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The Roles of Indoxyl Sulphate and p-Cresyl Sulphate in Patients with Chronic Kidney Disease: A Review of Therapeutic Options

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Abstract

Indoxyl sulphate (IS) and p-cresyl sulphate (PCS) are products of proteolytic bacterial fermentation by gut microbiota. They accumulate in the sera of patients with chronic kidney disease (CKD) and have been associated with CKD progression and cardiovascular and all-cause mortality. Therapeutic strategies for lowering IS and PCS include increased clearance (enhanced dialysis), gastrointestinal sequestration (oral adsorbents), reduced synthesis (dietary protein restriction, dietary fibre augmentation and pre-, pro- or synbiotics), antioxidants and organic anion transporter modulators. This review will discuss the roles of IS and PCS as therapeutic targets and examine the clinical evidence for different treatment options and their effects on CKD and cardiovascular disease risk. We will include our group's research with pre-, pro- and synbiotic interventions to mitigate serum uraemic toxin accumulation and modify cardiovascular and renal risk.

Keywords: indoxyl sulphate, p-cresyl sulphate, uraemic toxins, chronic kidney disease, gut microbiome

1. Introduction

The reciprocal relationship observed between gut microbiota and chronic kidney disease (CKD) has led to the recent recognition of the 'gut-kidney axis'. Patients with CKD, including those with end-stage kidney disease (ESKD), often experience impaired uraemic toxin clearance, salt and water retention, dietary restrictions, anorexia, dysgeusia and malnutrition,

which in turn leads to quantitative and qualitative alterations in gut microbiome composition (gut dysbiosis). Further effects include gut wall oedema, intestinal barrier impairment, translocation of bacteria and endotoxins across the intestinal wall and resultant systemic inflammation [1–3]. Gut dysbiosis may in turn lead to the production of various toxins and metabolites that contribute to uraemic toxicity, cardiovascular disease and progressive kidney scarring and failure [4–6]. The central role of the gut microbiome in kidney health therefore makes it an appealing therapeutic target in patients with CKD [7, 8].

Two key nephrovascular toxins produced by proteolytic bacterial fermentation in the gut are indoxyl sulphate (IS) and p-cresyl sulphate (PCS). IS is produced by tryptophan metabolism facilitated by *Escherichia coli* and *Clostridium sporogenes*, while PCS is generated by break down of tyrosine and phenylalanine by intestinal anaerobes, such as *Clostridium difficile*, *Faecalibacterium prausnitzii*, *Subdoligranulum* and selected strains within the *Bifidobacterium* and *Lactobacillus* genus [8]. IS and PCS are both solely produced by the gut microbiota [9–12] and accumulate in the serum of patients with CKD due to both increased intestinal production and reduced glomerular filtration and proximal tubular secretion [12–14]. Elevated serum levels of IS and PCS have been reported to be associated with CKD progression [13] and increased risks of cardiovascular events and all-cause mortality [15].

Although IS and PCS levels can be lowered with various therapeutic modalities, how this impacts on the risks of mortality and cardiovascular outcomes remains unclear. This review will discuss the roles of IS and PCS as therapeutic targets and examine the clinical evidence for different treatment options and their effects on CKD and cardiovascular disease risk.

2. Serum IS and PCS levels are elevated in CKD

Serum IS and PCS levels have been demonstrated to be elevated in patients with CKD, where IS levels may be more than 50 times and PCS levels more than 15 times the levels of those found in healthy people [12, 14]. Our group has demonstrated that IS and PCS levels are significantly elevated in patients with early-stage CKD compared with control subjects. These levels were seen to be progressively more elevated with advancing severity of CKD [13]. Increased circulating levels of IS and PCS have also been observed in living kidney donors, which were sustained at 2 years post-surgery [16]. Levels of IS and PCS appear to be most elevated in ESKD and are not effectively removed by haemodialysis [14]. In a sample of 45 haemodialysis patients, Itoh et al. observed IS and PCS levels were markedly elevated (2.99 ± 0.18 mg/dL and 3.71 ± 0.28 mg/dL, respectively) compared with the healthy subjects (0.05 ± 0.01 mg/dL and 0.22 ± 0.99 mg/dL, respectively), and these levels were only lowered by approximately 30% post-dialysis (2.02 ± 0.12 mg/dL and 2.60 ± 0.21 mg/dL, respectively). This degree of elevation and inefficient removal warrants exploration of the potential impact of these toxins in CKD.

3. Serum IS and PCS levels are associated with adverse renal, metabolic and cardiovascular effects

3.1. Renal effects

Elevation of serum IS and PCS levels in patients with CKD is associated with CKD progression [17]. The mechanisms underpinning the adverse renal effects of IS and PCS are thought to be at least partly mediated by the production of reactive oxygen species, which in turn activate the nuclear factor kappa B pathway (NF κ B) (**Figure 1**) [18]. *In vitro* studies have demonstrated that pro-inflammatory cytokine release and plasminogen activator inhibitor-1 upregulation via the NF κ B pathway subsequently led to inhibition of cell proliferation and induction of renal tubulointerstitial fibrosis [4, 5]. These observations have been similarly replicated in animal models, whereby oral administration of IS [6, 19] and PCS [20] caused renal function impairment, glomerular sclerosis and tubulointerstitial fibrosis. IS and PCS have also been shown both *in vitro* and *in vivo* to activate the intrarenal renin-angiotensin-aldosterone system and promote renal tubular epithelial-to-mesenchymal transition, possibly via increased expression of transforming growth factor- β and Snail [21].

In a prospective, observational study of 268 patients with varying stages of CKD, Wu and colleagues demonstrated a significant association between higher IS (hazard ratio [HR] 1.06, 95% confidence interval [CI] 1.04–1.09, $p < 0.001$) and PCS levels (HR 1.09, 95% CI 1.06–1.13, $p < 0.001$) and CKD progression, defined as greater than 50% reduction in estimated glomerular filtration rate (eGFR) or progression to ESKD [17]. Serum PCS and IS remained independently associated with CKD progression after adjustment for patient demographic characteristics (age, gender, diabetes mellitus, $p < 0.001$) or baseline renal function ($p < 0.001$). Additionally, IS and PCS levels at baseline were significantly higher in those patients who died during follow-up (serum PCS 12.07 [<1 –42.06] mg/L vs. 4.1 [<1 –36.24] mg/L in survivors, $p = 0.002$; serum IS 4.78 [0.7–12.54] mg/L vs. 2.07 [<0.225 –53.58] mg/dL, $p = 0.05$). Elevated serum total PCS was also found to be significantly associated with all-cause mortality on univariable analysis (HR 1.10, 95% CI 1.05–1.15, $p < 0.001$) and remained a predictor of mortality independent of other risk factors on multivariable analysis adjusted for patient demographic characteristics, baseline renal function and biomarkers including highly sensitive C-reactive protein [17].

