinary incontinence: what is the evidence for using them in 2010? *Arch Esp Urol* 2011; **64**: 339-346 [PMID: 21610278]
11 **Molden SM**, Lucente VR. New minimally invasive slings: TVT Secur. *Curr Urol Rep* 2008; **9**: 358-361 [PMID: 18702918 DOI: 10.1007/s11934-008-0062-8]
12 **Morton HC**, Hilton P. Urethral injury associated with minimally invasive mid-urethral sling procedures for the treatment of stress urinary incontinence: a case series and systematic literature search. *BJOG* 2009; **116**: 1120-1126 [PMID: 19438488 DOI: 10.1111/j.1471-0528.2009.02199.x]
13 **Siegel AL**. Urethral necrosis and proximal urethro-vaginal fistula resulting from tension-free vaginal tape. *Int Urogynecol J Pelvic Floor Dysfunct* 2006; **17**: 661-664 [PMID: 16944073 DOI: 10.1007/s00192-005-0031-4]
14 **Karram MM**, Segal JL, Vassallo BJ, Kleeman SD. Complications and untoward effects of the tension-free vaginal tape procedure. *Obstet Gynecol* 2003; **101**: 929-932 [PMID: 12738152 DOI: 10.1016/S0029-7844(03)00122-4]
15 **Deffieux X**, Bonnet K, Chevalier N, Gervaise A, Frydman R, Fernandez H. [Urinary complications in sub-urethral sling procedures]. *J Gynecol Obstet Biol Reprod* (Paris) 2005; **34**: 745-756 [PMID: 16319764 DOI: 10.1016/S0368-2315(05)82949-7]
16 **Ankardal M**, Heiwall B, Lausten-Thomsen N, Carnelid J, Milsom I. Short- and long-term results of the tension-free vaginal tape procedure in the treatment of female urinary incontinence. *Acta Obstet Gynecol Scand* 2006; **85**: 986-992 [PMID: 16862480 DOI: 10.1080/00016340600753091]
17 **Clemens JQ**, DeLancey JO, Faerber GJ, Westney OL, Mcguire EJ. Urinary tract erosions after synthetic pubovaginal slings: diagnosis and management strategy. *Urology* 2000; **56**: 589-594 [PMID: 11018611 DOI: 10.1016/S0090-4295(00)00740-8]
18 **Koelbl H**, Stoerer S, Seliger G, Wolters M. Transurethral penetration of a tension-free vaginal tape. *BJOG* 2001; **108**: 763-765 [PMID: 11467707 DOI: 10.1016/S0306-5456(00)00188-1]
19 **Deng DY**, Rutman M, Raz S, Rodriguez LV. Presentation and management of major complications of midurethral slings: Are complications under-reported? *Neurourol Urodyn* 2007; **26**: 46-52 [PMID: 17149713 DOI: 10.1002/nau.20357]
20 **Sweat SD**, Itano NB, Clemens JQ, Bushman W, Gruenenfelder J, McGuire EJ, Lightner DJ. Polypropylene mesh tape for stress urinary incontinence: complications of urethral erosion and outlet obstruction. *J Urol* 2002; **168**: 144-146 [PMID: 12050509 DOI: 10.1016/S0022-5347(05)64848-3]
21 **Baracat F**, Mitre AI, Kanashiro H, Montellato NI. Endoscopic treatment of vesical and urethral perforations after tension-free vaginal tape (TVT) procedure for female stress urinary incontinence. *Clinics* (Sao Paulo) 2005; **60**: 397-400 [PMID: 16254676 DOI: 10.1590/S1807-59322005000500008]
22 **McLennan MT**. Transurethral resection of transvaginal tape. *Int Urogynecol J Pelvic Floor Dysfunct* 2004; **15**: 360-362 [PMID: 15580426]
23 **Powers K**, Lazarou G, Greston WM. Delayed urethral erosion after tension-free vaginal tape. *Int Urogynecol J Pelvic Floor Dysfunct* 2006; **17**: 422-425 [PMID: 16741603 DOI: 10.1007/s00192-005-0008-3]
24 **Velemir L**, Amblard J, Jacquetin B, Fatton B. Urethral erosion after suburethral synthetic slings: risk factors, diagnosis, and functional outcome after surgical management. *Int Urogynecol J Pelvic Floor Dysfunct* 2008; **19**: 999-1006 [PMID: 18202812 DOI: 10.1007/s00192-007-0558-7]
25 **Wijffels SA**, Elzevier HW, Lycklama a Nijeholt AA. Transurethral mesh resection after urethral erosion of tension-free vaginal tape: report of three cases and review of literature. *Int Urogynecol J Pelvic Floor Dysfunct* 2009; **20**: 261-263 [PMID: 18716705 DOI: 10.1007/s00192-008-0705-9]
26 **Quiroz LH**, Cundiff GW. Transurethral resection of tension-free vaginal tape under tactile traction. *Int Urogynecol J Pelvic Floor Dysfunct* 2009; **20**: 873-875 [PMID: 18989606 DOI: 10.1007/s00192-008-0758-9]
27 **Daneshgari F**, Kong W, Swartz M. Complications of mid urethral slings: important outcomes for future clinical trials. *J Urol* 2008; **180**: 1890-1897 [PMID: 18801499 DOI: 10.1016/j.juro.2008.07.029]
28 **Petri E**, Ashok K. Complications of synthetic slings used in female stress urinary incontinence and applicability of the new IUGA-ICS classification. *Eur J Obstet Gynecol Reprod Biol* 2012; **165**: 347-351 [PMID: 22944381 DOI: 10.1016/j.ejogrb.2012.08.011]

P- Reviewer: Friedman EA, Markic D, Taheri S, Trkulja V
S- Editor: Ma YJ **L- Editor**: A **E- Editor**: Yan JL

World Journal of Nephrology

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Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.5527/wjn.v4.i3.406

World J Nephrol 2015 July 6; 4(3): 406-414
ISSN 2220-6124 (online)

ORIGINAL ARTICLE

Clinical Trial Study

Albuminuria as a marker of arterial stiffness in chronic kidney disease patients

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Author contributions: Kalaitzidis RG and Elisaf MS designed research; Karasavvidou DP, Pappas K, Katatsis G and Liontos A performed research; Tatsioni A analyzed data; Elisaf MS and Kalaitzidis RG wrote the paper.

Ethics approval statement: Our hospital Ethics Committee approved this study protocol with a registration identification number (ΑΔΑΒΛΓΦ46906Η-ΛΟΛ).

Informed consent statement: Informed consent was obtained from all patients.

Conflict-of-interest statement: The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Data sharing statement: Technical appendix, statistical code, and dataset available from the corresponding author at Moses Elisaf [melisaf54@gmail.com, egepi@cc.uoi.gr]. Participants gave informed consent for data sharing even though are anonymized and the risk of identification is low.

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Received: November 4, 2014
Peer-review started: November 9, 2014
First decision: November 27, 2014
Revised: December 22, 2014
Accepted: April 8, 2015
Article in press: April 9, 2015
Published online: July 6, 2015

Abstract

AIM: To access the association between albuminuria levels and arterial stiffness in non-diabetic patients with hypertension and chronic kidney disease (CKD) stages 1-2, treated with renin angiotensin blockade agents plus other hypertensive drugs when needed.

METHODS: One hundred fifteen patients [median age 52 years (68% males)] were consequently enrolled in the study. For each patient, we recorded gender, age, body mass index (BMI), peripheral systolic blood pressure (pSBP), peripheral diastolic blood pressure, peripheral pulse pressure, central systolic blood pressure (cSBP), central diastolic blood pressure (cDBP), central pulse pressure (cPP), hematocrit, hemoglobin, hsCRP, total cholesterol triglycerides, high-density lipoprotein-C, low-density lipoprotein-C, calcium, phosphorus, parathormone, and albumin, as well as 24 h urine albumin excretion. According to 24-h urine albumin collection, patients were then classified as those with moderately increased albuminuria (formerly called macroalbuminuria) ($\leqslant$ 300 mg/d) and those with severely increased albuminuria (formerly called macroaluminuria (> 300 mg/d). We considered aortic stiffness (AS) indices [carotid femoral pulse wave velocity (PWVc-f) and augmentation index (AIx)] as primary outcomes of

the study. We explored potential correlations between severely increased albuminuria and AS indices using a multiple linear regression model.

RESULTS: Fifty-eight patients were included in the moderately increased albuminuria group and 57 in the severely increased albuminuria. Blood pressure measurements of the study population were 138 ± 14/82 ± 1.3 mmHg (systolic/diastolic). There were no significant differences in age, sex, and BP measurements between the two groups. Patients with severely increased albuminuria had higher PWV and AIx than patients with moderately increased albuminuria ($P < 0.02$, $P < 0.004$, respectively). In addition these patients exhibited higher BMI ($P < 0.03$), hsCRP ($P < 0.001$), and fibrinogen levels ($P < 0.02$) compared to patients with moderately increased albuminuria. In multivariate linear regression analysis, severely increased albuminuria ($\beta = 1.038$, $P < 0.010$) pSBP ($\beta = 0.028$, $P < 0.034$) and Ht ($\beta = 0.171$, $P = 0.001$) remained independent determinants of the increased PWVc-f. Similarly, severely increased albuminuria ($\beta = 4.385$, $P < 0.012$), cSBP ($\beta = 0.242$, $P < 0.001$), cPP ($\beta = 0.147$, $P < 0.01$) and Ht levels ($\beta = 0.591$, $P < 0.013$) remained independent determinants of increased AIx.

CONCLUSION: These findings demonstrate an independent association between AS indices and severely increased albuminuria in non-diabetic, hypertensive patients with CKD stages 1-2 treated with renin angiotensin aldosterone system blockers.

Key words: Arterial stiffness; Pulse wave velocity; Augmentation index; Albuminuria

Core tip: Albuminuria heightened cardiovascular disease risk. Pulse wave velocity and augmentation index are markers of aortic stiffness (AS). However, whether severely increased albuminuria is a factor of AS elevation and its progressive deterioration in non-diabetic hypertensive patients treated with renin angiotensin aldosterone blockade agents (RAAS) has not be studied. In this study we aimed to access the association between albuminuria levels and AS, in chronic kidney disease (CKD) stage 1-2 non-diabetic patients with hypertension. All patients were already treated with RAAS blockade agents. Our findings demonstrate an independent association between AS indices and severely increased albuminuria in non-diabetic, hypertensive patients with CKD stages 1-2 treated with RAAS blockers.

Kalaitzidis RG, Karasavvidou DP, Tatsioni A, Pappas K, Katatsis G, Liontos A, Elisaf MS. Albuminuria as a marker of arterial stiffness in chronic kidney disease patients. *World J Nephrol* 2015; 4(3): 406-414 Available from: URL: http://www.wjgnet.com/2220-6124/full/v4/i3/406.htm DOI: http://dx.doi.org/10.5527/wjn.v4.i3.406

INTRODUCTION

The presence of albuminuria is associated with faster progression of renal failure and is also recognized as a marker of vascular dysfunction and heightened cardiovascular disease risk in hypertensive and chronic kidney disease (CKD) patients with or without diabetes[1,2].

Arterial stiffness (AS) assessment helps to predict cardiovascular events in patients with or without diabetes[3-5]. It is usually assessed with the aortic pulse wave velocity (PWV)[6,7] and peripheral pressure wave reflections, (Alx)[1,8]. These indices (PWV and AIx), as major determinants of the aortic pulse pressure, are early markers of atherosclerotic vascular changes and in CKD have been shown to be associated with renal micro vascular damage and kidney dysfunction[9]. On the other hand, AS was showed to be associated with incident albuminuria and the rate of decline in glomerular filtration rate (GFR)[10]. More recent information in patients with type 2 diabetes mellitus showed that levels of urinary albumin excretion, but not reduced estimated GFR, were associated with increased AS and atherosclerosis[11]. High normal albuminuria in the range (0-30 mg/g) is also associated with aortic stiffness[12] even in younger type 2 diabetic patients with shorter durations of disease[13], while in newly diagnosed patients with type 2 diabetes mellitus, moderately increased albuminuria was independently associated with AS and vascular inflammation[14].

However, limited data are available whether severely increased albuminuria is associated with AS in non-diabetic hypertensive patients already treated with renin angiotensin aldosterone blockade agents (RAAS)[15]. It has been suggested that endothelial dysfunction could be a possible mechanism involved in the remodeling of the arterial wall affecting AS and modifying glomerular permeability leading to increased albumin excretion[16,17]. In addition, AS could influence glomeruli function through an increased pulsatile stress, causing glomerular damage[18].

In this study we aimed to access the association between albuminuria levels and AS, in CKD stage 1-2 non-diabetic patients with hypertension. All patients were already treated with RAAS blockade agents plus other hypertensives when needed. RAAS blockers play an important role in the regulation of BP. These agents have demonstrated favorable effects beyond blood pressure control in situations, such as albuminuria, left ventricular hypertrophy and AS[16].

MATERIALS AND METHODS

We included 115 consecutive hypertensive patients with CKD stages 1-2 (Stage 1: kidney damage with normal or increased GFR, Stage 2: kidney damage with mild reduced GFR 60-89 mL/min). None of our patients had albuminuria > 1 g per 24 h. Albuminuria levels were stable for the past 6 mo. Patients with

Table 1 Demographic and aortic stiffness indices characteristics

Patients characteristics	Study population	Patients with moderately increased albuminuria	Patients with severely increased albuminuria	*P* value
No	115	58	57	-
Age (yr)	52.6 ± 14.6	51.4 ± 14.4	54.2 ± 15.07	NS
Sex (M/F), (*n*)	76/39	39/19	37/20	NS
BMI (kg/m^2)	30.9 ± 19.3	28.6 ± 4.5	33.9 ± 28.2	< 0.03
Smoking, *n* (%)	5	3	2	NS
pSBP (mmHg)	138.0 ± 14.2	139 ± 12.7	138.0 ± 15.5	NS
pDBP (mmHg)	84.2 ± 9.7	84.9 ± 10.7	82.6 ± 9.8	NS
pPP (mmHg)	57.9 ± 15.9	56.7 ± 15.1	59.4 ± 16.9	NS
cSBP (mmHg)	130.3 ± 14.6	130 ± 13.3	130.2 ± 16.5	NS
cDBP(mmH)	84.2 ± 9.7	84.9 ± 10.7	84.3 ± 8.6	NS
cPP (mmHg)	48.1 ± 17.6	47.9 ± 17.9	48.4 ± 17.8	NS
AIx (%)	21.1 ± 10.6	18.4 ± 10.0	24.5 ± 10.0	< 0.004
PWVc-f (m/sec)	8.7 ± 2.0	8.3 ± 2.0	9.1 ± 1.9	< 0.02
ACEIs, *n* (%) (ramipril or quinapril)	100 (86)	56 (96)	44 (77)	NS
AT1 RB, *n* (%) (valsartan or olmesartan)	20 (17.4)	9 (15.5)	11 (19.2)	NS
CCBs, *n* (%)	11 (9.5)	6 (10.3)	5 (8.7)	NS
Statins, *n* (%)	7 (6.08)	3 (5.1)	4 (7.01)	NS

Data are presented as mean value. M/F: Male/female; BMI: Body mass index; pSBP: Peripheral systolic blood pressure; pDBP: Peripheral Diastolic blood pressure; pPP: Peripheral pulse pressure; cSBP: Central systolic blood pressure; cDBP: Central diastolic blood pressure; cPP: Central pulse pressure; AIx: Augmentation index; AP: Augmentation pressure; PWVc-f: Pulse wave velocity carotid-femoral; ACEIs: Angiotensin converting enzyme inhibitors; AT1RB: Angiotensin type 1 receptor blockers; CCBs: Calcium channel blockers.

known glomerulopathy proven by biopsy were excluded. Most of our patients had hypertensive nephosclerosis with duration of hypertension for more than 10 years and none of them had diabetes mellitus. Furthermore we excluded patients with acute myocardial infarction, unstable angina, stroke, heart failure or transient ischemic attack within the past year. We recorded the demographic data of the patients including age, gender, body mass index (BMI), peripheral blood pressure measurements [(systolic, diastolic, pulse pressure (pSBP, pDBP, pPP) respectively] as well as central blood pressure measurements [(systolic, diastolic, pulse pressure (cSBP, cDBP, cPP) respectively].

All the patients were treated with RAAS agents (ACE inhibitors or angiotensin type 1 (AT1) receptor blockers) plus other antihypertensive drugs as needed. Our choice for the first agent was a RAAS blocker based on the favorable effects of these agents on albuminuria and AS reduction as well as for their anti-fibrotic and anti-inflammatory effects[17,18]. Furthermore, the reno- and cardio-protective effects beyond their hypotensive effects were also well established[19] and for our patients with moderately or severely albuminuria this treatment was already applied for at least 6 mo. Ramipril or quinapril were the ACE inhibitors used as well as valsartan or olmesartan were the AT1 receptor blockers, used in maximum doses. Ramipril and quinapril were prescribed at the doses of 20 mg OD and valsartan and olmesartan at the doses of 320 and 20 mg OD respectively. Six patients received valsartan 160 mg OD and 2 patients received quinapril 40 mg OD. A very small number of patients received amlodipine at a dose of 10 mg OD (Table 1).

Blood pressure measurements

The patient's representative peripheral BP levels at each visit, were the average of three consecutive BP measurements in a sitting position after 5 min rest, within 2 min intervals between them by an automated sphygmomanometer. Peripheral PP was defined as the difference between pSBP and pDBP.

Arterial stiffness measurements

For the assessment of the central aortic pressure were used the Sphygmocor system (Atcor, Sydney, Australia) and its software with an applanation tonometry (Millar tonometer, Millar Instruments, Houston, TX). The AIx was calculated as the increment in pressure from the first systolic shoulder to the peak pressure of the aortic pressure waveform expressed as a percentage[20]. Carotid-femoral pulse wave velocity (PWVc-f) was also calculated non-invasively by the aforementioned software of the Sphygmocor system (Atcor, Sydney, Australia) described elsewhere[20]. Primary outcomes of the study were considered arterial stiffness indices, AIx and PWVc-f.

Laboratory measurements

In all patients, blood samples were obtained after 12 h fasting. Serum samples were analyzed for haematocrit (Ht), haemoglobin (Hb), hsC-Reactive Protein (hsCRP), and serum lipids, such as total cholesterol (T-CHOL), triglycerides (TRG), and high-density lipoprotein-C (HDL-C). Low-density lipoprotein-C (LDL-C) was calculated using the Friedewald formula provided fasting TRG levels less than 400 mg/dL. In

patients with serum TRG values greater than 400 mg/dL, LDL-C concentrations were not determined. HsCRP was measured using a latex enhanced immunonephelometry assay on a Dade Behring BN Ⅱ nephelometer. We also measured serum levels of calcium, phosphorus, parathormone and albumin, as well as 24 h urine albumin excretion, using common commercial serological kits. Albuminuria was the mean value of 2 separate 24 h urinary collections. Patients were then classified as those with moderately increased albuminuria (formerly called macroalbuminuria) ($\leqslant$ 300 mg/d) and those with severely increased albuminuria (formerly called macroaluminuria (> 300 mg/d). Smoking status was defined as current or past smoker *vs* non-smoker.

All patients were received RAAS blockers (ACE inhibitors or AT1 receptor blockers and 5 patients (4.34%) were received double RAAS blockade agents (ACE inhibitor and AT1 receptor blocker). Our hospital Ethics Committee approved this study protocol. Informed consent was obtained from all patients.

Statistical analysis

We presented data separately for patients with moderately increased albuminuria and patients with severely increased albuminuria. Data were presented as absolute numbers and frequencies for binary and categorical variables, and as mean ± SD for continuous variables. Between the two groups, comparisons for binary variables were performed using χ^2 or Fisher's exact test. Comparisons for continuous variables were performed using Mann-Whitney *U* test. To investigate whether there was a potential relation between AS and patient characteristics, we first performed univariate linear regression analyses for each variable using the AS indices, *i.e.,* PWVc-f, and AIx as dependent variables.

We considered as independent covariates the following: albuminuria category (moderately *vs* severely increased albuminuria), age, sex, BMI, pSBP, pDBP, Ppp, cSBP, cDBP, cPP, anti-RAAS agents, Ht, Hb, T-CHOL, TRG, HDL-C, LDL-C, fibrinogen, and hsCRP.

Variables with a *P*-value < 0.1 from the univariate analysis were evaluated further in a multivariate regression analysis. For each variable beta (β) co-efficient with the corresponding confidence interval (CI) were calculated in the multivariate model. A two-sided P-value <0.05 was considered as statistically significant. The SPSS, version 16 (SPSS Inc.) statistical package was used for the statistical analysis. Statistically significant was considered a *P*-value < 0.05.

RESULTS

One-hundred fifteen hypertensive non diabetic patients were consequently enrolled in the study. The mean age of the patient's population was 52 years, and 68% of them were males. All enrolled patients exhibited estimated glomerular filtration rate (eGFR-MDRD) > 60 mL/min per 1.73 m^2. Fifty-eight patients were included in the moderately increased albuminuria group and 57 in the severely increased albuminuria group. Demographic and AS indices characteristics are reported in Table 1.

Patients with severely increased albuminuria compared to patients with moderately increased albuminuria had higher BMI ($P < 0.03$), and higher PWV and AIx values ($P < 0.02$, $P < 0.004$, respectively) (Table 1). No differences were found in the parameters of peripheral and central BP measurements between the two groups (Table 1).

Biochemical characteristics of the study populations are reported in Table 2. Patients with severely increased albuminuria compared to patients with moderately increased albuminuria had significantly lower values of haematocrit ($P < 0.01$) and haemoglobin ($P < 0.02$), as well as increased levels of fibrinogen ($P < 0.01$), hsCRP ($P < 0.001$), and phosphorus levels ($P < 0.01$), and lower eGFR-MDRD values ($P < 0.001$) (Table 2).

Univariate linear regression analyses for the association of the absolute values of PWV with other parameters are shown in Table 3. In multivariate linear regression analysis, only severely increased albuminuria ($\beta = 1.038$, $P < 0.010$), pSBP ($\beta = 0.028$, $P < 0.034$) and Ht ($\beta = 0.171$, $P = 0.001$) remained independent determinants of increased PWVc-f (Table 4).

Univariate linear regression analyses for the association of the absolute values of AIx with other parameters are shown in Table 5. Similarly, severely increased albuminuria ($\beta = 4.385$, $P < 0.012$), cSBP ($\beta = 0.242$, $P < 0.001$), cPP ($\beta = 0.147$, $P < 0.01$) and Ht levels ($\beta = 0.591$, $P < 0.013$) remained independent determinants of increased AIx values (Table 6). No other variables correlated significantly to AS indices.

DISCUSSION

The findings of our study demonstrate an independent association between AS indices (PWV and AIx) and severely increased albuminuria in hypertensive non-diabetic patients with moderate kidney dysfunction, CKD stages 1-2, treated with RAAS blockers.

Albuminuria is recognized as a marker of vascular dysfunction and heightened cardiovascular disease risk[1,2]. The presence of albuminuria is also an indicator of the underlying kidney disease with an increased probability of progressive kidney loss[18,21]. It is worth mentioning that the level of albuminuria and not the current level of GFR is the most relevant variable to predict CKD progression in those with a GFR of at least 30 mL/min[22].

In recent years, great emphasis has been placed on the role of AS in the development of cardiovascular diseases while this parameter has been used in the assessment of patients with hypertension and/or CKD, since AS measurement seems to have an additive value beyond traditional risk factors, including Framingham risk score[23]. Increased AS

Table 2 Biochemical characteristics of the study population

Patients characteristics	Study population	Patients with moderately increased albuminuria	Patients with severely increased albuminuria	*P* value
No	115	58	57	-
Ht (%)	42.3 ± 3.7	43.2 ± 3.7	41.4 ± 3.5	0.01
Hb (g/dL)	13.6 ± 1.3	14.1 ± 1.5	13.3 ± 1.5	0.02
Fe (μg/dL)	89 ± 35.5	90.2 ± 30	84.2 ± 38.5	NS
Ferritin (ng/L)	127.4 ± 90.1	127.8 ± 87.9	131.3 ± 96.5	NS
Fibrinogen (mg/dL)	336 ± 82.9	339 ± 86.5	394 ± 133.9	< 0.01
hsCRP (mg/dL)	0.19 ± 0.06	0.18 ± 0.05	0.23 ± 0.10	< 0.001
T-CHOL (mg/dL)	203 ± 37.5	208 ± 39.2	209 ± 46.8	NS
TRG (mg/dL)	126 ± 45.7	123 ± 46.3	145 ± 70.2	0.05
HDL-C (mg/dL)	53.4 ± 13.3	53 ± 12.9	54.2 ± 14.9	NS
LDL-C (mg/dL)	119 ± 32	120 ± 3.7	121 ± 3.6	NS
Ca^{2+} (mg/dL)	9.7 ± 0.4	9.7 ± 0.5	9.8 ± 0.4	NS
PO_4^{3-} (mg/dL)	3.42 ± 0.4	3.3 ± 0.4	3.5 ± 0.5	0.01
Ca^{2+} x PO_4^{3-}	33.7 ± 4.8	32.6 ± 4.5	33.9 ± 8.6	NS
PTH (pg/mL)	42.4 ± 15.4	39.8 ± 13.6	45.4 ± 29.9	NS
sAlb (gr/dL)	4.5 ± 0.2	4.5 ± 0.3	4.3 ± 0.5	0.03
eGFR-MDRD (mL/min per 1.73 m^2)	92 ± 0.5	91.5 ± 20.5	79.1 ± 15.3	< 0.001
CKD-EPI(mL/min per 1.73 m^2)	73.4 ± 1.25	73.1 ± 13.7	73.6 ± 11.2	NS

Data are presented as mean value. Ht: Haematocrit; Hb: Haemoglobin; Fe: Serum iron; Ferritin: Serum ferritin, hsCRP: High sensitive C-reactive protein; T-CHOL: Total cholesterol; TRG: Triglycerides; HDL-C: High density lipoprotein cholesterol; Ca^{2+}: Calcium; PO_4^{3-}: Phosphorus; PTH: Parathormone; sAlb: Serum albumin; eGFR-MDRD: Estimated-glomerular filtration rate-modification of diet in renal disease; RAAS-blocker: Renin angiotensin aldosterone system blocker; Alb: Albuminuria.

