The quest for a better understanding of chronic kidney disease complications: an update on uremic toxins

Em busca de uma melhor compreensão da doença renal crônica: uma atualização em toxinas urêmicas

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Abstract

Chronic kidney disease is characterized by a progressive reduction of glomerular filtration rate and/or the appearance of proteinuria, and subsequently the progressive retention of organic waste compounds called uremic toxins (UT). Over the last decades, a large number of such compounds have been identified and their effects on organs and tissues, especially the cardiovascular system, has been demonstrated. In this review, we present the current classification of UT, as proposed by the EUTox Group, and the effects of some of the probably most important UTs, such as phosphate, FGF-23, PTH, AGEs, indoxyl sulfate and para-cresyl sulfate. We provide an overview on therapeutic approaches aimed to increase their extracorporeal removal via convective and/or adsorptive strategies and to lower their intestinal production/absorption via dietetic and pharmacological interventions. The recognition that multiple toxins contribute to the uremia supports the need for new therapeutic targets, with a potentially positive impact on CKD progression and survival.

Keywords: cardiovascular diseases; dialysis; kidney failure, chronic; uremia.

Resumo

A doença renal crônica (DRC) caracteriza-se pela redução progressiva da filtração glomerular e/ou presença de proteinúria, e subsequente retenção progressiva de compostos orgânicos, denominados toxinas urêmicas. Nas últimas décadas, um grande número destes compostos foi identificado, assim como seus efeitos adversos no organismo, sobretudo no sistema cardiovascular. Nesta revisão, apresentamos a classificação das toxinas urêmicas, proposta pelo grupo europeu de estudo em toxinas urêmicas (EUTox), e discutiremos os efeitos de algumas das principais toxinas, como ADMA, fosfato, FGF-23, PTH, AGEs, indoxil sulfato e para-cresil sulfato. Além disso, abordaremos as principais estratégias terapêuticas para aumentar a remoção das toxinas urêmicas por métodos convectivos e/ou adsortivos; e para diminuir a produção e absorção intestinal dessas toxinas por meio de intervenções dietéticas e farmacológicas, respectivamente. A compreensão de que múltiplas toxinas contribuem para a uremia expõe a necessidade de novos alvos-terapêuticos, com potencial impacto positivo na progressão da DRC e na sobrevida dos pacientes.

Palavras-chave: diálise; doenças cardiovasculares; falência renal crônica; uremia.

Introduction

In the last two decades, renewed interest has emerged about the uremic syndrome and its negative impact on chronic kidney disease (CKD) patient outcomes. The syndrome is primarily caused by the progressive decline in kidney function that leads to an accumulation of organic waste products.[1] These waste products, not all identified as yet, are called "uremic toxins" or "uremic retention solutes". Under normal conditions, they are excreted by the kidneys. Thus, their concentrations increase progressively with the progression of CKD, interacting negatively with various biological functions. The clinical spectrum of this pathophysiological phenomenon is generally known as uremia (literally, "urine in the blood") and at present more frequently designed as the uremic state.[2,3]

The first publication in this field is from 1877,[4] and little was known regarding the nature of uremic toxins before the

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introduction of hemodialysis (HD) into clinical practice in the 1960s. The concept of uremic toxins has thus been developed at that time, and their identification and biological activities were actively investigated subsequently by a small number of research groups (Man and Funck-Brentano in France, Bergström in Sweden, etc). These research activities led to the "middle molecule" hypothesis.[5,6] However, studies in this field ceased subsequently because no convincing demonstration of their role in the uremic syndrome could be made at that time. The topic was resuscitated later on, in the early 1990ies, by the nephrology group in Gent, Belgium (Ringoir, Vanholder). They were able to provide new, scientifically valid evidence in favor of the biological activity of several uremic toxins. Finally, in 1999 Vanholder's group, together with other researchers in Europe, launched the European Uremic Toxins (EUTox) Work Group, which contributed substantially to the acceptance of the concept and the demonstration of the role of uremic toxins. Subsequently, the idea disseminated worldwide, as reflected by a remarkable increase in published reports devoted to this field.

According to EUTox, a compound can be considered as a uremic toxin if it fits a postulate, similar to Koch's postulate, as modified by Massry *et al.* in 1977 (Table 1).[3,7] At present, there are 152 solutes listed in the EUTox database (http://eutoxdb.odeesoft.com/index.php) and certainly an increase in this number in the following years can be expected.

Table 1 Requirements for a given compound be considered as a uremic toxin[4]

Chemically identified and accurately measured;
Total body/plasma levels should be higher than in non-uremic subjects;
High concentrations should be related whith specific dysfunctions/symptoms, which disappear when concentrations are reduced;
Biological activity of this compound should be proven in *ex vivo, in vivo* or *in vitro* studies, and finally, experimental concentrations of this molecule in those studies should match those found in body fluids or tissue from uremic patients.

The proper identification of new compounds that fulfill the criteria of uremic toxins has at least three main implications: firstly, to be able to explore the unknown pathophysiologic mechanisms of the uremic syndrome; secondly, to use a specific uremic toxin as a biomarker of a specific pathophysiologic process in CKD and thirdly, to explore clinical interventions directed to modulate the total body/plasma levels of uremic toxins.

Uremic toxins can be classified according to their physico-chemical characteristics and removal by dialysis into the following 3 classes:

I. Small water-soluble: compounds with a maximum molecular weight (MW) of 500 Daltons (Da). The main molecules in this group include urea, creatinine and guanidines, which are easily removed by dialysis. They do not necessarily have toxic effects.

II. Middle molecules: Compounds of moderately elevated MW (> 500 Da), with β2-microglobulin and leptin as prototypes. They can only be removed by dialysis membranes with pores large enough to allow their passage. Many of these compounds are peptides. They affect a large number of organs and systems.

III. Protein-bound compounds: They are generally of low MW. The prototypes of this group are phenols and indoles. They exert a variety of toxic effects and are difficult to remove by dialysis.

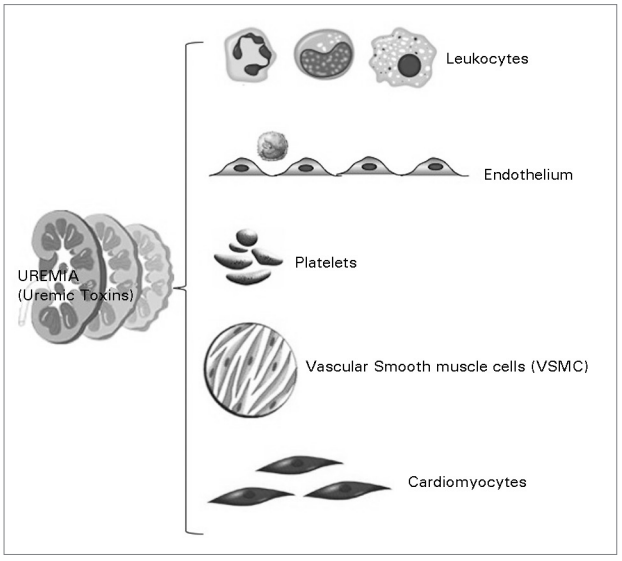
A large number of uremic toxins have deleterious effects on various organs and tissues in the body, mainly the cardiovascular system (Figure 1). The pathophysiological mechanisms involved are complex and far from being completely understood. They include reactive oxidative stress, inflammation, protein glycation and cellular transdifferentiation. In this review, we present the main toxin prototype groups, based on physico-chemical characteristics, clinical effects, and current or potential interventions aimed to modulate their concentration through extracorporeal removal methods and/or pharmacological approaches capable of opposing their toxic effects.

Small water-soluble compounds

Guanidines

An important group of small water-soluble compounds consists of guanidine compounds, metabolites of L-arginine that have long been known for their neurotoxic effects.[8] There are three types of methylated arginine residues: monomethyl arginine (MMA), symmetric dimethylarginine (SDMA) and asymmetric dimethylarginine (ADMA), the latter being the most abundant one. Several studies have shown an increase in serum ADMA levels in CKD, which may be particularly relevant to the presence of

Figure 1. Main targets of uremic toxins in the cardiovascular system.



nephrotic proteinuria, although its correlation with glomerular filtration rate (GFR) has been found to be poor.[9,10] Initially, it was assumed that this increase was the result of lower renal clearance. Later studies, however, suggested that the increase was due to both augmented synthesis and reduced catabolism of these compounds.[11] Of note, even though guanidines and urea are structurally similar, the distribution volume of the former is significantly greater, which results in a decrease in removal efficiency by dialysis procedures.[12]

ADMA has long been associated with endothelial dysfunction. In 1992, Vallence *et al.*[13] identified MMA and ADMA as endogenous inhibitors of nitric oxide synthase (eNOS). However, the intracellular concentration of MMA is very small, whereas ADMA is the most abundant eNOS inhibitor. ADMA has been further linked to cardiovascular disease (CVD) and mortality in both the general and CKD population ever since.[14,15]

SDMA, the structural counterpart of ADMA, was considered inert until recently. However, it is currently recognized as a pro-inflammatory and a pro-oxidant agent.[16,17] Unlike ADMA, SDMA was demonstrated to be capable of increasing TNF-α and interleukine-6 (IL-6) expression, and enhancing NFkB activation, in human monocytic cell line THP-1. In line with this *in vitro* data, SDMA has been associated with inflammatory markers, including TNF-α and IL-6, in pre-dialysis patients at different stages of CKD.[17]

Uric acid

Uric acid (MW: 168 Da) is considered a uremic toxin since a considerable body of evidence suggests that supraphysiologic uric acid concentrations have deleterious effects on the kidney and on the cardiovascular system. In CKD, factors like reduced GFR, increased renal vascular resistance and co-existent insulin resistance as well as the use of diuretics can lead to hyperuricemia, defined as the accumulation of serum uric acid beyond its solubility point in water (6.8 mg/dL).[18] Other factors such as high purine/protein diet, alcohol consumption and high cell turnover can also contribute to increase its serum concentration.

Classically, hyperuricemia could lead to hyperuricosuria and deposition of uric acid crystals in the kidney, increasing the risk of nephrolithiasis, renal inflammation and decrease of GFR by intraluminal obstruction and increased concentration of urate anion in renal tissue.[19,20] On the other hand, non-crystal dependent effects of uric acid have been proposed, but this remains a matter of debate. Though urate anion, the predominant form of uric acid under physiologic pH, has long been considered a powerful antioxidant at physiologic concentrations, experimental data have shown that at higher concentrations this anion can lead to endothelial dysfunction, increased systemic cytokine production, activation of the renin-angiotensin-aldosterone system and last, not least, glomerular lesions.[21]

In the clinical setting, observational studies have reported conflicting results. In the Cardiovascular Health Study, no association was observed between serum uric acid level and the incidence of CKD.[22] Similarly, in the patient cohort of the MDRD Study, uric acid was not found to be an independent risk factor for CKD progression.[23] In contrast, Hsu *et al.* evaluated a cohort of 177,570 participants followed over 25-years and found that increased serum uric acid levels were independently associated with an increased risk of end-stage kidney disease (ESKD).[24] A hypothesis to explain these apparent inconsistencies is that uric acid clearance is impaired in CKD, which in turn can act as a confounding factor.[21] Taking into account interventional studies aimed to decrease serum uric acid levels in CKD, the results, although encouraging,[25] are not strong enough to systematically recommend allopurinol as a therapy for halting or decreasing CKD progression.