3.2. Metabolic effects

Elevated PCS has been associated with insulin resistance and may therefore predispose to the metabolic syndrome and its complications. In mouse models, the administration of PCS for 4 weeks has been observed to induce hyperglycaemia, insulin resistance, hypercholesterolaemia and fat redistribution to muscle and liver, similar to the metabolic derangements observed in CKD [22] (**Figure 1**). These metabolic effects appeared to be ameliorated by uraemic toxin-reducing therapy, as the use of the prebiotic agent, arabino-xylo-oligosaccharide, reduced serum PCS concentration and improved glucose tolerance, insulin resistance, dyslipidaemia and ectopic fat distribution in uraemic, subtotal nephrectomised mice [22].

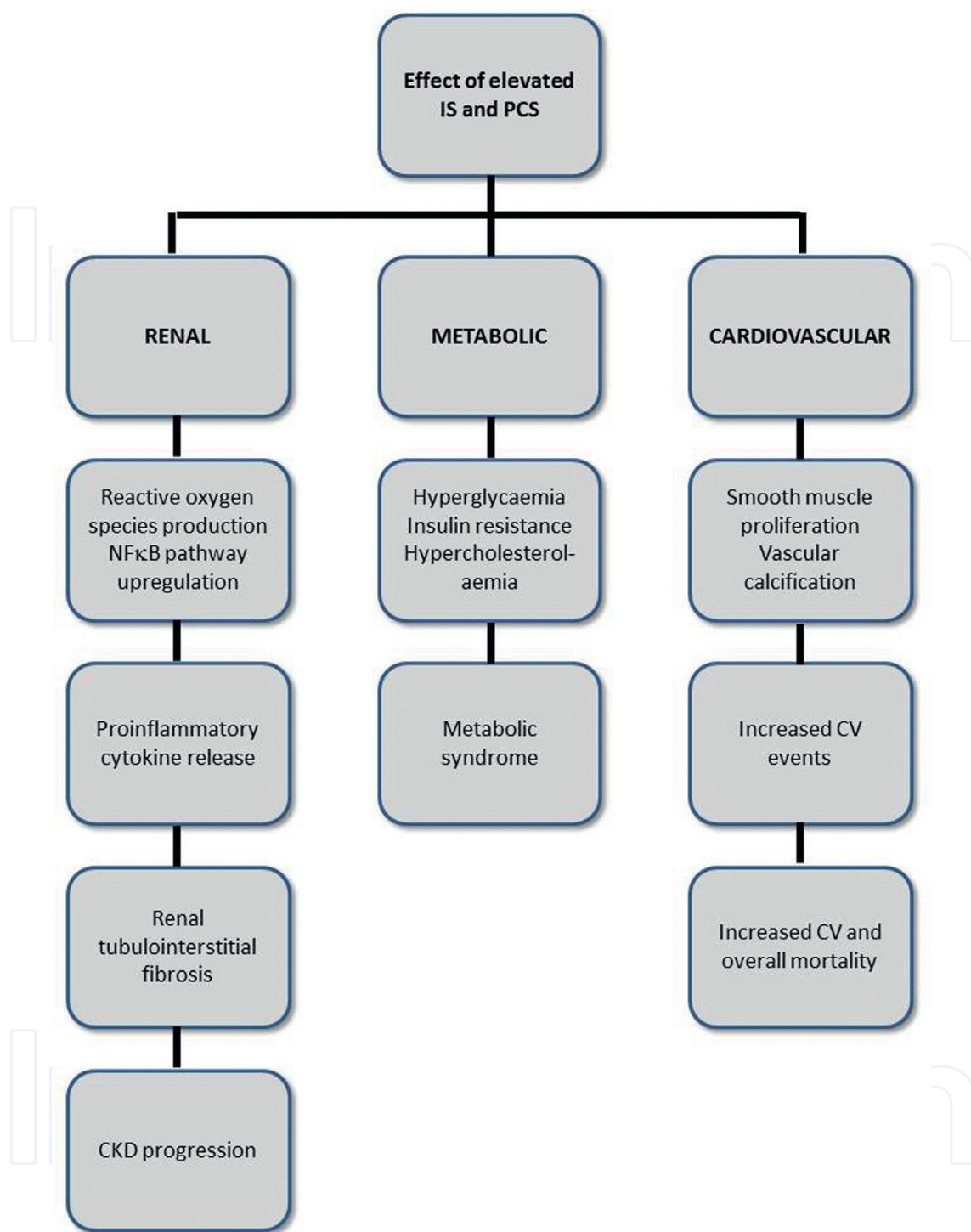


Figure 1. Mechanisms and potential effects of indoxyl sulphate (IS) and p-cresyl sulphate (PCS) on renal, metabolic and cardiovascular outcomes.

3.3. Cardiovascular effects and mortality

IS has been demonstrated to cause concentration-dependent vascular smooth muscle cell proliferation [23] and aortic calcification with aortic wall thickening in rats [6]. This appears to apply similarly to humans, such that elevated serum IS levels have been shown to be associated

with aortic calcification measured by multislice spiral computed tomography [24]. Likewise, total and free PCS levels have been linked with vascular disease [25]. Not surprisingly, elevated serum levels of both toxins have been reported to be predictors of cardiovascular events and mortality. Higher serum IS levels independently predicted overall mortality (HR 2.47, 95% CI 1.62–3.77), but not CV mortality, in 139 patients with stage 2–5 CKD participating in a study performed by the European Uraemic Toxin Work Group (EUTox) [24]. Similar results were reported in a prospective, observational cohort study of 521 US incident haemodialysis patients whereby serum IS concentrations above the median value of 1.6 mg/dL were independently associated with all-cause mortality (HR 1.30, 95% CI 1.01–1.69) after adjustment for age, sex, race, comorbidity score, baseline serum albumin, obesity and serum creatinine [26]. Elevated free PCS concentration has also been demonstrated to be an independent predictor of cardiovascular events [27, 28] and overall cardiovascular mortality [25] in CKD patients, including those ESKD receiving dialysis.

A meta-analysis by Lin and colleagues of 11 observational studies involving 1572 patients with stages 1–5 CKD followed for 0.83 to 5 years found that all-cause mortality was significantly associated with both free PCS (pooled odds ratio [OR] 1.16, 95% CI 1.03–1.30, $p = 0.013$) and free IS levels (pooled OR 1.10, 95% CI 1.03–1.17, $p = 0.03$) [15]. However, there was a moderate level of heterogeneity with I^2 values of 71.5% ($p = 0.004$), and 74.2% ($p = 0.004$) for PCS and IS, respectively. Furthermore, there was a concern about publication bias based on an asymmetrical funnel plot and significant Egger's test ($p = 0.005$). Following subsequent adjustment for the effect of publication bias, the adjusted point estimate of the OR reduced from 1.16 to 1.03 (95% CI 0.93–1.16), thereby raising concern about exaggeration of the observed effect size in the primary analysis. The study also reported a significantly increased risk of cardiovascular events with elevated levels of free PCS (pooled OR 1.28, 95% CI 1.10–1.50, $p = 0.002$), although this result was again limited by a high level of heterogeneity ($I^2 = 80.7\%$, $p < 0.001$). Furthermore, there was evidence of publication bias, such that when analysis was repeated using Duval and Tweedie's trim-and-fill method, the estimate was no longer statistically significant with an adjusted OR of 1.10 (95% CI 0.93–1.27).