Table 3 Univariate linear regression analysis of the parameters associated with the absolute values of pulse wave velocity

Covariates	β	*t*	β (95%CI)	*P* value
Alb	0.841	2.271	0.107-1.574	< 0.025
Age	0.026	2.047	0.001-0.051	< 0.043
BMI	0.009	0.880	0.011-0.028	0.381
pSBP	0.032	2.511	0.007-0.058	< 0.013
pDBP	0.005	0.285	-0.032-0.042	< 0.776
pPP	0.029	2.277	0.004-0.053	< 0.025
cSBP	0.031	2.298	0.004-0.508	< 0.024
cDBP	-0.004	-0.173	-0.045-0.038	0.963
cPP	0.014	1.257	0.008-0.037	0.212
Ht	0.103	2.077	0.005-0.202	0.040
Hb	0.227	1.812	0.020-0.476	0.070
T-CHOL	0.021	0.456	-0.011-0.007	0.649
TRG	0.002	0.752	0.009-0.004	0.454
HDL-C	-0.006	-0.417	-0.033-0.022	0.677
LDL-C	0.023	0.445	0.013-0.477	0.674
Fbrinogen	0.0001	0.524	-0.004-0.002	0.602
hsCRP	-0.011	0.046	-0.442-0.422	0.964
RAAS-blocker	0.081	-0.103	-1.650-1.487	0.918

Alb: Albumin; BMI: Body mass index; pSBP: Peripheral systolic blood pressure; pDBP: Peripheral diastolic blood pressure; pPP: Peripheral pulse pressure; cSBP: Central systolic blood pressure; cDBP: Central diastolic blood pressure; cPP: Central pulse pressure; Ht: Haematocrit; Hb: Haemoglobin; T-CHOL: Total cholesterol; TRG: Triglycerides; HDL-C: High density lipoprotein cholesterol; RAAS-blocker: Renin angiotensin aldosterone system blocker; LDL-C: Low density lipoprotein cholesterol; hsCRP: HsC-reactive protein.

provides prognostic information above traditional CV risk factors, such as BP itself, gender, age, smoking diabetes, and cholesterol[24,25]. It is an independent predictor of fatal stroke in patients with essential hypertension[3,4,26] and a powerful predictor of mortality in both diabetes mellitus and glucose-tolerance-tested multi-ethnic population samples[27]. In addition, PWVc-f is independently associated with a faster decline of kidney function in patients with type 2 diabetes mellitus[10]. The relationship between AS and events is continuous,

Table 4 Multivariate linear regression analysis of the parameters associated with the absolute values of pulse wave velocity

Covariates	β	*t*	β (95%CI)	*P* value
UAlb	1.038	2.638	0.257-1.820	< 0.010
pSBP	0.028	2.149	0.002-0.053	< 0.034
Ht	0.171	3.319	0.069-0.273	< 0.001

pSBP: Peripheral systolic blood pressure; Ht: Haematocrit.

Table 5 Univariate linear regression analysis of the parameters associated with absolute values of AIx

Covariates	β	*t*	β (95%CI)	*P* value
UAlb	6.201	2.977	2.065-10.337	< 0.004
Age	0.236	3.427	0.099-0.373	< 0.0001
BMI	0.036	0.667	0.070-0.142	0.507
pSBP	0.291	4.427	0.161-0.422	< 0.0001
pDBP	0.001	0.002	-0.216-0.216	< 0.002
pPP	0.278	4.536	0.156-0.400	< 0.0001
cSBP	0.349	5.518	0.223-0.474	< 0.0001
cDBP	-0.045	-0.401	0.265-0.176	0.689
cPP	0.265	4.912	0.558-0.372	< 0.0001
Ht	-0.848	0.002	-1.382-0.314	< 0.002
Hb	-0.599	-0.826	-2.040-0.841	0.411
T-CHOL	0.050	1.836	-0.041-0.042	0.069
TRG	0.021	1.164	0.015-0.057	0.247
HDL-C	0.104	1.318	-0.534-0.035	0.262
LDL-C	0.047	1.265	-0.045-0.052	0.243
Fbrinogen	0.007	0.749	-0.122-0.273	0.456
hsCRP	0.015	0.732	-1.738-3.768	0.466
RAAS-blocker	1.043	0.139	13.881-15.966	0.890

Alb: Albumin; BMI: Body mass index; pSBP: Peripheral systolic blood pressure; pDBP: Peripheral diastolic blood pressure; pPP: Peripheral pulse pressure; cSBP: Central systolic blood pressure; cDBP: Central diastolic blood pressure; cPP: Central pulse pressure; Ht: Haematocrit; Hb: Haemoglobin; T-CHOL: Total cholesterol; TRG: Triglycerides; HDL-C: High density lipoprotein cholesterol; RAAS-blocker: Renin angiotensin aldosterone system blocker; LDL-C: Low density lipoprotein cholesterol; hsCRP: HsC-reactive protein.

Table 6 Multivariate linear regression analysis of the parameters associated with the absolute values of AI

Covariates	β	*t*	β (95%CI)	*P* value
UAlb	4.385	2.557	1.023-8.146	< 0.012
cSBP	0.242	3.563	0.107-0.376	< 0.0001
cPP	0.147	2.623	0.036-0.259	< 0.0001
Ht	0.591	2.536	1.055-0.128	< 0.013

cSBP: Central systolic blood pressure; cPP: Central pulse pressure; Ht: Haematocrit.

however, a threshold of > 12 m/s has been suggested as a significant marker of vascular alterations and aortic dysfunction in middle-aged hypertensive patients[28]. A more recent expert consensus statement adjusted this threshold value to 10 m/s[29]. In fact in our study patients had mean PWV values < 10 m/s (Table 1).

Several cross-sectional studies demonstrated a relationship between AS and moderately increased albuminuria in the general population, in individuals with hypertension[30] and/or type 2 diabetes mellitus[5,14]. Additionally, epidemiologic evidence showed an independent association between AS, moderately increased albuminuria and other indices of subclinical target organ damage in non-hypertensive, non-diabetic individuals[28].

In our study we expanded this relationship in hypertensive patients with stage 1-2 CKD without diabetes already treated with RAAS blockers. Our results are in accordance with the results from the Framingham cross-sectional analyses in patients with moderate CKD that showed that PWVc-f was associated with both urinary albumin-to-creatinine ratio and moderately

increased albuminuria ($P < 0.0001$)[31]. In the study by Munakata *et al*[32] each 400 cm/s increase in brachial-ankle PWV, increased the incidence of new-onset moderately increased albuminuria about 2.4 times at 2-year follow-up, suggesting that higher brachial-ankle PWV could be an independent risk factor for the future development of moderately increased albuminuria in patients with hypertension[32]. A study by Kim *et al*[15] showed that AS is independently associated with moderately increased albuminuria, irrespectively of various covariates, in non-hypertensive, non-diabetic individuals. In our study we showed that these results are expanded to the patients with severely increased albuminuria.

The mechanisms linking AS and albuminuria are not fully established. However, it has been suggested that endothelial dysfunction could be a possible mechanism involved in the remodeling of the arterial wall causing structural and functional changes in the target vessels, resulting in the increase of the AS. On the other hand, endothelial dysfunction modifies glomerular permeability and as a consequence leads to increased albumin excretion[33,34]. Alternatively, an increased pulsatile stress mediated by an increased AS causes a pressure load on the glomeruli and could lead to their damage[35].

In our study the optimal BP control in our patients is believed to play a substantial role and contributed to a lower AS indices as well as lower levels of albuminuria. Furthermore all patients in our study were treated with a RAAS blocker.

In patients with arterial hypertension and albuminuria blockage of the RAAS is the treatment of choice[18]. These agents offer a cardio-renal protection which may be mediated, at least in part, by their beyond blood pressure control drug-specific effects. In the past, we showed that these agents improve AS and decrease significantly moderately increased albuminuria[36]. In the present study we showed that patients with severely increased albuminuria had a higher AIx which remained an independent determinant of increased AS. No significant differences in central aortic pressures between the groups were observed. It is known that AIx and central aortic pressures reflect different arterial wall properties compared to PWV. The latest, reflect changes in pressure wave reflections from the large arteries at the distal sites. In contrast, AIx reflect functional properties from the small arteries. In the Framingham study AIx was not associated with urinary albumin excretion[31]. In contrast, in our study, the association of AIx with severely increased albuminuria remains significant in multivariate analysis despite the administration of RAAS blockade agents.

In patients with severely increased albuminuria lower Ht levels were found, which were independently associated with PWV and AIx. In this group of patients, despite the near normal degree of Ht levels along with increased inflammatory indices, such as fibrinogen and hsCRP, Ht might be related to the kidney dysfunction as suggested by Hiramoto *et al*[37]. Of note, in hypertensive patients, increased levels of inflammatory biomarkers, such hs-CRP, are associated with AS indices (*i.e.*, PWV and AIx)[38]. Furthermore, these markers are also increased with the deterioration of renal function[38]. Thus, our results, emphasize the possibility of a common pathophysiologic mechanism affecting renal dysfunction, anemia and AS deterioration: inflammation and endothelial dysfunction may play a prominent role in the interrelation of these entities[37,39].

The cross sectional design, which does not allow to establish cause-effect relationships as well as the small number of patients involved are potential limitations of our study. Subsequently, further prospective studies are required to verify whether albuminuria is a contributing factor and/or a consequence of the increased AS independently of the RAAS blockade and BP control.

Our findings suggest an independent association between AS and severely increased albuminuria in non-diabetic, hypertensive patients, already treated with RAAS agents, who exhibited early renal dysfunction.

COMMENTS

Background

Albuminuria is associated with higher cardiovascular risk. Pulse wave velocity (PWV) and augmentation index (AIx) are early markers of vascular changes and aortic stiffness (AS) in patients with chronic kidney disease (CKD).

Research frontiers

The current research hotspot is the association between albuminuria levels and arterial stiffness. Patients participated in the study were non-diabetic with hypertension and CKD stages 1-2. All patients were treated with renin angiotensin blockade agents plus other hypertensive drugs for a rational period of time. Limited data are available whether severely increased albuminuria is associated with AS in non-diabetic hypertensive patients already treated with renin angiotensin aldosterone blockade agents.

Innovations and breakthroughs

Previous studies showed that in patients with type 2 diabetes mellitus the levels of urinary albumin excretion, but not reduced estimated glomerular filtration rate, were associated with increased AS and atherosclerosis. Even high normal albuminuria in the range (0-30 mg/g) is also associated with aortic stiffness even in type 2 diabetic patients. Limited data are available whether severely increased albuminuria is associated with AS in non-diabetic hypertensive patients already treated with renin angiotensin aldosterone blockade agents. This association even in treated patients with agents that could reduce albuminuria or could reduce arterial stiffness still exists.

Applications

The study results suggest an independent association between AS indices and severely increased albuminuria in non-diabetic, hypertensive patients with CKD stages 1-2 treated with renin angiotensin aldosterone system (RAAS) blockers. Despite the treatment of these patients with RAAS blockers still an association between arterial stiffness and severe increased albuminuria exist.

Terminology

Albuminuria is associated with faster progression of renal failure and is also recognized as a marker of vascular dysfunction and heightened cardiovascular disease risk. Arterial stiffness assessment helps to predict cardiovascular events in patients with or without diabetes. It is usually assessed with the aortic PWV and peripheral pressure wave reflections. These indices (PWV and AIx), as major determinants of the aortic pulse pressure, are early markers of atherosclerotic vascular changes and in CKD have been shown to be associated with renal micro vascular damage and kidney dysfunction.

Peer-review

This is a cross sectional study in which the authors demonstrate an independent association between AS indices and severely increased albuminuria in non-diabetic, hypertensive patients with CKD stages 1-2 treated with RAAS blockers. The study is interesting because all the patients were treated with

RAAS agents [ACE inhibitors or angiotensin type 1 (AT1) receptor blockers] plus other antihypertensive drugs as needed. The choice for the first agent was a RAAS blocker based on the favorable effects of these agents on albuminuria and AS reduction as well as for their anti-fibrotic and anti-inflammatory effects and this treatment was already applied for at least 6 mo. The results suggest an independent association between AS indices (PWV and AIx) and severely increased albuminuria in hypertensive non-diabetic patients with moderate kidney dysfunction, CKD stages 1-2, even though these patients were treated with RAAS agents (ACE inhibitors or AT1 receptor blockers) for this period of time and the BP levels were well controlled.

REFERENCES

1 **Cotter J**, Oliveira P, Cunha P, Polónia J. Risk factors for development of microalbuminuria in diabetic and nondiabetic normoalbuminuric hypertensives with high or very high cardiovascular risk - a twelve-month follow-up study. *Nephron Clin Pract* 2009; **113**: c8-c15 [PMID: 19590230 DOI: 10.1159/000228070]

2 **Sarnak MJ**, Levey AS, Schoolwerth AC, Coresh J, Culleton B, Hamm LL, McCullough PA, Kasiske BL, Kelepouris E, Klag MJ, Parfrey P, Pfeffer M, Raij L, Spinosa DJ, Wilson PW. Kidney disease as a risk factor for development of cardiovascular disease: a statement from the American Heart Association Councils on Kidney in Cardiovascular Disease, High Blood Pressure Research, Clinical Cardiology, and Epidemiology and Prevention. *Circulation* 2003; **108**: 2154-2169 [PMID: 14581387 DOI: 10.1161/01.CIR.0000095676.90936.80]

3 **Laurent S**, Katsahian S, Fassot C, Tropeano AI, Gautier I, Laloux B, Boutouyrie P. Aortic stiffness is an independent predictor of fatal stroke in essential hypertension. *Stroke* 2003; **34**: 1203-1206 [PMID: 12677025 DOI: 10.1161/01.STR.0000065428.03209.64]

4 **Laurent S**, Cockcroft J, Van Bortel L, Boutouyrie P, Giannattasio C, Hayoz D, Pannier B, Vlachopoulos C, Wilkinson I, Struijker-Boudier H. Expert consensus document on arterial stiffness: methodological issues and clinical applications. *Eur Heart J* 2006; **27**: 2588-2605 [PMID: 17000623 DOI: 10.1093/eurheartj/ehl254]

5 **Yokoyama H**, Aoki T, Imahori M, Kuramitsu M. Subclinical atherosclerosis is increased in type 2 diabetic patients with microalbuminuria evaluated by intima-media thickness and pulse wave velocity. *Kidney Int* 2004; **66**: 448-454 [PMID: 15200455 DOI: 10.1111/j.1523-1755.2004.00752.x]

6 **Ford ML**, Tomlinson LA, Chapman TP, Rajkumar C, Holt SG. Aortic stiffness is independently associated with rate of renal function decline in chronic kidney disease stages 3 and 4. *Hypertension* 2010; **55**: 1110-1115 [PMID: 20212269 DOI: 10.1161/HYPERTENSIONAHA.109.143024]

7 **Ishikawa T**, Hashimoto J, Morito RH, Hanazawa T, Aikawa T, Hara A, Shintani Y, Metoki H, Inoue R, Asayama K, Kikuya M, Ohkubo T, Totsune K, Hoshi H, Satoh H, Imai Y. Association of microalbuminuria with brachial-ankle pulse wave velocity: the Ohasama study. *Am J Hypertens* 2008; **21**: 413-418 [PMID: 18369360 DOI: 10.1038/ajh.2007.77]

8 **Fesler P**, du Cailar G, Ribstein J, Mimran A. Glomerular hemodynamics and arterial function in normal individuals. *J Hypertens* 2010; **28**: 2462-2467 [PMID: 20720500 DOI: 10.1097/HJH.0b013e32833e2a21]

9 **Hashimoto J**, Ito S. Central pulse pressure and aortic stiffness determine renal hemodynamics: pathophysiological implication for microalbuminuria in hypertension. *Hypertension* 2011; **58**: 839-846 [PMID: 21968753 DOI: 10.1161/HYPERTENSIONAHA.111.177469]

10 **Bouchi R**, Babazono T, Mugishima M, Yoshida N, Nyumura I, Toya K, Hanai K, Tanaka N, Ishii A, Uchigata Y, Iwamoto Y. Arterial stiffness is associated with incident albuminuria and decreased glomerular filtration rate in type 2 diabetic patients. *Diabetes Care* 2011; **34**: 2570-2575 [PMID: 21972413]

11 **Sjöblom P**, Nystrom FH, Länne T, Engvall J, Östgren CJ. Microalbuminuria, but not reduced eGFR, is associated with cardiovascular subclinical organ damage in type 2 diabetes. *Diabetes Metab* 2014; **40**: 49-55 [PMID: 24200881 DOI: 10.1016/j.diabet.2013.09.008]

12 **Liu JJ**, Tavintharan S, Yeoh LY, Sum CF, Ng XW, Pek SL, Lee SB, Tang WE, Lim SC. High normal albuminuria is independently associated with aortic stiffness in patients with Type 2 diabetes. *Diabet Med* 2014; **31**: 1199-1204 [PMID: 24702136 DOI: 10.1111/dme.12461]

13 **Huang L**, Yang L, Zhang S, Liu D, Yan X, Yan S. Low-grade albuminuria associated with brachial-ankle pulse wave velocity in young adults with type 2 diabetes mellitus in China. *Diabetes Metab Res Rev* 2015; **31**: 262-268 [PMID: 25139614 DOI: 10.1002/dmrr.2598]

14 **Shin DI**, Seung KB, Yoon HE, Hwang BH, Seo SM, Shin SJ, Kim PJ, Chang K, Baek SH. Microalbuminuria is independently associated with arterial stiffness and vascular inflammation but not with carotid intima-media thickness in patients with newly diagnosed type 2 diabetes or essential hypertension. *J Korean Med Sci* 2013; **28**: 252-260 [PMID: 23400641 DOI: 10.3346/jkms.2013.28.2.252]

15 **Kim BJ**, Lee HA, Kim NH, Kim MW, Kim BS, Kang JH. The association of albuminuria, arterial stiffness, and blood pressure status in nondiabetic, nonhypertensive individuals. *J Hypertens* 2011; **29**: 2091-2098 [PMID: 21881520]

16 **Pende A**, Dallegri F. Renin-angiotensin antagonists: therapeutic effects beyond blood pressure control? *Curr Pharm Des* 2012; **18**: 1011-1020 [PMID: 22283770 DOI: 10.2174/138161212799436575]

17 **Sciarretta S**, Paneni F, Palano F, Chin D, Tocci G, Rubattu S, Volpe M. Role of the renin-angiotensin-aldosterone system and inflammatory processes in the development and progression of diastolic dysfunction. *Clin Sci* (Lond) 2009; **116**: 467-477 [PMID: 19200056 DOI: 10.1042/CS20080390]

18 **Kalaitzidis RG**, Bakris GL. Should proteinuria reduction be the criterion for antihypertensive drug selection for patients with kidney disease? *Curr Opin Nephrol Hypertens* 2009; **18**: 386-391 [PMID: 19561494 DOI: 10.1097/MNH.0b013e32832edc99]

19 Randomised placebo-controlled trial of effect of ramipril on decline in glomerular filtration rate and risk of terminal renal failure in proteinuric, non-diabetic nephropathy. The GISEN Group (Gruppo Italiano di Studi Epidemiologici in Nefrologia) *Lancet* 1997; **349**: 1857-1863 [PMID: 9217756 DOI: 10.1016/S0140-6736(96)11445-8]

20 **Chen CH**, Nevo E, Fetics B, Pak PH, Yin FC, Maughan WL, Kass DA. Estimation of central aortic pressure waveform by mathematical transformation of radial tonometry pressure. Validation of generalized transfer function. *Circulation* 1997; **95**: 1827-1836 [PMID: 9107170 DOI: 10.1161/01.CIR.95.7.1827]

21 **Wachtell K**, Olsen MH. Is it time to change the definition of normal urinary albumin excretion? *Nat Clin Pract Nephrol* 2008; **4**: 650-651 [PMID: 18852732 DOI: 10.1038/ncpneph0971]

22 **Lea J**, Greene T, Hebert L, Lipkowitz M, Massry S, Middleton J, Rostand SG, Miller E, Smith W, Bakris GL. The relationship between magnitude of proteinuria reduction and risk of end-stage renal disease: results of the African American study of kidney disease and hypertension. *Arch Intern Med* 2005; **165**: 947-953 [PMID: 15851648 DOI: 10.1001/archinte.165.8.947]

23 **Mancia G**, Fagard R, Narkiewicz K, Redón J, Zanchetti A, Böhm M, Christiaens T, Cifkova R, De Backer G, Dominiczak A, Galderisi M, Grobbee DE, Jaarsma T, Kirchhof P, Kjeldsen SE, Laurent S, Manolis AJ, Nilsson PM, Ruilope LM, Schmieder RE, Sirnes PA, Sleight P, Viigimaa M, Waeber B, Zannad F. 2013 ESH/ESC Guidelines for the management of arterial hypertension: the Task Force for the management of arterial hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC). *J Hypertens* 2013; **31**: 1281-1357 [PMID: 23817082 DOI: 10.1097/01.hjh.0000431740.32696.cc]

24 **Mattace-Raso FU**, van der Cammen TJ, Hofman A, van Popele NM, Bos ML, Schalekamp MA, Asmar R, Reneman RS, Hoeks AP, Breteler MM, Witteman JC. Arterial stiffness and risk of coronary heart disease and stroke: the Rotterdam Study. *Circulation* 2006; **113**: 657-663 [PMID: 16461838 DOI: 10.1161/CIRCULATIONAHA.105.555235]

25 **Willum-Hansen T**, Staessen JA, Torp-Pedersen C, Rasmussen

S, Thijs L, Ibsen H, Jeppesen J. Prognostic value of aortic pulse wave velocity as index of arterial stiffness in the general population. *Circulation* 2006; **113**: 664-670 [PMID: 16461839 DOI: 10.1161/CIRCULATIONAHA.105.579342]

26 **Boutouyrie P**, Tropeano AI, Asmar R, Gautier I, Benetos A, Lacolley P, Laurent S. Aortic stiffness is an independent predictor of primary coronary events in hypertensive patients: a longitudinal study. *Hypertension* 2002; **39**: 10-15 [PMID: 11799071 DOI: 10.1161/hy0102.099031]

27 **Cruickshank K**, Riste L, Anderson SG, Wright JS, Dunn G, Gosling RG. Aortic pulse-wave velocity and its relationship to mortality in diabetes and glucose intolerance: an integrated index of vascular function? *Circulation* 2002; **106**: 2085-2090 [PMID: 12379578 DOI: 10.1161/01.CIR.0000033824.02722.F7]

28 **Mancia G**, De Backer G, Dominiczak A, Cifkova R, Fagard R, Germano G, Grassi G, Heagerty AM, Kjeldsen SE, Laurent S, Narkiewicz K, Ruilope L, Rynkiewicz A, Schmieder RE, Boudier HA, Zanchetti A, Vahanian A, Camm J, De Caterina R, Dean V, Dickstein K, Filippatos G, Funck-Brentano C, Hellemans I, Kristensen SD, McGregor K, Sechtem U, Silber S, Tendera M, Widimsky P, Zamorano JL, Erdine S, Kiowski W, Agabiti-Rosei E, Ambrosioni E, Lindholm LH, Viigimaa M, Adamopoulos S, Agabiti-Rosei E, Ambrosioni E, Bertomeu V, Clement D, Erdine S, Farsang C, Gaita D, Lip G, Mallion JM, Manolis AJ, Nilsson PM, O'Brien E, Ponikowski P, Redon J, Ruschitzka F, Tamargo J, van Zwieten P, Waeber B, Williams B. 2007 Guidelines for the Management of Arterial Hypertension: The Task Force for the Management of Arterial Hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC). *J Hypertens* 2007; **25**: 1105-1187 [PMID: 17563527 DOI: 10.1097/HJH.0b013e3281fc975a]

29 **Safar ME**, Levy BI, Struijker-Boudier H. Current perspectives on arterial stiffness and pulse pressure in hypertension and cardiovascular diseases. *Circulation* 2003; **107**: 2864-2869 [PMID: 12796414 DOI: 10.1161/01.CIR.0000069826.36125.B4]

30 **Tsioufis C**, Tzioumis C, Marinakis N, Toutouzas K, Tousoulis D, Kallikazaros I, Stefanadis C, Toutouzas P. Microalbuminuria is closely related to impaired arterial elasticity in untreated patients with essential hypertension. *Nephron Clin Pract* 2003; **93**: c106-c111 [PMID: 12660419 DOI: 10.1159/000069546]

31 **Upadhyay A**, Hwang SJ, Mitchell GF, Vasan RS, Vita JA, Stantchev PI, Meigs JB, Larson MG, Levy D, Benjamin EJ, Fox CS. Arterial stiffness in mild-to-moderate CKD. *J Am Soc Nephrol* 2009; **20**: 2044-2053 [PMID: 19608701 DOI: 10.1681/ASN.2009010074]

32 **Munakata M**, Miura Y, Yoshinaga K. Higher brachial-ankle pulse wave velocity as an independent risk factor for future microalbuminuria in patients with essential hypertension: the J-TOPP study. *J Hypertens* 2009; **27**: 1466-1471 [PMID: 19412131]

33 **Stehouwer CD**, Smulders YM. Microalbuminuria and risk for cardiovascular disease: Analysis of potential mechanisms. *J Am Soc Nephrol* 2006; **17**: 2106-2111 [PMID: 16825333 DOI: 10.1681/ASN.2005121288]

34 **Deen WM**. What determines glomerular capillary permeability? *J Clin Invest* 2004; **114**: 1412-1414 [PMID: 15545991 DOI: 10.1172/JCI23577]

35 **O'Rourke MF**, Safar ME. Relationship between aortic stiffening and microvascular disease in brain and kidney: cause and logic of therapy. *Hypertension* 2005; **46**: 200-204 [PMID: 15911742 DOI: 10.1161/01.HYP.0000168052.00426.65]

36 **Spanos G**, Kalaitzidis R, Karasavvidou D, Pappas K, Siamopoulos KC. Efficacy of aliskiren and valsartan in hypertensive patients with albuminuria: a randomized parallel-group study. *J Renin Angiotensin Aldosterone Syst* 2013; **14**: 315-321 [PMID: 23132848 DOI: 10.1177/1470320312465219]

37 **Hiramoto JS**, Katz R, Peralta CA, Ix JH, Fried L, Cushman M, Siscovick D, Palmas W, Sarnak M, Shlipak MG. Inflammation and coagulation markers and kidney function decline: the Multi-Ethnic Study of Atherosclerosis (MESA). *Am J Kidney Dis* 2012; **60**: 225-232 [PMID: 22560844 DOI: 10.1053/j.ajkd.2012.02.335]

38 **Elias MF**, Davey A, Dore GA, Gillespie A, Abhayaratna WP, Robbins MA. Deterioration in renal function is associated with increased arterial stiffness. *Am J Hypertens* 2014; **27**: 207-214 [PMID: 24080989 DOI: 10.1093/ajh/hpt179]

39 **Barbaro NR**, Fontana V, Modolo R, De Faria AP, Sabbatini AR, Fonseca FH, Anhê GF, Moreno H. Increased arterial stiffness in resistant hypertension is associated with inflammatory biomarkers. *Blood Press* 2015; **24**: 7-13 [PMID: 25061978 DOI: 10.3109/08037051.2014.940710]

P- Reviewer: Bellomo G, Chang CC, Fujigaki Y **S- Editor**: Tian YL
L- Editor: A **E- Editor**: Yan JL

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DOI: 10.5527/wjn.v4.i3.415

World J Nephrol 2015 July 6; 4(3): 415-422
ISSN 2220-6124 (online)

ORIGINAL ARTICLE

Prospective Study

Low T3 syndrome and long-term mortality in chronic hemodialysis patients

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Author contributions: All authors contributed to this manuscript.