Inorganic phosphate (Pi)

The regulatory mechanisms responsible for Pi homeostasis, such as $1,25(OH)_2$-vitamin D, parathyroid hormone (PTH) and fibroblast growth factor-23 (FGF23), together with Klotho,[26] are disrupted in the CKD setting. Pi overload may occur early on in the course of CKD, while hyperphosphatemia appears only when GFR falls below 30 ml/min/1.73 m^2 of body surface.[27] When CKD progresses and dialysis treatment is required, the positive balance of Pi becomes more pronounced due to (i) loss of kidney function and (ii) insufficient removal by conventional hemodialysis (HD) or peritoneal dialysis (PD) (Table 2). A detailed discussion of Pi homeostasis and hyperphosphatemia treatment can be found elsewhere.[26,28]

Table 2 Estimated weekly removal of phosphate by dialytic methods

Dialytic methods	Duration/ frequency	Phosphate removal (mg/wk)
Conventional HD*	4h; 3x/wk	2.356 ± 864
Short daily HD*	2-3h; 6x/wk	2.452 ± 720
Nocturnal HD*	6-8h; 6x/wk	8.000 ± 2.800
Hemodiafiltration	4h; 3x/wk	3.570 ± 270
Peritoneal dialysis		
APD		2.739 ± 1.042
CAPD		2.790 ± 1.022

HD: Hemodialysis; APD: Automated peritoneal dialysis; CAPD: Continuous ambulatory peritoneal dialysis. * Using high-flux membranes.

Hyperphosphatemia has been classically linked to the pathogenesis of secondary hyperparathyroidism (sHPT).[29] Furthermore, since the end of the 1990's, the role of Pi in uremia-related cardiovascular complications and mortality has gained growing interest. Several observational studies showed an association of hyperphosphatemia with overall and cardiovascular mortality, both in dialysis and pre-dialysis patients.[30,31] Vascular calcification (VC) has been proposed as one of the possible mechanisms linking hyperphosphatemia to mortality. *In vitro* studies have shown that Pi enters vascular smooth muscle cells (VSMCs) through sodium-phosphate co-transporter type III (PiT-1), activate Cbfa-1/Runx-2 genes and thereby promote VSMC transdifferentiation into osteoblast/osteochondrocyte-like cells.[32] Even though animal studies have demonstrated very clearly that lowering serum Pi can decrease the progression of VC,[33,34] this remains yet to be confirmed by appropriate clinical trials in patients with CKD.[35,36]

More recently, the role of Pi as a potential cardiovascular toxin has received further support. Several observational studies have suggested that high Pi levels, even within the normal range, may be associated with an increased risk of incident CVD,[37] left ventricular hypertrophy (LVH) and increased risk of heart failure,[38] coronary disease,[39] and higher mortality[40] in the general population.

Experimental studies have shed some light on the mechanisms by which Pi acts as a cardiovascular toxin, above and beyond the promotion of VC. Apolipoprotein E knockout mice fed an atherogenic diet with high (1.6%) Pi content had significantly more atheroma at the site of the aortic sinus than mice fed a low (0.2%) or standard (0.6%) Pi diet, while lipid profile and blood pressure were not affected.[41] Furthermore, it has been demonstrated in two different mouse models of CKD that lowering serum Pi levels with sevelamer, a phosphate-binding agent, improved VC and atherosclerotic lesions,[34] aortic stiffness, diastolic dysfunction and prevented ventricular hypertrophy.[42] *In vivo* studies have demonstrated that Pi overload may lead to endothelial dysfunction, by inducing apoptosis,[43] impaired acethylcholine-induced vasodilation,[44,45] inhibition of nitric oxide production[44] and downregulation of annexin II.[46] Interestingly, lowering serum Pi levels (at least in animal models of CKD) by either dietary Pi restriction or sevelamer administration ameliorates endothelial dysfunction.[43,47]

A major limitation in the interpretation of Pi effects *in vivo* is the difficulty to know whether they are the result of a direct action of Pi or rather due to indirect mechanisms, e.g. via an increase in serum PTH levels. In two studies using a rat model of CKD, in which the animals underwent parathyroidectomy and continuous controlled infusion of PTH, the authors were able to demonstrate a direct Pi effect on the heart, inducing myocardial hypertrophy, cardiomyocyte hyperplasia and interstitial fibrosis, and vessels in the absence of changes in serum PTH concentration.[48-50]

Recent clinical evidence attributing Pi an important role as a culprit in CVD in both CKD and general population has brought this compound to the forefront of uremic toxins. It is also worth mentioning that the amount of Pi in Western diet, especially due to its use as a food additive, is probably much higher than the one recommended by health authorities, resulting in the excessive exposure to a continuous

Pi overload. Therefore, given the increased CVD and mortality risk associated with hyperphosphatemia, the proposition has been made to label food products with high Pi content in the interest of public health, similarly to what is currently recommended for food NaCl content.

Middle molecules

Fibroblast growth factor-23

FGF23 was described more than 10 years ago by two independent groups.[51,52] This hormone, secreted by osteocytes and osteoblasts, is a protein constituted by 251-amino-acids (MW: 30,000 Da). Its major function is the control of mineral metabolism, particularly serum Pi and calcitriol levels, through inhibition of renal tubular Pi reabsorption and 1α-hydroxylase activity. FGF23 also acts on the parathyroid gland inhibiting PTH secretion. All these actions are mediated by FGF23 binding to FGF receptors (FGFR) and its co-receptor Klotho, which greatly increases the binding affinity of FGF23 for FGFR.[53,54]

The serum FGF23 level increases early in the course of CKD, probably due to a state of primary FGF23 excess, resulting from a combination of increased production and decreased degradation.[55] As GFR declines, FGF23 increases and can reach levels > 1,000-fold above normal in patients with CKD stage 5D. Furthermore, residual clearance by the kidney and removal by dialysis do not appear to modify serum FGF23 to a significant extent.[56]

Numerous reports showed a positive association between elevated FGF23 levels and adverse clinical outcomes, such as CKD progression,[57,58] LVH,[59] VC[60] and mortality in pre-dialysis and dialysis CKD populations.[61,62] Considering the above effects of FGF23 on biological functions, one might expect to see evidence in support of a causal relationship in this respect. However, available experimental findings do not allow a clear conclusion. For example, complete neutralization of FGF23 action by a specific antibody has been shown to be harmful since it aggravated hyperphosphatemia, VC, and mortality in a rat model of CKD and mineral bone disorder (MBD).[63] This observation suggests that FGF23, like PTH, exerts beneficial effects in early stages of CKD but that its excessive rise with the progression of CKD to ESKD is a maladaptation syndrome with highly deleterious consequences.

Regarding LVH, convincing experimental evidence has recently been provided by Faul *et al*. in favor of a direct noxious effect of FGF23.[64] The authors showed that the intramyocardial or intravenous injection of FGF23 in wild-type mice induced LVH, independent of Klotho which is not expressed in the myocardium.[64] In addition, the authors reported that elevated FGF23 levels were independently associated with LVH in a large, racially diverse CKD cohort, in line with findings by others in the general population.[65] However, not all authors have been able to find an association of high FGF23 levels with vascular calcification[66] or mortality[59,67] Apparent inconsistencies can be explained by small sample size, different approaches to adjust for confounding factors, imaging of different arterial beds, lack of prospective data, and finally, different FGF23 mechanisms of toxicity.

Therefore, it is not yet clear if FGF23 is only a surrogate marker of CKD-MBD, CKD progression and cardiovascular disease or whether it is also an active player and hence a potential therapeutic target. For a more detailed review of FGF23 the reader should refer to recent comprehensive reviews.[68,69]

Leptin

The hormone leptin is a 167-amino-acid secreted protein (MW: 16,000 Da), expressed mainly in adipose tissue. It was originally proposed to be an antiobesity factor. However, the majority of obese healthy individuals have elevated serum leptin levels. Therefore, the hypothesis of leptin resistance in human obesity emerged.[70] The classical actions of leptin include the control of feeding behavior, energy balance, fertility and immune function. Recently, other actions of leptin have been described, such as its influence on bone mass and the cardiovascular system.[71] At the cellular level, leptin stimulates alkaline phosphatase activity, as well as the proliferation, migration and calcification of VSMCs.[72,73]

Although clinical studies in the general population have shown a link between leptin and CVD,[74,75] leptin's role in CKD seems to be more complex. A group of authors have suggested that dialysis patients with high, rather that low, serum leptin levels have better outcomes,[76] However, there was no association between serum leptin and body mass index (BMI). Clearly, the relation between serum leptin and BMI is complex in ESKD, and other group did not find any survival advantage in obese HD patients as compared to lean HD patients.[77]

Apart from the unsolved issue of the role of leptin in clinical outcomes of CKD patients, leptin appears to be related to the metabolic syndrome. In a clinical study of 142 patients with CKD stages 2-5D, followed for a minimum of 20 months, plasma leptin was an

independent predictor of metabolic syndrome but not of clinical outcomes. Interestingly, PTH was an independent predictor of plasma leptin levels.[78] Taking into account these two findings, one could speculate that leptin is related to factors that exert a more close impact on the clinical outcomes of CKD patients, such as mineral disturbances and metabolic factors. This hypothesis remains to be proven in future clinical long-term studies.