4. Therapeutic opportunities for reducing serum IS and PCS levels

Given the numerous deleterious, multi-system effects that have been associated with elevated serum IS and PCS concentrations, much interest has been generated in developing therapeutic options to reduce the levels of these nephrovascular toxins with the aim of improving clinical outcomes in patients with CKD. Potential therapeutic strategies to reduce IS and PCS levels in patients with CKD may involve reducing gut synthesis, gastrointestinal sequestration, reduced proximal tubular retention and increased dialytic clearance (**Table 1**).

4.1. Reduced gut synthesis

Since increased dietary protein load can result in heightened generation of uraemic toxins by the gut microbiota, prescription of very low-protein diets has experienced a resurgence of interest. Marzocco and colleagues performed a post-hoc analysis of a very low vs. low-protein

Strategy	Intervention	Outcome
Reduced gut synthesis	Very low-protein diet [29]	Reduced serum IS levels
	Dietary fibre [10, 33, 34]	Reduced serum IS and PCS levels
	Pre-, pro- and synbiotics [35–41]	Reduced IS and PCS levels
Gastrointestinal sequestration	AST-120 (Kremezin) [51–56]	Reduced renal disease progression
	Ai Xi Te [54]	Reduced renal disease progression
	Niaoduqing granules [54]	Reduced renal disease progression
Reduced proximal tubular retention	OAT [^] modulators [11, 58, 61]	Reduced proximal tubular uptake of IS
Increased dialytic clearance	Extended dialysis (long dialysis, short daily dialysis) [65, 66]	No clear benefit
	Haemodiafiltration [67, 68]	Reduced serum IS and PCS levels
	Super-flux cellulose triacetate membranes [69]	Reduced serum IS levels
	Nanoporous monolith dialysis [70]	Reduced serum IS and PCS levels

[^]OAT: organic anion transporters

Table 1. Potential therapeutic interventions targeting indoxyl sulphate (IS) and p-cresyl sulphate (PCS).

diet cross-over study [29]. Thirty-two patients with a creatinine clearance between 20 and 55 ml/min were included and randomized to receive either a very low-protein diet (VLPD; 0.3 g/kg/day) or a low-protein diet (LPD; 0.6 g/kg/day) in the first week, then switched to the other in the second week. There was no wash-out period. The authors found that patients treated with a VLPD experienced a significant 36% reduction in serum IS levels compared with those treated with a LPD ($7.12 \pm 3.89 \mu\text{M}$ during VLPD vs. $11.1 \pm 6.6 \mu\text{M}$ during LPD, $p < 0.0001$). Although a meta-analysis has identified reduction in the occurrence of renal death with a low-protein intake in CKD patients, the overall value of these diets remains a subject of debate, given that the risks of malnutrition may present a greater danger [30–32]. Furthermore, poor compliance is also likely to be an issue, as participants often did not meet dietary targets even with the intensive support provided within a trial setting.

There is newer evidence to suggest that dietary fibre may in fact be more important than dietary protein intake in terms of managing uraemic toxin levels. A single-centre, cross-sectional study of 40 patients with CKD measured baseline total and free serum IS and PCS levels and correlated this with dietary factors including dietary fibre, protein and protein-fibre index [10]. In this study, dietary fibre was found to be inversely associated with free and total serum PCS ($r = -0.42$ and $r = -0.44$, both $p < 0.01$) whereas dietary protein was not ($r = -0.14$, $p = 0.38$). Protein-fibre index was significantly associated with both total PCS ($r = 0.43$, $p = 0.005$) and total IS ($r = 0.40$, $p = 0.012$) levels. Increased dietary fibre as an intervention has been shown to result in significantly reduced free plasma IS in haemodialysis patients [33]. Moreover, a prospective cohort study of 390 Swedish men between the age of 70 and 71 years found an association between protein-fibre intake ratio and cardiovascular events (adjusted HR 1.33, 95% CI 1.08–1.64). These findings suggest that dietary intervention focusing on protein-fibre ratio has the potential to influence clinical outcomes [34], mediated via uraemic toxin production.

Probiotics and prebiotics represent another strategy for reducing uraemic toxin synthesis. Preparations of lactic-acid bacteria, simulating a probiotic, have been shown to decrease serum IS concentrations by 30% and also reverse aerobic bacterial overgrowth [35]. Their use has been demonstrated to result in a significant decrease in urinary PCS and an increase in faecal bifidobacteria [36]. Synbiotics, which represent a combination of pre- and probiotics, have similarly been demonstrated to reduce serum IS and PCS levels in CKD and haemodialysis patients [37–39]. More recently, the use of synbiotics for reducing uraemic toxin levels has been evaluated in the SYNbiotics Easing Renal failure by improving Gut microbiology (SYNERGY) trial [40, 41]. In this single-centre, double-blind, placebo-controlled, cross-over trial, 37 pre-dialysis patients with stage 4 or 5 CKD were randomized to receive either synbiotic supplements or placebo for 6 weeks, followed by a 4-week wash-out period, followed by treatment with the alternative therapy for a further 6 weeks. Thirty-one participants completed both treatments. Although the study failed to demonstrate a significant change in total serum IS levels (−2 mmol/L, 95% CI −5 to 1 mmol/L, $p = 0.12$), the change in serum PCS levels did reach a level of statistical significance, with a 13% reduction in the treatment group (−14 mmol/L, 95% CI −27 to −2 mmol/L, $p = 0.03$). Furthermore, after excluding the 10 participants who had received antibiotic therapy during the trial, which is known to affect the balance of bacterial species in the gut [8, 42], the changes in serum levels with synbiotic therapy for both total IS (−5 mmol/L, 95% CI −8 to −1 mmol/L, $p = 0.03$) and PCS (−25 mmol/L, 95% CI −38 to −12 mmol/L, $p = 0.001$) were significant. The changes in free IS and PCS levels were also significant amongst antibiotic-free completers. Synbiotic therapy additionally had an effect on the stool microbiome, with significantly increased abundance of *Bifidobacterium* spp. (3.2%, $p = 0.003$) and *Lachnospiraceae* (2.1%, $p = 0.01$) and decreased abundance of *Ruminococcaceae* (4.3%, $p = 0.01$). Interestingly, albuminuria was observed to significantly increase with synbiotic therapy, which contradicted the reports of a beneficial effect on proteinuria from animal studies using other uraemic toxin-lowering therapies, such as AST-120 [43, 44]. Due to the short duration and small participant numbers of synbiotic trials to date, the effects of treatment on patient-level clinical outcomes remain unknown [2].