Ethics approval statement: The study was reviewed and approved by the Institutional Review Board of both the Dimocritus University Hospital in Alexandroupoli and the "George Papanikolaou" General Hospital in Thessaloniki, as part of my PhD thesis (Registration No: 1221/12-12-2006).

Clinical trial registration: The study was not interventional, randomized or controlled. All patients during the study were receiving routine examination and laboratory evaluation and were followed-up for mortality as part of their routine care. Thus a registration identification number of the study was not necessary.

Informed consent statement: All involved patients gave a verbal informed consent for the legal use of their blood samples and handling of their personal data before their enrollment.

Conflict-of-interest statement: All authors declare no conflict of interest.

Data sharing statement: There are no additional data available.

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Received: December 22, 2014
Peer-review started: December 23, 2014
First decision: January 8, 2015
Revised: March 9, 2015
Accepted: April 10, 2015
Article in press: April 14, 2015
Published online: July 6, 2015

Abstract

AIM: To investigate the predictive value of low freeT3 for long-term mortality in chronic hemodialysis (HD) patients and explore a possible causative role of chronic inflammation.

METHODS: One hundred fourteen HD patients (84 males) consecutively entered the study and were assessed for thyroid function and two established markers of inflammation, high sensitivity C-reactive protein (hsCRP) and interleukin-6 (IL-6). Monthly blood samples were obtained from all patients for three consecutive months during the observation period for evaluation of thyroid function and measurement of inflammatory markers. The patients were then divided in two groups based on the cut-off value of 1.8 pg/mL for mean plasma freeT3, and were prospectively studied for a mean of 50.3 ± 30.8 mo regarding cumulative survival. The prognostic power of low serum fT3 levels for mortality was assessed using the Kaplan-Meier method and univariate and multivariate regression analysis.

RESULTS: Kaplan-Meier survival curve showed a negative predictive power for low freeT3. In Cox regression analysis low freeT3 remained a significant predictor of mortality after adjustment for age, diabetes mellitus, hypertension, hsCRP, serum creatinine and albumin. Regarding the possible association with inflammation, freeT3 was correlated with hsCRP, but not IL-6, and only at the first month of the study.

CONCLUSION: In chronic hemodialysis patients, low plasma freeT3 is a significant predictor of all-cause mortality. Further studies are required to identify the underlying mechanisms of this association.

Key words: C-reactive protein; Hemodialysis; Inflammation; Interleukin-6; Low T3 syndrome; Mortality

Core tip: Monthly blood samples were obtained from 114 patients for three consecutive months during the observation period for evaluation of thyroid function and measurement of inflammatory markers high sensitivity C-reactive protein (hsCRP) and interleukin-6 (IL-6). Patients were then followed-up for 7-years. Low mean freeT3 (< 1.8 pg/mL) emerged as a significant predictor of all-cause mortality after adjustment for age, diabetes mellitus, hypertension, hsCRP, serum creatinine and albumin. However, freeT3 was correlated with hsCRP, but not IL-6, and only at the first month suggesting that further studies are required to identify the underlying pathogenetic mechanisms of the association between thyroid function and survival.

Fragidis S, Sombolos K, Thodis E, Panagoutsos S, Mourvati E, Pikilidou M, Papagianni A, Pasadakis P, Vargemezis V. Low T3 syndrome and long-term mortality in chronic hemodialysis patients. *World J Nephrol* 2015; 4(3): 415-422 Available from: URL: http://www.wjgnet.com/2220-6124/full/v4/i3/415.htm DOI: http://dx.doi.org/10.5527/wjn.v4.i3.415

INTRODUCTION

Despite the significant advances in hemodialysis (HD) techniques during the last decades, morbidity and mortality of HD patients remains unacceptably high and it is attributed mainly to the excess prevalence of cardiovascular disease (CVD)[1]. Beyond traditional risk factors (Framingham risk factors), which are highly prevalent in end-stage renal disease (ESRD) patients, patients on renal replacement therapy appear to be subject to the deleterious effect of a number of other harmful factors, including uremic milieu, anemia, bone-mineral disorders and hyperhomocysteinemia[2]. Moreover, recent studies suggested that oxidative stress, endothelial dysfunction and inflammation, also exacerbate cardiovascular disease[3,4]. So far, there is a lack of a strong marker of disease severity in ESRD, easy to perform and with high availability and low cost, that could guide treatment or even serve as a prognostic indicator of mortality[5]. Presumably, a multifactorial approach of traditional markers combined with novel biomarkers could fulfill this necessity, though this hypothesis needs to be confirmed in large prospective trials[6,7].

Low free triiodothyronin (freeT3) has emerged as a potent biomarker in ESRD in several studies and represents the main finding of non thyroidal illness syndrome (NTIS) in renal disease[2,8-10]. This syndrome, has been a debate for several years, as changes in hormone levels have been considered either a laboratory pitfall or an adoptive response to chronic stress aimed to spare calories[11,12]. It is important to emphasize the significance of values range, especially in differentiating incidents of subclinical hypothyroidism that could be mistaken as low T3 syndrome cases[13]. More recently, a narrower range of TSH values was proposed, although not widely accepted[14,15].

The exact pathogenetic mechanisms of NTIS are not yet fully elucidated but iodine retention, alterations to protein binding, derangements to deiodinases activity and dysregulation at the hypothalamic level appear to play a major role[16]. In addition, some studies demonstrated a significant negative correlation between interleukin-6 (IL-6) and serum T3 levels and suggested that NTIS is an acute phase response, yielded by activation of a cytokine network[17,18]. However, a causal role of chronic inflammation in the development of low T3 syndrome remains to be identified.

The aim of this prospective study was to investigate the predictive power of low freeT3 for long-term mortality in chronic hemodialysis patients; moreover the correlation between thyroid function and inflammation, assessed by measurements of high sensitivity CRP (hsCRP) and IL-6, was studied.

MATERIALS AND METHODS

Design

Prospective, observational study in 118 clinically stable chronic hemodialysis patients.

Patients

One hundred eighteen chronic hemodialysis patients from two large dialysis centers in Northern Greece, the Renal Unit at "G. Papanikolaou" General Hospital of Thessaloniki and the Department of Nephrology at University Hospital of Alexandroupolis, consecutively entered the study. Both centers had similar methods regarding hemodialysis and treatment strategies in ESRD, which are in accordance with the Kidney Disease Outcomes Quality Initiative (K/DOQI) guidelines[19]. All patients had been stabilized on renal replacement therapy for > 3 mo prior to enrollment (mean HD duration 113.4 ± 65.1 mo, range 15-427 mo) and were clinically stable and free of active infection.

Patients with known history of thyroid disorders or taking medications with possible effect in thyroid hormone values, like amiodarone or lithium, were excluded from the study[20]. None of the patients was receiving antibiotics at the time of the study or had required hospitalization up to 3 mo prior to study entry. All patients were on a 4hr three times weekly hemodialysis schedule. The dialysis modalities were on-line hemodiafiltration (OL-HDF) or conventional hemodialysis (C-HD) with high-flux or low-flux dialyzer membranes respectively. Both dialysis centers used semisynthetic membranes (polyamide or polysulphone) with surface ranging from 1.6-2.1 m^2. The dialysis solution consisted of standard bicarbonate preparations ($HCO3^-$: 32-35 mmol/L, Na: 138 mmol/L, K: 1-3 mmol/L, Mg: 0.5-0.75 mmol/L, Ca: 1.25-1.75 mmol/L). Low-molecular-weight or unfractionated heparin were used as standard anticoagulation. Dialysis prescription was guided by a goal of achieving a value of $\geqslant$ 0.65 for the urea reduction ratio and a value of Kt/V $\geqslant$ 1.2. The above indices of adequacy of dialysis were calculated by the formula [(pre-dialysis urea)-(post-dialysis urea)/predialysis urea], and by the second generation Daugirdas equation, respectively.

Body mass index (BMI) was calculated by dividing the dry weight in kilograms by the square of the height in meters. Blood pressure (BP) was measured in a supine position after 15 min of recumbency before a routine midweek dialysis session with standard mercury sphygmomanometers. Patients were considered hypertensive if they had pre-dialysis BP $\geqslant$ 160/90 mmHg or if they were receiving one or more anti-hypertensive drugs at the time of the study. History of cardiovascular disease was defined as history of myocardial infarction, coronary artery bypass or clinical signs of angina pectoris, stroke or transient ischemic attack or peripheral vascular disease.

The study protocol was conformed to the ethical guidelines of the Declaration of Helsinki and was approved both by the institutional review board and the ethics committee of each participating centre. Furthermore, all patients gave informed consent for the legal use of their blood samples and handling of their personal data before their enrollment.

Laboratory methods

Monthly blood samples were taken before a midweek routine hemodialysis session, for three consecutive months. Blood was drawn from the "arterial" lumen of fistula needle or the arterial port of the central hemodialysis catheter after extracting heparin lock in order to avoid artifactual alterations of measured freeT3 and the other studied parameters[21]. All samples were centrifuged for 15 min at 3500 rpm and the supernatant was immediately separated into vials (SST Ⅱ BD Vacutainer). Blood samples from Alexandroupoli were transferred to General Hospital "G. Papanikolaou" in Thessaloniki in dry ice. We were tied in with all appropriate scientific measures and legislation about transportation of biologic fluids. Samples from both institutions were eventually gathered and stored at G.H. "G. Papanikolaou" at -70 ℃.

All laboratory parameters were measured in the Central Biochemical Department of General Hospital "G. Papanikolaou". Complete blood cell count, urea, creatinine, total cholesterol, triglyceride, total protein and albumin, were determined by routine techniques using an automated analyser. Serum thyroid parameters (freeT3, freeT4, TSH), were measured by chemiluminescent, immunometric assay, according to the routine laboratory methods, using an IMMULITE-2000 analyzer, Laboratory reference values were 0.4 to 4.0 mIU/mL for TSH, 1.8 to 4.2 pg/mL for freeT3 and 0.89 to 1.76 ng/dL for freeT4.

Serum CRP levels were measured by high sensitivity nephelometrry ("Beckman Coulter" Ireland Inc). The detection limit was 0.1 mg/dL, with intra-assay and inter-assay coefficient of variation of 5% and 6.5%, respectively. Values above the threshold of 0.15 mg/dL were considered to be abnormal.

Serum IL-6 concentrations were measured by sandwich ELISA immunoassay using commercially available standard kits (AMS Biotechnology, United Kingdom). The concentrations of IL-6 were calculated by reference to standard curves performed with the corresponding recombinant molecule. All serum samples were tested in duplicate. The detection limit was 0.92 pg/mL, with intra-assay and inter-assay coefficient of variation of 3.4% and 5.2%, respectively.

Seven-year follow-up study

After the initial assessment and the determination of laboratory parameters for three consecutive months, all patients were followed-up for up to 7 years. During follow-up deaths were recording accurately by reviewing patient's hospital records.

Statistical analysis

Normality of variable distribution was tested using Kolmogorov-Smirnov test. Data are reported as mean ± SD (normally distributed data), median and interquartile range (non-normally distributed data) or as percentage frequency, as appropriate. Friedman's test was used to detect significant variations between non-parametric variables during the three month blood sampling period. According to the conducted power analysis each group had to include a minimum of 30 subjects in order to test differences in survival after a 7 year follow-up period. The significance of differences in means between the two groups was assessed by Student's *t* test or Mann-Whitney test. Differences in proportions were tested with the use of χ^2 test. Correlations were tested by Pearson's r or Spearman test for parametric and non-paramertric data analysis respectively. Non-normally distributed variables were log-transformed before entering regression analysis. For the survival analyses, patients were divided in low and normal freeT3 groups according to mean 3 mo freeT3. Patients included in the low freeT3 group had mean 3 mo values below the

Table 1 Baseline demographic, hemodynamic and clinical characteristics of low and normal freeT3 patient groups

Variable	Normal freeT3 (n = 79)	Low freeT3 (n = 35)	P value
Age	59.96 ± 14.74	66.84 ± 13.11	0.019
Female gender	18 (22.78%)	12 (34.28%)	0.237
BMI (kg/m^2)	25.65 ± 2.92	26.49 ± 4.02	0.622
HD duration (mo)	66.42 ± 59.69	56.49 ± 41.08	0.302
HD morning shift	75 (94.93%)	32 (91.42%)	0.418
OL-HDF	22 (27.84%)	11 (31.42%)	0.484
CVC access	10 (12.65%)	9 (25.71%)	0.160
Kt/V	1.27 ± 0.39	1.29 ± 0.42	0.822
Smoking	16 (20.25%)	9 (25.71%)	0.606
History of CVD	24 (30.37%)	17(48.57%)	0.048
Diabetes	16 (20.25%)	16 (45.71%)	0.017
Hypertension	60 (75.94%)	23 (65.71%)	0.488
SBP (mmHg)	131.06 ± 19.94	130.50 ± 21.94	0.819
DBP (mmHg)	68.42 ± 13.87	71.22 ± 14.47	0.357

Values are expressed as means ± SD or numbers (%) as appropriate. BMI: Body mass index; CVC: Central venous catheter; CVD: Cardiovascular disease; DBP: Diastolic blood pressure; HD: Hemodialysis; OL- HDF: On-line hemodiafiltration; SBP: Systolic blood pressure.

Table 2 Baseline laboratory parameters of low and normal freeT3 patient groups

Variable	Normal freeT3 (n = 79)	Low freeT3 (n = 35)	P value
Creatinine (mg/dL)	9.50 ± 2.75	8.44 ± 2.35	0.041
Urea (mg/dL)	146.59 ± 36.73	140.33 ± 51.06	0.556
Cholesterol (mg/dL)	180.67 ± 47.3	175.8 ± 52.52	0.176
Triglycerides (mg/dL)	183.86 ± 94.69	171.20 ± 130.36	0.215
Hematocrit (%)	37.41 ± 3.74	36.63 ± 3.86	0.860
Hemoglobin (g/dL)	12.04 ± 1.33	11.52 ± 1.31	0.262
WBC (/mm^3)	7.33 ± 2.20	7.61 ± 2.18	0.406
Albumin (g/dL)	3.99 ± 0.34	3.74 ± 0.497	0.002
TSH (μIU/mL)	2.77 ± 8.45	1.87 ± 1.02	0.354
FreeT4 (ng/dL)	1.13 ± 0.20	1.11 ± 0.3	0.170
IL-6 (pg/mL)	13.73 ± 13.29	16.49 ± 16.85	0.948
hsCRP (mg/dL)	1.56 ± 3.37	1.99 ± 2.73	0.156

Values are expressed as means ± SD or interquartile range as appropriate. hsCRP: High sensitive C-reactive protein; freeT4: Thyroxine; IL-6: Interleukin-6; TSH: Thyroid-stimulating hormone; WBC: White blood count.

lower laboratory's normal range (1.8 pg/mL). Time was calculated from the end of the 3 mo observation and blood sampling period until death or censoring. The data were censored if a patient underwent renal transplantation, switched from hemodialysis to peritoneal dialysis during the study period, died and at the end of follow-up. Furthermore, patients were censored if they were diagnosed with thyroid disease, primary or as a complication of treatment. Survival curves according to mean freeT3 values were calculated using the Kaplan-Meier method. Differences in survival were assessed with the use of log-rank test. Survival analyses were also made with the Cox proportional hazards model. The relative risks for mortality were determined by univariate and multivariate Cox regression analysis and presented as hazard ratio (HR; 95%CI). Covariates tested in the Cox model were age, diabetes mellitus, hypertension, hsCRP, creatinine, history of CVD, duration of hemodialysis and hemoglobin. Variables were included in the multivariate analyses if they had a P value < 0.05 in the univariate analysis or if they were clinically important confounders. The calculations were performed using SPSS 19.0 (Statistical Package for Social Sciences, IBM SPSS, Chicago, Ill). Statistical review of the study was performed by a biomedical statistician. A two-tailed P value < 0.05 was considered to be statistically significant.

The statistical review of the study was performed by a biomedical statistician.

RESULTS

During the blood sampling period four patients that required hospitalization or died were excluded from the analyses. Thus finally 114 patients (84 males) consecutively entered the study and were followed-up for 7 years (mean ± SD: 55.3 ± 2.9 mo). Mean age was 62.3 ± 14.3 years and mean HD duration 114.3 ± 91 mo (range 15-427 mo). Primary disease was diabetic nephropathy in 23.9% of the patients, glomerulonephritis in 19.7% and unknown in 27.4%. Three-month mean freeT3 was 2.17 ± 1.25 pg/mL, freeT4 was 0.956 ± 0.19 ng/dL, TSH was 1.94 ± 0.65 mIU/mL, hsCRP was 1.57 ± 2.98 mg/dL and IL-6 was 14.12 ± 11.52 pg/mL. Patients were divided into two groups based on the mean freeT3 values; the low freeT3 group consisted of thirty five patients (30.7%) with freeT3 below the lower normal laboratory range (1.8 pg/mL) and the normal freeT3 group consisted of 79 patients with freeT3 ⩾ 1.8 pg/mL).

Baseline (at the beginning of follow-up period) demographic, hemodynamic and clinical characteristics of low and normal freeT3 groups are shown in Table 1 and laboratory parameters in Table 2. Compared with patients that had normal freeT3, patients with low freeT3 were significantly older (P = 0.019) and had higher prevalence of diabetes mellitus (P = 0.017) and history of cardiovascular disease (P = 0.048). Gender, HD duration and morning shift, dialysis modality, Kt/V, type of vascular access, prevalence of smoking and hypertension and systolic and diastolic blood pressure did not differ significantly between the two groups (Table 1). Moreover, compared with patients with normal freeT3, patients with low freeT3 had at baseline significantly lower serum creatinine (P = 0.041) and albumin (P = 0.002). Low freeT3 group had also higher hsCRP and above the upper normal range (1.5 mg/dL) but the difference failed to reach statistical significance (P = 0.156). Hemoglobin and hematocrit, white blood cell count, blood urea, lipid levels, IL-6, freeT4 and TSH did not show significant differences in the two patient groups (Table 2).

Correlations of freeT3 levels with clinical and laboratory parameters

No statistically significant variation was observed in

Table 3 Demographic, clinical and laboratory significantly associated with survival in univariate Cox regression analyses

Variable	Spearman's rho	*P* value
Age (yr)	0.345	< 0.001
Diabetes mellitus (yes/no)	0.199	0.050
Hemodialysis vintage (mo)	-0.435	0.017
Hemoglobin (g/dL)	-0.329	0.001
Hematocrit (%)	-0.270	0.007
Hypertension (yes/no)	0.275	0.006
Free T3 (pg/mL)	-0.257	0.011
Previous CVD events (yes/no)	0.305	0.002
hsCRP (mg/dL)	0.240	0.017
Creatinine (mg/dL)	-0.400	< 0.001

hsCRP: High sensitive C-reactive protein; freeT3: Free triiodothyronine; CVD: Cardiovascular disease.

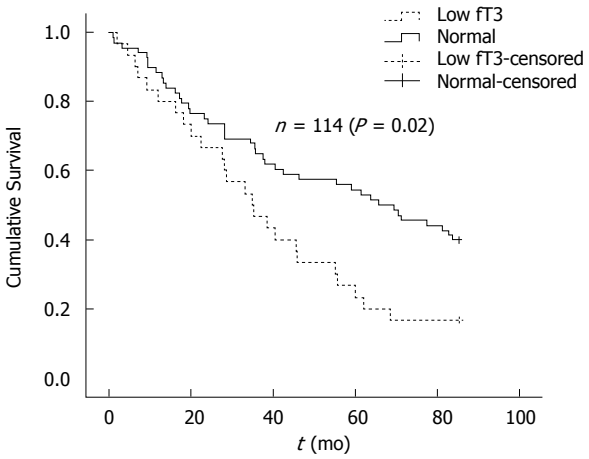

Figure 1 Kaplan-Meier estimate of survival in patients with low freeT3 (*n* = 35) and normal freeT3 (*n* = 79) levels.

hsCRP or IL-6 values within the 3 monthly samples (Friedman test, $P = 0.088$ and $P = 0.168$ respectively). In contrast, freeT3 levels showed a statistical significant variation during the three months ($P < 0.001$).

A linear statistically significant association was noted between age and mean freeT3, which resulted in the following algorithm: Mean freeT3 = 2.80-0.01*Age. In addition, serum albumin showed a strong correlation with freeT3 in all three consecutive measurements (meanAlb - meanfreeT3: Spearman's rho = 0.318, $P = 0.001$). No statistically significant correlations were observed between hsCRP or IL-6 with the other studied clinical parameters.

In the first month, significant correlations were found between TSH and freeT4 (Spearman's rho = -0.259, $P = 0.010$), hsCPR and freeT3 (Spearman's rho = -0.323, $P = 0.001$) and hsCRP and IL-6 (Spearman's rho = 0.312, $P = 0.001$). These associations remained significant in the following 2 mo except from the correlation between hsCRP and freeT3.

Correlation of freeT3 with mortality

During the 7 year follow-up period, 69 patients (60.5%) died. In addition, 11 patients were transplanted, one switched from hemodialysis to peritoneal dialysis and three patients were lost to follow-up. During follow-up 41 deaths (59.4%) were recorded in the normal freeT3 group and 25 deaths (83.3%) in the low freeT3 group ($P = 0.036$). Also, compared with patients that had normal freeT3 levels, patients with low freeT3 had a lower survival (54.9 ± 3.4 mo *vs* 39.8 ± 2.7 mo, $P = 0.019$). Furthermore, Kaplan-Meier survival curves differed significantly between patients with low and normal freeT3 levels [Log Rank (Mantel-cox), $P = 0.02$] (Figure 1).

In univariate (unadjusted) Cox regression analysis low freeT3 was a significant predictor of mortality [HR = 1.89 (1.146-3.124), $P = 0.011$]. Other parameters associated significantly with mortality were age, HD duration, history of CVD, hypertension, hemoglobin and hematocrit, serum creatinine and hsCRP. Moreover, diabetes mellitus showed a correlation of borderline significance with mortality (Table 3). In contrast, no association was observed between serum albumin and survival ($P = 0.178$). In addition, in a Cox Regression model, low freeT3 remained a significant independent predictor of mortality after adjustment for age, diabetes, hypertension, hsCRP, serum creatinine and albumin. However, in a multivariate stepwise Cox Regression analysis, including also hemoglobin, dialysis vintage and history of CVD, plasma freeT3 values failed to retain their predictive power for all-cause mortality. In the fully adjusted model, factors independently associated with mortality were age (3.2% higher odds of death for every year) and history of CVD (83.5% likelihood of death). Moreover, for every 1 g/dL of raise in hemoglobin levels there was a risk reduction for mortality of 43%. Diabetes mellitus and hsCRP values were not also associated with mortality in the fully adjusted model (Table 4).

DISCUSSION

Chronic uremia, similar to other chronic illnesses, may cause a variety of nonspecific wasting syndromes including protein loss, accumulation of fat stores, hyperglycemia and insulin resistance, hypoproteinemia, and hypertriglyceridemia[22]. Thyroid abnormalities are also very often in ESRD patients, with low freeT3 levels observed in the majority of cases[23,24]. The latter represents the main finding of euthyroid sick syndrome or non thyroidal illness syndrome which has been a debate for several year. Interestingly, recent studies in other models of metabolic derangements, investigated the causal link between circadian misalignment and metabolic homeostasis using a controlled simulation of "shift-work" in the clinical laboratory[25]. Circadian dysregulation caused decreased leptin levels and resulted in hyperglycemia and hyperinsulinemia. Moreover, daily cortisol excretion was reversed, arterial pressure was increased and sleep efficiency decreased[26]. Thus, it was hypothesized that neuroendocrine derangements should

Table 4 Adjusted and unadjusted relative risks of low freeT3 for all cause mortality

Variables	All cause mortality relative risks, (95%CI), *P* values	
	Model 1 (unadjusted)	Model 2 (fully adjusted)
LowT3_1.8 (pg/mL)	1.89 (1.146-3.124) *P* = 0.013	1.61 (0.88-2.92) *P* = 0.115
Age (1 yr)	-	1.031 (1.00-1.05) *P* = 0.008
DM (yes)	-	1.22 (0.68-2.18) *P* = 0.50
History of CVD (yes)	-	1.878 (1.09-3.21) *P* = 0.021
Mean_Hb (1 g/dL)	-	0.561 (0.426-0.73) *P* = 0.000
hsCRP (0.1 mg/dL)	-	1.076 (0.91-1.27) *P* = 0.388
HD duration (1 mo)	-	0.990 (0.98-0.99) *P* = 0.004

DM: Diabetus mellitus; CVD: Cardiovascular disease; Hb: Hemoglobin; hsCRP: High sensitive C-reactive protein; HD: Hemodialysis.

be crucial in ESRD patients and might play an important role in NTIS while also explain the possible association with inflammation and cardiometabolic syndrome[27].