Parathyroid hormone

Secondary hyperparathyroidism, characterized by an increase in PTH (MW: 9.4 kDa) synthesis and secretion and by parathyroid gland hyperplasia, is a common complication of CKD. Elevated PTH levels are generally found since the early stages of CKD (GFR > 60 ml/min/1.73 m^2), in any case much sooner than hyperphosphatemia.[27] As one of the most important regulators of bone metabolism, PTH in excess induces significant changes in bone structure and function, leading to the development of high turnover bone disease which, in association with CKD, is called *osteitis fibrosa*.[29,79] It is characterized by increased bone fragility, which may explain, at least in part, the association between sHPT and increased fracture risk.[80] In addition, sHPT may cause bone marrow fibrosis and impair erythropoiesis. It has been suggested that very high PTH levels contribute to the polyneuropathy, glucose intolerance, dyslipidemia and inflammation of the uremic state.[81]

It is important to recognize that toxic PTH levels may lead to deleterious effects on many other organs and tissues due to the ubiquitous expression of its main receptor, PTH1R, including the cardiovascular system. Observational studies have found associations between high PTH levels and CVD in the setting of CKD, such as VC,[82] disturbed left ventricular function[83] and mortality.[84] Observational studies have pointed toward an association between PTH and cardiovascular mortality in the general population as well.[85] CKD patients with severe sHPT often develop severe VC, which are particularly located in the arterial media such as the ones observed in digitial arteries of the hands and the feet, and which can entirely regress after surgical parathyroidectomy.[86]

The effect of PTH on the CV system is an issue under continuous investigation. Experimental studies using a rat model of CKD (5/6 nephrectomy) in which the animals underwent parathyroidectomy, the continuous infusion of supraphysiological rates of synthetic PTH was associated with the development of extensive VC - independently of serum Pi levels or the presence of uremia.[48] In another study using the same CKD rat model, higher PTH levels were associated with myocardial hypertrophy and fibrosis along with higher myocardial expression of inflammation markers and oxidative stress.[49] Furthermore, studies have reported the interplay between PTH on the one hand and aldosterone and norepinephrine release on the other, suggesting additional pathophysiologic pathways by which sHPT may lead to cardiovascular damage.[87,88]

Finally, there is increasing evidence that circulating fragments of PTH, which are elevated in the uremic state, are hormonally active, exerting partially opposing effects to those of intact PTH. Thus, it has been suggested that 7-84 PTH fragments may be involved in the skeletal resistance to PTH observed in CKD.[89]

There is an intricate relationship between PTH and other factors involved in the disturbances of mineral metabolism in CKD, which may fog the importance of PTH per se as a risk factor for uremia-related complications. The inconclusive, non-definitive results of the recent EVOLVE trial have further contributed to this uncertainty, since in intent-to-treat analysis there was no significant advantage of cinacalcet treatment over best presently available standard treatment in the combined primary endpoint (cardiovascular events plus death) despite a marked decrease in serum PTH.[90] However, when performing prespecified corrections of major confounders such as age and study drug discontinuation the better control of hyperparathyroidism was associated with a nominally significant superiority in hard outcomes. Of note, numerous previous clinical and experimental studies support the hypothesis that PTH acts as a systemic uremic toxin, with direct and indirect effects on a variety of tissues and organs, and severe sHPT is a major threat to CKD patient outcomes. Therefore, PTH remains an important therapeutic target to avoid bone and cardiovascular complications in such patients.

Advanced glycation end products (AGEs) and advanced oxidized protein products (AOPPs)

AGEs are a heterogeneous group of molecules formed by non-enzymatic glycosylation reactions with sugars, lipids and nucleic acids. Humans are exposed to two

main sources of AGEs. The exogenous forms come from diet whereas the endogenous ones are formed in the body. AGE transformation of nutrients occurs when foods are processed at high temperature. In contrast, endogenous transformation results from exposure to high glucose levels such as in diabetic patients, from aging, and from uremia.[91,92] AGEs result in a first step from non-enzymatic glycation of proteins by aldehyde and ketones to form a Schiff base. After rearrangement of the these structures, the intermediary products are formed (Amadori products), and finally, AGEs in a second step.[93] There are more than 20 AGE compounds, including 1,2-dicarbonyl precursor compounds glyoxal, methylglyoxal, and the end products, N-carboxymethyl-lysine, pentosidine, and hydroimidazolone as the best characterized compounds, which serve as markers of AGE accumulation in a range of tissues.[94] AGEs main pathway of elimination from the body is through the urine, or through dialysis in case of renal function replacement.[95] Patients under PD are more susceptible than HD patients to systemic and local formation of AGEs, not only as a result of the uremic state but also of the constant exposure of the peritoneum to high levels of glucose and glucose degradation products generated during sterilization of dialysis fluid by heat.[95] Moreover, AGEs accumulate progressively in the peritoneal mesothelial layer with increasing dialysate dwell time.[96] Another cause of increased AGE formation in the uremic state is oxidative stress, generated by an imbalance between pro-oxidant forces (such as an increase in the ratio of oxidized glutathione to reduced glutathione) and anti-oxidant defense system (such as reduced superoxide dismutase/peroxidase activity).

AGEs exert several potentially deleterious effects in the body. In the cardiovascular system, their accumulation contributes to myocardial changes, endothelial dysfunction, arterial stiffness, and atherosclerotic plaque formation. When these molecules bind to collagen and elastin, they accumulate in the matrix of blood vessels in a non functional and disorderly way, changing endothelial vasomotor tone modulation, platelet adhesion, and cell proliferation.[97] AGEs exert their actions in the vascular system by binding to a specific receptor, called RAGE. Activation of RAGE induces an inflammatory response, leading to the effects described above and also increasing the production of adhesion molecules, increasing proliferation of the vessel intimal layer, angiogenesis and oxidative stress.[98] RAGE is expressed in all cells involved in atherogenesis, including monocytes, macrophages, endothelial cells and VSMC. Interestingly, these cells do not express significant amounts of RAGE under physiological conditions, but can be induced to express RAGE more vigorously in conditions where their ligands and/or transcription factors accumulate, like in uremia.[99]

Advanced oxidation protein products (AOPPs) are a class of dityrosine-containing protein products, which arise from the reaction between chlorinated oxidants and plasma proteins. Increased levels of AOPPs are detected in uremic serum since predialysis stage. AOPPs have been suggested to be not only reliable markers of oxidative stress but also mediators of inflammation, by triggering monocyte activation.[100] They have been associated with podocyte injury,[101] incident thrombo-occlusive atherosclerotic CV events in the pre-dialysis setting[102] and carotid atherosclerosis in ESKD patients.[103] These findings support the hypothesis that AOPPs should be considered as uremic toxins.

Protein-bound uremic toxins

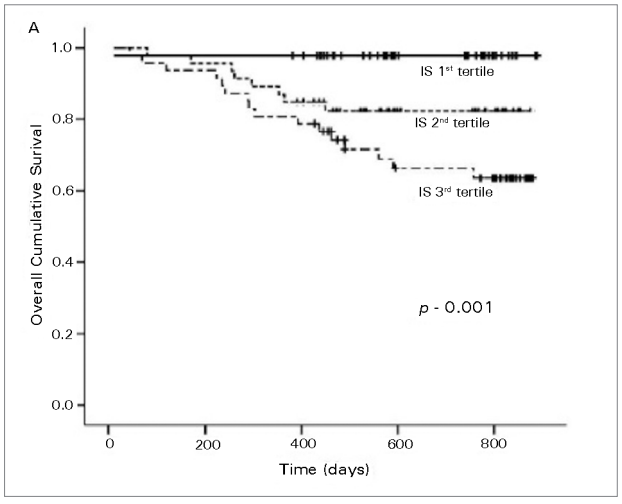
Indoxyl sulfate

Indoxyl sulfate (IS) is a low-MW (213.21 Da) uremic toxin derivated from dietary protein. It is an indol derived from the amino acid tryptophan by action of intestinal bacteria.[104] It is normally excreted into the urine, with its mean urinary excretion rate ranging from 50-70 mg/day in healthy persons. In CKD patients, as renal function declines it accumulates in serum due to its reduced renal clearance.[105] The main part of IS in the blood of CKD patients is bound to serum albumin, which means that its urinary excretion occurs mainly by tubular secretion, mainly by proximal tubular cells, and secondarily by glomerular filtration. Organic anion transporter (OAT) 1, the prototypic para-amino hippurate transporter, is localized in renal tubular cells and interacts with a wide range of small endogenous substances, including IS. IS is taken up from the blood by OAT1 and OAT3 at the basolateral membrane of tubular cells and accumulates in the cells at high concentrations.[106]

Several studies have shown an impact of IS accumulation in CKD patients. Increased IS concentrations compete for protein binding or excretion with other molecules. IS probably mediates its toxicity by direct induction of a host of genes involved in inflammation and fibrosis.[107] IS plays a key role

in endothelial damage and triggers the production of pro-inflammatory molecules, inhibition of endothelial regeneration and repair, and endothelial free radical production.[108] In addition, the participation of IS in VC, endothelial microparticle release, disruption of adherent junctions of endothelial cells, proliferation of VSMCs, renal and cardiac fibrosis, and impairement of osteoclast differentiation and function has been reported as well.[109-112] It has been proposed that IS may affect the remnant nephrons, especially proximal tubular cells, and stimulate tubulointerstitial fibrosis, glomerular sclerosis, and the progression of CKD by increasing the gene expression of TGF-β1, TIMP-1, and pro-α1collagen, leading to a further loss of nephrons, completing the vicious circle of progressive renal injury.[104] Several clinical studies have shown that high IS levels are associated with high IL-6 levels, coronary artery disease, vascular damage, progression of CKD and mortality (Figure 2).[112-116]

Figure 2. Kaplan-Meyer estimates of several survival probability for CKD patients as a function of tertiles of serum indoxyl sulfate levels. Footnote: reprinted with permission from Barreto FC (ref.115).



Para-cresol (p-cresol) and p-cresyl sulfate (PCS)

P-cresol (4-methylphenol) is another uremic toxin (MW: 108 Da) linked to serum proteins, with various deleterious effects. This molecule originates from tyrosine and phenylalanine metabolism by bacterial microbiota fermentation in the large intestine. During its passage through the colonic mucosa and the liver, it is metabolized by conjugation processes (sulfation and glucuronidation) forming two compounds, PCS and ρ-cresylglucoronidate. In patients with CKD it is possible to find these two derivatives of ρ-cresol, both in conjugated and unconjugated form.[117] The earliest studies with phenol compounds involved ρ-cresol; however, it was discovered subsequently that this compound is found in only tiny concentrations in the body since it is rapidly metabolized to its conjugates by the intestinal microbiota. Of note, it has been shown that the biochemical impact of ρ-cresol is not necessarily the same as the impact of its conjugates.[118] Previous studies have demonstrated that these uremic toxins are first taken up by the kidneys, blood vessels, bones, and across the blood-brain barrier via OATs and then induce the production of oxygen free radicals and inflammatory cytokines in the respective organs.[119-122] ρ-cresol affects the inflammatory response, interfering with the activation of polymorphonuclear leukocytes and endothelial response to cytokines.[123] However, neither in uremic patients nor in normal people this molecule can be detected in the circulation in its unconjugated form, in contrast to PCS.[124] Meijers *et al.* demonstrated that PCS induces the release of endothelial microparticles, even in the absence of endothelial injury, suggesting that this toxin is involved in endothelial dysfunction.[125] Furthermore, Schepers *et al.* observed a pro-inflammatory effect of PCS, measured by increased formation of free radicals produced by leukocytes, contributing to vascular damage in patients with CKD.[126] Importantly, high PCS levels have been associated with mortality in CKD.[124] In addition, Koppe *et al.* observed that normal mice treated with PCS for 4 weeks, developed insulin resistance, loss of fat mass, and ectopic redistribution of lipid in muscle and liver, mimicking features associated with CKD.[127]

Therapeutic strategies

As discussed above, the uremic syndrome is a complex condition resulting from the effects of wide variety of toxins. Current strategies aimed to decrease their serum concentration are dietary and pharmacological interventions, mainly via modulation of intestinal absorption capacity through binding effects and/or reduction of ingested amounts of the toxins or their precursors, and extra-corporeal removal via PD and HD. Notably, removal of middle and protein-bound uremic toxins by conventional HD and PD is insufficient. The main therapeutic strategies aimed to reduce the level of these compounds and possible advantages are briefly discussed below.