Lastly, the use of acarbose for lowering serum levels of gut-derived uraemic toxins has been investigated. Acarbose, an alpha-glucosidase inhibitor, causes increased delivery of undigested carbohydrate to the colon, which may drive gut bacterial fermentation towards a saccharolytic pathway and away from proteolytic fermentation and toxin production. In a pilot pre-test/post-test study involving nine healthy volunteers, Evanepoel et al. demonstrated that treatment with oral acarbose 300 mg per day for 3 weeks resulted in significant reductions in both serum p-cresol concentration (1.14–1.11 mg/L, $p = 0.047$) and urinary excretion of p-cresol (29.93–10.54 mg/day, $p = 0.03$), suggesting reduced colonic generation of p-cresol, the precursor of PCS [45]. Further studies confirming this finding are required.

4.2. Gastrointestinal sequestration

IS and PCS absorption from the gut may also be prevented by the use of oral intestinal adsorbents, such as AST-120 (Kremezin), which bind uraemic toxins and their precursors thereby sequestering them in the gut and allowing them to be excreted via the faeces. Oral administration of AST-120 has been shown to result in a dose-dependent decrease in serum IS and PCS

in both human [46–48] and animal studies [19, 49], and its use is associated with slower progression of renal dysfunction [44, 50] and reduction of proteinuria [43, 44] in animal models of CKD. It has also been demonstrated to slow progression of renal dysfunction in early non-randomized and randomized studies in pre-dialysis patients [51–53]. In a subsequent Cochrane systematic review and meta-analysis of eight randomized controlled trials (RCTs) of AST-120 plus routine care compared with routine care alone in patients with stages 1–5 (non-dialysis) CKD, Wu et al. [54] reported that AST-120 treatment resulted in a significant reduction in the rate of decline in creatinine clearance (2 studies, 486 participants; standardized mean difference [SMD] 0.39, 95% CI 0.21–0.57; $I^2 = 0\%$), but did not significantly affect reciprocal serum creatinine slope over time (2 studies, 76 participants; mean difference [MD] 0.07 dL/mg/month, 95% CI –0.12 to 0.26; $I^2 = 69\%$), doubling of serum creatinine concentration (1 study, 460 participants; relative risk [RR] 0.55, 95% CI 0.19 to 1.62), ESKD incidence (3 studies, 504 participants; RR 0.70, 95% CI 0.15–3.35; $I^2 = 11\%$) or all-cause mortality (1 study, 460 participants; RR 0.70, 95% CI 0.19–1.62). In three separate placebo-controlled RCTs, AST-120 treatment did not significantly affect changes in serum creatinine, slope of reciprocal serum creatinine over time or creatinine clearance [54].

In the following year, the Evaluating Prevention of Progression in CKD (EPPIC)-1 and EPPIC-2 trials [55] reported on the effects of AST-120 (9 g/day) or placebo on CKD progression in 2035 patients with non-dialysis-dependent CKD treated at 239 sites in 13 countries. No significant difference was observed in time to primary end point (composite of doubling of serum creatinine, dialysis initiation and kidney transplantation) between treatment arms (pooled analysis HR 0.97, 95% CI 0.83–1.12, $p = 0.64$). Furthermore, the treatment group did not experience any difference in proteinuria or quality of life compared with the placebo group. Similarly, a subsequent prospective, open-label, randomized controlled trial of 579 patients with stage 3 or 4 CKD from 11 Korean centres reported that oral administration of AST (6 g/day of AST-120 in 3 divided daily doses) did not significantly affect time to the primary composite outcome of doubling of serum creatinine, eGFR decrease $>50\%$, or initiation of renal replacement therapy (HR 1.12, 95% CI 0.85–1.48) [56]. There was no significant difference in change in serum IS levels over time between the intervention and control group ($p = 0.29$). The treatment also did not result in a significant difference in mortality, health-related quality of life or serious adverse effects.

A Cochrane systematic review of alternative oral adsorbents, Ai Xi Te and Niaoduqing granules, reported positive effects on CKD progression, but were limited by small samples sizes and poor methodologic quality with unclear or high risks of bias [54].

4.3. Reduced proximal tubular retention

Renal proximal tubular cells contain multiple transporters that perform basolateral uptake or luminal excretion of various substances, including uraemic toxins. Such transporters include the organic anion transporters (OAT)1, OAT3 and OATP4C1, as well as the organic cation transporter (OCT)2, the multidrug and toxin extrusion proteins (MATEs), the breast cancer resistance protein (BCRP) and the adenosine triphosphate (ATP)-binding cassette transporter family [57]. Anionic substances, such as IS, enter renal proximal tubule cells via basolateral

OAT, particularly OAT1 and OAT3, and are excreted into the tubular lumen by luminal OATs [58, 59]. Using cultured kidney tubule cells (LLC-PK1) and rat kidney slices, Deguchi et al. demonstrated that p-aminohippurate (OAT1 inhibitor), pravastatin (OAT3 inhibitor) and benzylpenicillin (OAT3 inhibitor) inhibited the renal tubular uptake of indoxyl sulphate to comparable extents [60]. In a 5/6-nephrectomized rat model of CKD, Enomoto et al. demonstrated that administration of IS resulted in IS accumulation in proximal tubule cells expressing OAT1 and OAT3, and was associated with more rapid CKD progression, as measured by creatinine clearance [58]. Furthermore, addition of IS to cultured rat proximal tubule (S2) cells reduced their viability, although this nephrotoxicity was abrogated by administration of the OAT1 inhibitor, probenecid [58]. Thus, OAT inhibitors, such as probenecid and statins, might be a potential strategy for preventing proximal tubule cell accumulation of IS and ensuing nephrotoxicity and CKD progression. In addition, as OATs are expressed widely throughout the body, these transporters may play a role in uraemic toxin-induced pathology in various organs. For example, Liu and colleagues demonstrated that administration of 10 μ M IS to cultured Sprague-Dawley cardiac myocytes and fibroblasts stimulated myocyte hypertrophy and collagen synthesis, which was abrogated by probenecid (OAT1 antagonist) and cilastatin (OAT3 antagonist) [61].

Therapeutic manipulation of efflux transporters, such as OAT polypeptide 4C1 (SLCO4C1), may also lead to enhanced excretion of uraemic retention solutes into the urine [62]. For example, Toyohara et al. demonstrated that overexpression of SLCO4C1 in rat kidney decreased plasma levels of uraemic toxins and reduced inflammation, hypertension and cardiomegaly [11]. Moreover, renal clearance of uraemic toxins was also increased by pravastatin, which is known to upregulate proximal tubular SLCO4C1 [11].