In the present study, the prognostic value of NTIS for long-term mortality in chronic hemodialysis patients and its probable association with low-grade inflammation, a common feature of chronic uremia, was investigated[5]. Our results showed no association of freeT4 or TSH with either hsCRP or IL-6. Moreover, freeT3 had significant negative correlation with hsCRP but only at baseline. Some studies previously examined the latter association but the number is limited and their results are rather inconsistent. Zoccali *et al*[8] first reported that freeT3 had a significant negative correlation with CRP and IL-6. Meuwese *et al*[9] investigated the trimestal variation of thyroid hormones and IL-6; in their study, IL-6 was positively associated with TSH and negatively with T3 whereas CRP levels were positively associated with T4 but only at baseline**.** Finally, and in accordance with our results, in the study of Carrero *et al*[28], only T3 and not freeT3 showed a correlation with CRP and IL-6. The above discrepant results could be, at least partially, explained by differences in patient's characteristics, exclusion criteria and laboratory methods used. It should be noted that in our study patients with recent infection or chronic inflammatory diseases were carefully excluded as well as patients who presented with infection during the 3 mo observation-sampling period. However, based on the above it appears reasonable to conclude that in HD patients, chronic low grade inflammation does not play a major role in the development of NTIS.

During the 7 year follow-up period, 60.5% of patients died, mostly from cardiovascular causes. Compared with patients who remained alive, patients who died had significantly lower freeT3 values. Moreover, Kaplan Meier survival curves differed significantly between patients with low and normal freeT3. In univariate Cox regression analysis survival was also associated with freeT3. Other factors significantly affecting outcome were age, HD duration, diabetes mellitus, history of CVD, presence of hypertension, hemoglobin, serum creatinine and hsCRP. In contrast, no association was observed between serum albumin and survival either in univariate or multivariate analysis. However, it should be mentioned that although the low T3 group had significantly lower serum albumin compared to normal T3 group, only 8 patients (14%) of the former group had serum albumin below the lower laboratory normal range and none had albumin lower than 3 g/dL. Given the well known association between malnutrition, inflammation and atherosclerosis in uremia, the above findings are consistent with the presence of a very low grade of inflammation in our patient population and relatively good nutritional status. Interestingly, in multivariate Cox Regression analyses, low freeT3 remained a significant independent predictor of mortality after adjustment for some traditional and uremia-related risk factors including age, diabetes, hypertension, hsCRP, serum creatinine and albumin. Of note, compared with patients with normal freeT3, those with low freeT3 had increased incidence of fatal and non-fatal CVD events during follow up (data not shown). However, in the fully adjusted model including also mean hemoglobin, history of CVD event, and HD duration, low freeT3 lost its power as a predictor of mortality. Several previous studies have also suggested a correlation of NTIS with mortality although they have important differences with our report. Zoccali *et al*[10] observed an independent association between freeT3 and mortality during an average follow-up of 42 mo; however, they included both incident and prevalent HD patients whereas freeT3 values were significantly higher compared to those in our patients. Horáček *et al*[29] in unselected HD patients found that survival curves differed between patients with low and normal freeT3 values during a five-year follow-up; however, more detailed survival analyses are not reported. In their study in incident dialysis patients, Carrero *et al*[28] found an independent association between only T3, but not freeT3, with survival during a 20 mo median follow-up. Fernández-Reyes *et al*[30] were unable to detect any association between freeT3 and mortality in patients who survived at least 12 mo on dialysis during an almost 34 mo follow-up. Finally, in the study of Ozen *et al*[31], in chronic HD patients, freeT3 was also found to be correlated with survival only in unadjusted analysis. Taken together, the above studies argue for a role of thyroid function in the outcome of hemodialysis patients although in some cases the above association could be confounded by other factors which are commonly present in uremia, including inflammation and malnutrition. Nevertheless, further studies are needed to identify the exact nature of the association between low freeT3 and mortality in ESRD patients. Moreover, it remains to be investigated whether or not

routine measurement of freeT3 in hemodialysis would add significantly greater predictive power for mortality to models based on traditional and uremia-related risk factors including inflammation. Nevertheless, although the use of thyroid hormone therapy in NTIS is controversial, design of interventional studies in hemodialysis patients aimed to investigate whether normalization of T3 values would actually reduce mortality is a tempting idea[32-34]. However, a study in ESRD patients with NTIS, showed that administration of T3 resulted in excess protein turnover, therefore increasing the need for dialysis[35].

Our study has some strengths. Firstly, only one previous study examined thyroid hormone variation. Secondly, patients were carefully examined to rule out any occult infection before study entry and moreover, patients were excluded if they presented with an acute infection during the three month observation-sampling period. Finally, the present report has the longest follow-up. However, to properly address the implications of the present study some limitations should be considered. It is known that thyroid hormone production follows a circadian rhythm and blood samples were taken at two different time points according to the patient's shifts. However, only a very small percentage of the patients (6%) underwent hemodialysis in the evening and moreover, no association was observed between HD shift and either freeT3 or freeT4 values. Nevertheless, we believe that circadian rhythms may present alterations in patients on hemodialysis similar to other incidents of biological clock disarrangements like shift workers, although time of sampling was not an issue in relevant studies[29]. These metabolic changes could actually be interpreted as an adaptive mechanism to the high energy expenditure during hemodialysis treatment. In addition, our study included only chronic hemodialysis (prevalent) patients and the results would not necessarily apply to incident dialysis patients.

In conclusion, in this prospective study in chronic hemodialysis patients, low freeT3 levels had emerged as a significant predictor of mortality independently of traditional and some uremia-related risk factors including age, diabetes mellitus, hypertension, serum albumin and hsCRP. However, an association between NTIS and inflammation could not be documented and the exact mechanisms underlying the above association remain to be identified in future mechanistic and interventional trials.

COMMENTS

Background

Non thyroidal illness syndrome (NTIS) has been associated with several chronic severe illnesses. This syndrome has been a debate for several years, as alterations in thyroid hormone levels have been considered either a laboratory pitfall or an adoptive response to chronic stress. In end stage renal disease the main finding of the syndrome is low free triiodothyronin (freeT3) which recently has emerged as a potent biomarker of cardiovascular risk and a predictor of mortality.

Research frontiers

Previous studies have suggested a probable association between low freeT3 and inflammation in end stage renal disease and a link between NTIS and cardiorenal syndrome. However, the predictive power of NTIS for long-term mortality and the mechanisms underlying the above associations remain unclear.

Innovations and breakthroughs

In the present study, the predictive value of low NTIS for long-term mortality in chronic hemodialysis patients and its link with low-grade inflammation was investigated. Our results showed that low freeT3 was a significant predictor of mortality independently of traditional and some uremia-related risk factors including inflammatory markers. However, an association between NTIS and inflammation was not documented and the exact mechanisms underlying the above association remained unclear. In this regard, there is a limited number of previous relevant studies and their results are rather inconsistent. This study has several strengths. Firstly, only one previous study examined trimestral thyroid hormone variation. Secondly, patients were carefully examined to rule out any occult infection before their enrollment and moreover, patients were excluded if they presented with an acute infection during the three month observation-sampling period. Finally, the present report has the longest follow-up.

Application

So far, there is a lack of a strong marker of cardiovascular disease in end-stage renal disease, easy to perform and with high availability and low cost, that could guide treatment or even serve as a predictor of mortality. Presumably, a multifactorial approach of traditional markers combined with novel biomarkers could fulfill this necessity. Among the latter, low freeT3 has emerged as a potent predictor of adverse clinical outcome, which is easy to perform, inexpensive and widely available.

Terminology

Euthyroid sick syndrome or non thyroidal illness syndrome refers to patients with severe chronic illnesses like starvation, sepsis, end stage renal disease, myocardial infarction and others, in whom a decrease in serum thyroid hormone levels is observed without any identifiable primary thyroid disease.

Peer-review

It is a very good study well conducted.

REFERENCES

1 **Collins AJ**, Foley RN, Chavers B, Gilbertson D, Herzog C, Ishani A, Johansen K, Kasiske BL, Kutner N, Liu J, St Peter W, Guo H, Hu Y, Kats A, Li S, Li S, Maloney J, Roberts T, Skeans M, Snyder J, Solid C, Thompson B, Weinhandl E, Xiong H, Yusuf A, Zaun D, Arko C, Chen SC, Daniels F, Ebben J, Frazier E, Johnson R, Sheets D, Wang X, Forrest B, Berrini D, Constantini E, Everson S, Eggers P, Agodoa L. US Renal Data System 2013 Annual Data Report. *Am J Kidney Dis* 2014; **63**: A7 [PMID: 24360288 DOI: 10.1053/j.ajkd.2011.11.015]

2 **Stenvinkel P**, Carrero JJ, Axelsson J, Lindholm B, Heimbürger O, Massy Z. Emerging biomarkers for evaluating cardiovascular risk in the chronic kidney disease patient: how do new pieces fit into the uremic puzzle? *Clin J Am Soc Nephrol* 2008; **3**: 505-521 [PMID: 18184879 DOI: 10.2215/CJN.03670807]

3 **Honda H**, Qureshi AR, Heimbürger O, Barany P, Wang K, Pecoits-Filho R, Stenvinkel P, Lindholm B. Serum albumin, C-reactive protein, interleukin 6, and fetuin a as predictors of malnutrition, cardiovascular disease, and mortality in patients with ESRD. *Am J Kidney Dis* 2006; **47**: 139-148 [PMID: 16377395 DOI: 10.1053/j.ajkd.2005.09.014]

4 **Carrero JJ**, Stenvinkel P. Persistent inflammation as a catalyst for other risk factors in chronic kidney disease: a hypothesis proposal. *Clin J Am Soc Nephrol* 2009; **4** Suppl 1: S49-S55 [PMID: 19996005 DOI: 10.2215/CJN.02720409]

5 **Ortiz A**, Massy ZA, Fliser D, Lindholm B, Wiecek A, Martínez-Castelao A, Covic A, Goldsmith D, Süleymanlar G, London GM, Zoccali C. Clinical usefulness of novel prognostic biomarkers in patients on hemodialysis. *Nat Rev Nephrol* 2012; **8**: 141-150 [PMID: 22045239 DOI: 10.1038/nrneph.2011.170]

6 **Lameire N**, Van Biesen W, Vanholder R. Did 20 years of technological innovations in hemodialysis contribute to better patient outcomes? *Clin J Am Soc Nephrol* 2009; **4** Suppl 1: S30-S40 [PMID: 19996003 DOI: 10.2215/CJN.04000609]

7 **Tripepi G**, Mallamaci F, Zoccali C. Inflammation markers, adhesion molecules, and all-cause and cardiovascular mortality in patients with ESRD: searching for the best risk marker by multivariate modeling. *J Am Soc Nephrol* 2005; **16** Suppl 1: S83-S88 [PMID: 15938042 DOI: 10.1681/ASN.2004110972]

8 **Zoccali C**, Tripepi G, Cutrupi S, Pizzini P, Mallamaci F. Low triiodothyronine: a new facet of inflammation in end-stage renal disease. *J Am Soc Nephrol* 2005; **16**: 2789-2795 [PMID: 16033857 DOI: 10.1681/ASN.2005040356]

9 **Meuwese CL**, Dekker FW, Lindholm B, Qureshi AR, Heimburger O, Barany P, Stenvinkel P, Carrero JJ. Baseline levels and trimestral variation of triiodothyronine and thyroxine and their association with mortality in maintenance hemodialysis patients. *Clin J Am Soc Nephrol* 2012; **7**: 131-138 [PMID: 22246282 DOI: 10.2215/CJN.05250511]

10 **Zoccali C**, Mallamaci F, Tripepi G, Cutrupi S, Pizzini P. Low triiodothyronine and survival in end-stage renal disease. *Kidney Int* 2006; **70**: 523-528 [PMID: 16775599 DOI: 10.1038/sj.ki.5001566]

11 **Warner MH**, Beckett GJ. Mechanisms behind the non-thyroidal illness syndrome: an update. *J Endocrinol* 2010; **205**: 1-13 [PMID: 20016054 DOI: 10.1677/JOE-09-0412]

12 **Chopra IJ**, Hershman JM, Pardridge WM, Nicoloff JT. Thyroid function in nonthyroidal illnesses. *Ann Intern Med* 1983; **98**: 946-957 [PMID: 6407376 DOI: 10.7326/0003-4819-98-6-946]

13 **Dietrich JW**, Stachon A, Antic B, Klein HH, Hering S. The AQUA-FONTIS study: protocol of a multidisciplinary, cross-sectional and prospective longitudinal study for developing standardized diagnostics and classification of non-thyroidal illness syndrome. *BMC Endocr Disord* 2008; **8**: 13 [PMID: 18851740 DOI: 10.1186/1472-6823-8-13]

14 **Wartofsky L**, Dickey RA. The evidence for a narrower thyrotropin reference range is compelling. *J Clin Endocrinol Metab* 2005; **90**: 5483-5488 [PMID: 16148345 DOI: 10.1210/jc.2005-0455]

15 **Biondi B**. The normal TSH reference range: what has changed in the last decade? *J Clin Endocrinol Metab* 2013; **98**: 3584-3587 [PMID: 24014812 DOI: 10.1210/jc.2013-2760]

16 **St Germain DL**, Galton VA, Hernandez A. Minireview: Defining the roles of the iodothyronine deiodinases: current concepts and challenges. *Endocrinology* 2009; **150**: 1097-1107 [PMID: 19179439 DOI: 10.1210/en.2008-1588]

17 **Boelen A**, Platvoet-Ter Schiphorst MC, Wiersinga WM. Association between serum interleukin-6 and serum 3,5,3'-triiodothyronine in nonthyroidal illness. *J Clin Endocrinol Metab* 1993; **77**: 1695-1699 [PMID: 8263160]

18 **Rozing MP**, Westendorp RG, Maier AB, Wijsman CA, Frölich M, de Craen AJ, van Heemst D. Serum triiodothyronine levels and inflammatory cytokine production capacity. *Age (Dordr)* 2012; **34**: 195-201 [PMID: 21350816 DOI: 10.1007/s11357-011-9220-x]

19 **Hemodialysis Adequacy 2006 Work Group**. Clinical practice guidelines for hemodialysis adequacy, update 2006. *Am J Kidney Dis* 2006; **48** Suppl 1: S2-90 [PMID: 16813990 DOI: 10.1053/j.ajkd.2006.03.051]

20 **Trainer TD**, Howard PL. Thyroid function tests in thyroid and nonthyroid disease. *Crit Rev Clin Lab Sci* 1983; **19**: 135-171 [PMID: 6426859 DOI: 10.3109/10408368309165761]

21 **Jaume JC**, Mendel CM, Frost PH, Greenspan FS, Laughton CW. Extremely low doses of heparin release lipase activity into the plasma and can thereby cause artifactual elevations in the serum-free thyroxine concentration as measured by equilibrium dialysis. *Thyroid* 1996; **6**: 79-83 [PMID: 8733876 DOI: 10.1089/thy.1996.6.79]

22 **De Groot LJ**. Dangerous dogmas in medicine: the nonthyroidal illness syndrome. *J Clin Endocrinol Metab* 1999; **84**: 151-164 [PMID: 9920076 DOI: 10.1210/jcem.84.1.5364]

23 **Lim VS**. Thyroid function in patients with chronic renal failure. *Am J Kidney Dis* 2001; **38**: S80-S84 [PMID: 11576928 DOI: 10.1053/ajkd.2001.27410]

24 **Song SH**, Kwak IS, Lee DW, Kang YH, Seong EY, Park JS. The prevalence of low triiodothyronine according to the stage of chronic kidney disease in subjects with a normal thyroid-stimulating hormone. *Nephrol Dial Transplant* 2009; **24**: 1534-1538 [PMID: 19106286 DOI: 10.1093/ndt/gfn682]

25 **Maury E**, Ramsey KM, Bass J. Circadian rhythms and metabolic syndrome: from experimental genetics to human disease. *Circ Res* 2010; **106**: 447-462 [PMID: 20167942 DOI: 10.1161/CIRCRESAHA.109.208355]

26 **Scheer FA**, Hilton MF, Mantzoros CS, Shea SA. Adverse metabolic and cardiovascular consequences of circadian misalignment. *Proc Natl Acad Sci USA* 2009; **106**: 4453-4458 [PMID: 19255424 DOI: 10.1073/pnas.0808180106]

27 **Van den Berghe G**. The neuroendocrine response to stress is a dynamic process. *Best Pract Res Clin Endocrinol Metab* 2001; **15**: 405-419 [PMID: 11800514 DOI: 10.1053/beem.2001.0160]

28 **Carrero JJ**, Qureshi AR, Axelsson J, Yilmaz MI, Rehnmark S, Witt MR, Bárány P, Heimbürger O, Suliman ME, Alvestrand A, Lindholm B, Stenvinkel P. Clinical and biochemical implications of low thyroid hormone levels (total and free forms) in euthyroid patients with chronic kidney disease. *J Intern Med* 2007; **262**: 690-701 [PMID: 17908160 DOI: 10.1111/j.1365-2796.2007.01865.x]

29 **Horáček J**, Dusilová Sulková S, Kubišová M, Safránek R, Malířová E, Kalousová M, Svilias I, Malý J, Sobotka L, Zák P. Thyroid hormone abnormalities in hemodialyzed patients: low triiodothyronine as well as high reverse triiodothyronine are associated with increased mortality. *Physiol Res* 2012; **61**: 495-501 [PMID: 22881233]

30 **Fernández-Reyes MJ**, Diez JJ, Collado A, Iglesias P, Bajo MA, Estrada P, Del Peso G, Heras M, Molina A, Selgas R. Are low concentrations of serum triiodothyronine a good marker for long-term mortality in hemodialysis patients? *Clin Nephrol* 2010; **73**: 238-240 [PMID: 20178724]

31 **Ozen KP**, Asci G, Gungor O, Carrero JJ, Kircelli F, Tatar E, Sevinc Ok E, Ozkahya M, Toz H, Cirit M, Basci A, Ok E. Nutritional state alters the association between free triiodothyronine levels and mortality in hemodialysis patients. *Am J Nephrol* 2011; **33**: 305-312 [PMID: 21389695 DOI: 10.1159/000324883]

32 **Bartalena L**. The dilemma of non-thyroidal illness syndrome: to treat or not to treat? *J Endocrinol Invest* 2003; **26**: 1162 [PMID: 15055465]

33 **Brent GA**, Hershman JM. Thyroxine therapy in patients with severe nonthyroidal illnesses and low serum thyroxine concentration. *J Clin Endocrinol Metab* 1986; **63**: 1-8 [PMID: 3011834 DOI: 10.1210/jcem-63-1-1]

34 **Pingitore A**, Galli E, Barison A, Iervasi A, Scarlattini M, Nucci D, L'abbate A, Mariotti R, Iervasi G. Acute effects of triiodothyronine (T3) replacement therapy in patients with chronic heart failure and low-T3 syndrome: a randomized, placebo-controlled study. *J Clin Endocrinol Metab* 2008; **93**: 1351-1358 [PMID: 18171701 DOI: 10.1210/jc.2007-2210]

35 **Lim VS**, Tsalikian E, Flanigan MJ. Augmentation of protein degradation by L-triiodothyronine in uremia. *Metabolism* 1989; **38**: 1210-1215 [PMID: 2593833]

P- Reviewer: Bernieh B, Hammes M, Trimarchi H **S- Editor**: Ji FF
L- Editor: A **E- Editor**: Yan JL

World Journal of Nephrology

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Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.5527/wjn.v4.i3.423

World J Nephrol 2015 July 6; 4(3): 423-437
ISSN 2220-6124 (online)

ORIGINAL ARTICLE

Randomized Controlled Trial

Changes in urinary excretion of water and sodium transporters during amiloride and bendroflumethiazide treatment

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Author contributions: All authors had contributed to the manuscript; Jensen JM, Mose FH and Pedersen EB designed the project; Jensen JM, Mose FH and Kulik AEO performed the experiments and statistical analyzes; Fenton RA performed the NKCC2 antibody characterization; Jensen JM, Mose FH, Bech JN, Fenton RA and Pedersen EB wrote and edited the manuscript; all authors read and approved the final manuscript.

Supported by Grants from The Lundbeck Foundation, Aase and Ejnar Danielsens Foundation, Helen and Ejnar Bjoernows Foundation and Region Midjutlands Research Fund.

Ethics approval statement: This study was review and approved by the Regional Committees on Health Research Ethics, Skottenborg 26, 8600 Viborg, Denmark (j.no 1-10-72-178-12).

Clinical trial registration: This study is registered at clinical trials. The registration identification number is: NCT 01635231 http://clinicaltrials.gov/ct2/show/NCT01635231.

Informed consent statement: All study participants provided informed written consent prior to study enrolment.

Conflict-of-interest statement: The authors declare that they have no competing interests.

Data sharing statement: No additional data are available.

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Received: November 13, 2014
Peer-review started: November 18, 2014
First decision: February 7, 2015
Revised: March 8, 2015
Accepted: April 28, 2015
Article in press: April 30, 2015
Published online: July 6, 2015

Abstract

AIM: To quantify changes in urinary excretion of aquaporin2 water channels (u-AQP2), the sodium-potassium-chloride co-transporter (u-NKCC2) and the epithelial sodium channels (u-ENaC) during treatment with bendroflumethiazide (BFTZ), amiloride and placebo.

METHODS: In a randomized, double-blinded, placebo-controlled, 3-way crossover study we examined 23 healthy subjects on a standardized diet and fluid intake. The subjects were treated with amiloride 5 mg, BFTZ 1.25 mg or placebo twice a day for 4.5 d before each examination day. On the examination day, glomerular filtration rate was measured by the constant infusion clearance technique with ^{51}Cr-EDTA as reference substance. To estimate the changes in water transport *via* AQP2 and sodium transport *via* NKCC2 and ENaC, u-NKCC2, the gamma fraction of ENaC (u-ENaCγ), and

u-AQP2 were measured at baseline and after infusion with 3% hypertonic saline. U-NKCC2, u-ENaCγ, u-AQP2 and plasma concentrations of vasopressin (p-AVP), renin (PRC), angiotensin Ⅱ (p-ANG Ⅱ) and aldosterone (p-Aldo) were measured, by radioimmunoassay. Central blood pressure was estimated by applanation tonometry and body fluid volumes were estimated by bio-impedance spectroscopy. General linear model with repeated measures or related samples Friedman's two-way analysis was used to compare differences. Post hoc Bonferroni correction was used for multiple comparisons of post infusion periods to baseline within each treatment group.

RESULTS: At baseline there were no differences in u-NKCC2, u-ENaCγ and u-AQP2. PRC, p-Ang Ⅱ and p-Aldo were increased during active treatments (P < 0.001). After hypertonic saline, u-NKCC2 increased during amiloride (6% ± 34%; P = 0.081) and increased significantly during placebo (17% ± 24%; P = 0.010). U-AQP2 increased significantly during amiloride (31% ± 22%; P < 0.001) and placebo (34% ± 27%; P < 0.001), while u-NKCC2 and u-AQP2 did not change significantly during BFTZ (-7% ± 28%; P = 0.257 and 5% ± 16%; P = 0.261). U- ENaCγ increased in all three groups (P < 0.050). PRC, Ang Ⅱ and p-Aldo decreased to the same extent, while AVP increased, but to a smaller degree during BFTZ (P = 0.048). cDBP decreased significantly during BFTZ (P < 0.001), but not during amiloride or placebo. There were no significant differences in body fluid volumes.

CONCLUSION: After hypertonic saline, u-NKCC2 and u-AQP2 increased during amiloride, but not during BFTZ. Lower p-AVP during BFTZ potentially caused less stimulation of NKCC2 and AQP2 and subsequent lower reabsorption of water and sodium.

Key words: Amiloride; Thiazide; Sodium-potassium-chloride co-transporter; Aquaporin2; Epithelial sodium channels; Sodium; Water; Sodium transporters; Hypertonic saline; Urine

Core tip: Measurements of urinary sodium-potassium chloride co-transporter (NKCC2), epithelial sodium channel (ENaC) and aquaporin2 (AQP2) can be used as biomarkers of water- and sodium transport in the nephron. However, it has never been studied to what extent the function of NKCC2, ENaC and AQP2 is simultaneously affected in response to diuretics. The present study showed that infusion of 3% saline increased u-NKCC2 and u-AQP2 during amiloride and placebo, while u-NKCC2 and u-AQP2 remained unchanged during bendroflumethiazide. Therefor, in contrast to amiloride, bendroflumethiazide caused the absence of a compensatory reabsorption of sodium *via* NKCC2 and water *via* AQP2.

Jensen JM, Mose FH, Kulik AEO, Bech JN, Fenton RA, Pedersen EB. Changes in urinary excretion of water and sodium transporters during amiloride and bendroflumethiazide treatment. *World J Nephrol* 2015; 4(3): 423-437 Available from: URL: http://www.wjgnet.com/2220-6124/full/v4/i3/423.htm DOI: http://dx.doi.org/10.5527/wjn.v4.i3.423

INTRODUCTION

During normal conditions, approximately 60% of filtered sodium is absorbed in the proximal tubules and 30% of sodium is absorbed in the kidneys *via* the sodium-potassium chloride co-transporter (NKCC2) in the thick ascending limb of Henle's loop (TAL). The distal convoluted tubules are responsible for 5%-10% of sodium reabsorption *via* the sodium chloride co-transporter (NCC)[1]. Thiazides inhibit NCC in distal tubules and decrease sodium reabsorption[2]. In the collecting duct the epithelial sodium channel (ENaC) is responsible for the reabsorption of 3%-5% of filtered sodium[1]. Amiloride is a potassium sparing selective inhibitor of ENaC channels[3]. Water is predominantly reabsorbed in the proximal tubules and thin descending limb of Henle's loop[1]. In the collecting ducts water absorption depends on passive transport *via* AQP2 water channels and is regulated by vasopressin (AVP)[4]. AQP2 can be excreted into urine[5,6], and may be used as a biomarker of collecting duct water transport[7-9]. Similarly, urinary excretion of beta ENaC correlates with changes in urinary sodium excretion[10]. Recently, our group documented changes in transport of water *via* AQP2 and sodium *via* ENaC in healthy subjects after infusion of isotonic glucose or hypertonic and isotonic saline, by measurements of urinary excretion of AQP2 (u-AQP2) and gamma ENaC (u-ENaCγ)[11] and abnormal urinary excretion of NKCC2 (u-NKCC2) and u-AQP2 in patients with chronic kidney disease[12].