Dietary intervention

Protein-bound uremic toxins are generally produced from the metabolism of amino acids in the intestine. Thus, low-protein diet is often considered as a possible

dietary approach to reduce the serum concentration of these toxins. Animal experiments have shown that rats fed a low-protein diet had lower concentration of protein-bound solutes.[128] Recently, it was demonstrated that a reduced protein and Pi intakes associated with a very low protein diet, supplemented with ketoanalogues and essential amino acids, significantly lowered IS in CKD patients.[129] However, nephrologists should be aware of the fact that reducing protein intake excessively may deteriorate the nutritional status of CKD patients and that continuous nutritional evaluations by an expert dietitian are required. Another interesting issue that needs further investigation is the influence of vegetarian diets on the production of uremic toxins. The urinary excretion of PCS and IS was 62% and 59% lower, respectively, in vegetarians than in participants consuming an unrestricted diet, which was associated with higher fiber intake and lower protein intake.[130] Finally, the use of pre and probiotics has been suggested as interesting approach to reduce IS and PCS serum levels as well.[131,132]

Pharmacological intervention

The rationale for using oral sorbents to achieve a reduction of protein-bound toxins in the circulation is that they are derived from the intestinal metabolism of amino acids. Clinical studies have demonstrated that the oral sorbent AST-120 (Kremezin®) is associated with lower levels of IS,[133] slower progression of CKD[134] and improved survival after dialysis was started.[135] Experimental studies had also shown potentially useful therapeutic effects of AST-120 in preventing IS-induced pathological alterations, such as VC.[136,137] Results of two EPPIC trials, that evaluated the effectiveness of AST-120 added to standard-of-care therapy in moderate to severe CKD, did not support efficacy of this drug in slowing CKD progression. However, a subgroup analysis indicated a trend for AST-120 associated reduction of CKD progression in compliant patients with rapid decline of renal function.[138] Further trials are required to confirm the reality of this trend.

The use of specific types of phosphate binders has also been proposed as a possible means to decrease the concentrations of several uremic toxins, apart from their main therapeutic utilization in the control of hyperphosphatemia. Recently, two small clinical studies pointed to the possibility of achieving a pharmacologic modulation of leptin[139] and FGF23[140] levels in a cohort of early stage CKD patients via the use of sevelamer. Moreover, Vlassara *et al*. demonstrated in patients with diabetes and early CKD that sevelamer reduced markers of inflammantion and oxidative stress, independently of changes in Pi, possibly due to sevelamer's capacity to bind AGEs in the intestinal lumen.[141] In a recent observational cross-sectional study, in PD patients, sevelamer use was associated with lower levels of ρ-cresol as well.[142] In a pilot study in healthy volunteers treatment with acarbose, an alpha-glucosidase inhibitor, decreased the generation and serum concentrations of the protein bound uremic solute ρ-cresol.[143] Whether these actions may lead to an improvement of specific uremia-related complications and overall outcome of patients with CKD has yet to be shown.

Hemodialysis with high-flux membranes

Several randomized controlled trials (RCT) aimed to demonstrate that an increase in the clearance of higher weight molecules via the use of high-flux dialysis membranes led to a survival benefit as compared to standard low-flux membranes. The first large-scale RCT was the HEMO study.[144] It failed to find a difference in mortality between the two groups. It also was unable to demonstrate a benefit of high dialysis dose as compared to standard dialysis dose. Post hoc secondary analyses of the HEMO study pointed to a benefit of using high-flux membranes in terms of cardiac outcomes,[145] and decreased cerebro-vascular disease mortality in patient subcategories,[145] possibly as the result of better middle molecule removal due to the larger pore size of this type of membrane. It is worth mentioning that high-flux HD has no effect on protein-bound uremic toxin levels. Similarly to the HEMO trial, the subsequently done MPO trial also failed to demonstrate a survival advantage with the use of high-flux as compared to low-flux dialysis membranes.[146] It must be pointed out that positive results obtained by secondary analyses may strengthen, but do not prove, the hypothesis that high-flux treatment may improve cardiovascular events and survival in certain subpopulations of HD patients.

Convective strategies (hemofiltration or hemodiafiltration)

It has been demonstrated that convective strategies improve the removal of middle molecules and

protein-bound uremic toxins.[147,148] By comparing three main convective strategies in parallel, named pre-dilution hemodiafiltration (HDF), post-dilution HDF and pre-hemofiltration (HF), Meert *et al.* reported that under similar convective volumes, post- and pre-dilution HDF had similar effects on protein-bound molecules removal, though the former appeared to be better for small water soluble compounds and β2-microgobulin. Pre-HF was superior to pre-HDF only for β2-microgobulin removal and not at all superior to post-HDF.[148] In summary, post-HDF was found to be the most effective convective strategy for solute removal. Mid-dilution HDF, a new strategy that allows simultaneous infusion in a pre- and post-dilution fashion, appears to be as efficient as post-dilution HDF for small water-soluble and protein-bound solutes removal.[149]

A beneficial impact of convective therapies on hard clinical outcomes is however far from being firmly established. Although benefit has been suggested by observational studies,[150,151] the results obtained in recent RCTs are less clear.[152,153] Thus, Grooteman *et al.* found in a recent multicenter RCT that dialysis patients assigned to high-efficiency post-dilution on-line HDF had less cardiovascular events but there was no difference between the two groups in the primary endpoint, namely all-cause mortality.[152] Ok *et al.* set up an RCT comparing the effect of on-line HDF to that of high-flux HD.[153] The primary outcome (composite of death from any cause and nonfatal cardiovascular events) was not different between the two groups. However, in adjusted Cox-regression analysis treatment with high-efficiency on-line HD was associated with a 46% risk reduction for overall mortality. In a very recent RCT, Asci *et al.* failed to find an advantage in fatal or non-fatal cardiovascular event-free survival with high flux HD as compared to standard HD treatment.[154] Only Maduell *et al.* were able to demonstrate that dialysis patients randomized to on-line HDF had a better survival than those randomized to standard HD.[155] Whether the superiority of HDF was due to a better clearance of uremic toxins in the middle-to-large MW range remains to be demonstrated.

Adsorption

Other possible therapeutic strategies that could be used to improve dialysis removal of protein-bound uremic toxins are: addition of activated charcoal or 5% albumin to the dialysate; use of highly permeable dialysis membranes to promote albumin leakage; fractionated plasma separation and adsorption.[156] They have not yet been used in large clinical trials, though. The risk of aggravating malnutrition, limited effectiveness due to saturation of adsorption sites and thrombotic complications are potential limitations of these therapies.[157] Additional studies are required before recommending these strategies in routine clinical use.

Peritoneal dialysis

Data on uremic toxins in PD is scarce. In spite of the serum concentration of protein-bound uremic toxins being lower in PD compared to HD patients, their removal is worse in the former group.[158] These observations suggest that factors beyond the dialysis method, such as intestinal generation and/or metabolism, may play a role to determine their serum levels.

Although several studies have suggested a benefit of therapeutic intervention on middle and protein-bound uremic toxins levels, most of these data came from pos hoc analysis or small studies. Larger, prospective, well-designed RCT aimed to identify the impact of reducing the levels of different uremic toxins on hard outcomes, such as overall and cardiovascular mortality, are required.

General conclusions

The study of uremic toxins is of growing interest in nephrology. Evidence from clinical and experimental studies have demonstrated the impact of these compounds on a variety of organs and systems. Notably, uremic toxins have recently been claimed as new cardiovascular risk factors, attracting interest from other fields of medicine.[159] Understanding the effects of uremic toxins may provide a more comprehensive view of CKD and its complications and, most likely, new therapeutic targets to retard the progression of CKD and to counteract its cardiovascular complications.

References

1. Meyer TW, Hostetter TH. Uremia. N Engl J Med 2007;357:1316-25. PMID: 17898101 DOI: http://dx.doi.org/10.1056/NEJMra071313
2. Vanholder R. Uremic toxins. Nephrologie 2003;24:373-6.
3. Glassock RJ. Uremic toxins: what are they? An integrated overview of pathobiology and classification. J Ren Nutr 2008;18:2-6. DOI: http://dx.doi.org/10.1053/j.jrn.2007.10.003
4. Mahomed FA. On the Pathology of Uraemia and the Socalled Uraemic Convulsions. Br Med J 1877;2:10-2. DOI: http://dx.doi.org/10.1136/bmj.2.862.10