The activities of multidrug resistance protein (MRP) 4 and BRCP efflux transporters have also been demonstrated to be downregulated by PCS *in vitro* [63] and may be potential therapeutic targets.

4.4. Increased dialytic clearance

IS and PCS are highly (>90%) protein bound and are therefore not easily removed with conventional haemodialysis and peritoneal dialysis [14, 59, 64]. Long dialysis, short daily dialysis and high-flux haemodialysis have been investigated as potential methods of improving clearance of protein-bound molecules, but have failed to show clear benefit [59, 65, 66].

In contrast to conventional haemodialysis, which mainly depends on diffusion to clear solutes, haemodiafiltration combines convection and diffusion, which is potentially very useful in facilitating removal of larger molecules, such as protein-bound solutes. Haemodiafiltration has been shown in prospective cross-over studies to be superior to high-flux haemodialysis in removing IS and PCS [67, 68]. In this respect, the effectiveness of pre- and post-dilution haemodiafiltration was comparable [67, 68]. The mechanism for the improved clearance of protein-bound solutes is not well understood but seemed to be dependent on a combination of both diffusion and convection since haemofiltration (which does not involve diffusion) reduced the serum levels of protein-bound solutes but not to the same extent as haemodiafiltration [67].

The use of super-flux cellulose triacetate membranes has also been evaluated and found to be superior to low-flux haemodialysis with respect to removing IS and most protein-bound compounds, although this might be at least partly explained by an increase in removal of albumin [69]. Similarly, dialysis with the use of a nanoporous carbon monolith (pores 2–100 nm) was able to almost completely remove IS and PCS, whereas the use of a microporous monolith (<2 nm) resulted in only partial removal, and standard high-flux haemodialysis resulted in insignificant removal [70]. A potential issue with enhanced dialysis of toxins is the rebound release of further toxins from tissues, which is observed with water-based solutes. However, Martinez and colleagues demonstrated that the rebound movement of PCS and protein solutes in the first 30 minutes post-dialysis appeared to be negligible [71].

Eloot and colleagues utilised kinetic modelling to try to determine optimal dialysis parameters to facilitate protein-bound solute removal, and found that regardless of longer or more frequent dialysis, increased volume of blood processing per week was required to increase clearance [72].

In a cross-over study of 14 patients, high-clearance dialysis (high dialysate flow rate and large dialyzer) resulted in significantly greater PCS and IS clearance compared with low-clearance dialysis (PCS 23 ± 4 ml/min vs. 12 ± 3 ml/min, $p < 0.001$; IS 30 ± 5 ml/min vs. 17 ± 4 ml/min, $p < 0.001$). However, there was no significant change in serum PCS levels with high-clearance dialysis although there was a significant decrease in IS levels [73]. The authors suggested that this lack of reduction in serum PCS levels may be due to concurrent PCS generation, and thus treatment to suppress PCS production would be required in order to achieve significant reductions in serum PCS.

5. Summary and future directions

In summary, IS and PCS are products of bacterial metabolism within the gut. Serum IS and PCS levels are increased in patients with CKD and have been associated with CKD progression, vascular disease acceleration, adverse metabolic profile and poorer cardiovascular and overall mortality. There are several methods of lowering serum IS and PCS levels, including reduced intestinal bacterial production through dietary modification of protein and/or fibre intake or pre-, pro- and synbiotic use, gastrointestinal sequestration through oral adsorbent use, reduced cellular uptake of IS through OAT inhibition, and increased clearance through enhanced dialysis. Though these treatments have been shown in some studies to successfully reduce IS and PCS levels in sera and/or cells, it is less clear whether this translates into meaningful and sustained improvements in clinical outcomes. The studies conducted to date have been limited by small patient numbers, relatively short follow-up duration and poor methodologic quality. Given the biological plausibility and clinical importance of the adverse health outcomes thought to be mediated by these toxins, further high-quality studies are needed to evaluate the short- and long-term effects of IS and PCS lowering treatments on patient-level clinical outcomes.

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References

- [1] Mafra D, Barros AF, Fouque D. Dietary protein metabolism by gut microbiota and its consequences for chronic kidney disease patients. *Future Microbiology*. 2013;**8**(10):1317-1323
- [2] Vaziri ND. Effect of synbiotic therapy on Gut-Derived uremic toxins and the intestinal microbiome in patients with CKD. *Clinical Journal of the American Society of Nephrology*. 2016;**11**(2):199-201
- [3] Vaziri ND, Yuan J, Rahimi A, Ni Z, Said H, Subramanian VS. Disintegration of colonic epithelial tight junction in uremia: A likely cause of CKD-associated inflammation. *Nephrology Dialysis Transplantation*. 2012;**27**(7):2686-2693
- [4] Shimizu H, Bolati D, Adijiang A, Enomoto A, Nishijima F, Dateki M, Niwa T. Senescence and dysfunction of proximal tubular cells are associated with activated p53 expression by indoxyl sulfate. *American Journal of Physiology Cell Physiology*. 2010;**299**(5):C1110-C1117
- [5] Shimizu H, Bolati D, Adijiang A, Muteliefu G, Enomoto A, Nishijima F, Dateki M, Niwa T. NF-kappaB plays an important role in indoxyl sulfate-induced cellular senescence, fibrotic gene expression, and inhibition of proliferation in proximal tubular cells. *American journal of physiology Cell Physiology*. 2011;**301**(5):C1201-C1212
- [6] Adijiang A, Goto S, Uramoto S, Nishijima F, Niwa T. Indoxyl sulphate promotes aortic calcification with expression of osteoblast-specific proteins in hypertensive rats. *Nephrology Dialysis Transplantation*. 2008;**23**(6):1892-1901
- [7] Evenepoel P, Meijers BK, Bammens BR, Verbeke K. Uremic toxins originating from colonic microbial metabolism. *Kidney International Supplements*. 2009;**76**(114):S12-S19
- [8] Briskey D, Tucker P, Johnson DW, Coombes JS. The role of the gastrointestinal tract and microbiota on uremic toxins and chronic kidney disease development. *Clin Exp Nephrol*. 2017 Feb;**21**(1):7-15. DOI: 10.1007/s10157-016-1255-y. pub 2016 Mar 10. Review. PMID: 26965149