In the present randomised, placebo-controlled study present study in healthy young subjects, we hypothesize that excretion of NKCC2 will not be effected but compensatory increases in distal transporter activity will occur during thiazide treatment but not during amiloride. This is compared to placebo both at baseline and in response to a saline load. Therefore, the aim was to quantify changes in urinary excretion of NKCC2, u-ENaCγ and u-AQP2 as estimates of tubular water and sodium handling at baseline conditions and after 3% saline infusion, during treatment with bendroflumethiazide (BFTZ), amiloride and placebo. In addition, changes in renal tubular function, vasoactive hormones, body fluid volumes and blood pressure were measured. The novelty of this study is due to; measurements of u-NKCC2 and the interplay with ENaC, AQP2 and the regulating mechanisms involved in water and sodium homeostasis, while simultaneously antagonizing NCC with BFTZ and ENaC with amiloride. Quantification of sodium- and water channel proteins in urine during different conditions may provide important information of the mechanisms involved in water and

sodium balance in the nephron.

MATERIALS AND METHODS

Design

The trial was conducted as a randomized, double-blinded, placebo-controlled, 3-way crossover study. Subjects were randomized to tablet BFTZ, amiloride or placebo for 4.5 d. Each treatment period was followed by an examination day, separated by at least 3 wk.

Participants

Eligible participants were healthy non-smoking men and women aged between 18-45 years. Exclusion criteria were clinical signs or history of heart, lung, kidney, endocrine or malignant disease; abnormal findings in ECG, urine dipstick or biochemistry [blood cell count, plasma concentrations of glucose, bilirubin, alanine aminotransferase, alkaline phosphatase, sodium, potassium, creatinine, albumin, cholesterol and haemoglobin; arterial hypertension (24 h ambulatory BP > 130/80 mmHg); medical treatment (except oral contraceptives); alcohol and substance abuse, *i.e.*, more than 21 alcoholic drinks per week for males and 14 drinks for females]; current smoking; pregnancy, breast feeding; donation of blood within one month prior to the study and obesity (BMI > 32 kg/m^2). Withdrawal criteria were, development of conditions given in exclusion criteria during the study, withdrawal of informed consent and poor compliance.

Participants were recruited through advertisement at public institutions in Holstebro, Denmark.

Study settings

The study took place at Department of Medical Research, University Clinic of Nephrology and Hypertension, Regional Hospital Holstebro, Denmark, from 1st of August 2012 until 13th of September 2013.

Ethics

This study was reviewed and approved by the Regional Committees on Health Research Ethics, Skottenborg 26, Viborg, Denmark (j.no 1-10-72-178-12) and was carried out in accordance with the Helsinki Declaration. All study participants provided informed written consent prior to study enrolment.

Effect variables

The main effect variable was u-NKCC2. Secondary effect variables were: u-AQP2, u- ENaCγ, glomerular filtration rate (GFR), free water clearance (C_{H2O}), urine output (UO), urinary excretion of sodium (u-Na) and potassium (u-K), fractional excretion of sodium (FE_{Na}) and potassium (FE_K), plasma sodium (p-Na) and potassium (p-K), plasma osmolality (p-osm) and plasma albumin (p-alb), plasma concentration of renin (PRC), angiotensin Ⅱ (p-AngⅡ), aldosterone (p-Aldo), vasopressin (p-AVP), extracellular fluid volume (ECV), intracellular fluid volume (ICV), total body water (TBW), brachial systolic- (bSBP) and diastolic blood pressure (bDBP), pulse wave velocity (PWV) and central systolic- (cSBP) and diastolic blood pressure (cDBP).

Number of subjects

Using a significance level of 5% and a power of 80% it was calculated that the number of subjects should be 16, when the minimal relevant difference in u-NKCC2 was 0.3 ng/min and SD was 0.3 ng/min. In this study, incomplete voiding during study days was expected in some subjects; therefore, 20 subjects were included as a minimum.

Randomisation

Subjects were randomized to treatment using block randomization conducted at www.randomization.com. Aarhus Hospital Pharmacy, Denmark, generated the randomization sequence into five blocks of six from 01-30 and labeled the bottles. Five days prior to each examination day, participants received a numbered bottle containing BFTZ, amiloride or placebo tablets. BFTZ, amiloride and placebo were capsulated in grey DB Caps® size B (Capsugel) with click effect to obtain blinding. The randomization code was kept at Aarhus Hospital Pharmacy during the trial. Individual randomization codes were kept in sealed envelopes at Department of Medical Research if necessary for the investigator to know the given treatment. Investigators, participants and other study personnel were blinded to treatment assignment for the duration of the study.

Experimental procedures

Experimental procedure prior to the study day: Four days prior to each study day, subjects consumed a standardized diet regarding calories, sodium and fluid. The diet consisted of 11000 (kJ/d) with an energy distribution of 55% carbohydrates, 30% fat and 15% protein in accordance to general dietary guidelines. The sodium content was approximately 120-150 mmol pr. day. The subjects were asked to drink 2500 mL/d. No alcohol or soft drink consumption was allowed while on the standardized diet. A maximum of two cups (6 oz.) of coffee or tea was allowed daily. Subjects were instructed to keep their usual physical activity during the experiments but to abstain from hard training the day prior to the examination. A 24-h urine collection from 7:00 AM to 7:00 AM on the examination day was used to assess water and sodium balance. A 24-h ambulatory BP measurement was performed to evaluate the effect of the intervention on blood pressure (Table 1).

Interventions

During the four-day diet and the morning of the examination day, participants were randomized to capsules containing either 1.25 mg BFTZ, 5 mg amiloride or matching placebo twice daily at 7 AM and 6 PM.

Table 1 Experimental procedures

	Before the study day				On the study day											
	Day-4	Day-3	Day-2	Day-1	6:00-8:00	8:00-08:30	8:30-09:00	9:00-9:30	9:30-10:00	10:00-10:30	10:30-11:00	11:00-11:30	11:30-12:00	12:00-12:30	12:30-13:00	13:00-13:30
Periods										Baseline			Infusion		Post Infusion	
Time									0	30	60	90	120	150	180	210	240
Diet	x	x	x	x													
Study drug	xx	xx	xx	xx	x												
24-h BP				x------	-----x												
24-h urine				x------	-----x												
IV access						x											
Weight						x					x		x			x	
Water load						x----------					175 mL every 30 min		----------				x
Urine sample							(x)	x	x	x	x		x	x	x	x	
Blood samples								x	x	x	x		x	x	x	x	
Blood pressure						x		x	x	x	x		x	x	x	x	
^{51}Cr-EDTA								x	----------								x
IV. fluid												x----------					
App.Ton											x		x				
BIS						x					x		x			x	

24-h BP: 24-h ambulatory blood pressure measurements; 24-h urine: 24-h urine collection; App.Ton: Applanation tonometry; BIS: Bioimpedance spectroscopy.

Experimental procedure on the study day

Table 1 shows the time points of study interventions. Following an overnight fast, subjects arrived at our research facility at 8:00 AM. Two indwelling catheters for blood sampling and administration of ^{51}Cr-EDTA and fluid were placed in both cubital veins. Every 30 min, starting at arrival, participants received an oral water load of 175 mL. Urine was collected in standing or sitting position. Otherwise, subjects were kept in a supine position in a quiet temperature-controlled room (22 ℃-25 ℃). At 9:00 AM a priming dose of ^{51}Cr-EDTA was administered, followed by sustained infusion. Three 30-min baseline clearance periods were obtained from 9:30 AM to 11:00 AM. The baseline periods were followed by an infusion period from 11:00 AM to 12:00 PM during which a sustained infusion of 3% hypertonic saline was administered. The post infusion period consisted of three 30-min periods from 12:00 PM to 1:30 PM. Blood and urine samples were collected every 30 min from 8:30 AM to 1:30 PM.

Blood samples were drawn and analyzed for ^{51}Cr-EDTA, p-sodium, p-potassium, p-albumin and p-osmolality. Analysis of PRC, p-Ang Ⅱ, p-Aldo and p-AVP were conducted from blood samples drawn at 11:00 AM, 12:00 PM and 1:30 PM.

Urine samples were analyzed for u-^{51}Cr-EDTA, u-sodium, u-potassium, u-creatinine and u-osmolality. Analysis of u-AQP2, u-NKCC2 and u-ENaCγ was conducted from the 24-h urine collection and clearance period 10:30-11:00 AM (basal); 11:00-12:00 AM (fluid infusion), 12:00-12:30 PM (30 min after cessation of fluid infusion) and 1:00-1:30 PM (90 min after cessation of fluid infusion). For data analysis, the 30-min periods from 9:30 AM to 1:30 PM were subdivided into: baseline (0-90 min), infusion period (90-150 min) and three post infusion period 150-180 min, 180-210 min and 210-240 min).

Measurements of PWV, augmentation index (Aix) and cBP were performed at 11:00 AM (before infusion) and 12:00 AM (after infusion). Body composition was measured at 8:30 AM, 11:00 AM, 12:00 PM and 1:30 PM (end of examination day).

Measurements

Renal function: Glomerular filtration rate was measured by the constant infusion clearance technique with ^{51}Cr-EDTA as reference substance. More than 15% variation in GFR between the three baseline periods led to the exclusion of clearance related analysis.

Fractional excretion of sodium and potassium was calculated as: [Sodium/potassium clearance ($C_{Na/K}$)/GFR x 100%]. Free water clearance was calculated as: [Urine output (UO) - osmolar clearance (C_{OSM})]. C_{OSM} was calculated as: [Urine osmolality/plasma osmolality x UO].

Blood samples: were centrifuged for 10 min at 2200 x *g* at 4 ℃. Plasma hormone samples were kept frozen at -20 ℃ (Ang Ⅱ) and -80 ℃ (PRC, Aldo, and AVP) until assayed. Renin in plasma was determined using an immunoradiometric assay (CIS Bio International, Gif-Sur-Yvette Cedex, France). Minimal detection level was 1 pg/mL. The coefficients of variation were 14.5% (interassay) and 4.5% (intra assay). Aldosterone in plasma was determined by radioimmunoassay (Demeditec Diagnostics Systems Laboratories Inc., Webster, TX, United States). Minimal detection level was 22 pmol/L. The coefficients of variation were 8.2% (inter-assay) and 3.9% (intra-assay). Arginine vasopressin and Angiotensin Ⅱ were extracted from plasma with C_{18} Sep-Pak (Water associates, Milford, MA, United States) and subsequently measured using radioimmunoassay as previously described[13]. The antibody against angiotensin Ⅱ was obtained from the Department of Clinical Physiology, Glostrup Hospital, Glostrup, Denmark. Minimal detection level was 2 pmol/L. The coefficients of variation were 12% (inter-assay) and 8% (intra-assay). The antibody against AVP was a gift from Professor Jacques Dürr (Miami, FL, United States). Minimal detection level was 0.2 pmol/L. The coefficients of variation were 13% (inter-assay) and 9% (intra-assay).

Generation of NKCC2 specific antibody: A novel rabbit polyclonal antiserum against human NKCC2 (*Slc12a2*) was generated against the following peptide: CNITKTTPKKDGSIN by Genscript® (New Jersey, United States). The N-terminal cysteine was added for conjugation to carrier protein and for attaching the peptide to the affinity purification column. The immune serum from two rabbits (#593 and #594) was affinity purified using immunizing peptides, resulting in NKCC2-specific antibodies. NKCC2 antibody characterization has previously been described[12].

Urine sample immunoassays: Urines were stored frozen at -20 ℃ until assayed.

U-NKCC2 was measured in urine by a newly developed radioimmunoassay[12]. Antibodies were raised in rabbits against human NKCC2 (Slc12a2) against the peptide CNITKTTPKKDGSIN. The N-terminal cysteine was added for conjugation to carrier protein and affinity purification. Minimal detection level was 0.5 ng/tube. The coefficients of variation were 14% (inter-assay) and 6.8% (intra-assay).

U-AQP2 was measured by radioimmunoassay as previously described[9,14]. Antibodies were raised in rabbits to a synthetic peptide corresponding to the 15 COOH-terminal amino acids in human AQP2 to which was added an NH_2-terminal cysteine for conjugation and affinity purification. Minimal detection level was 34 pg/tube per tube. The coefficients of variation were 11.7% (inter-assay) and 5.9% (intra-assay).

U-ENaCγ was measured by radioimmunoassay as previously described[15,16]. Antibodies were raised against a synthetic ENaCγ peptide in rabbits and affinity purified[17]. Minimal detection level was 48 pg/tube. The coefficients of variation were 14% (inter-assay) and 6.7% (intra-assay).

Blood pressure measurement: Twenty-four hours BP was measured using Kivex TM-2430 (Kivex, Hoersholm, Denmark). Measurements were taken every 15 min during daytime and every 30 min overnight. Brachial blood pressure was recorded using a semiautomatic oscillometric device (Omron 705IT, Omron Matsusaka, Japan).

Plasma and urine: Concentrations of sodium, potassium, creatinine and albumin were measured using routine methods at the Department of Clinical Biochemistry, Holstebro Hospital.

Plasma and urine osmolality was measured by freezing point depression (Advanced Model 3900 multisampling osmometer).

Bioimpedance spectroscopy: Was performed at 50 frequencies, from 5 to 1000 kHz using the Fresenius Body Composition Monitor and the Fluid Management Tool, version 3.

Applanation tonometry: Recordings of PWA and carotid-femoral PWV were obtained by applanation tonometry (SphygmoCor® CPV system®, AtCor Medical, Sydney, Australia) as double-recordings by a trained observer. Only duplicate recording meeting the quality requirements were included in the final analysis. An operator index of 80 or more was required to accept recordings of a peripheral pulse-wave form[18].

Study drug

Bendroflumethiazide [Tablet Salures 2.5 mg (1/2 tablet)] were obtained from Pfizer AB, Sollentuna, Sweden. Amiloride (Tablet Amilorid Mylan 5 mg) were obtained from Mylan AB, Stockholm, Sweden *via* Tjellesen Max Jenne A/S, Medilink A/S, Roedovre, Denmark.

Statistical methods

Statistical analyses were performed by the authors using IBM SPSS statistics version 20.0.0 (IBM Corp.; Armonk, NY, United States).

As clearance data from the three baseline periods were very similar, single baseline values were obtained by taking the average of the measurements from the three baseline periods. Parametric data are presented as

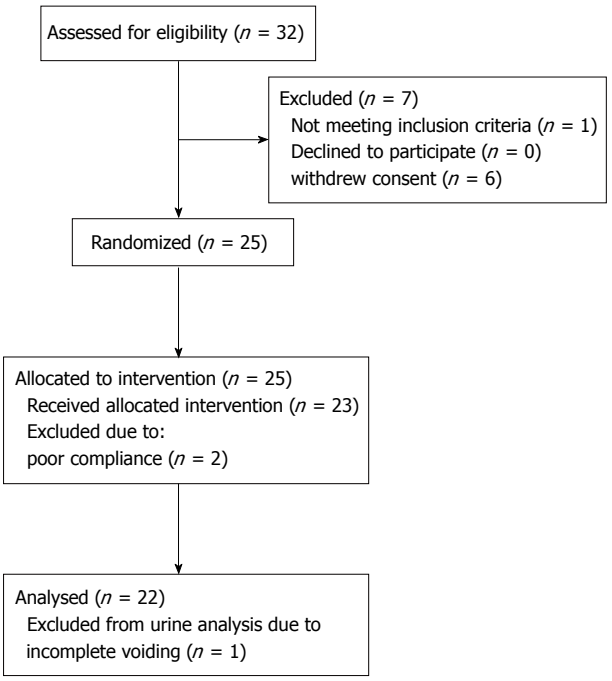

Figure 1 Flow chart.

means ± SD and nonparametric data as medians with 25th and 75th percentiles. General linear model (GLM) with repeated measures was performed, with time as within-subject factor and intervention as between subject factor, to test for differences within and between groups. One-way ANOVA was used for comparison of means between groups when differences were found. For non-parametric data, related samples Friedman's two-way analysis was used. Post hoc Bonferroni correction was used for multiple comparisons of post infusion periods to baseline within each treatment group. Statistical significance was defined as $P < 0.050$ in all analyses.

RESULTS

Demographics

Thirty-two healthy women and men were assessed for eligibility. Nine were excluded due to: withdrawal of informed consent (6), non-compliance (2) or 24-h BP above 130/80 mmHg (1). Thus, 23 were initially allocated to and completed the study. One was not able to void satisfactorily during baseline clearance experiments and was excluded from urine analysis (Figure 1).

The 23 subjects (8 males; 15 females) who completed the trial had a mean age of 26 ± 4 years, BMI 24 ± 3 kg/m^2, 24-h BP 117/69 ± 7/4 mmHg. Screening blood values were b-haemoglobin 8.4 ± 0.7 mmol/L, p-sodium 141 ± 1 mmol/L, p-potassium 3.7 ± 0.4 mmol/L, p-creatinine 79 ± 14 μmol/L, eGFR 93 ± 16 mL/min, p-albumin 43 ± 3 g/L, p-glucose 5± 1 mmol/L, p-alanine transaminase 25 ± 19 U/L and p-cholesterol 4.4 ± 0.8 mmol/L. Baseline values did not differ between males and females, apart from p-crea (males: 91 ± 15 μmol/L *vs* females: 73 ± 7 μmol/L, $P < 0.012$), b-haemoglobin (males: 8.9 ± 0.6 mmol/L *vs* females: 8.2 ± 0.5 mmol/L, $P < 0.016$) and p-albumin (males: 45 ± 2 g/L *vs* females: 42 ± 3 g/L, $P < 0.003$).

Effects of BFTZ and amiloride on 24-h urine and ambulatory BP

UO, u-osm, C_{H2O}, Creatinine-Clearance, u-Na, u-AQP2 and ENaCγ in 24-h urine were not significantly different between treatments. During BFTZ treatment u-NKCC2 and u-K were significant higher than both amiloride and placebo treatment (Table 2). Twenty-four hour ambulatory bSBP did not differ between treatments, however there was a small but significant lower bDBP during amiloride treatment (Table 2).

Effects of BFTZ and amiloride on u-NKCC2, u-ENaCγ and u-AQP2

Figure 2 shows the changes in urinary excretion of AQP2, NKCC2 and ENaCγ during basal, infusion and post-infusion periods.

At baseline, u-NKCC2 did not differ between groups. U-NKCC2 decreased during the infusion period and increased during the first post infusion period in all three treatments. U-NKCC2 increased further during amiloride (6% ± 34%; $P = 0.081$) and placebo (17% ± 24%; $P = 0.010$), whereas u-NKCC2 declined in the BFTZ treated group (-7% ± 28%; $P = 0.257$), during the two last post infusion periods. By the end of the examination day there was a significant difference between BFTZ *vs* amiloride ($P < 0.001$) and *vs* placebo ($P = 0.033$). There was no significant difference between amiloride and placebo groups ($P = 0.407$).

At baseline, u-ENaCγ was similar. In response to 3% saline, u-ENaCγ increased significant to a maximum after the first post infusion period. Although u-ENaCγ tended to be lower during amiloride treatment, there was no statistical difference between the three treatment groups throughout the examination day.

There was no significant difference in u-AQP2 at baseline. In response to 3% saline, u-AQP2 increased significantly and similarly during amiloride (31% ± 22%; $P < 0.001$) and placebo treatment (34 ± 27%; $P < 0.001$), but did not change during BFTZ (5% ± 16%; $P = 0.261$). By the end of the examination day there was a significant difference between BFTZ *vs* amiloride and placebo ($P < 0.001$), but there was no difference between amiloride and placebo.

Divided by gender, the creatinine adjusted excretion of u-AQP2, u-NKCC2 and u-ENaCγ tended to be higher in females compared to males in all three treatment groups, but the difference is attributed to a lower urinary excretion of creatinine in females (data not shown).

Effects of BFTZ and amiloride on GFR and tubular function

Table 3 shows the absolute values of C_{H2O}, UO, FE_{Na},

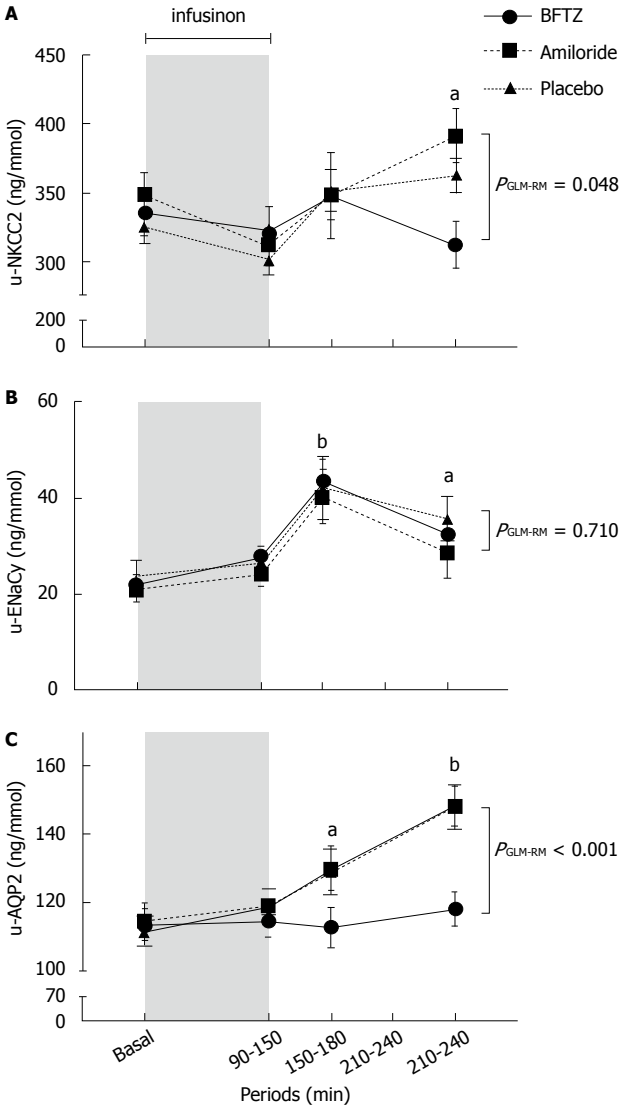

Figure 2 Effects of 3% hypertonic saline on urinary excretion of sodium-potassium-2chloride co-transporter (A), gamma fraction of epithelial sodium channels (B) and aquaporin2 (C) in 22 healthy subjects treated with bendroflumethiazide, amiloride or placebo. Values are means ± SEM. General linear model with repeated measurements (GLM-RM) was performed to test for differences between groups. A: U-NKCC2 increased during amiloride (P = 0.081) and placebo (P = 0.010) treatments. The increase in u-NKCC2 was however only significant during placebo. U-NKCC2 did not change during BFTZ; B: U-ENaCγ increased significantly and to the same extent during all three treatments; C: U-AQP2 increased significantly during amiloride and placebo (P < 0.001), but not during BFTZ. Paired t-test was used for comparison of post-infusion periods *vs* baseline. [a]P < 0.050; [b]P < 0.001. AQP2: Aquaporin2; U-NKCC2: Urinary excretion of sodium-potassium-2chloride co-transporter; ENaC: Epithelial sodium channels; BFTZ: Bendroflumethiazide.

u-Na, FE_K, u-K and ^{51}Cr-EDTA clearance.

C_{H2O} and UO decreased significantly in all three treatments. At baseline, C_{H2O} was lower during BFTZ and showed an attenuated decrease at post infusion period 210-240 min compared to amiloride (P = 0.207) and placebo (P = 0.005).

At baseline, FE_{Na} and u-Na were higher during amiloride compared to BFTZ and placebo. After 3% saline infusion there was a significant increase in u-Na and FE_{Na} in all three treatments, but less pronounced during BFTZ (P = 0.001).

There was no difference in u-K at baseline. In response to 3% saline, u-K and FE_K decreased during BFTZ and increased during amiloride compared to placebo. There was a significant difference between all three treatments (P = 0.001). GFR did not change significantly.

Effects of BFTZ and amiloride on plasma hormones

Figure 3 shows the changes in PRC, AngⅡ, p-Aldo and p-AVP during the examination day. PRC, AngⅡ and p-Aldo were significantly increased during active treatment compared to placebo. PRC and p-AngⅡ were highest during BFTZ treatment (P < 0.001), whereas p-Aldo was highest during amiloride treatment (P < 0.001). PRC, AngⅡ and p-Aldo declined significantly in response to 3% saline, in all three treatments, with no relative differences between treatments.

P-AVP was similar at baseline. P-AVP increased in all three groups, in response to 3% saline. Although, p-AVP was lower during BFTZ at 150 min (P = 0.048), the relative increase in p-AVP, after 3% saline, was not significantly different between BFTZ *vs* placebo (82% ± 100% *vs* 116% ± 67%; P = 0.072)

Effects of BFTZ and amiloride on plasma

Table 4 shows the absolute values of p-Na, p-K, p-Osm and p-Alb during basal-, infusion- and post infusion periods. During baseline conditions p-osm and p-Na were significantly lower during BFTZ and amiloride compared to placebo. P-K was higher in the amiloride group compared to placebo and BFTZ, and p-K was lower during BFTZ compared to placebo.