5. Man NK, Terlain B, Paris J, Werner G, Sausse A, Funck-Brentano JL. An approach to "middle molecules" identification in artificial kidney dialysate, with reference to neuropathy prevention. Trans Am Soc Artif Intern Organs 1973;19:320-4.
6. Babb AL, Johansen PJ, Strand MJ, Tenckhoff H, Scribner BH. Bi-directional permeability of the human peritoneum to middle molecules. Proc Eur Dial Transplant Assoc 1973;10:247-62.
7. Vanholder R, De Smet R, Glorieux G, Argilés A, Baurmeister U, Brunet P, et al.; European Uremic Toxin Work Group (EUTox). Review on uremic toxins: classification, concentration, and interindividual variability. Kidney Int 2003;63:1934-43. PMID: 12675874
8. D'Hooge R, Pei YQ, Marescau B, De Deyn PP. Convulsive action and toxicity of uremic guanidino compounds: behavioral assessment and relation to brain concentration in adult mice. J Neurol Sci 1992;112:96-105. PMID: 1469446 DOI: http://dx.doi.org/10.1016/0022-510X(92)90138-B
9. Eloot S, Schepers E, Barreto DV, Barreto FC, Liabeuf S, Van Biesen W, et al. Estimated glomerular filtration rate is a poor predictor of concentration for a broad range of uremic toxins. Clin J Am Soc Nephrol 2011;6:1266-73. DOI: http://dx.doi.org/10.2215/CJN.09981110
10. Kielstein JT, Böger RH, Bode-Böger SM, Frölich JC, Haller H, Ritz E, et al. Marked increase of asymmetric dimethylarginine in patients with incipient primary chronic renal disease. J Am Soc Nephrol 2002;13:170-6.
11. Baylis C. Nitric oxide deficiency in chronic kidney disease. Am J Physiol Renal Physiol 2008;294:F1-9. PMID: 17928410
12. Eloot S, van Biesen W, Dhondt A, de Smet R, Marescau B, De Deyn PP, et al. Impact of increasing haemodialysis frequency versus haemodialysis duration on removal of urea and guanidino compounds: a kinetic analysis. Nephrol Dial Transplant 2009;24:2225-32. DOI: http://dx.doi.org/10.1093/ndt/gfp059
13. Vallance P, Leone A, Calver A, Collier J, Moncada S. Endogenous dimethylarginine as an inhibitor of nitric oxide synthesis. J Cardiovasc Pharmacol 1992;20:S60-2. PMID: 1282988 DOI: http://dx.doi.org/10.1097/00005344-199204002-00018
14. Böger RH, Bode-Böger SM, Szuba A, Tsao PS, Chan JR, Tangphao O, et al. Asymmetric dimethylarginine (ADMA): a novel risk factor for endothelial dysfunction: its role in hypercholesterolemia. Circulation 1998;98:1842-7. PMID: 9799202
15. Zoccali C, Bode-Böger S, Mallamaci F, Benedetto F, Tripepi G, Malatino L, et al. Plasma concentration of asymmetrical dimethylarginine and mortality in patients with end-stage renal disease: a prospective study. Lancet 2001;358:2113-7. PMID: 11784625 DOI: http://dx.doi.org/10.1016/S0140-6736(01)07217-8
16. Bode-Böger SM, Scalera F, Kielstein JT, Martens-Lobenhoffer J, Breithardt G, Fobker M, et al. Symmetrical dimethylarginine: a new combined parameter for renal function and extent of coronary artery disease. J Am Soc Nephrol 2006;17:1128-34. DOI: http://dx.doi.org/10.1681/ASN.2005101119
17. Schepers E, Barreto DV, Liabeuf S, Glorieux G, Eloot S, Barreto FC, et al. Symmetric dimethylarginine as a proinflammatory agent in chronic kidney disease. Clin J Am Soc Nephrol 2011;6:2374-83. DOI: http://dx.doi.org/10.2215/CJN.01720211
18. Jalal DI, Chonchol M, Chen W, Targher G. Uric acid as a target of therapy in CKD. Am J Kidney Dis 2013;61:134-46. DOI: http://dx.doi.org/10.1053/j.ajkd.2012.07.021
19. Umekawa T, Chegini N, Khan SR. Increased expression of monocyte chemoattractant protein-1 (MCP-1) by renal epithelial cells in culture on exposure to calcium oxalate, phosphate and uric acid crystals. Nephrol Dial Transplant 2003;18:664-9. DOI: http://dx.doi.org/10.1093/ndt/gfg140
20. Spencer HW, Yarger WE, Robinson RR. Alterations of renal function during dietary-induced hyperuricemia in the rat. Kidney Int 1976;9:489-500. DOI: http://dx.doi.org/10.1038/ki.1976.63
21. Johnson RJ, Kang DH, Feig D, Kivlighn S, Kanellis J, Watanabe S, et al. Is there a pathogenetic role for uric acid in hypertension and cardiovascular and renal disease? Hypertension 2003;41:1183-90.
22. Chonchol M, Shlipak MG, Katz R, Sarnak MJ, Newman AB, Siscovick DS, et al. Relationship of uric acid with progression of kidney disease. Am J Kidney Dis 2007;50:239-47. PMID: 17660025 DOI: http://dx.doi.org/10.1053/j.ajkd.2007.05.013
23. Madero M, Sarnak MJ, Wang X, Greene T, Beck GJ, Kusek JW, et al. Uric acid and long-term outcomes in CKD. Am J Kidney Dis 2009;53:796-803. DOI: http://dx.doi.org/10.1053/j.ajkd.2008.12.021
24. Hsu CY, Iribarren C, McCulloch CE, Darbinian J, Go AS. Risk factors for end-stage renal disease: 25-year follow-up. Arch Intern Med 2009;169:342-50. PMID: 19237717 DOI: http://dx.doi.org/10.1001/archinternmed.2008.605
25. Goicoechea M, de Vinuesa SG, Verdalles U, Ruiz-Caro C, Ampuero J, Rincón A, et al. Effect of allopurinol in chronic kidney disease progression and cardiovascular risk. Clin J Am Soc Nephrol 2010;5:1388-93. DOI: http://dx.doi.org/10.2215/CJN.01580210
26. Uribarri J. Phosphorus homeostasis in normal health and in chronic kidney disease patients with special emphasis on dietary phosphorus intake. Semin Dial 2007;20:295-301. DOI: http://dx.doi.org/10.1111/j.1525-139X.2007.00309.x
27. Levin A, Bakris GL, Molitch M, Smulders M, Tian J, Williams LA, et al. 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;71:31-8. DOI: http://dx.doi.org/10.1038/sj.ki.5002009
28. Barreto FC, de Oliveira RA, Oliveira RB, Jorgetti V. Pharmacotherapy of chronic kidney disease and mineral bone disorder. Expert Opin Pharmacother 2012;12:2627-40. DOI: http://dx.doi.org/10.1517/14656566.2011.626768
29. Sampaio EA, Lugon JR, Barreto FC. Fisopatologia do hiperparatireoidismo secundário J Bras Nefrol 2008;30:6-10.
30. Block GA, Hulbert-Shearon TE, Levin NW, Port FK. Association of serum phosphorus and calcium x phosphate product with mortality risk in chronic hemodialysis patients: a national study. Am J Kidney Dis 1998;31:607-17. DOI: http://dx.doi.org/10.1053/ajkd.1998.v31.pm9531176
31. Kestenbaum B, Sampson JN, Rudser KD, Patterson DJ, Seliger SL, Young B, et al. Serum phosphate levels and mortality risk among people with chronic kidney disease. J Am Soc Nephrol 2005;16:520-8. DOI: http://dx.doi.org/10.1681/ASN.2004070602
32. Giachelli CM. Vascular calcification: in vitro evidence for the role of inorganic phosphate. J Am Soc Nephrol 2003;14:S300-4. DOI: http://dx.doi.org/10.1097/01.ASN.0000081663.52165.66
33. Phan O, Ivanovski O, Nguyen-Khoa T, Mothu N, Angulo J, Westenfeld R, et al. Sevelamer prevents uremia-enhanced atherosclerosis progression in apolipoprotein E-deficient mice. Circulation 2005;112:2875-82. PMID: 16267260 DOI: http://dx.doi.org/10.1161/CIRCULATIONAHA105.541854
34. Phan O, Ivanovski O, Nikolov IG, Joki N, Maizel J, Louvet L, et al. Effect of oral calcium carbonate on aortic calcification in apolipoprotein E-deficient (apoE-/-) mice with chronic renal failure. Nephrol Dial Transplant 2008;23:82-90. DOI: http://dx.doi.org/10.1093/ndt/gfm699
35. Barreto DV, Barreto Fde C, de Carvalho AB, Cuppari L, Draibe SA, Dalboni MA, et al. Phosphate binder impact on bone remodeling and coronary calcification-results from the BRiC study. Nephron Clin Pract 2008;110:c273-83. DOI: http://dx.doi.org/10.1159/000170783
36. Chertow GM, Burke SK, Raggi P; Treat to Goal Working Group. Sevelamer attenuates the progression of coronary and aortic calcification in hemodialysis patients. Kidney Int 2002;62:245-52. DOI: http://dx.doi.org/10.1046/j.1523-1755.2002.00434.x
37. Dhingra R, Sullivan LM, Fox CS, Wang TJ, D'Agostino RB Sr, Gaziano JM, et al. Relations of serum phosphorus and calcium levels to the incidence of cardiovascular disease in the community. Arch Intern Med 2007;167:879-85. PMID: 17502528 DOI: http://dx.doi.org/10.1001/archinte.167.9.879