- [9] Meijers BK Evenepoel P. The gut-kidney axis: Indoxyl sulfate, p-cresyl sulfate and CKD progression. *Nephrology Dialysis Transplantation*. 2011;**26**(3):759-761
- [10] Rossi M, Johnson DW, Xu H, Carrero JJ, Pascoe E, French C, Campbell KL. Dietary protein-fiber ratio associates with circulating levels of indoxyl sulfate and p-cresyl sulfate in chronic kidney disease patients. *Nutrition, Metabolism & Cardiovascular Diseases*. 2015;**25**(9):860-865
- [11] Toyohara T, Suzuki T, Morimoto R, Akiyama Y, Souma T, Shiwaku HO, Takeuchi Y, Mishima E, Abe M, Tanemoto M, Masuda S, Kawano H, Maemura K, Nakayama M, Sato H, Mikkaichi T, Yamaguchi H, Fukui S, Fukumoto Y, Shimokawa H, Inui K, Terasaki T, Goto J, Ito S, Hishinuma T, Rubera I, Tauc M, Fujii-Kuriyama Y, Yabuuchi H, Moriyama Y, Soga T, Abe T. SLCO4C1 transporter eliminates uremic toxins and attenuates hypertension and renal inflammation. *Journal of the American Society of Nephrology*. 2009;**20**(12):2546-2555
- [12] Vanholder R, De Smet R, Glorieux G, Argiles A, Baurmeister U, Brunet P, Clark W, Cohen G, De Deyn PP, Deppisch R, Descamps-Latscha B, Henle T, Jorres A, Lemke HD, Massy ZA, Passlick-Deetjen J, Rodriguez M, Stegmayr B, Stenvinkel P, Tetta C, Wanner C, Zidek W. Review on uremic toxins: Classification, concentration, and interindividual variability. *Kidney International*. 2003;**63**(5):1934-1943
- [13] Rossi M, Campbell K, Johnson D, Stanton T, Pascoe E, Hawley C, Dimeski G, McWhinney B, Ungerer J, Isbel N. Uraemic toxins and cardiovascular disease across the chronic kidney disease spectrum: An observational study. *Nutrition, Metabolism & Cardiovascular Diseases*. 2014;**24**(9):1035-1042
- [14] Itoh Y, Ezawa A, Kikuchi K, Tsuruta Y, Niwa T. Protein-bound uremic toxins in hemodialysis patients measured by liquid chromatography/tandem mass spectrometry and their effects on endothelial ROS production. *Analytical and Bioanalytical Chemistry*. 2012;**403**(7):1841-1850
- [15] Lin CJ, Wu V, Wu PC, Wu CJ. Meta-Analysis of the associations of p-Cresyl Sulfate (PCS) and indoxyl sulfate (IS) with cardiovascular events and All-Cause mortality in patients with chronic renal failure. *PLoS One*. 2015;**10**(7):e0132589
- [16] Rossi M, Campbell KL, Johnson DW, Stanton T, Haluska BA, Hawley CM, Dimeski G, McWhinney BC, Ungerer JP, Kaisar OM, Isbel NM. Uremic toxin development in living kidney donors: A longitudinal study. *Transplantation*. 2014;**97**(5):548-554
- [17] Wu IW, Hsu KH, Lee CC, Sun CY, Hsu HJ, Tsai CJ, Tzen CY, Wang YC, Lin CY, Wu MS. p-Cresyl sulphate and indoxyl sulphate predict progression of chronic kidney disease. *Nephrology Dialysis Transplantation*. 2011;**26**(3):938-947
- [18] Motojima M, Hosokawa A, Yamato H, Muraki T, Yoshioka T. Uremic toxins of organic anions up-regulate PAI-1 expression by induction of NF-kappaB and free radical in proximal tubular cells. *Kidney International*. 2003;**63**(5):1671-1680
- [19] Niwa T, Ise M. Indoxyl sulfate, a circulating uremic toxin, stimulates the progression of glomerular sclerosis. *Journal of Laboratory and Clinical Medicine* 1994;**124**(1):96-104

- [20] Watanabe H, Miyamoto Y, Honda D, Tanaka H, Wu Q, Endo M, Noguchi T, Kadowaki D, Ishima Y, Kotani S, Nakajima M, Kataoka K, Kim-Mitsuyama S, Tanaka M, Fukagawa M, Otagiri M, Maruyama T. p-Cresyl sulfate causes renal tubular cell damage by inducing oxidative stress by activation of NADPH oxidase. *Kidney International*. 2013;**83**(4):582-592
- [21] Sun CY, Chang SC, Wu MS. Uremic toxins induce kidney fibrosis by activating intrarenal renin-angiotensin-aldosterone system associated epithelial-to-mesenchymal transition. *PLoS One*. 2012;**7**(3):e34026
- [22] Koppe L, Pillon NJ, Vella RE, Croze ML, Pelletier CC, Chambert S, Massy Z, Glorieux G, Vanholder R, Dugenet Y, Soula HA, Fouque D, Soulage CO. p-Cresyl sulfate promotes insulin resistance associated with CKD. *Journal of the American Society of Nephrology*. 2013;**24**(1):88-99
- [23] Yamamoto H, Tsuruoka S, Ioka T, Ando H, Ito C, Akimoto T, Fujimura A, Asano Y, Kusano E. Indoxyl sulfate stimulates proliferation of rat vascular smooth muscle cells. *Kidney International*. 2006;**69**(10):1780-1785
- [24] Barreto FC, Barreto DV, Liabeuf S, Meert N, Glorieux G, Temmar M, Choukroun G, Vanholder R, Massy ZA. Serum indoxyl sulfate is associated with vascular disease and mortality in chronic kidney disease patients. *Clinical Journal of the American Society of Nephrology*. 2009;**4**(10):1551-1558
- [25] Liabeuf S, Barreto DV, Barreto FC, Meert N, Glorieux G, Schepers E, Temmar M, Choukroun G, Vanholder R, Massy ZA. Free p-cresylsulphate is a predictor of mortality in patients at different stages of chronic kidney disease. *Nephrology Dialysis Transplantation*. 2010;**25**(4):1183-1191
- [26] Melamed ML, Plantinga L, Shafi T, Parekh R, Meyer TW, Hostetter TH, Coresh J, Powe NR. Retained organic solutes, patient characteristics and all-cause and cardiovascular mortality in hemodialysis: Results from the retained organic solutes and clinical outcomes (ROSCO) investigators. *BMC Nephrology*. 2013;**14**:134
- [27] Meijers BK, Claes K, Bammens B, de Loor H, Viaene L, Verbeke K, Kuypers D, Vanrenterghem Y, Evenepoel P. p-Cresol and cardiovascular risk in mild-to-moderate kidney disease. *Clinical Journal of the American Society of Nephrology*. 2010;**5**(7):1182-1189
- [28] Bammens B, Evenepoel P, Keuleers H, Verbeke K, Vanrenterghem Y. Free serum concentrations of the protein-bound retention solute p-cresol predict mortality in hemodialysis patients. *Kidney International*. 2006;**69**(6):1081-1087
- [29] Marzocco S, Dal Piaz F, Di Micco L, Torraca S, Sirico ML, Tartaglia D, Autore G, Di Iorio B. Very low protein diet reduces indoxyl sulfate levels in chronic kidney disease. *Blood Purification*. 2013;**35**(1-3):196-201
- [30] Fouque D, Laville M. Low protein diets for chronic kidney disease in non diabetic adults. *Cochrane Database Syst Rev*. 2009 Jul 8;**3**:CD001892. DOI: 10.1002/14651858.CD001892.pub3. Review. PMID: 19588328
- [31] Johnson DW. Dietary protein restriction as a treatment for slowing chronic kidney disease progression: The case against. *Nephrology (Carlton)*. 2006;**11**(1):58-62