In response to 3% saline infusion, p-Na and p-osm increased to the same extent in all three treatments, but remained highest in the placebo group. P-K decreased significant in the amiloride group compared to BFTZ and placebo. P-alb decreased significantly in all three treatments, in response to 3% saline.

Effects of BFTZ and amiloride on blood pressure

Table 5 shows the absolute values of bSBP, bDBP, pulse rate, cSBP, cDBP, PWV and AIx. At baseline there was no difference in bSBP or bDBP. In response to 3% hypertonic saline, bSBP increased and bDBP decreased. At the end of the day the decrease in bDBP was more pronounced during BFTZ (-6% ± 6%) compared to amiloride (-2% ± 6%; P = 0.030) and placebo (-2% ± 5%; P = 0.021).

There was no difference in cSBP at baseline or in response to 3% saline between treatments. At baseline cDBP was the same in all three treatments, however cDBP decreased significant in the BFTZ group (P < 0.001) but not during amiloride and placebo. PWV followed the same pattern, however the decrease during BFTZ treatment was not significant.

Effects of BFTZ and amiloride on body fluid volumes

Figure 4 shows the changes in ICV, ECV and TBW during the examination day.

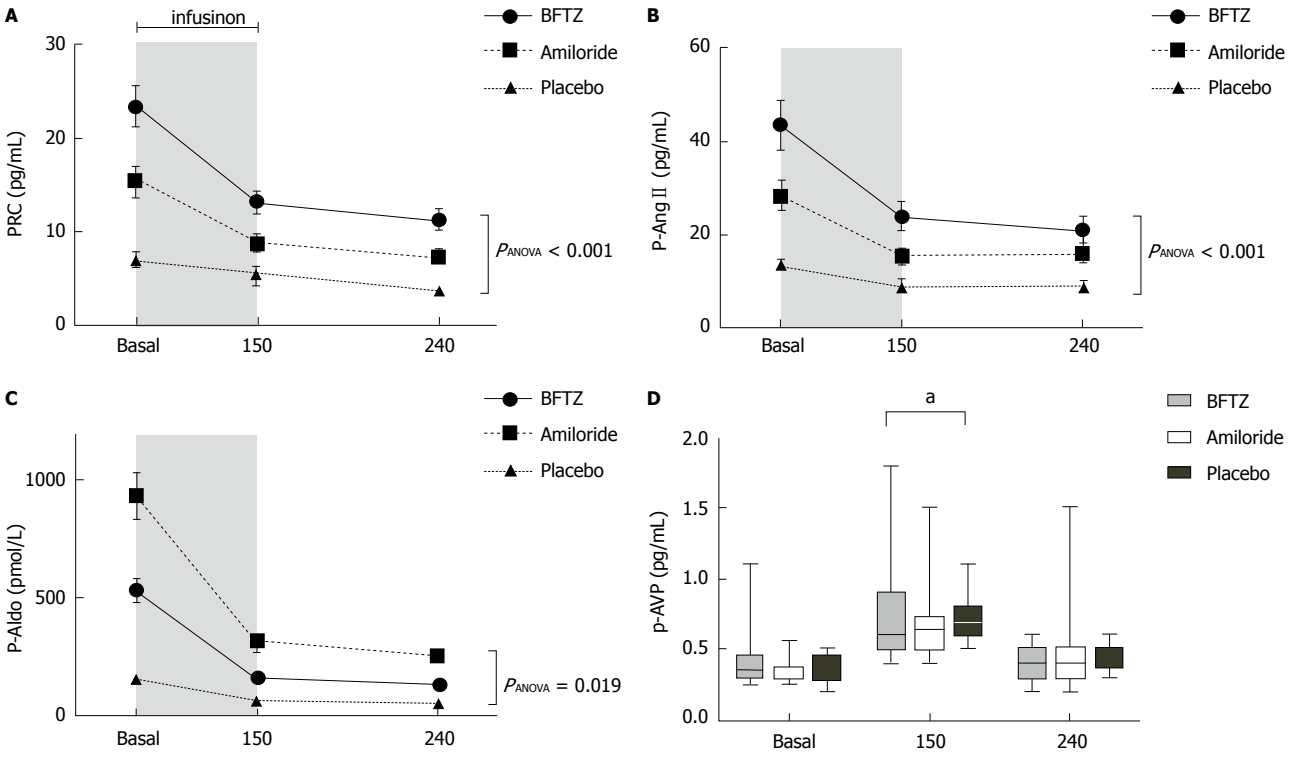

Figure 3 Effects of 3% hypertonic saline on plasma concentrations of renin (A), angiotensin II (B), aldosterone (C) and arginine vasopressin (D) in 23 healthy subjects pre-treated with bendroflumethiazide, amiloride or placebo. A-C: There was a significant difference between PRC, p-Ang II and p-Aldo plasma levels throughout the study day. Values are means ± SEM. One-way ANOVA was used to test for differences between treatments; D: P-AVP increased significantly at 150 min with a borderline significant difference between treatments ($^{a}P = 0.048$). Values are medians with upper and lower limits. Friedman's test was used to test for differences between treatments. BFTZ: Bendroflumethiazide; PRC: Plasma renin concentration.

Table 2 Twenty-four hours brachial blood pressure and urine collection with fluid deprivation (12 PM to 8.00 AM) in 23 healthy subjects

	Examination day			*P* (ANOVA)
	Thiazide	Amilorid	Placebo	
Urine output (mL/24 h)	2527 ± 728	2418 ± 469	2316 ± 700	0.481
u-osm (mosm/24 h)	865 ± 158	835 ± 176	761 ± 187	0.087
C_{H2O} (mL/min)	-0.40 ± 0.33	-0.37 ± 0.47	-0.24 ± 0.53	0.534
Cr.Cl (mL/min per m^2)	113 ± 25	118 ± 32	110 ± 26	0.549
u-NKCC (ng/mmol)	0.35 ± 0.07	0.30 ± 0.05	0.32 ± 0.06	0.025
u-AQP2 (ng/mmol)	113.2 ± 39.2	103.3 ± 25.5	98.5 ± 17.4	0.244
u-ENaCγ (ng/mmol)	37.8 ± 26.7	30.8 ± 17.3	32.7 ± 15.6	0.867
u-Na (mmol/24 h)	108 ± 34	121 ± 27	106 ± 37	0.263
u-K (mmol/24 h)	80 ± 20	64 ± 21	60 ± 19	0.002
bSBP (mmHg)	119 ± 6	114 ± 7	116 ± 7	0.213
bDBP (mmHg)	72 ± 4	69 ± 3	70 ± 4	0.034

Values are means ± SD. One-way ANOVA was used for comparison between groups. *P*-values represent the possibility of a difference between groups. u-osm: Urine output, urine osmolality; C_{H2O}: Free water clearance; Cr.Cl: Creatinin clearance; u-NKCC2: Urinary NKCC2; u-AQP2: Urinary excretion of AQP2; u-ENaCγ: ENaCγ excretion adjusted for creatinin; u-Na: Urinary excretion of sodium; u-K: Potassium; bSBP: Brachial systolic blood pressure; bDBP: Brachial diastolic blood pressure.

At baseline, ECV and TBW tended to be lower during amiloride ($P = 0.515$) and BFTZ ($P = 0.951$) compared to placebo. However, it did not reach statistical significance. ICV did not differ between treatments. As expected, after administering 3% saline, ICV decreased while ECV and TBW increased reaching a maximum at the end of the study day. Although there was a tendency towards a lower ECV and TBW in the two diuretic groups there were no statistically significant differences in volume status between the three treatments.

DISCUSSION

In the present study, the aim was to investigate the

Table 3 Effect of 3% hypertonic saline on urinary parameters in 22 healthy subjects treated with bendroflumethiazide or amiloride

Periods	Baseline	Infusion	Post infusion			$P_{GLM\ RM}$
	0-90 min	90-150 min	150-180 min	180-210 min	210-240 min	
C_{H2O}						< 0.001
BFTZ	3.1 ± 1.6[a]	-0.2 ± 0.5[d]	-1.6 ± 0.6[d]	-1.6 ± 0.7[d]	-0.6 ± 1.6[d]	
Amiloride	3.7 ± 0.9	-0.6 ± 0.7[d]	-2.1 ± 0.6[d]	-2.4 ± 0.8[d]	-1.4 ± 1.7[d]	
Placebo	4.4 ± 1.1	-0.6 ± 0.6[d]	1.8 ± 0.7[d]	-2.5 ± 1.0[d]	-2.0 ± 0.5[d]	
$P_{GLM\ between\ subjects}$			0.061			
P_{ANOVA}	0.006	NS	NS	0.001	0.007	
UO (mL/min)						0.029
BFTZ	6.1 ± 1.6	2.6 ± 0.6[d]	1.4 ± 0.5[d]	1.7 ± 1.0[d]	2.7 ± 1.9[d]	
Amiloride	6.8 ± 1.3	2.5 ± 0.6[d]	1.6 ± 0.5[d]	2.0 ± 0.7[d]	3.2 ± 1.2[d]	
Placebo	7.3 ± 1.2	2.3 ± 0.9[d]	1.7 ± 0.8[d]	2.2 ± 1.2[d]	2.4 ± 1.1[d]	
$P_{GLM\ between\ subjects}$			0.245			
P_{ANOVA}	0.019	NS	NS	NS	NS	
u-Na (mmol/min)						< 0.001
BFTZ	1.4 ± 0.4	1.7 ± 0.4[b]	1.9 ± 0.7[b]	2.1 ± 0.6[d]	2.0 ± 0.6[b]	
Amiloride	1.6 ± 0.5	2.0 ± 0.7	2.5 ± 1.0[b]	2.9 ± 1.2[d]	3.0 ± 1.0[d]	
Placebo	1.3 ± 0.3	1.9 ± 1.1[a]	2.5 ± 1.3[b]	3.3 ± 1.7[d]	3.0 ± 1.0[d]	
$P_{GLM\ between\ subjects}$			0.020			
P_{ANOVA}	0.028	NS	NS	0.007	<0.001	
FENa (%)						< 0.001
BFTZ	1.5 ± 0.4	1.8 ± 0.5[a]	2.0 ± 0.6[b]	2.2 ±0.6[d]	2.1 ± 0.6[d]	
Amiloride	1.8 ± 0.6	2.1 ± 0.6[a]	2.6 ± 1.0[b]	2.9 ± 1.2[d]	3.0 ± 1.1[d]	
Placebo	1.4 ± 0.4	2.1 ± 1.0[b]	2.7 ± 1.2[d]	3.0 ± 1.1[d]	3.1 ± 1.1[d]	
$P_{GLM\ between\ subjects}$			0.036			
P_{ANOVA}	0.022	NS	NS	0.019	0.001	
u-K (mmol/min)						
BFTZ	20.3 ± 6.7	17.7 ± 5.3	15.8 ± 4.4[b]	14.2 ± 5.6[b]	13.3 ± 6.3[b]	< 0.001
Amiloride	18.5 ± 8.4	15.7 ± 9.3	18.6 ± 11.1	22.4 ± 12.0	23.3 ± 10.2[a]	
Placebo	22.3 ± 9.3	15.6 ± 6.9[d]	16.4 ± 8.5[a]	22.3 ± 12.3	20.4 ± 7.9	
$P_{GLM\ between\ subjects}$			0.255			
P_{ANOVA}	NS	NS	NS	0.015	0.001	
FEK (%)						
BFTZ	21.7 ± 7.4	20.0 ± 6.6	16.1 ± 4.8[b]	15.0 ± 5.9[b]	14.4 ± 6.9[b]	< 0.001
Amiloride	20.8 ± 9.8	18.7 ± 10.8	20.5 ± 12.0	23.6 ± 12.4	25.0 ± 11.0[a]	
Placebo	23.7 ± 9.3	18.1 ± 8.2[b]	17.5 ± 8.9[a]	20.6 ± 9.9	21.4 ± 8.8	
$P_{GLM\ between\ subjects}$			0.254			
P_{ANOVA}	NS	NS	NS	0.018	0.001	
^{51}Cr-EDTA (mL/min per 1.73m^2)						0.271
BFTZ	92.1 ± 10.8	91.3 ± 11.9	96.0 ± 16.7	96.9 ± 16.8	96.5 ± 21.2	
Amiloride	92.7 ± 13.7	93.2 ± 12.8	94.1 ± 12.1	96.7 ± 14.1	100.2 ± 15.6	
Placebo	96.5 ± 9.5	90.0 ± 13.3	94.7 ± 15.1	102.4 ± 14.3	98.1 ± 15.9	
$P_{GLM\ between\ subjects}$			0.887			

Free water clearance (C_{H2O}), urinary output (OU), excretion of sodium (u-Na) and fractional excretion of sodium (FENa), urinary excretion of potassium (u-K) and fractional excretion of potassium (FEK) and ^{51}Cr-EDTA clearance in a randomized, placebo-controlled, crossover study of 23 healthy subjects. Values are mean ± SD. General linear model (GLM) with repeated measures was performed for comparison within the group and intervention as between subjects factor. One-way ANOVA was performed when differences were found between interventions. Post hoc Bonferroni correction was used for multiple comparisons of post infusion periods to baseline within each treatment group. [a]$P < 0.05$; [b]$P < 0.01$; [d]$P < 0.001$.

effect of five days BFTZ and amiloride treatment on the urinary excretion of NKCC2, ENaCγ and AQP2 during baseline conditions and after an acute intravenous volume load of 3% hypertonic saline in healthy subjects. To our knowledge, this study is the first randomized, placebo-controlled trial that measured the changes in u-NKCC2, u-ENaCγ and u-AQP2 during inhibition of the NCC cotransporter with BFTZ and ENaC with amiloride in humans.

This study showed that, in response to 3% saline, u-NKCC2, u-ENaCγ and u-AQP2 increased to the same extent during amiloride and placebo treatment, but neither u-NKCC2 nor u-AQP2 changed significantly during BFTZ.

Sodium and tubular sodium transporters

Thiazides predominantly inhibit NCC along the distal convoluted tubules[2]. Animal studies have shown that when thiazide was administered chronically, urinary sodium returned to normal within 2-3 d[19]. This is in accordance with our findings in 24 h urine collection. Further, a study documented that longer term NCC

Table 4 Effect of 3% hypertonic saline on plasma in 23 healthy subjects treated with bendroflumethiazide or amiloride

Time	Baseline	Infusion	Post infusion			$P_{GLM\text{-}RM}$
	0-90 min	150 min	180 min	210 min	240 min	
p-Na						0.281
BFTZ	137 ± 2	141 ± 2[d]	141 ± 2[d]	139 ± 2[d]	139 ± 2[d]	
Amilorid	137 ± 2	141 ± 2[d]	141 ± 2[d]	140 ± 2[d]	139 ± 2[d]	
Placebo	139 ± 1	43 ± 2[d]	142 ± 1[d]	141 ± 1[d]	140 ± 1[d]	
$P_{GLM\ between\ subjects}$		0.003				
P_{ANOVA}	0.001	0.019	0.004	0.007	0.005	
p-K						0.001
BFTZ	3.35 ± 0.22	3.32 ± 0.23	3.43 ± 0.26	3.42 ± 0.21	3.40 ± 0.20	
Amilorid	4.32 ± 0.30	4.17 ± 0.23[d]	4.26 ± 0.27	4.27 ± 0.25	4.20 ± 0.20[a]	
Placebo	3.89 ± 0.18	3.83 ± 0.22	3.97 ± 0.23	3.94 ± 0.22	3.92 ± 0.20	
$P_{GLM\ between\ subjects}$		< 0.0001				
P_{ANOVA}	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	
p-Osm						0.600
BFTZ	281 ± 5	288 ± 4[d]	288 ± 6[d]	286 ± 5[d]	284 ± 4[d]	
Amilorid	283 ± 4	290 ± 4[d]	290 ± 3[d]	287 ± 3[d]	285 ± 3[a]	
Placebo	286 ± 3	294 ± 4[d]	292 ± 4[d]	290 ± 4[d]	289 ± 3[b]	
$P_{GLM\ between\ subjects}$		< 0.0001				
P_{ANOVA}	< 0.001	< 0.001	0.005	0.002	< 0.001	
p-Alb (g/L)						0.007
BFTZ	40.7 ± 3.1	35.5 ± 2.2[d]	35.9 ± 2.7[d]	36.0 ± 2.8[d]	36.0 ± 2.7[d]	
Amilorid	40.9 ± 2.9	35.5 ± 2.3[d]	36.4 ± 2.6[d]	36.4 ± 2.7[d]	36.3 ± 2.5[d]	
Placebo	39.0 ± 2.4	34.5 ± 2.0[d]	35.1 ± 2.2[d]	35.1 ± 2.3[d]	35.3 ± 2.4[d]	
$P_{GLM\ between\ subjects}$		0.203				

Plasma concentrations of sodium (p-Na), potassium (p-K) and albumin (p-Alb) and plasma osmolality (p-osm). Values are mean ± SD. General linear model (GLM) with repeated measures was performed for comparison within the group and intervention as between subjects factor. One-way ANOVA was performed when differences were found between interventions. Bonferroni correction was used for multiple comparisons between study-periods *vs* baseline. [a]$P < 0.05$; [b]$P < 0.01$; [d]$P < 0.001$.

inhibition might cause a structural adaption, which will activate ENaC and cause increased distal sodium reabsorption and kaliuresis[20]. Twenty-four hours urine collections, demonstrated a small, but significantly increased u-K, and increased u-ENaCγ during BFTZ compared to amiloride and placebo; which supports the theory of a compensatory increase in sodium reabsorption *via* ENaC during longer-term thiazide treatment.

In this present study, 3% hypertonic saline induced an increase in u-NKCC2 when subjects were treated with amiloride and placebo. It was probably related to a counter regulatory mechanism to compensate for temporarily impaired lower fractional sodium reabsorption in proximal tubules during volume expansion[21-23]. It has previously been described in healthy humans that u-NKCC2 decreased after 3% saline[12]. However, the subjects' average age was approximately 35 years older in the aforementioned study. Tian *et al*[24] showed a blunting in the up regulation of sodium transport proteins in response to water restriction in aged rats, which seemed to be particularly apparent with regards to NKCC2. The age difference might explain the discrepancy in the u-NKCC2 response between the two studies.

During BFTZ treatment, u-NKCC2 ceased to increase. In rats, chronic thiazide treatment produces a compensatory fractional increased reabsorption of sodium in proximal tubules[19], which might explain why u-NKCC2 ceased to increase in the late post infusion periods during BFTZ. Thus, during BFTZ, there was no need of a compensatory reabsorption *via* NKCC, which is supported by the relative lower increase in FE_{Na} during BFTZ compared to both amiloride and placebo, in response to 3% saline. In animals, AVP has been demonstrated to increase NKCC2 activity, mediated by V2 receptors *via* adenylate-cyclase-6 to facilitate phosphorylation and trafficking of NKCC2 to the apical membrane[25,26]. As p-AVP was lower during BTFZ treatment, it cannot be excluded that the decline in u-NKCC2 might also reflect a lack of stimulation from AVP.

Thus, our findings reflect a more profound change in glomerular tubular balance during BFTZ treatment, than the more distal acting diuretic, amiloride.

In the collecting ducts, sodium transport occurs *via* the ENaC located in the luminal membrane of principal cells[27,28]. ENaC can be regulated by aldosterone[29,30], but is also regulated by AVP, that binds to the V2 receptors and induces a rapid change in channel activity *via* ENaC opening[31-36]. Recently our group demonstrated an increased u-ENaCγ after hypertonic saline infusion in healthy young subjects. The increase in u-ENaCγ was explained by an increased sodium load to the distal tubules caused by a decrease in renal sodium absorption in the proximal tubules[11,21,22]. In the present study, we measured a similar increase during all three treatments. As amiloride inhibits

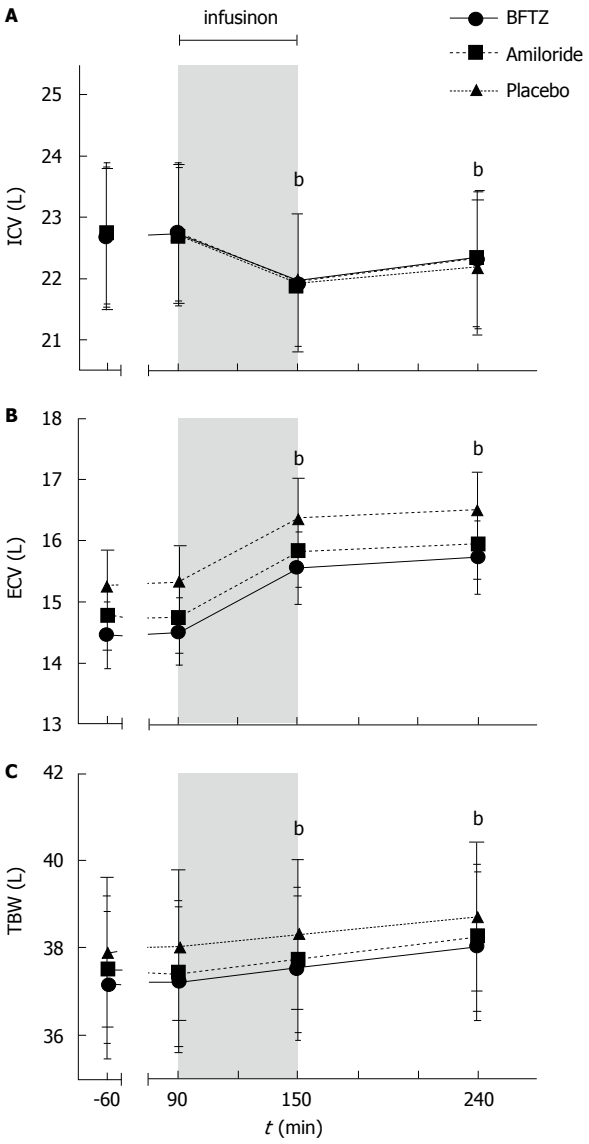

Figure 4 Effects of 3% hypertonic saline on (A) intracellular and (B) extracellular volume and (C) total body water in 23 healthy subjects pretreated with bendroflumethiazide, amiloride or placebo. Values are means ± SEM. General linear model with repeated measures was non-significant between treatments. Paired *t*-test was used for comparison of post infusion periods *vs* baseline $^{b}P < 0.001$. BFTZ: Bendroflumethiazide; ECV: Extracellular volume; ICV: Intracellular volume; TBW: Total body water.

ENaC, we expected to find a decrease in u-ENaCγ both at baseline and in response to 3% saline treatment during amiloride treatment, especially as we had also found an increased FE_{Na} at baseline. There are several possible explanations: Firstly, as ENaC only controls as little as 2%-5% of sodium reabsorption, perhaps a small decrease in the fractional reabsorption of sodium *via* ENaC would cause a significant rise in excretion of sodium. Secondly, p-Aldo was highest during amiloride treatment and its stimulation on the principal cells might have increased the amount of ENaC within the apical membrane, and thus antagonized the effect of amiloride. Thirdly, amiloride treatment has been shown to increase whole cell channel abundance caused by an intracellular sodium feedback mechanism[37,38]. These intracellular counter regulatory mechanisms might also be involved.

As expected, during the acute hypertonic sodium load, PRC, p-AngⅡ and p-Aldo decreased and urinary sodium excretion increased[9,11,39]. The increase in urinary sodium excretion is preceded by a decrease in ENaC expression and activity[40]. Meanwhile, p-AVP increased due to increased p-osm, and likely caused ENaC channels to be inserted in the apical membrane and thus increased reabsorption of sodium[41].

Thus, despite increased FE_{Na} at baseline during amiloride treatment, u-ENaCγ did not differ significantly between treatments neither at baseline nor after 3% saline. These findings do not appear to be dependent on aldosterone, but more to reflect a compensatory role of ENaC to adjust for the decreased reabsorption of sodium in proximal parts of the nephron during an acute sodium load.

Water and AQP2

Vasopressin (AVP) regulates AQP2 function by binding to V2 receptors in the basolateral membrane of principal cells, increasing the delivery of intracellular vesicles containing AQP2 to the apical membrane and thus increasing water reabsorption[42,43]. AQP2 is also excreted into urine[5-9,44-46]. Volume expansion with 3% hypertonic saline increases plasma osmolality, p-AVP, reabsorption of water and u-AQP2[11,14,47]. In the present study, there was an increase in u-AQP2, in response to 3% saline, during amiloride and placebo treatment, but not during BFTZ. The changes in u-AQP2 during BFTZ correspond to the attenuated decrease in CH2O. Different explanations include: (1) an increased water load to the collecting tubules due to inhibition of NCC in distal collecting ducts, resulting in higher water excretion; (2) Decreased p-osm and p-AVP during BFTZ treatment and thus a reduced effect on V2R in the collecting ducts; and (3) A reduced need for at counter regulatory water reabsorption in the collecting ducts due to the lower reabsorption of sodium *via* NKCC2.

Potassium

Potassium is freely excreted in glomerulus and it is reabsorbed and secreted across the nephron[48]. Intracellular signalling networks, volume status, p-K status and aldosterone tightly regulate the balance of potassium excretion[49,50]. Thiazides do not affect potassium transport directly, but induce adaption primarily along the connecting and collecting tubules where enhanced sodium reabsorption stimulates potassium secretion *via* renal outer medullary K+ (ROMK) and large-conductance K+ (BK) channels[20,51].