38. Dhingra R, Gona P, Benjamin EJ, Wang TJ, Aragam J, D'Agostino RB Sr, et al. Relations of serum phosphorus levels to echocardiographic left ventricular mass and incidence of heart failure in the community. Eur J Heart Fail 2010;12:812-8. DOI: http://dx.doi.org/10.1093/eurjhf/hfq106
39. Cancela AL, Santos RD, Titan SM, Goldenstein PT, Rochitte CE, Lemos PA, et al. Phosphorus is associated with coronary artery disease in patients with preserved renal function. PLoS One 2012;7:e36883. DOI: http://dx.doi.org/10.1371/journal.pone.0036883
40. Tonelli M, Sacks F, Pfeffer M, Gao Z, Curhan G; Cholesterol And Recurrent Events Trial Investigators. Relation between serum phosphate level and cardiovascular event rate in people with coronary disease. Circulation 2005;112:2627-33. PMID: 16246962
41. Ellam T, Wilkie M, Chamberlain J, Crossman D, Eastell R, Francis S, et al. Dietary phosphate modulates atherogenesis and insulin resistance in apolipoprotein E knockout mice-brief report. Arterioscler Thromb Vasc Biol 2011;31:1988-90. DOI: http://dx.doi.org/10.1161/ATVBAHA.111.231001
42. Maizel J, Six I, Dupont S, Secq E, Dehedin B, Barreto FC, et al. Effects of sevelamer treatment on cardiovascular abnormalities in mice with chronic renal failure. Kidney Int 2013;84:491-500. DOI: http://dx.doi.org/10.1038/ki.2013.110
43. Di Marco GS, Hausberg M, Hillebrand U, Rustemeyer P, Wittkowski W, Lang D, et al. Increased inorganic phosphate induces human endothelial cell apoptosis in vitro. Am J Physiol Renal Physiol 2008;294:F1381-7. PMID: 18385273 DOI: http://dx.doi.org/10.1152/ajprenal.00003.2008
44. Shuto E, Taketani Y, Tanaka R, Harada N, Isshiki M, Sato M, et al. Dietary phosphorus acutely impairs endothelial function. J Am Soc Nephrol 2009;20:1504-12. DOI: http://dx.doi.org/10.1681/ASN.2008101106
45. Six I, Maizel J, Barreto FC, Rangrez AY, Dupont S, Slama M, et al. Effects of phosphate on vascular function under normal conditions and influence of the uraemic state. Cardiovasc Res 2012;96:130-9. PMID: 22822101 DOI: http://dx.doi.org/10.1093/cvr/cvs240
46. Di Marco GS, König M, Stock C, Wiesinger A, Hillebrand U, Reiermann S, et al. High phosphate directly affects endothelial function by downregulating annexin II. Kidney Int 2013;83:213-22. DOI: http://dx.doi.org/10.1038/ki.2012.300
47. Van TV, Watari E, Taketani Y, Kitamura T, Shiota A, Tanaka T, et al. Dietary phosphate restriction ameliorates endothelial dysfunction in adenine-induced kidney disease rats. J Clin Biochem Nutr 2012;51:27-32. DOI: http://dx.doi.org/10.3164/jcbn.11-96
48. Neves KR, Graciolli FG, dos Reis LM, Pasqualucci CA, Moysés RM, Jorgetti V. Adverse effects of hyperphosphatemia on myocardial hypertrophy, renal function, and bone in rats with renal failure. Kidney Int 2004;66:2237-44. PMID: 15569312 DOI: http://dx.doi.org/10.1111/j.1523-1755.2004.66013.x
49. Custódio MR, Koike MK, Neves KR, dos Reis LM, Graciolli FG, Neves CL, et al. Parathyroid hormone and phosphorus overload in uremia: impact on cardiovascular system. Nephrol Dial Transplant 2012;27:1437-45. DOI: http://dx.doi.org/10.1093/ndt/gfr447
50. Graciolli FG, Neves KR, dos Reis LM, Graciolli RG, Noronha IL, Moysés RM, et al. Phosphorus overload and PTH induce aortic expression of Runx2 in experimental uraemia. Nephrol Dial Transplant 2009;24:1416-21. DOI: http://dx.doi.org/10.1093/ndt/gfn686
51. Yamashita T, Yoshioka M, Itoh N. Identification of a novel fibroblast growth factor, FGF-23, preferentially expressed in the ventrolateral thalamic nucleus of the brain. Biochem Biophy Res Comm 2000;277:494-8. DOI: http://dx.doi.org/10.1006/bbrc.2000.3696
52. ADHR Consortium. Autossomal dominant hypophosphataemic rickets is associated with mutations in FGF23. Nat Genet 2000; 26:345-8. DOI: http://dx.doi.org/10.1038/81664
53. Urakawa I, Yamazaki Y, Shimada T, Iijima K, Hasegawa H, Okawa K, et al. Klotho converts canonical FGF receptor into a specific receptor for FGF23. Nature 2006;444:770-4. PMID: 17086194 DOI: http://dx.doi.org/10.1038/nature05315
54. Ben-Dov IZ, Galitzer H, Lavi-Moshayoff V, Goetz R, Kuro-o M, Mohammadi M, et al. The parathyroid is a target organ for FGF23 in rats. J Clin Invest 2007;117:4003-8. PMID: 17992255
55. Larsson T, Nisbeth U, Ljunggren O, Jüppner H, Jonsson KB. Circulating concentration of FGF-23 increases as renal function declines in patients with chronic kidney disease, but does not change in response to variation in phosphate intake in healthy volunteers. Kidney Int 2003;64:2272-9. PMID: 14633152 DOI: http://dx.doi.org/10.1046/j.1523-1755.2003.00328.x
56. Isakova T, Xie H, Barchi-Chung A, Vargas G, Sowden N, Houston J, et al. Fibroblast growth factor 23 in patients undergoing peritoneal dialysis. Clin J Am Soc Nephrol 2011;6:2688-95. DOI: http://dx.doi.org/10.2215/CJN.04290511
57. Fliser D, Kollerits B, Neyer U, Ankerst DP, Lhotta K, Lingenhel A, et al. Fibroblast growth factor 23 (FGF23) predicts progression of chronic kidney disease: the Mild to Moderate Kidney Disease (MMKD) Study. J Am Soc Nephrol 2007;18:2600-8. DOI: http://dx.doi.org/10.1681/ASN.2006080936
58. Titan SM, Zatz R, Graciolli FG, dos Reis LM, Barros RT, Jorgetti V, et al. FGF-23 as a predictor of renal outcome in diabetic nephropathy. Clin J Am Soc Nephrol 2011;6:241-7. DOI: http://dx.doi.org/10.2215/CJN.04250510
59. Hsu HJ, Wu MS. Fibroblast growth factor 23: a possible cause of left ventricular hypertrophy in hemodialysis patients. Am J Med Sci 2009;337:116-22. PMID: 19214027 DOI: http://dx.doi.org/10.1097/MAJ.0b013e3181815498
60. Desjardins L, Liabeuf S, Renard C, Lenglet A, Lemke HD, Choukroun G, et al.; European Uremic Toxin (EUTox) Work Group. FGF23 is independently associated with vascular calcification but not bone mineral density in patients at various CKD stages. Osteoporos Int 2012;23:2017-25. PMID: 22109743
61. Gutiérrez OM, Mannstadt M, Isakova T, Rauh-Hain JA, Tamez H, Shah A, et al. Fibroblast growth factor 23 and mortality among patients undergoing hemodialysis. N Engl J Med 2008;359:584-92. PMID: 18687639 DOI: http://dx.doi.org/10.1056/NEJMoa0706130
62. Isakova T, Xie H, Yang W, Xie D, Anderson AH, Scialla J, et al. Fibroblast growth factor 23 and risks of mortality and end-stage renal disease in patients with chronic kidney disease. JAMA 2011;305:2432-9. PMID: 21673295 DOI: http://dx.doi.org/10.1001/jama.2011.826
63. Shalhoub V, Shatzen EM, Ward SC, Davis J, Stevens J, Bi V, et al. FGF23 neutralization improves chronic kidney disease-associated hyperparathyroidism yet increases mortality. J Clin Invest 2012;122:2543-53. PMID: 22728934 DOI: http://dx.doi.org/10.1172/JCI61405
64. Faul C, Amaral AP, Oskouei B, Hu MC, Sloan A, Isakova T, et al. FGF23 induces left ventricular hypertrophy. J Clin Invest 2011;121:4393-408. PMID: 21985788 DOI: http://dx.doi.org/10.1172/JCI46122
65. Mirza MA, Larsson A, Melhus H, Lind L, Larsson TE. Serum intact FGF23 associate with left ventricular mass, hypertrophy and geometry in an elderly population. Atherosclerosis 2009;207:546-1. DOI: http://dx.doi.org/10.1016/j.atherosclerosis.2009.05.013
66. Scialla JJ, Lau WL, Reilly MP, Isakova T, Yang HY, Crouthamel MH, et al. Fibroblast growth factor 23 is not associated with and does not induce arterial calcification. Kidney Int 2013;83:1159-68. DOI: http://dx.doi.org/10.1038/ki.2013.3
67. Olauson H, Qureshi AR, Miyamoto T, Barany P, Heimburger O, Lindholm B, et al. Relation between serum fibroblast growth factor-23 level and mortality in incident dialysis patients: are gender and cardiovascular disease confounding the relationship? Nephrol Dial Transplant 2010;25:3033-8.

68. Oliveira RB, Moysés RM. FGF-23: state of the art. J Bras Nefrol 2010;32:323-1. DOI: http://dx.doi.org/10.1590/S0101-28002010000300015
69. Wolf M. Update on fibroblast growth factor 23 in chronic kidney disease. Kidney Int 2012;82:737-47. PMID: 22622492 DOI: http://dx.doi.org/10.1038/ki.2012.176
70. Considine RV, Sinha MK, Heiman ML, Kriauciunas A, Stephens TW, Nyce MR, et al. Serum immunoreactive-leptin concentrations in normal-weight and obese humans. N Engl J Med 1996;334:292-5. PMID: 8532024 DOI: http://dx.doi.org/10.1056/NEJM199602013340503
71. Friedman JM, Halaas JL. Leptin and the regulation of body weight in mammals. Nature 1998;395:763-70. PMID: 9796811 DOI: http://dx.doi.org/10.1038/27376
72. Parhami F, Tintut Y, Ballard A, Fogelman AM, Demer LL. Leptin enhances the calcification of vascular cells: artery wall as a target of leptin. Circ Res 2001;88:954-60. PMID: 11349006 DOI: http://dx.doi.org/10.1161/hh0901.090975
73. Oda A, Taniguchi T, Yokoyama M. Leptin stimulates rat aortic smooth muscle cell proliferation and migration. Kobe J Med Sci 2001;47:141-50. PMID: 11729375
74. Singhal A, Farooqi IS, Cole TJ, O'Rahilly S, Fewtrell M, Kattenhorn M, et al. Influence of leptin on arterial distensibility: a novel link between obesity and cardiovascular disease? Circulation 2002;106:1919-24. PMID: 12370213
75. Wallace AM, McMahon AD, Packard CJ, Kelly A, Shepherd J, Gaw A, et al. Plasma leptin and the risk of cardiovascular disease in the west of Scotland coronary prevention study (WOSCOPS). Circulation 2001;104:3052-6. PMID: 11748099 DOI: http://dx.doi.org/10.1161/hc5001.101061
76. Scholze A, Rattensperger D, Zidek W, Tepel M. Low serum leptin predicts mortality in patients with chronic kidney disease stage 5. Obesity (Silver Spring) 2007;15:1617-22. DOI: http://dx.doi.org/10.1038/oby.2007.191
77. de Mutsert R, Snijder MB, van der Sman-de Beer F, Seidell JC, Boeschoten EW, Krediet RT, et al. Association between body mass index and mortality is similar in the hemodialysis population and the general population at high age and equal duration of follow-up. J Am Soc Nephrol 2007;18:967-74. DOI: http://dx.doi.org/10.1681/ASN.2006091050
78. de Oliveira RB, Liabeuf S, Okazaki H, Lenglet A, Desjardins L, Lemke HD, et al. The clinical impact of plasma leptin levels in a cohort of chronic kidney disease patients. Clin Kidney J 2013;6:63-70. DOI: http://dx.doi.org/10.1093/ckj/sfs176
79. Mac Way F, Lessard M, Lafage-Proust MH. Pathophysiology of chronic kidney disease-mineral and bone disorder. Joint Bone Spine 2012;79:544-9. PMID: 23177912 DOI: http://dx.doi.org/10.1016/j.jbspin.2012.09.014
80. Jadoul M, Albert JM, Akiba T, Akizawa T, Arab L, Bragg-Gresham JL, et al. Incidence and risk factors for hip or other bone fractures among hemodialysis patients in the Dialysis Outcomes and Practice Patterns Study. Kidney Int 2006;70:1358-66. PMID: 16929251 DOI: http://dx.doi.org/10.1038/sj.ki.5001754
81. Rodriguez M, Lorenzo V. Parathyroid hormone, a uremic toxin. Semin Dial 2009;22:363-8. DOI: http://dx.doi.org/10.1111/j.1525-139X.2009.00581.x
82. Hernandes FR, Barreto FC, Rocha LA, Draibe SA, Canziani ME, Carvalho AB. Evaluation of the role of severe hyperparathyroidism on coronary artery calcification in dialysis patients. Clin Nephrol 2007;67:89-95. PMID: 17338428 DOI: http://dx.doi.org/10.5414/CNP67089
83. Drüeke T, Fauchet M, Fleury J, Lesourd P, Toure Y, Le Pailleur C, et al. Effect of parathyroidectomy on left--ventricular function in haemodialysis patients. Lancet 1980;1:112-4. DOI: http://dx.doi.org/10.1016/S0140-6736(80)90602-9
84. Block GA, Klassen PS, Lazarus JM, Ofsthun N, Lowrie EG, Chertow GM. Mineral metabolism, mortality, and morbidity in maintenance hemodialysis. J Am Soc Nephrol 2004;15:2208-18. DOI: http://dx.doi.org/10.1097/01.ASN.0000133041.27682.A2
85. van Ballegooijen AJ, Reinders I, Visser M, Dekker JM, Nijpels G, Stehouwer CD, et al. Serum parathyroid hormone in relation to all-cause and cardiovascular mortality: the Hoorn study. J Clin Endocrinol Metab 2013;98:E638-45. DOI: http://dx.doi.org/10.1210/jc.2012-4007
86. Rostand SG, Drüeke TB. Parathyroid hormone, vitamin D, and cardiovascular disease in chronic renal failure. Kidney Int 1999;56:383-92. PMID: 10432376 DOI: http://dx.doi.org/10.1046/j.1523-1755.1999.00575.x
87. Tomaschitz A, Ritz E, Pieske B, Fahrleitner-Pammer A, Kienreich K, Horina JH, et al. Aldosterone and parathyroid hormone: a precarious couple for cardiovascular disease. Cardiovasc Res 2012;94:10-9. PMID: 22334595 DOI: http://dx.doi.org/10.1093/cvr/cvs092
88. Potthoff SA, Janus A, Hoch H, Frahnert M, Tossios P, Reber D, et al. PTH-receptors regulate norepinephrine release in human heart and kidney. Regul Pept 2011;171:35-42. PMID: 21756942 DOI: http://dx.doi.org/10.1016/j.regpep.2011.06.002
89. Iwasaki Y, Yamato H, Nii-Kono T, Fujieda A, Uchida M, Hosokawa A, et al. Insufficiency of PTH action on bone in uremia. Kidney Int Suppl 2006:S34-6. DOI: http://dx.doi.org/10.1038/sj.ki.5001600
90. EVOLVE Trial Investigators; Chertow GM, Block GA, Correa-Rotter R, Drüeke TB, Floege J, Goodman WG, et al. Effect of cinacalcet on cardiovascular disease in patients undergoing dialysis. N Engl J Med 2012;367:2482-94. PMID: 23121374 DOI: http://dx.doi.org/10.1056/NEJMoa1205624
91. Semba RD, Nicklett EJ, Ferrucci L. Does accumulation of advanced glycation and products contribute to the aging phenotype? J Gerontol A Biol Sci Med Sci 2010;65:963-75. PMID: 20478906
92. Schwedler S, Schinzel R, Vaith P, Wanner C. Inflammation and advanced glycation end products in uremia: simple coexistence, potentiation or causal relationship? Kidney Int Suppl 2001;78:S32-6. PMID: 11168979
93. Piperi C, Adamopoulos C, Dalagiorgou G, Diamanti-Kandarakis E, Papavassiliou AG. Crosstalk between advanced glycation and endoplasmic reticulum stress: emerging therapeutic targeting for metabolic diseases. J Clin Endocrinol Metab 2012;97:2231-42. PMID: 22508704 DOI: http://dx.doi.org/10.1210/jc.2011-3408
94. Thornalley PJ, Rabbani N. Highlights and hotspots of protein glycation in end-stage renal disease. Semin Dial 2009;22:400-4. DOI: http://dx.doi.org/10.1111/j.1525-139X.2009.00589.x
95. Alhamdani MS, Al-Azzawie HF, Abbas FK. Decreased formation of advanced glycation end-products in peritoneal fluid by carnosine and related peptides. Perit Dial Int 2007;27:86-9.
96. Brunkhorst R, Kruse M, Ehlerding G, Mahiout A. Advanced glycosylated end-products (AGEs) in non-diabetic patients undergoing dialysis. Clin Nephrol 1996;46:265-6.
97. Zieman S, Kass D. Advanced glycation end product cross-linking: pathophysiologic role and therapeutic target in cardiovascular disease. Congest Heart Fail 2004;10:144-9. DOI: http://dx.doi.org/10.1111/j.1527-5299.2004.03223.x
98. Xu Y, Wang S, Feng L, Zhu Q, Xiang P, He B. Blockade of PKC-beta protects HUVEC from advanced glycation end products induced inflammation. Int Immunopharmacol 2010;10:1552-9. DOI: http://dx.doi.org/10.1016/j.intimp.2010.09.006
99. Figarola JL, Shanmugam N, Natarajan R, Rahbar S. Anti-inflammatory effects of the advanced glycation end product inhibitor LR-90 in human monocytes. Diabetes 2007;56:647-55. PMID: 17327432 DOI: http://dx.doi.org/10.2337/db06-0936
100. Witko-Sarsat V, Friedlander M, Capeillère-Blandin C, Nguyen-Khoa T, Nguyen AT, Zingraff J, et al. Advanced oxidation protein products as a novel marker of oxidative stress in uremia. Kidney Int 1996;49:1304-13. PMID: 8731095 DOI: http://dx.doi.org/10.1038/ki.1996.186
101. Zhou LL, Hou FF, Wang GB, Yang F, Xie D, Wang YP, et al. Accumulation of advanced oxidation protein products induces podocyte apoptosis and deletion through NADPH-dependent mechanisms Kidney Int 2009;76:1148-60. PMID: 19727064