- [32] Johnson DW, Atai E, Chan M, Phoon RK, Scott C, Toussaint ND, Turner GL, Usherwood T, Wiggins KJ. KHA-CARI guideline: Early chronic kidney disease: Detection, prevention and management. *Nephrology (Carlton)*. 2013;**18**(5):340-350
- [33] Sirich TL, Plummer NS, Gardner CD, Hostetter TH, Meyer TW. Effect of increasing dietary fiber on plasma levels of colon-derived solutes in hemodialysis patients. *Clinical Journal of the American Society of Nephrology*. 2014;**9**(9):1603-1610
- [34] Xu H, Rossi M, Campbell KL, Sencion GL, Arnlov J, Cederholm T, Sjogren P, Riserus U, Lindholm B, Carrero JJ. Excess protein intake relative to fiber and cardiovascular events in elderly men with chronic kidney disease. *Nutrition, Metabolism & Cardiovascular Diseases*. 2016;**26**(7):597-602
- [35] Hida M, Aiba Y, Sawamura S, Suzuki N, Satoh T, Koga Y. Inhibition of the accumulation of uremic toxins in the blood and their precursors in the feces after oral administration of Lebenin, a lactic acid bacteria preparation, to uremic patients undergoing hemodialysis. *Nephron*. 1996;**74**(2):349-355
- [36] Francois IE, Lescroart O, Veraverbeke WS, Marzorati M, Possemiers S, Evenepoel P, Hamer H, Houben E, Windey K, Welling GW, Delcour JA, Courtin CM, Verbeke K, Broekaert WF. Effects of a wheat bran extract containing arabinoxylan oligosaccharides on gastrointestinal health parameters in healthy adult human volunteers: A double-blind, randomised, placebo-controlled, cross-over trial. *British Journal of Nutrition*. 2012;**108**(12):2229-2242
- [37] Nakabayashi I, Nakamura M, Kawakami K, Ohta T, Kato I, Uchida K, Yoshida M. Effects of synbiotic treatment on serum level of p-cresol in haemodialysis patients: A preliminary study. *Nephrology, Dialysis, Transplantation*. 2011;**26**(3):1094-1048
- [38] Guida B, Germano R, Trio R, Russo D, Memoli B, Grumetto L, Barbato F, Cataldi M. Effect of short-term synbiotic treatment on plasma p-cresol levels in patients with chronic renal failure: A randomized clinical trial. *Nutrition, Metabolism & Cardiovascular Diseases*. 2014;**24**(9):1043-1049
- [39] 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. *International Journal of Nephrology*. 2012;**2012**:673631
- [40] Rossi M, Johnson DW, Morrison M, Pascoe E, Coombes JS, Forbes JM, McWhinney BC, Ungerer JP, Dimeski G, Campbell KL. SYNbiotics easing renal failure by improving gut microbiology (SYNERGY): A protocol of placebo-controlled randomised cross-over trial. *BMC Nephrology*. 2014;**15**:106
- [41] Rossi M, Johnson DW, Morrison M, Pascoe EM, Coombes JS, Forbes JM, Szeto CC, McWhinney BC, Ungerer JP, Campbell KL. Synbiotics easing renal failure by improving gut microbiology (SYNERGY): A randomized trial. *Clinical Journal of the American Society of Nephrology*. 2016;**11**(2):223-231
- [42] Buffie CG, Jarchum I, Equinda M, Lipuma L, Gobourne A, Viale A, Ubeda C, Xavier J, Pamer EG. Profound alterations of intestinal microbiota following a single dose of

- clindamycin results in sustained susceptibility to *Clostridium difficile*-induced colitis. *Infection and Immunity*. 2012;**80**(1):62-73
- [43] Okada K, Takahashi S, Nagura Y, Hatano M, Shimamura T. Early morphological changes of tubules in rats with chronic renal failure. *Nihon Jinzo Gakkai Shi*. 1992;**34**(1):65-70
- [44] Horike K, Usami T, Kamiya Y, Kamiya T, Yoshida A, Itoh S, Yamato H, Ise M, Kimura G. Oral carbonaceous adsorbent modifies renal function of renal ablation model without affecting plasma renin-angiotensin system or protein intake. *Clinical and Experimental Nephrology*. 2003;**7**(2):120-124
- [45] 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 International*. 2006;**70**(1):192-198
- [46] 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. *American Journal of Kidney Diseases*. 2006;**47**(4):565-577
- [47] Wu IW, Hsu KH, Sun CY, Tsai CJ, Wu MS, Lee CC. Oral adsorbent AST-120 potentiates the effect of erythropoietin-stimulating agents on Stage 5 chronic kidney disease patients: A randomized crossover study. *Nephrology, Dialysis, Transplantation*. 2014;**29**(9):1719-1727
- [48] Yamamoto S, Kazama JJ, Omori K, Matsuo K, Takahashi Y, Kawamura K, Matsuto T, Watanabe H, Maruyama T, Narita I. Continuous reduction of Protein-Bound uraemic toxins with improved oxidative stress by using the oral charcoal adsorbent AST-120 in haemodialysis patients. *Scientific Reports*. 2015;**5**:14381
- [49] Kikuchi K, Itoh Y, Tateoka R, Ezawa A, Murakami K, Niwa T. Metabolomic search for uremic toxins as indicators of the effect of an oral sorbent AST-120 by liquid chromatography/tandem mass spectrometry. *Journal of Chromatography B*. 2010;**878**(29):2997-3002
- [50] Miyazaki T, Aoyama I, Ise M, Seo H, Niwa T. An oral sorbent reduces overload of indoxyl sulphate and gene expression of TGF-beta1 in uraemic rat kidneys. *Nephrology, Dialysis, Transplantation*. 2000;**15**(11):1773-1781
- [51] Takahashi N, Kawaguchi T, Suzuki T. Therapeutic effects of long-term administration of an oral adsorbent in patients with chronic renal failure: Two-year study. *International Journal of Urology*. 2005;**12**(1):7-11
- [52] Sanaka T, Akizawa T, Koide K, Koshikawa S. Protective effect of an oral adsorbent on renal function in chronic renal failure: Determinants of its efficacy in diabetic nephropathy. *Therapeutic Apheresis and Dialysis*. 2004;**8**(3):232-240
- [53] Shoji T, Wada A, Inoue K, Hayashi D, Tomida K, Furumatsu Y, Kaneko T, Okada N, Fukuhara Y, Imai E, Tsubakihara Y. Prospective randomized study evaluating the efficacy of the spherical adsorptive carbon AST-120 in chronic kidney disease patients with moderate decrease in renal function. *Nephron Clinical Practice*. 2007;**105**(3):c99-c107