It has recently been shown that angiotensinⅡ directly inhibits ROMK in potassium-depleted animals, and thereby contributes to potassium conservation[52,53]. In this present study, p-Ang Ⅱ was highest during BFTZ treatment, and may have inhibited ROMK, and explains the increased sodium reabsorption during 3% saline

Table 5 Effect of 3% hypertonic saline on brachial blood pressure, central blood pressure and pulse wave velocity in 23 healthy subjects treated with bendroflumethiazide or amiloride

Periods	Baseline	Infusion	Post infusion			$P_{GLM\ RM}$
	0-90 min	150 min	180 min	210 min	240 min	
bSBP						
BFTZ	112 ± 9	118 ± 9[d]	116 ± 10	116 ± 11	117 ± 13	0.825
Amilorid	110 ± 8	115 ± 9[d]	114 ± 10[d]	114 ± 10[b]	114 ± 10[b]	
Placebo	113 ± 10	117 ± 10[d]	115 ± 9	115 ± 8	115 ± 10	
$P_{GLM\ between\ subjects}$			0.679			
bDBP						
BFTZ	66 ± 7	63 ± 7[b]	62 ± 6[d]	62 ± 7[d]	62 ± 7[b]	0.055
Amilorid	64 ± 4	62 ± 5	60 ± 5[b]	61 ± 5	62 ± 6	
Placebo	64 ± 6	63 ± 5	63 ± 5	62 ± 6	63 ± 6	
$P_{GLM\ between\ subjects}$			0.695			
Pulse Rate						
BFTZ	58 ± 10	61 ± 11[a]	61 ± 11[b]	62 ± 10[b]	62 ± 10[b]	0.782
Amilorid	57 ± 10	61 ± 11[d]	60 ± 10[d]	61 ± 11[d]	61 ± 11[d]	
Placebo	55 ± 10	59 ± 12[a]	59 ± 12[b]	59 ± 12[b]	59 ± 13[a]	
$P_{GLM\ between\ subjects}$			0.712			
cSBP						
BFTZ	99 ± 7	98 ± 7				NS
Amilorid	96 ± 5	97 ± 7				NS
Placebo	98 ± 6	98 ± 8				NS
P_{ANOVA}	NS	NS				
cDBP						
BFTZ	67 ± 5	63 ± 6				< 0.001
Amilorid	65 ± 5	65 ± 6				NS
Placebo	65 ± 5	64 ± 5				NS
P_{ANOVA}	NS	NS				
PWV						
BFTZ	5.5 ± 0.6	5.3 ± 0.4				0.055
Amilorid	5.3 ± 0.5	5.3 ± 0.5				NS
Placebo	5.3 ± 0.7	5.3 ± 0.6				NS
P_{ANOVA}	NS	NS				
AI						
BFTZ	-2.2 ± 14.6	-5.9 ±17.7				NS
Amilorid	0.4 ± 12.5	-1.4 ± 13.4				NS
Placebo	-1.6 ± 14.6	-4.9 ± 18.6				0.034
P_{ANOVA}	NS	NS				

Values are mean ± SD. General linear model (GLM) with repeated measures was performed for comparison within the group and intervention as between subjects factor for brachial systolic (bSBP), brachial diastolic blood pressure (bDBP) and pulse rate. Bonferroni correction was used for multiple comparisons between study-periods *vs* baseline. [a]$P < 0.05$; [b]$P < 0.001$; [d]$P < 0.001$; Paired *t*-test was used for comparison between post infusion *vs* baseline for central systolic (cSBP), central diastolic (cDBP), pulse wave velocity (PWV) and augmentation index (AIx).

while potassium secretion decreased.

During amiloride treatment there was a decrease in u-K at baseline. Amiloride exerts a direct effect on potassium excretion due to the blocking of ENaC. If the influx of sodium does not occur, there will be no lumen negative potential to drive potassium excretion[48]. In response to 3% hypertonic saline however, the excretion of potassium increased most during amiloride. This phenomenon might have been due to prolonged effect of aldosterone to increase sodium reabsorption *via* ENaC and secretion *via* ROMK, despite current amiloride blockage.

Blood pressure

Thiazide decreases ECV and peripheral vascular resistance[2,54]. Amiloride is an antihypertensiva that exhibits its effects by significant natriuresis[55]. In this study the lack of difference in 24 h ambulatory blood pressure might partly be explained by the fact that the subjects were young and healthy with normal BP before entering the study. Moreover, BFTZ and amiloride are relatively weak antihypertensives and a very negligible blood pressure lowering effect was expected in these normohypertensive subjects.

Data showed that bDBP decreased significant in all three treatments, but bDBP decreased relatively more during BFTZ treatment compared to amiloride and placebo. As brachial office BP was also used to calibrate the SphygmoCor cDBP this may explain the reduction in cDBP during BFTZ. A negative augmentation index (AIx) has been reported in healthy young subjects, but is of limited use due to normal cardiovascular elasticity in this age group[56].

Body fluid volumes

The determination of body fluid volumes *via* bioimpedance

spectroscopy (BIS) is an accurate method for estimating total body water and the distribution of water between the intracellular and extracellular spaces[57]. In this present study, we measured no statistical difference between the groups, but as expected TBV was lower during both diuretic treatments compared to placebo, due to a decrease in ECV followed by sodium deficit. This reduction in ECV, during BFTZ treatment, is in agreement with current knowledge[2]. We did not expect a major decrease in ECV after amiloride, being a weak diuretic agent[55]. However the decrease in ECV was very similar to BFTZ. A significant difference in body fluid volumes between treatment groups was not detected in the present study, possibly due to the small number of subjects in each group.

Strengths and limitations

The major strength of this study was the design as a randomized, placebo controlled, double-blinded crossover study with a homogenous group of healthy young men and women. The test conditions were very well defined regarding diet, sodium and fluid intake. Thus, the results are not confounded by differences in sodium or water intake. However, as the study group was healthy humans the conclusions is limited to this population group and may not be extracted to patients with disturbances in water and sodium balance. Also the excretion of NCC was not measured, which would have provided us with even more information on renal handling of sodium.

Conclusion

In this study of healthy humans, amiloride and placebo clearly increased u-NKCC2, u-ENaCγ and u-AQP2 in response to 3% hypertonic saline, while u-NKCC2 and u-AQP2 were unchanged during BFTZ. In contrast to amiloride, BFTZ treatment seemed to have changed glomerular-tubular balance, which caused the absence of a compensatory reabsorption of sodium *via* NKCC2 after hypertonic saline. It is possible that the lower p-AVP during BFTZ treatment resulted in a relatively less stimulation of NKCC2 and AQP2, with subsequent reduced transport of sodium and water *via* the transporters. During all three treatments, the increase in u-ENaCγ might reflect a compensatory reabsorption to adjust for the decreased reabsorption of sodium in the proximal part of the nephron.

ACKNOWLEDGEMENTS

The authors greatly acknowledge the skilful assistance of our laboratory technicians: Inger-Merete Paulsen, Anne Mette Ravn, Kirsten Nygaard, Henriette Vorup Simonsen and Susan Milton Rasmussen.

COMMENTS

Background

The discovery that urine contains proteins from renal epithelia of proximal tubule, Henle's loop, distal convoluted tubule and the collecting ducts has provided us with urinary biomarkers as a tool to investigate physiological and pathophysiological processes in renal sodium and water homeostasis.

Research frontiers

Urinary excretion of the aquaporin2 water channel (u-AQP2) is a biomarker that has been investigated in numerous studies of various water-balance disorders. It has also been demonstrated that the urinary excretion of epithelial sodium channels (u-ENaC) can be used as biomarkers of sodium transport *via* excretion of epithelial sodium channels (ENaC). However the exact physiological mechanisms are still unknown, and studies are needed to address the complete physiological handling of sodium and water in humans.

Innovations and breakthroughs

In animals, volume expansion and diuretics changes proximal water and sodium reabsorption and the expression of AQP2 and sodium transporters along the nephron. In addition, changes in transport activity of the sodium-potassium-2chloride cotransporter (NKCC2); ENaC and AQP2 may also be involved in the abnormal tubular function in patients with chronic kidney disease. However, it has never been studied to what extent the function of NKCC2, ENaC and AQP2 is simultaneously affected in response to amiloride and bendroflumethiazide (BFTZ) in humans. In the present study, the u-NKCC2 and u-AQP2 increased during amiloride and placebo, while u-NKCC2 and u-AQP2 remained unchanged during BFTZ, in response to infusion of 3% saline.

Applications

Thus, measurements of water- and sodium transporters in urine, as biomarkers of water-and sodium transport *via* NKCC2, ENaC and AQP2 may provide important information of the mechanisms involved in water and sodium balance in the kidney.

Terminology

AQP2, NKCC2 and ENaC are transporters in the nephron that play essential roles in regulating water and sodium homeostasis, extracellular volume and controlling blood pressure by reabsorbing water and sodium. BFTZ is a diuretic that inhibit the sodium-chloride co-transporter and amiloride is a diuretic that block the ENaC channels. These diuretics, which were developed empirically to treat patients with edema and hypertension, can be used as tools to characterize sodium transport pathways.

Peer-review

This is an interesting paper.

REFERENCES

1 **Esteva-Font C**, Ballarin J, Fernández-Llama P. Molecular biology of water and salt regulation in the kidney. *Cell Mol Life Sci* 2012; **69**: 683-695 [PMID: 21997386 DOI: 10.1007/s00018-011-0858-4]
2 **Duarte JD**, Cooper-DeHoff RM. Mechanisms for blood pressure lowering and metabolic effects of thiazide and thiazide-like diuretics. *Expert Rev Cardiovasc Ther* 2010; **8**: 793-802 [PMID: 20528637 DOI: 10.1586/erc.10.27]
3 **Kleyman TR**, Cragoe EJ. Amiloride and its analogs as tools in the study of ion transport. *J Membr Biol* 1988; **105**: 1-21 [PMID: 2852254]
4 **Nielsen S**, Agre P. The aquaporin family of water channels in kidney. *Kidney Int* 1995; **48**: 1057-1068 [PMID: 8569067]
5 **Kanno K**, Sasaki S, Hirata Y, Ishikawa S, Fushimi K, Nakanishi S, Bichet DG, Marumo F. Urinary excretion of aquaporin-2 in patients with diabetes insipidus. *N Engl J Med* 1995; **332**: 1540-1545 [PMID: 7537863 DOI: 10.1056/NEJM199506083322303]
6 **Rai T**, Sekine K, Kanno K, Hata K, Miura M, Mizushima A, Marumo F, Sasaki S. Urinary excretion of aquaporin-2 water channel protein in human and rat. *J Am Soc Nephrol* 1997; **8**: 1357-1362 [PMID: 9294826]
7 **Elliot S**, Goldsmith P, Knepper M, Haughey M, Olson B. Urinary excretion of aquaporin-2 in humans: a potential marker of collecting duct responsiveness to vasopressin. *J Am Soc Nephrol* 1996; **7**: 403-409 [PMID: 8704105]
8 **Saito T**, Ishikawa SE, Sasaki S, Nakamura T, Rokkaku K, Kawakami A, Honda K, Marumo F, Saito T. Urinary excretion of aquaporin-2 in the diagnosis of central diabetes insipidus. *J Clin Endocrinol Metab* 1997; **82**: 1823-1827 [PMID: 9177390]
9 **Pedersen RS**, Bentzen H, Bech JN, Pedersen EB. Effect of water

deprivation and hypertonic saline infusion on urinary AQP2 excretion in healthy humans. *Am J Physiol Renal Physiol* 2001; **280**: F860-F867 [PMID: 11292629]
10 **Lauridsen TG**, Vase H, Starklint J, Graffe CC, Bech JN, Nielsen S, Pedersen EB. Increased renal sodium absorption by inhibition of prostaglandin synthesis during fasting in healthy man. A possible role of the epithelial sodium channels. *BMC Nephrol* 2010; **11**: 28 [PMID: 21029429 DOI: 10.1186/1471-2369-11-28]
11 **Jensen JM**, Mose FH, Bech JN, Nielsen S, Pedersen EB. Effect of volume expansion with hypertonic- and isotonic saline and isotonic glucose on sodium and water transport in the principal cells in the kidney. *BMC Nephrol* 2013; **14**: 202 [PMID: 24067081 DOI: 10.1186/1471-2369-14-202]
12 **Jensen JM**, Mose FH, Kulik AE, Bech JN, Fenton RA, Pedersen EB. Abnormal urinary excretion of NKCC2 and AQP2 in response to hypertonic saline in chronic kidney disease: an intervention study in patients with chronic kidney disease and healthy controls. *BMC Nephrol* 2014; **15**: 101 [PMID: 24970686 DOI: 10.1186/1471-2369-15-101]
13 **Pedersen EB**, Eiskjaer H, Madsen B, Danielsen H, Egeblad M, Nielsen CB. Effect of captopril on renal extraction of renin, angiotensin II, atrial natriuretic peptide and vasopressin, and renal vein renin ratio in patients with arterial hypertension and unilateral renal artery disease. *Nephrol Dial Transplant* 1993; **8**: 1064-1070 [PMID: 8272217]
14 **Graffe CC**, Bech JN, Pedersen EB. Effect of high and low sodium intake on urinary aquaporin-2 excretion in healthy humans. *Am J Physiol Renal Physiol* 2012; **302**: F264-F275 [PMID: 21993890 DOI: 10.1152/ajprenal.00442.2010]
15 **Lauridsen TG**, Vase H, Starklint J, Bech JN, Pedersen EB. Protein-enriched diet increases water absorption via the aquaporin-2 water channels in healthy humans. *Nephrol Dial Transplant* 2010; **25**: 2502-2510 [PMID: 20237060 DOI: 10.1093/ndt/gfq111]
16 **Matthesen SK**, Larsen T, Vase H, Lauridsen TG, Jensen JM, Pedersen EB. Effect of amiloride and spironolactone on renal tubular function and central blood pressure in patients with arterial hypertension during baseline conditions and after furosemide: a double-blinded, randomized, placebo-controlled crossover trial. *Clin Exp Hypertens* 2013; **35**: 313-324 [PMID: 22966789 DOI: 10.3109/10641963.2012.721843]
17 **Hager H**, Kwon TH, Vinnikova AK, Masilamani S, Brooks HL, Frøkiaer J, Knepper MA, Nielsen S. Immunocytochemical and immunoelectron microscopic localization of alpha-, beta-, and gamma-ENaC in rat kidney. *Am J Physiol Renal Physiol* 2001; **280**: F1093-F1106 [PMID: 11352848]
18 **Matthesen SK**, Larsen T, Vase H, Lauridsen TG, Pedersen EB. Effect of potassium supplementation on renal tubular function, ambulatory blood pressure and pulse wave velocity in healthy humans. *Scand J Clin Lab Invest* 2012; **72**: 78-86 [PMID: 22149452 DOI: 10.3109/00365513.2011.635216]
19 **Walter SJ**, Shirley DG. The effect of chronic hydrochlorothiazide administration on renal function in the rat. *Clin Sci* (Lond) 1986; **70**: 379-387 [PMID: 3698514]
20 **Hunter RW**, Craigie E, Homer NZ, Mullins JJ, Bailey MA. Acute inhibition of NCC does not activate distal electrogenic Na+ reabsorption or kaliuresis. *Am J Physiol Renal Physiol* 2014; **306**: F457-F467 [PMID: 24402096 DOI: 10.1152/ajprenal.00339.2013]
21 **Dirks JH**, Cirksena WJ, Berliner RW. The effects of saline infusion on sodium reabsorption by the proximal tubule of the dog. *J Clin Invest* 1965; **44**: 1160-1170 [PMID: 14328393 DOI: 10.1172/JCI105223]
22 **Svensén CH**, Waldrop KS, Edsberg L, Hahn RG. Natriuresis and the extracellular volume expansion by hypertonic saline. *J Surg Res* 2003; **113**: 6-12 [PMID: 12943804]
23 **Häberle DA**, von Baeyer H. Characteristics of glomerulotubular balance. *Am J Physiol* 1983; **244**: F355-F366 [PMID: 6340526]
24 **Tian Y**, Riazi S, Khan O, Klein JD, Sugimura Y, Verbalis JG, Ecelbarger CA. Renal ENaC subunit, Na-K-2Cl and Na-Cl cotransporter abundances in aged, water-restricted F344 x Brown Norway rats. *Kidney Int* 2006; **69**: 304-312 [PMID: 16408120 DOI: 10.1038/sj.ki.5000076]
25 **Rieg T**, Tang T, Uchida S, Hammond HK, Fenton RA, Vallon V. Adenylyl cyclase 6 enhances NKCC2 expression and mediates vasopressin-induced phosphorylation of NKCC2 and NCC. *Am J Pathol* 2013; **182**: 96-106 [PMID: 23123217 DOI: 10.1016/j.ajpath.2012.09.014]
26 **Giménez I**, Forbush B. Short-term stimulation of the renal Na-K-Cl cotransporter (NKCC2) by vasopressin involves phosphorylation and membrane translocation of the protein. *J Biol Chem* 2003; **278**: 26946-26951 [PMID: 12732642 DOI: 10.1074/jbc.M303435200]
27 **Edinger RS**, Bertrand CA, Rondandino C, Apodaca GA, Johnson JP, Butterworth MB. The epithelial sodium channel (ENaC) establishes a trafficking vesicle pool responsible for its regulation. *PLoS One* 2012; **7**: e46593 [PMID: 23029554 DOI: 10.1371/journal.pone.0046593]
28 **Loffing J**, Korbmacher C. Regulated sodium transport in the renal connecting tubule (CNT) via the epithelial sodium channel (ENaC). *Pflugers Arch* 2009; **458**: 111-135 [PMID: 19277701 DOI: 10.1007/s00424-009-0656-0]
29 **Garty H**, Palmer LG. Epithelial sodium channels: function, structure, and regulation. *Physiol Rev* 1997; **77**: 359-396 [PMID: 9114818]
30 **Masilamani S**, Kim GH, Mitchell C, Wade JB, Knepper MA. Aldosterone-mediated regulation of ENaC alpha, beta, and gamma subunit proteins in rat kidney. *J Clin Invest* 1999; **104**: R19-R23 [PMID: 10510339 DOI: 10.1172/JCI7840]
31 **Bankir L**, Fernandes S, Bardoux P, Bouby N, Bichet DG. Vasopressin-V2 receptor stimulation reduces sodium excretion in healthy humans. *J Am Soc Nephrol* 2005; **16**: 1920-1928 [PMID: 15888562 DOI: 10.1681/ASN.2004121079]
32 **Ecelbarger CA**, Kim GH, Wade JB, Knepper MA. Regulation of the abundance of renal sodium transporters and channels by vasopressin. *Exp Neurol* 2001; **171**: 227-234 [PMID: 11573975 DOI: 10.1006/exnr.2001.7775]
33 **Bugaj V**, Pochynyuk O, Stockand JD. Activation of the epithelial Na+ channel in the collecting duct by vasopressin contributes to water reabsorption. *Am J Physiol Renal Physiol* 2009; **297**: F1411-F1418 [PMID: 19692483 DOI: 10.1152/ajprenal.00371.2009]
34 **Ecelbarger CA**, Kim GH, Terris J, Masilamani S, Mitchell C, Reyes I, Verbalis JG, Knepper MA. Vasopressin-mediated regulation of epithelial sodium channel abundance in rat kidney. *Am J Physiol Renal Physiol* 2000; **279**: F46-F53 [PMID: 10894786]
35 **Perucca J**, Bichet DG, Bardoux P, Bouby N, Bankir L. Sodium excretion in response to vasopressin and selective vasopressin receptor antagonists. *J Am Soc Nephrol* 2008; **19**: 1721-1731 [PMID: 18596120 DOI: 10.1681/ASN.2008010021]
36 **Stockand JD**. Vasopressin regulation of renal sodium excretion. *Kidney Int* 2010; **78**: 849-856 [PMID: 20736986 DOI: 10.1038/ki.2010.276]
37 **Patel AB**, Frindt G, Palmer LG. Feedback inhibition of ENaC during acute sodium loading in vivo. *Am J Physiol Renal Physiol* 2013; **304**: F222-F232 [PMID: 23171553 DOI: 10.1152/ajprenal.00596.2012]
38 **Frindt G**, Silver RB, Windhager EE, Palmer LG. Feedback regulation of Na channels in rat CCT. II. Effects of inhibition of Na entry. *Am J Physiol* 1993; **264**: F565-F574 [PMID: 8384418]
39 **Andersen LJ**, Jensen TU, Bestle MH, Bie P. Isotonic and hypertonic sodium loading in supine humans. *Acta Physiol Scand* 1999; **166**: 23-30 [PMID: 10372975]
40 **Pácha J**, Frindt G, Antonian L, Silver RB, Palmer LG. Regulation of Na channels of the rat cortical collecting tubule by aldosterone. *J Gen Physiol* 1993; **102**: 25-42 [PMID: 8397276]
41 **Butterworth MB**. Regulation of the epithelial sodium channel (ENaC) by membrane trafficking. *Biochim Biophys Acta* 2010; **1802**: 1166-1177 [PMID: 20347969 DOI: 10.1016/j.bbadis.2010.03.010]
42 **DiGiovanni SR**, Nielsen S, Christensen EI, Knepper MA. Regulation of collecting duct water channel expression by vasopressin in Brattleboro rat. *Proc Natl Acad Sci USA* 1994; **91**: 8984-8988 [PMID: 7522327]
43 **Nielsen S**, Chou CL, Marples D, Christensen EI, Kishore BK, Knepper MA. Vasopressin increases water permeability of kidney

collecting duct by inducing translocation of aquaporin-CD water channels to plasma membrane. *Proc Natl Acad Sci USA* 1995; **92**: 1013-1017 [PMID: 7532304]

44 **Wen H**, Frokiaer J, Kwon TH, Nielsen S. Urinary excretion of aquaporin-2 in rat is mediated by a vasopressin-dependent apical pathway. *J Am Soc Nephrol* 1999; **10**: 1416-1429 [PMID: 10405197]

45 **Pisitkun T**, Shen RF, Knepper MA. Identification and proteomic profiling of exosomes in human urine. *Proc Natl Acad Sci USA* 2004; **101**: 13368-13373 [PMID: 15326289 DOI: 10.1073/pnas.0403453101]

46 **Pedersen RS**, Bentzen H, Bech JN, Nyvad O, Pedersen EB. Urinary aquaporin-2 in healthy humans and patients with liver cirrhosis and chronic heart failure during baseline conditions and after acute water load. *Kidney Int* 2003; **63**: 1417-1425 [PMID: 12631357 DOI: 10.1046/j.1523-1755.2003.00858.x]

47 **Starklint J**, Bech JN, Pedersen EB. Down-regulation of urinary AQP2 and unaffected response to hypertonic saline after 24 hours of fasting in humans. *Kidney Int* 2005; **67**: 1010-1018 [PMID: 15698439 DOI: 10.1111/j.1523-1755.2005.00164.x]

48 **Castañeda-Bueno M**, Arroyo JP, Gamba G. Independent regulation of Na+ and K+ balance by the kidney. *Med Princ Pract* 2012; **21**: 101-114 [PMID: 22042004 DOI: 10.1159/000332580]

49 **Arroyo JP**, Ronzaud C, Lagnaz D, Staub O, Gamba G. Aldosterone paradox: differential regulation of ion transport in distal nephron. *Physiology* (Bethesda) 2011; **26**: 115-123 [PMID: 21487030 DOI: 10.1152/physiol.00049.2010]

50 **Hoorn EJ**, Ellison DH. WNK kinases and the kidney. *Exp Cell Res* 2012; **318**: 1020-1026 [PMID: 22405999 DOI: 10.1016/j.yexcr.2012.02.029]

51 **Ellison DH**, Loffing J. Thiazide effects and adverse effects: insights from molecular genetics. *Hypertension* 2009; **54**: 196-202 [PMID: 19564550 DOI: 10.1161/HYPERTENSIONAHA.109.129171]

52 **Wei Y**, Zavilowitz B, Satlin LM, Wang WH. Angiotensin II inhibits the ROMK-like small conductance K channel in renal cortical collecting duct during dietary potassium restriction. *J Biol Chem* 2007; **282**: 6455-6462 [PMID: 17194699 DOI: 10.1074/jbc.M607477200]

53 **Yue P**, Sun P, Lin DH, Pan C, Xing W, Wang W. Angiotensin II diminishes the effect of SGK1 on the WNK4-mediated inhibition of ROMK1 channels. *Kidney Int* 2011; **79**: 423-431 [PMID: 20927043 DOI: 10.1038/ki.2010.380]

54 **Jessup JA**, Brosnihan KB, Gallagher PE, Chappell MC, Ferrario CM. Differential effect of low dose thiazides on the Renin Angiotensin system in genetically hypertensive and normotensive rats. *J Am Soc Hypertens* 2008; **2**: 106-115 [PMID: 19343087 DOI: 10.1016/j.jash.2007.10.005]

55 **Bull MB**, Laragh JH. Amiloride. A potassium-sparing natriuretic agent. *Circulation* 1968; **37**: 45-53 [PMID: 5634728]

56 **Hughes AD**, Park C, Davies J, Francis D, McG Thom SA, Mayet J, Parker KH. Limitations of augmentation index in the assessment of wave reflection in normotensive healthy individuals. *PLoS One* 2013; **8**: e59371 [PMID: 23544061 DOI: 10.1371/journal.pone.0059371]

57 **Moissl UM**, Wabel P, Chamney PW, Bosaeus I, Levin NW, Bosy-Westphal A, Korth O, Müller MJ, Ellegård L, Malmros V, Kaitwatcharachai C, Kuhlmann MK, Zhu F, Fuller NJ. Body fluid volume determination via body composition spectroscopy in health and disease. *Physiol Meas* 2006; **27**: 921-933 [PMID: 16868355 DOI: 10.1088/0967-3334/27/9/012]

P- Reviewer: McNally RJQ, Su M **S- Editor**: Ji FF
L- Editor: A **E- Editor**: Yan JL

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DOI: 10.5527/wjn.v4.i3.438

World J Nephrol 2015 July 6; 4(3): 438-443
ISSN 2220-6124 (online)

CASE REPORT

Unexpected hypercalcemia in a diabetic patient with kidney disease

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Author contributions: Lupica R, Buemi M and Santoro D designed the report; Santoro D performed the biopsy analyses; Trimboli D, Canale V, Cernaro V and Campennì A collected the patient's clinical data; Lupica R analyzed the data and wrote the paper.

Ethics approval statement: The study was reviewed and approved by the Institutional Review Board.

Informed consent statement: All study participants, or their legal guardian, provided informed written consent prior to study enrolment.

Conflict-of-interest statement: The authors declare that they have no relevant financial interests.