102. Descamps-Latscha B, Witko-Sarsat V, Nguyen-Khoa T, Nguyen AT, Gausson V, Mothu N, et al. Advanced oxidation protein products as risk factors for atherosclerotic cardiovascular events in nondiabetic predialysis patients. Am J Kidney Dis 2005;45:39-47. PMID: 15696442 DOI: http://dx.doi.org/10.1053/j.ajkd.2004.09.011
103. Drüeke T, Witko-Sarsat V, Massy Z, Descamps-Latscha B, Guerin AP, Marchais SJ, et al. Iron therapy, advanced oxidation protein products, and carotid artery intima-media thickness in end-stage renal disease. Circulation 2002;106:2212-7. PMID: 12390950 DOI: http://dx.doi.org/10.1161/01.CIR.0000035250.66458.67
104. Niwa T. Uremic toxicity of indoxyl sulfate. Nagoya J Med Sci 2010;72:1-11.
105. Bolati D, Shimizu H, Yisireyili M, Nishijima F, Niwa T. Indoxyl sulfate, a uremic toxin, downregulates renal expression of Nrf2 through activation of NF-κB. BMC Nephrol 2013;14:56. DOI: http://dx.doi.org/10.1186/1471-2369-14-56
106. Taki K, Nakamura S, Miglinas M, Enomoto A, Niwa T. Accumulation of indoxyl sulfate in OAT1/3-positive tubular cells in kidneys of patients with chronic renal failure. J Ren Nutr 2006;16:199-203. DOI: http://dx.doi.org/10.1053/j.jrn.2006.04.020
107. Lowenstein J. The anglerfish and uremic toxins. FASEB J 2011;25:1781-5. DOI: http://dx.doi.org/10.1096/fj.11-0602ufm
108. Dou L, Bertrand E, Cerini C, Faure V, Sampol J, Vanholder R, et al. The uremic solutes p-cresol and indoxyl sulfate inhibit endothelial proliferation and wound repair. Kidney Int 2004;65:442-51. DOI: http://dx.doi.org/10.1111/j.1523-1755.2004.00399.x
109. Bammens B, Evenepoel P, Verbeke K, Vanrenterghem Y. Removal of middle molecules and protein-bound solutes by peritoneal dialysis and relation with uremic symptoms. Kidney Int 2003;64:2238-43. DOI: http://dx.doi.org/10.1046/j.1523-1755.2003.00310.x
110. Neirynck N, Vanholder R, Schepers E, Eloot S, Pletinck A, Glorieux G. An update on uremic toxins. Int Urol Nephrol 2013;45:139-50. DOI: http://dx.doi.org/10.1007/s11255-012-0258-1
111. Mozar A, Louvet L, Godin C, Mentaverri R, Brazier M, Kamel S, et al. Indoxyl sulphate inhibits osteoclast differentiation and function. Nephrol Dial Transplant 2012;27:2176-81. DOI: http://dx.doi.org/10.1093/ndt/gfr647
112. Mozar A, Louvet L, Morlière P, Godin C, Boudot C, Kamel S, et al. Uremic toxin indoxyl sulfate inhibits human vascular smooth muscle cell proliferation. Ther Apher Dial 2011;15:135-9. DOI: http://dx.doi.org/10.1111/j.1744-9987.2010.00885.x
113. Lee CT, Kuo CC, Chen YM, Hsu CY, Lee WC, Tsai YC, et al. Factors associated with blood concentrations of indoxyl sulfate and p-cresol in patients undergoing peritoneal dialysis. Perit Dial Int 2010;30:456-63. DOI: http://dx.doi.org/10.3747/pdi.2009.00092
114. Chiu CA, Lu LF, Yu TH, Hung WC, Chung FM, Tsai IT, et al. Increased levels of total P-Cresylsulphate and indoxyl sulphate are associated with coronary artery disease in patients with diabetic nephropathy. Rev Diabet Stud 2010;7:275-84. DOI: http://dx.doi.org/10.1900/RDS.2010.7.275
115. Barreto FC, Barreto DV, Liabeuf S, Meert N, Glorieux G, Temmar M, et al.; European Uremic Toxin Work Group (EUTox). Serum indoxyl sulfate is associated with vascular disease and mortality in chronic kidney disease patients. Clin J Am Soc Nephrol 2009;4:1551-8. DOI: http://dx.doi.org/10.2215/CJN.03980609
116. Wu IW, Hsu KH, Lee CC, Sun CY, Hsu HJ, Tsai CJ, et al. p-Cresyl sulphate and indoxyl sulphate predict progression of chronic kidney disease. Nephrol Dial Transplant 2010;26:938-47.
117. de Loor H, Bammens B, Evenepoel P, De Preter V, Verbeke K. Gas chromatographic-mass spectrometric analysis for measurement of p-cresol and its conjugated metabolites in uremic and normal serum. Clin Chem 2005;51:1535-8. DOI: http://dx.doi.org/10.1373/clinchem.2005.050781
118. Vanholder R, Van Laecke S, Glorieux G. What is new in uremic toxicity? Pediatr Nephrol 2008;23:1211-21.
119. Deguchi T, Ohtsuki S, Otagiri M, Takanaga H, Asaba H, Mori S, et al. Major role of organic anion transporter 3 in the transport of indoxyl sulfate in the kidney. Kidney Int 2002;61:1760-8. PMID: 11967025 DOI: http://dx.doi.org/10.1046/j.1523-1755.2002.00318.x
120. Tsutsumi Y, Deguchi T, Takano M, Takadate A, Lindup WE, Otagiri M. Renal disposition of a furan dicarboxylic acid and other uremic toxins in the rat. J Pharmacol Exp Ther 2002;303:880-7. DOI: http://dx.doi.org/10.1124/jpet.303.2.880
121. Enomoto A, Niwa T. Roles of organic anion transporters in the progression of chronic renal failure. Ther Apher Dial 2007;1:S27-31. DOI: http://dx.doi.org/10.1111/j.1744-9987.2007.00515.x
122. Miyamoto Y, Watanabe H, Noguchi T, Kotani S, Nakajima M, Kadowaki D, et al. Organic anion transporters play an important role in the uptake of p-cresyl sulfate, a uremic toxin, in the kidney. Nephrol Dial Transplant 2011;26:2498-502. DOI: http://dx.doi.org/10.1093/ndt/gfq785
123. Dou L, Cerini C, Brunet P, Guilianelli C, Moal V, Grau G, et al. P-cresol, a uremic toxin, decreases endothelial cell response to inflammatory cytokines. Kidney Int 2002;62:1999-2009. PMID: 12427124 DOI: http://dx.doi.org/10.1046/j.1523-1755.2002.t01-1-00651.x
124. Liabeuf S, Barreto DV, Barreto FC, Meert N, Glorieux G, Schepers E, et al.; European Uraemic Toxin Work Group (EUTox). Free p-cresylsulphate is a predictor of mortality in patients at different stages of chronic kidney disease. Nephrol Dial Transplant 2010;25:1183-91. DOI: http://dx.doi.org/10.1093/ndt/gfp592
125. Meijers BK, Van Kerckhoven S, Verbeke K, Dehaen W, Vanrenterghem Y, Hoylaerts MF, et al. The uremic retention solute p-cresyl sulfate and markers of endothelial damage. Am J Kidney Dis 2009;54:891-901. PMID: 19615803 DOI: http://dx.doi.org/10.1053/j.ajkd.2009.04.022
126. Schepers E, Meert N, Glorieux G, Goeman J, Van der Eycken J, Vanholder R. P-cresylsulphate, the main in vivo metabolite of p-cresol, activates leucocyte free radical production. Nephrol Dial Transplant 2007;22:592-6. DOI: http://dx.doi.org/10.1093/ndt/gfl584
127. Koppe L, Pillon NJ, Vella RE, Croze ML, Pelletier CC, Chambert S, et al. p-Cresyl sulfate promotes insulin resistance associated with CKD. J Am Soc Nephrol 2013;24:88-99. DOI: http://dx.doi.org/10.1681/ASN.2012050503
128. Brunet P, Dou L, Cerini C, Berland Y. Protein-bound uremic retention solutes. Adv Ren Replace Ther 2003;10:310-20. DOI: http://dx.doi.org/10.1053/j.arrt.2003.08.002
129. Marzocco S, Dal Piaz F, Di Micco L, Torraca S, Sirico ML, Tartaglia D, et al. Very low protein diet reduces indoxyl sulfate levels in chronic kidney disease. Blood Purif 2013;35:196-201. DOI: http://dx.doi.org/10.1159/000346628
130. Patel KP, Luo FJ, Plummer NS, Hostetter TH, Meyer TW. The production of p-cresol sulfate and indoxyl sulfate in vegetarians versus omnivores. Clin J Am Soc Nephrol 2012;7:982-8. DOI: http://dx.doi.org/10.2215/CJN.12491211
131. Meijers BK, De Preter V, Verbeke K, Vanrenterghem Y, Evenepoel P. p-Cresyl sulfate serum concentrations in haemodialysis patients are reduced by the prebiotic oligofructose-enriched inulin. Nephrol Dial Transplant 2010;25:219-24. DOI: http://dx.doi.org/10.1093/ndt/gfp414
132. Rossi M, Klein K, Johnson DW, Campbell KL. Pre-, pro-, and synbiotics: do they have a role in reducing uremic toxins? A systematic review and meta-analysis. Int J Nephrol 2012;2012:673631. PMID: 23316359
133. Schulman G, Agarwal R, Acharya M, Berl T, Blumenthal S, Kopyt N. A multicenter, randomized, double-blind, placebo-controlled, dose-ranging study of AST-120 (Kremezin) in patients with moderate to severe CKD. Am J Kidney Dis 2006;47:565-77. PMID: 16564934 DOI: http://dx.doi.org/10.1053/j.ajkd.2005.12.036