- [54] Wu HM, Sun HJ, Wang F, Yang M, Dong BR, Liu GJ. Oral adsorbents for preventing or delaying the progression of chronic kidney disease. *Cochrane Database Syst Rev*. 2014 Oct 15;(10):CD007861. DOI: 10.1002/14651858.CD007861.pub2. Review. PMID: 25317905
- [55] Schulman G, Berl T, Beck GJ, Remuzzi G, Ritz E, Arita K, Kato A, Shimizu M. Randomized Placebo-Controlled EPPIC trials of AST-120 in CKD. *Journal of the American Society of Nephrology*. 2015;**26**(7):1732-1746
- [56] Cha RH, Kang SW, Park CW, Cha DR, Na KY, Kim SG, Yoon SA, Han SY, Chang JH, Park SK, Lim CS, Kim YS. A Randomized, controlled trial of oral intestinal sorbent AST-120 on renal function deterioration in patients with advanced renal dysfunction. *Clinical Journal of the American Society of Nephrology*. 2016;**11**(4):559-567
- [57] Masereeuw R, Mutsaers HA, Toyohara T, Abe T, Jhawar S, Sweet DH, Lowenstein J. The kidney and uremic toxin removal: Glomerulus or tubule? *Seminars in Nephrology*. 2014;**34**(2):191-208
- [58] Enomoto A, Takeda M, Tojo A, Sekine T, Cha SH, Khamdang S, Takayama F, Aoyama I, Nakamura S, Endou H, Niwa T. Role of organic anion transporters in the tubular transport of indoxyl sulfate and the induction of its nephrotoxicity. *Journal of the American Society of Nephrology*. 2002;**13**(7):1711-1720
- [59] Neiryneck N, Glorieux G, Schepers E, Pletinck A, Dhondt A, Vanholder R. Review of protein-bound toxins, possibility for blood purification therapy. *Blood Purification*, 2013;**35**(Suppl 1):45-50
- [60] Deguchi T, Kusuvara H, Takadate A, Endou H, Otagiri M, Sugiyama Y. Characterization of uremic toxin transport by organic anion transporters in the kidney. *Kidney International*. 2004;**65**(1):162-174
- [61] Liu S, Wang BH, Kompa AR, Lekawanvijit S, Krum H. Antagonists of organic anion transporters 1 and 3 ameliorate adverse cardiac remodelling induced by uremic toxin indoxyl sulfate. *International Journal of Cardiology*. 2012;**158**(3):457-458
- [62] Glorieux G, Tattersall J. Uraemic toxins and new methods to control their accumulation: Game changers for the concept of dialysis adequacy. *Clinical Kidney Journal*. 2015;**8**(4):353-362
- [63] Mutsaers HA, Caetano-Pinto P, Seegers AE, Dankers AC, van den Broek PH, Wetzels JF, van den Brand JA, van den Heuvel LP, Hoenderop JG, Wilmer MJ, Masereeuw R. Proximal tubular efflux transporters involved in renal excretion of p-cresyl sulfate and p-cresyl glucuronide: Implications for chronic kidney disease pathophysiology. *Toxicology In Vitro*. 2015;**29**(7):1868-1877
- [64] Viaene L, Annaert P, de Loo H, Poesen R, Evenepoel P, Meijers B. Albumin is the main plasma binding protein for indoxyl sulfate and p-cresyl sulfate. *Biopharmaceutics & Drug Disposition*. 2013;**34**(3):165-175
- [65] Fagugli RM, De Smet R, Buoncristiani U, Lameire N, Vanholder R. Behavior of non-protein-bound and protein-bound uremic solutes during daily hemodialysis. *American Journal of Kidney Diseases*. 2002;**40**(2):339-347

- [66] Basile C, Libutti P, Di Turo AL, Casino FG, Vernaglion L, Tundo S, Maselli P, De Nicolo EV, Ceci E, Teutonico A, Lomonte C. Removal of uraemic retention solutes in standard bicarbonate haemodialysis and long-hour slow-flow bicarbonate haemodialysis. *Nephrology, Dialysis, Transplantation*. 2011;**26**(4):1296-1303
- [67] Meert N, Eloot S, Schepers E, Lemke HD, Dhondt A, Glorieux G, Van Landschoot M, Waterloos MA, Vanholder R. Comparison of removal capacity of two consecutive generations of high-flux dialysers during different treatment modalities. *Nephrology, Dialysis, Transplantation*. 2011;**26**(8):2624-2630
- [68] Meert N, Eloot S, Waterloos MA, Van Landschoot M, Dhondt A, Glorieux G, Ledebø I, Vanholder R. Effective removal of protein-bound uraemic solutes by different convective strategies: A prospective trial. *Nephrology, Dialysis, Transplantation*. 2009;**24**(2):562-570
- [69] De Smet R, Dhondt A, Eloot S, Galli F, Waterloos MA, Vanholder R. Effect of the super-flux cellulose triacetate dialyser membrane on the removal of non-protein-bound and protein-bound uraemic solutes. *Nephrology, Dialysis, Transplantation*. 2007;**22**(7):2006-2012
- [70] Sandeman SR, Howell CA, Phillips GJ, Zheng Y, Standen G, Pletzenauer R, Davenport A, Basnayake K, Boyd O, Holt S, Mikhalovsky SV. An adsorbent monolith device to augment the removal of uraemic toxins during haemodialysis. *Journal of Materials Science: Materials in Medicine*. 2014;**25**(6):1589-1597
- [71] Martinez AW, Recht NS, Hostetter TH, Meyer TW. Removal of P-cresol sulfate by hemodialysis. *Journal of the American Society of Nephrology*. 2005;**16**(11):3430-3436
- [72] Eloot S, Schneditz D, Cornelis T, Van Biesen W, Glorieux G, Dhondt A, Kooman J, Vanholder R. Protein-Bound uremic toxin profiling as a tool to optimize hemodialysis. *PLoS One*. 2016;**11**(1):e0147159
- [73] Camacho O, Rosales MC, Shafi T, Fullman J, Plummer NS, Meyer TW, Sirich TL. Effect of a sustained difference in hemodialytic clearance on the plasma levels of p-cresol sulfate and indoxyl sulfate. *Nephrology, Dialysis, Transplantation*. 2016;**31**(8):1335-1341