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Received: November 13, 2014
Peer-review started: November 13, 2014
First decision: December 26, 2014
Revised: January 14, 2015
Accepted: April 28, 2015
Article in press: April 30, 2015
Published online: July 6, 2015

Abstract

We report a case of a diabetic patient with progressive chronic kidney disease and unexplained hypercalcemia. This unusual presentation and the investigation of all possible causes led us to perform a renal biopsy. The systemic sarcoidosis diagnosis was confirmed by the presence of interstitial multiple granulomas composed of epithelioid and multinucleated giant cells delimited by a thin fibrous reaction, and by pulmonary computed tomography finding of numerous lumps with ground-glass appearance. Sarcoidosis most commonly involves lungs, lymph nodes, skin and eyes, whilst kidney is less frequently involved. When it affects males it is characterized by hypercalcemia, hypercalciuria, and progressive loss of renal function. Early treatment with steroids allows for a gradual improvement in renal function and normalization of calcium serum values. Otherwise, the patient would quickly progress to end stage renal disease. Finding of hypercalcemia in a patient with renal failure must alert physicians because it may be a sign of several pathological entities.

Key words: Biopsy; Granulomatous; Hypercalcemia; Kidney; Sarcoidosis

Core tip: This report not only describes a case of kidney sarcoidosis, but also explains the diagnostic algorithm that led to the correct diagnosis of a case wrongly labelled

as chronic kidney disease (CKD) secondary to diabetic nephropathy. The absence of other microangiopathic alterations such as retinopathy and secondly the presence of hypercalcemia with hypoparathyroidism in a patient with CKD need to be further explored.

Lupica R, Buemi M, Campennì A, Trimboli D, Canale V, Cernaro V, Santoro D. Unexpected hypercalcemia in a diabetic patient with kidney disease. *World J Nephrol* 2015; 4(3): 438-443 Available from: URL: http://www.wjgnet.com/2220-6124/full/v4/i3/438.htm DOI: http://dx.doi.org/10.5527/wjn.v4.i3.438

INTRODUCTION

Sarcoidosis is a multisystem granulomatous disease with unknown etiology. The diagnosis requires histological confirmation by the discovery of noncaseating granulomas, the exclusion of other diseases with similar symptoms and the clinical evidence of multiple organ involvement[1]. It is often subclinical or self-limited or the symptoms are shaded[2]. The presentation can be various: respiratory symptoms are not specific and may be mistakenly attributed to other lung diseases, for which empiric therapy may be attempted before other diagnostic tests are performed. Moreover we can find dermatological or ocular abnormalities and totally nonspecific systemic symptoms (fever, weight loss, night sweats). It usually has a benign course with spontaneous resolution in up to two-thirds of cases. However, in one third of cases it is a progressive disorder with significant organ impairment[3].

CASE REPORT

In October 2013, a caucasian 59-year-old man was admitted to Nephrology division. The history was notable for non-insulin diabetes mellitus (ten years), with poor glycemic control (glycosylated haemoglobin A1c = 10.1%). Two months before he had been hospitalized at the internal medicine ward and insulin therapy had been started. Because of increased creatinine levels with lower limb oedema, the patient was referred to the Nephrology Unit. On admission, physical examination revealed normal blood pressure (120/70 mmHg) and heart rate (100 beats/min), moderate leg oedema; laboratory data showed serum creatinine equal to 2.9 mg/dL, calcium 10.4 mg/dL, proteinuria 156 mg/24 h, calculated creatinine clearance (cCrCl) 30.69 mL/min per 1.73 m^2 (other blood tests are shown in Table 1); electrocardiogram and echocardiogram were normal. Renal ultrasound (Figure 1) and Colour Doppler showed normal kidneys [antero-posterior diameter of right kidney = 11.17 cm, left kidney = 11.48 cm; normal cortico-medullary representation; resistance index (RI) = 0.70 to right, 0.73 to left]. Fundus oculi was negative for typical lesions of diabetic retinopathy. The patient was discharged with diagnosis of "stage Ⅲ chronic kidney disease (CKD)" and insulin and antibiotic therapy was recommended. After approximately 1 mo, the patient was hospitalized again due to the worsening of his general conditions and hypercalcemia. He complained of loss of appetite and weakness, with decrease in body weight of about 2 kg. General physical examination was normal except for the presence of right eye hyperaemia, more represented peripheral oedema and reduced breath sounds over the entire pulmonary area. The laboratory tests on the admission are shown in Table 1.

The patient was studied for other secondary causes of hypercalcemia by using an appropriate algorithm[4]. Because of the non-PTH mediated further increase in serum calcium (PTH = 5.5 pg/mL), normal level of 25D (21.73 ng/mL) and absence of vitamin D therapy, hematologic screening was performed to exclude cancer. Chest X-ray (Figure 2) detected a parenchymal consolidation in the right hilum, two pseudo nodular thickenings in the base with a diffuse micronodular pattern. Pulmonary computed tomography (CT) (Figure 3) showed multiple ground-glass like lumps involving both lungs, but not in the basal regions, associated with a thickening of the central and peripheral interstitium and multiple reactive mediastinal lymph nodes. Finally, since data were not suggestive for diabetic nephropathy we decided to perform a renal biopsy. Formalin-fixed and paraffin embedded tissue was processed using standard techniques. Light microscopy showed the presence of interstitial multiple granulomas composed of epithelioid and multinucleated giant cells delimited by a thin fibrous reaction (Figures 4 and 5). Edema was present in the interstitium with mild to moderate lymphocyte-monocyte infiltration and tubular atrophy affecting about 15%-20% of the core. Some tubules showed detachment of necrotic cells in the tubular lumen and in some cases rupture of the basement membrane. Arteriolar hyalinosis and arterial intimal fibrosis were also present. Immunofluorescence staining of frozen sections was negative for IgG, IgM, IgA, C3, C1q, fibrinogen, kappa, and lambda light chains.

Diagnosis

Interstitial Granulomatous nephritis secondary to Sarcoidosis. Arteriosclerosis and arteriolosclerosis.

Clinical follow up

The patient was treated with oral steroids at the dose of 1 mg/kg per day that was progressively tapered.

Normalization in calcium values and improvement of renal function were observed after the first month of corticosteroid therapy (Table 1). There was also resolution of peripheral oedema and asthenia, and improvement of appetite with progressive body weight increase. At the same time, chest CT showed amelioration of pulmonary alterations and disappearance of lumps.

DISCUSSION

Sarcoidosis most commonly involves lungs, lymph

Table 1 October 2013: laboratory data on first hospitalization; December 2013: laboratory data on second hospitalization before kidney biopsy; January 2014: laboratory data after 1 mo of treatment; February 2014: laboratory data after 2 mo of treatment

Serum chemistries	October 2013	December 2013	January 2014	February 2014
Sodium (mmol/L)	141	136	139	140
Potassium (mmol/L)	38	4.4	4	4.6
Glucose (mg/dL)	139	140	117	145
HbA1c (%)	10.1	9	6.30	7
Proteinuria (mg/24 h)	156	408	328	394
Creatinine (mg/dL)	2.9	3.6	1.83	1.82
eGFR (mL/min per 1.73 m^2)	30.69	20.69	42	64
Calcium (mg/dL)	10.05	13.01	8.3	8.1
Phosphorus (mg/dL)	4.6	4.2	3.9	4
PTH (pg/mL)	3.50	5.50	59.76	61.38
VIT D3 (ng/mL)	30.67	21.73	20.50	19.83
AST (U/L)	12	15	17	12
ALT (U/L)	9	8	24	9
ALP (U/L)	89	80	79	86
Hematologic studies				
WBC count (x 10^3/μL)	10.4	13.6	11.2	10.90
Hemoglobin (g/dL)	10	10.6	13.60	12.2
Platelet count (x 10^3/μL)	448	456	432	

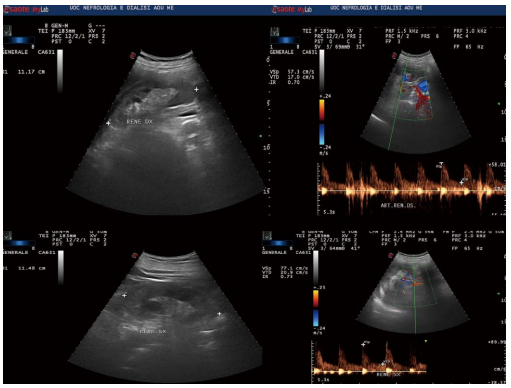

Figure 1 Renal ultrasound.

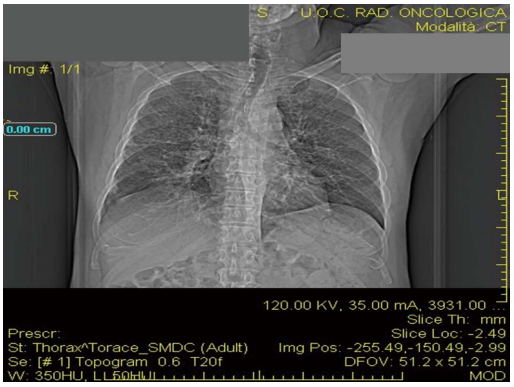

Figure 2 Chest X-ray.

nodes, skin and eyes, whilst kidney is less usually affected[5]. The disease typically occurs in young and middle-aged black race women. Clinical presentation is different depending on the race. Pulmonary involvement covers 90% of patients, of which 61%-63% are women[6]. Among black subjects besides lungs, eyes and skin are often affected. Curiously, calcium metabolism abnormalities are more frequent and more severe in caucasic men with renal interstitial granulomatosis[6] The incidence of renal involvement remains unclear: in autoptic studies, granulomatous infiltrate is found in up to 23% of kidneys, and even up to 48% in small series of biopsies[7]. In our case, the characteristic lesions were related to the presence of multiple granulomas consisting of epithelioid and multinucleated giant cells, delimited by a thin fibrous reaction, interstitial oedema with a mild to moderate lymphocyte and monocyte infiltration and tubular atrophy, involving about 15%-20% of the renal tissue. Our patient was initially classified as a case of CKD secondary to diabetic nephropathy in a context of poor glycemic control. However, first of all the absence of other microangiopathic alterations such as diabetic retinopathy and secondly the presence of hypercalcemia with hypoparathyroidism in a patient with CKD needed to be further explored[8]. On the second admission, the persistence of hypercalcemia despite the suspension of vitamin D therapy, weight loss, anorexia and asthenia suggested for a tubular damage. Differential diagnosis for hypercalcemia at that time was betweeen malignant disease and systemic sarcoidosis. In particular, hypercalcemia characterizes paraneoplastic syndromes[9-12] secondary to bronchial carcinoma, small cell lung cancer, breast, gastric and uterus cancer, myeloma, lymphoma. It could be the first clinical manifestation of a parathyroid adenoma; in an autonomous and independent way, neoplastic tissue produces parathyroid-related hormone (PTHrP), which stimulates bone turnover and calcium reabsorption in the kidney, thus increasing serum calcium values[13]. However, in these situations not only calcium but also PTH is elevated, while in our patient PTH was suppressed. We then considered other causes of hypercalcemia, especially an inappropriate intake or increased activation of vitamin D. In the first hypothesis, suspension of oral vitamin D should have led to a normalization of calcium values, but it was not our case. Moreover, the subject was also suffering from CKD, a condition characterized by reduced production of 1-α-hydroxylase and consequently low concentrations of 1,25 dihydroxycholecalciferol[8] due to progressive replacement of renal parenchyma with fibrotic tissue. We therefore concluded that hypercalcemia was sustained by an abnormal production of 1-α-hydroxylase from granulocyte monocyte-macrophage cells, secondary to a chronic granulomatous disease without any feed-back mechanism of control: this may lead to calcium retention, hypercalciuria and nephrocalcinosis[14]. These situations may be consistent with a diagnosis of sarcoidosis.

Our patient was wrongly categorized as chronic renal failure secondary to diabetes mellitus, whilst it was a case of acute renal injury secondary to sarcoidosis, the identification and treatment of which led to the improvement of renal function. The

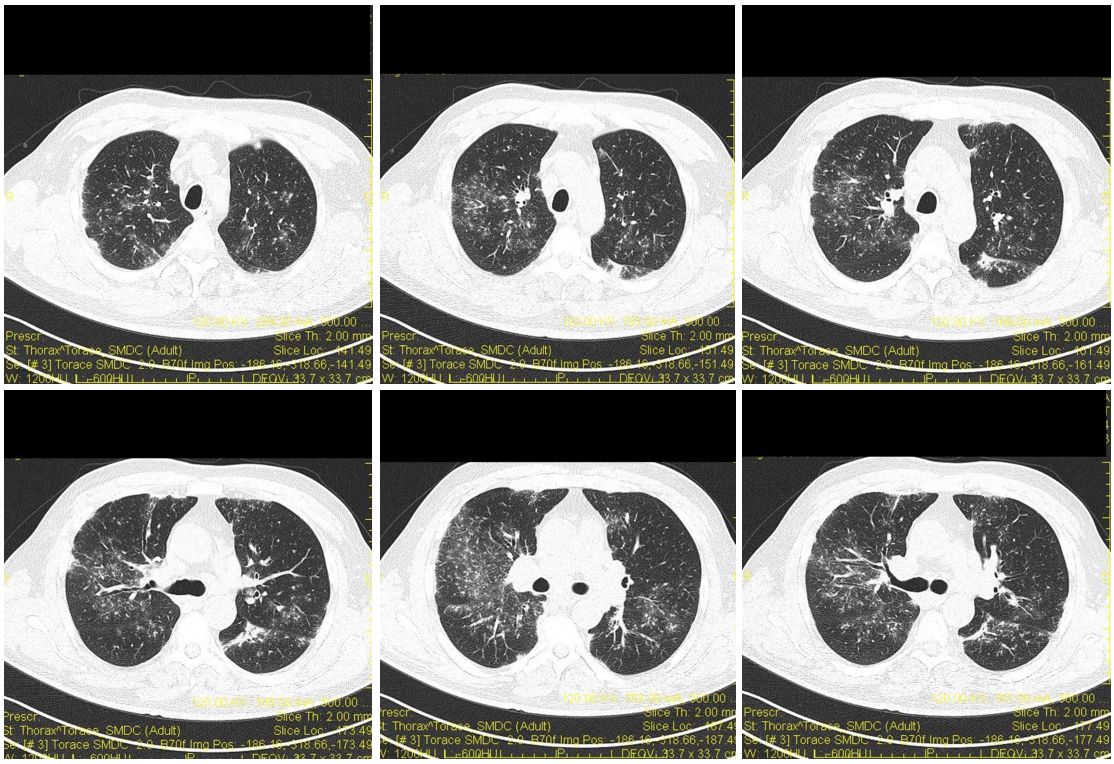

Figure 3 Pulmonary computed tomography.

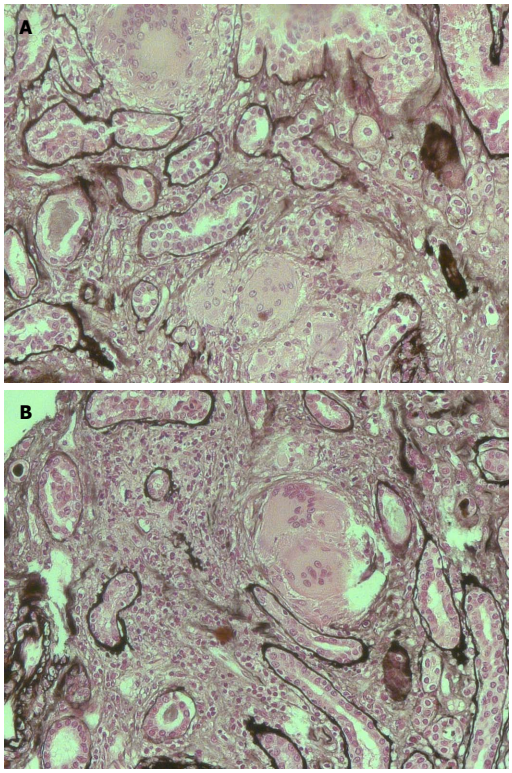

Figure 4 Histological findings. A: Presence of interstitial multiple granulomas composed of epithelioid cells and moderate lymphocyte and monocyte infiltration (silver stain); B: Multinucleated giant cells (silver stain).

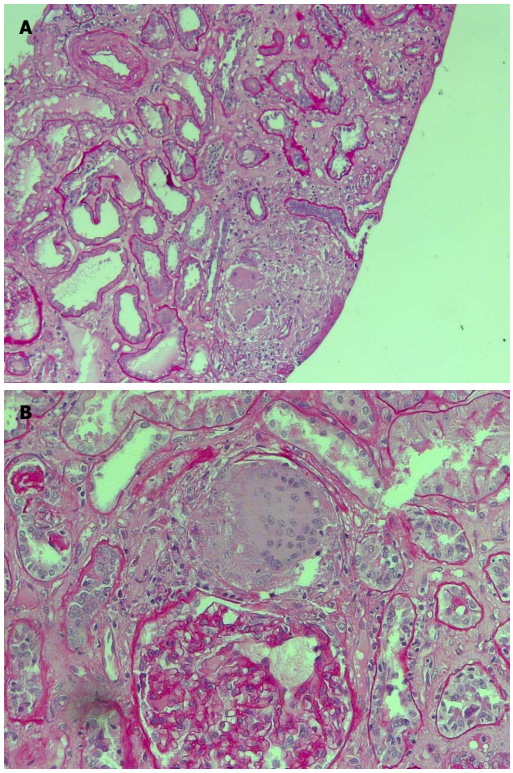

Figure 5 Histological findings. A: Moderate lymphocyte and monocyte infiltration; B: Multinucleated giant cells [periodic acid schiff (PAS) stain].

investigation of all possible causes of "hypercalcemia" allowed to diagnose systemic sarcoidosis confirmed by the evidence of granulomas in renal biopsy. A comprehensive evaluation should be performed in all patients with suspected sarcoidosis, including history, physical examination, chest radiography, pulmonary

function tests, peripheral blood counts, complete serum chemistries. Pulmonary evaluation starts with function tests (such as spirometry, diffusing capacity, *etc.*) reveal a restrictive defect with reduced gas exchange and reduced functional status; chest X-ray. Bilateral hilar lymphadenopathy is the most typical sign at chest radiography. Computed tomography (CT) is essentially face for atypical manifestations of the disease,in order to avoid confusion with differential diagnoses and, sometimes, comorbidities. CT typically shows diffuse pulmonary perilymphatic micronodules, with a perilobular and fissural distribution and upper and posterior predominance, even when an atypical CT pattern is predominant. CT allows deciphering pulmonary lesions in cases of pulmonary fibrosis, pulmonary hypertension, and airflow limitation[15]. Gallium-67-citrate scintigraphy is another imaging method that can be employed both in diagnosis, staging and in the follow-up. Use of fluorine-18-fluorodeoxyglucose positron emission/computed tomography (18F-FDG-PET/CT) seems to have higher diagnostic accuracy with respect to Gallium-67-citrate but the high fee of the method limits its employ. Diagnosis relies on compatible clinical and radiological presentation, evidence of noncaseating granulomas and exclusion of other diseases with a similar presentation or histology. However, there are important variations in diagnostic work-up due to diverse expressions of sarcoidosis and differences in clinical practices among physicians. In our case for example, Gallium-67-citrate scintigraphy, 18F-FDG-PET/CT and serum angiotensin-converting enzyme (ACE) dosage were not made as the result of histological examinations performed on renal tissue was diagnostics. On the other hand, rapid worsening of renal function has been made it necessary to perform biopsy.

In conclusion, we can assume that clinical, laboratory and instrumental data should not be ignored because they are all pieces of a greater puzzle that once rebuilt allows to reach the correct diagnosis and give the right treatment. Like in our case, in a diabetic patient, the presence of hypercalcemia with CKD and the absence of other microangiopathic alterations needs to be further explored because the diagnosis of cancer, paraneoplastic syndrome or parathyroid adenoma has to be excluded as the cause of hypercalcemia.

COMMENTS

Case characteristics

A caucasian 59-year-old man with non-insulin diabetes mellitus and poor glycemic control, increased serum creatinine level and hypercalcemia.

Clinical diagnosis

The patient was initially classified as a case of chronic kidney disease (CKD) secondary to diabetic nephropathy; however, the absence of other microangiopathic alterations in a diabetic patient with CKD needed to be further explored.

Differential diagnosis

Differential diagnosis for hypercalcemia was with cancer, paraneoplastic syndromes and parathyroid adenoma.

Laboratory diagnosis

Laboratory data showed serum creatinine 2.9 mg/dL, calcium 10.4 mg/dL, proteinuria 156 mg/24 h, cCrCl 30.69 mL/min per 1.73 m^2, PTH 5.5 pg/mL, 25D 21.73 ng/mL.

Imaging diagnosis

Ultrasound showed normal kidneys. Pulmonary computed tomography revealed multiple ground-glass like lumps involving both lungs.

Pathological diagnosis

Histological examination showed the presence of interstitial multiple granulomas composed of epithelioid and multinucleated giant cells delimited by a thin fibrous reaction. Oedema was present in the interstitium with mild to moderate lymphocyte and monocyte infiltration and tubular atrophy affecting about 15%-20% of the core.

Treatment

The patient was treated with oral steroids at the dose of 1 mg/kg per day that was progressively tapered. Normalization in calcium values and improvement of renal function were observed after the first month of corticosteroid therapy.

Related reports

Sarcoidosis most commonly involves lungs, lymph nodes, skin and eyes, whilst kidney is less frequently affected. The incidence of renal involvement remains unclear: in autoptic studies, granulomatous infiltrate is found in up to 23% of kidneys, and even up to 48% in small series of biopsies.

Experiences and lessons

The patient was wrongly categorized as chronic renal failure secondary to diabetes mellitus, whilst it was an interesting case of acute kidney injury secondary to sarcoidosis, the identification and treatment of which led to the improvement of renal function.

Peer-review

It is a good article.

REFERENCES

1 **Hunninghake GW**, Costabel U, Ando M, Baughman R, Cordier JF, du Bois R, Eklund A, Kitaichi M, Lynch J, Rizzato G, Rose C, Selroos O, Semenzato G, Sharma OP. ATS/ERS/WASOG statement on sarcoidosis. American Thoracic Society/European Respiratory Society/World Association of Sarcoidosis and other Granulomatous Disorders. *Sarcoidosis Vasc Diffuse Lung Dis* 1999; **16**: 149-173 [PMID: 10560120]

2 **Newman LS**, Rose CS, Maier LA. Sarcoidosis. *N Engl J Med* 1997; **336**: 1224-1234 [PMID: 9110911]

3 **Iannuzzi MC**, Fontana JR. Sarcoidosis: clinical presentation, immunopathogenesis, and therapeutics. *JAMA* 2011; **305**: 391-399 [PMID: 21266686 DOI: 10.1001/jama.2011.10]

4 **Crowley R**, Gittoes N. How to approach hypercalcaemia. *Clin Med* 2013; **13**: 287-290 [PMID: 23760705 DOI: 10.7861/clinmedicine.13-3-287]

5 **Mayock RL**, Bertrand P, Morrison CE, Scott JH. Manifestations of sarcoidosis. Analysis of 145 patients, with a review of nine series selected from the literature. *Am J Med* 1963; **35**: 67-89 [PMID: 14046006]

6 **Baughman RP**, Teirstein AS, Judson MA, Rossman MD, Yeager H, Bresnitz EA, DePalo L, Hunninghake G, Iannuzzi MC, Johns CJ, McLennan G, Moller DR, Newman LS, Rabin DL, Rose C, Rybicki B, Weinberger SE, Terrin ML, Knatterud GL, Cherniak R. Clinical characteristics of patients in a case control study of sarcoidosis. *Am J Respir Crit Care Med* 2001; **164**: 1885-1889 [PMID: 11734441]

7 **Berliner AR**, Haas M, Choi MJ. Sarcoidosis: the nephrologist's perspective. *Am J Kidney Dis* 2006; **48**: 856-870 [PMID: 17060009]

8 **Levin A**, Bakris GL, Molitch M, Smulders M, Tian J, Williams LA, Andress DL. Prevalence of abnormal serum vitamin D, PTH, calcium, and phosphorus in patients with chronic kidney disease: results of the study to evaluate early kidney disease. *Kidney Int* 2007; **71**: 31-38 [PMID: 17091124]

9 **Nielsen PK**, Rasmussen AK, Feldt-Rasmussen U, Brandt M, Christensen L, Olgaard K. Ectopic production of intact parathyroid hormone by a squamous cell lung carcinoma in vivo and in vitro. *J Clin Endocrinol Metab* 1996; **81**: 3793-3796 [PMID: 8855839]

10 **Nussbaum SR**, Gaz RD, Arnold A. Hypercalcemia and ectopic secretion of parathyroid hormone by an ovarian carcinoma with rearrangement of the gene for parathyroid hormone. *N Engl J*

Med 1990; **323**: 1324-1328 [PMID: 2215618]
11 **Sargent JT**, Smith OP. Haematological emergencies managing hypercalcaemia in adults and children with haematological disorders. *Br J Haematol* 2010; **149**: 465-477 [PMID: 20377591 DOI: 10.1111/j.1365-2141.2010.08173.x]
12 **Stewart AF**. Clinical practice. Hypercalcemia associated with cancer. *N Engl J Med* 2005; **352**: 373-379 [PMID: 15673803]
13 **Rosner MH**, Dalkin AC. Onco-nephrology: the pathophysiology and treatment of malignancy-associated hypercalcemia. *Clin J Am Soc Nephrol* 2012; **7**: 1722-1729 [PMID: 22879438 DOI: 10.2215/CJN.02470312]
14 **Reichel H**, Koeffler HP, Barbers R, Norman AW. Regulation of 1,25-dihydroxyvitamin D3 production by cultured alveolar macrophages from normal human donors and from patients with pulmonary sarcoidosis. *J Clin Endocrinol Metab* 1987; **65**: 1201-1209 [PMID: 3119653]
15 **Valeyre D**, Bernaudin JF, Uzunhan Y, Kambouchner M, Brillet PY, Soussan M, Nunes H. Clinical presentation of sarcoidosis and diagnostic work-up. *Semin Respir Crit Care Med* 2014; **35**: 336-351 [PMID: 25007086 DOI: 10.1055/s-0034-1381229]

P- Reviewer: Prakash J, Turgut A **S- Editor**: Ji FF **L- Editor**: A
E- Editor: Yan JL

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