134. Shimizu H, Okada S, Shinsuke OI, Mori M. Kremezin (AST-120) delays the progression of diabetic nephropathy in Japanese type 2 diabetic patients. Diabetes Care 2005;28:2590. DOI: http://dx.doi.org/10.2337/diacare.28.10.2590
135. Ueda H, Shibahara N, Takagi S, Inoue T, Katsuoka Y. AST-120 treatment in pre-dialysis period affects the prognosis in patients on hemodialysis. Ren Fail 2008;30:856-60. DOI: http://dx.doi.org/10.1080/08860220802356531
136. Fujii H, Nishijima F, Goto S, Sugano M, Yamato H, Kitazawa R, et al. Oral charcoal adsorbent (AST-120) prevents progression of cardiac damage in chronic kidney disease through suppression of oxidative stress. Nephrol Dial Transplant 2009;24:2089-95. DOI: http://dx.doi.org/10.1093/ndt/gfp007
137. Iwasaki Y, Yamato H, Nii-Kono T, Fujieda A, Uchida M, Hosokawa A, et al. Administration of oral charcoal adsorbent (AST-120) suppresses low-turnover bone progression in uraemic rats. Nephrol Dial Transplant 2006;21:2768-74. DOI: http://dx.doi.org/10.1093/ndt/gfl311
138. Schulman G, Bel T, Beck GJ, Remuzzi G, Ritz E. EPPIC (Evaluating Prevention of Progression in Chronic Kidney Disease): Results from 2 Phase III, Randomized, Placebo-Controlled, Double-Blind Trials of AST-120 in Adults with CKD (Abstract). J Am Soc Nephrol 2012;23:7.
139. de Oliveira RB, Graciolli FG, dos Reis LM, Cancela AL, Cuppari L, Canziani ME, et al. Disturbances of Wnt/β-catenin pathway and energy metabolism in early CKD: effect of phosphate binders. Nephrol Dial Transplant 2013;28:2510-7. DOI: http://dx.doi.org/10.1093/ndt/gft234
140. Oliveira RB, Cancela AL, Graciolli FG, Dos Reis LM, Draibe SA, Cuppari L, et al. Early control of PTH and FGF23 in normophosphatemic CKD patients: a new target in CKD-MBD therapy? Clin J Am Soc Nephrol 2010;5:286-91.
141. Vlassara H, Uribarri J, Cai W, Goodman S, Pyzik R, Post J, et al. Effects of sevelamer on HbA1c, inflammation, and advanced glycation and products in diabetic kidney disease. Clin J Am Soc Nephrol 2012;7:934-42. DOI: http://dx.doi.org/10.2215/CJN.12891211
142. Guida B, Cataldi M, Riccio E, Grumetto L, Pota A, Borrelli S, et al. Plasma p-cresol lowering effect of sevelamer in peritoneal dialysis patients: evidence from a Cross-Sectional Observational Study. PLoS One 2013;8:e73558. DOI: http://dx.doi.org/10.1371/journal.pone.0073558
143. Evenepoel P, Bammens B, Verbeke K, Vanrenterghem Y. Acarbose treatment lowers generation and serum concentrations of the protein-bound solute p-cresol: a pilot study. Kidney Int 2006;70:192-8. PMID: 16688114 DOI: http://dx.doi.org/10.1038/sj.ki.5001523
144. Eknoyan G, Beck GJ, Cheung AK, Daugirdas JT, Greene T, Kusek JW, et al.; Hemodialysis (HEMO) Study Group. Hemodialysis (HEMO) Study Group. Effect of dialysis dose and membrane flux in maintenance hemodialysis. N Engl J Med 2002;347:2010-9. DOI: http://dx.doi.org/10.1056/NEJMoa021583
145. Cheung AK, Levin NW, Greene T, Agodoa L, Bailey J, Beck G, et al. Effects of high-flux hemodialysis on clinical outcomes: results of the HEMO study. J Am Soc Nephrol 2003;14:3251-63. DOI: http://dx.doi.org/10.1097/01.ASN.0000096373.13406.94
146. Locatelli F, Martin-Malo A, Hannedouche T, Loureiro A, Papadimitriou M, Wizemann V, et al.; Membrane Permeability Outcome (MPO) Study Group. Effect of membrane permeability on survival ofhemodialysis patients. J Am Soc Nephrol 2009;20:645-54. DOI: http://dx.doi.org/10.1681/ASN.2008060590
147. Ward RA, Schmidt B, Hullin J, Hillebrand GF, Samtleben W. A comparison of on-line hemodiafiltration and high-flux hemodialysis: a prospective clinical study. J Am Soc Nephrol 2000;11:2344-50.
148. Meert N, Eloot S, Waterloos MA, Van Landschoot M, Dhondt A, Glorieux G, et al. Effective removal of protein-bound uraemic solutes by different convective strategies: a prospective trial. Nephrol Dial Transplant 2009;24:562-70. DOI: http://dx.doi.org/10.1093/ndt/gfn522
149. Eloot S, Dhondt A, Van Landschoot M, Waterloos MA, Vanholder R. Removal of water-soluble and protein-bound solutes with reversed mid-dilution versus post-dilution haemodiafiltration. Nephrol Dial Transplant 2012;27:3278-83. DOI: http://dx.doi.org/10.1093/ndt/gfs060
150. Canaud B, Bragg-Gresham JL, Marshall MR, Desmeules S, Gillespie BW, Depner T, et al. Mortality risk for patients receiving hemodiafiltration versus hemodialysis: European results from the DOPPS. Kidney Int 2006;69:2087-93. PMID: 16641921 DOI: http://dx.doi.org/10.1038/sj.ki.5000447
151. Vilar E, Fry AC, Wellsted D, Tattersall JE, Greenwood RN, Farrington K. Long-term outcomes in online hemodiafiltration and high-flux hemodialysis: a comparative analysis. Clin J Am Soc Nephrol 2009;4:1944-53. DOI: http://dx.doi.org/10.2215/CJN.05560809
152. Grooteman MP, van den Dorpel MA, Bots ML, Penne EL, van der Weerd NC, Mazairac AH, et al.; CONTRAST Investigators. Effect of online hemodiafiltration on all--cause mortality and cardiovascular outcomes. J Am Soc Nephrol 2012;23:1087-96. DOI: http://dx.doi.org/10.1681/ASN.2011121140
153. Ok E, Asci G, Toz H, Ok ES, Kircelli F, Yilmaz M, et al.; Turkish Online Haemodiafiltration Study. Mortality and cardiovascular events in online haemodiafiltration (OL-HDF) compared with high-flux dialysis: results from the Turkish OL-HDF Study. Nephrol Dial Transplant 2013;28:192-202. DOI: http://dx.doi.org/10.1093/ndt/gfs407
154. Asci G, Tz H, Ozkahya M, Duman S, Demirci MS, Cirit M, et al.; EGE Study Group. The impact of membrane permeability and dialysate purity on cardiovascular outcomes. J Am Soc Nephrol 2013;24:1014-23. DOI: http://dx.doi.org/10.1681/ASN.2012090908
155. Maduell F, Moreso F, Pons M, Ramos R, Mora-Macià J, Carreras J, et al.; ESHOL Study Group. High--efficiency postdilution online hemodiafiltration reduces all-cause mortality in hemodialysis patients. J Am Soc Nephrol 2013;24:487-97. DOI: http://dx.doi.org/10.1681/ASN.2012080875
156. Neirynck N, Glorieux G, Schepers E, Pletinck A, Dhondt A,Vanholder R. Review of protein-bound toxins, possibility for blood purification therapy. Blood Purif 2013;35:45-50. DOI: http://dx.doi.org/10.1159/000346223
157. Meijers BK, Verhamme P, Nevens F, Hoylaerts MF, Bammens B, Wilmer A, et al. Major coagulation disturbances during fractionated plasma separation and adsorption. Am J Transplant 2007;7:2195-9. DOI: http://dx.doi.org/10.1111/j.1600-6143.2007.01909.x
158. Pham NM, Recht NS, Hostetter TH, Meyer TW. Removal of the protein-bound solutes indican and p-cresol sulfate by peritoneal dialysis. Clin J Am Soc Nephrol 2008;3:85-90. DOI: http://dx.doi.org/10.2215/CJN.02570607
159. Massy ZA, Barreto DV, Barreto FC, Vanholder R. Uraemic toxins for consideration by the cardiologist-Beyond traditional and non-traditional cardiovascular risk factors. Atherosclerosis 2010;211:381-3. PMID: 20471651 DOI: http://dx.doi.org/10.1016/j.atherosclerosis.2010.04.010