

REVIEW

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# Removal of uremic toxins by renal replacement therapies: a review of current progress and future perspectives

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## Abstract

Accumulation of uremic toxins induces various uremia-related complications in patients with chronic kidney disease, particularly those undergoing dialysis treatment. Direct interactions of uremic toxins with organ tissues are thought to be a major pathophysiological mechanism for disease; for example, indoxyl sulfate reacts directly with macrophages and accelerates atherosclerosis. The removal of sufficient volume of uremic toxins will prevent uremia-related complications in dialysis patients. Hemodialysis with the use of a high-flux dialysis membrane, long or frequent treatment, and increased blood/dialysate flow has improved removal of water-soluble small molecular weight uremic toxins. Middle molecular weight molecules are removed more effectively with hemodialysis using a high-flux membrane, hemodiafiltration and hemofiltration, and a direct hemoperfusion method using  $\beta_2$ -microglobulin adsorption column, which is useful in reducing serum  $\beta_2$ -microglobulin levels as well as improving dialysis-related amyloidosis-induced clinical symptoms. With improvements in dialysis therapies, removal of low and middle molecular weight water-soluble molecules has improved; however, conventional dialysis treatment is limited in its ability to remove protein-bound uremic toxins (PBUTs). Recent findings suggest that adsorption treatments using oral charcoal adsorbent, mixed matrix membrane hollow fiber, and additive charcoal in the dialysate, in addition to conventional dialysis treatment, may effectively remove a substantial amount of PBUTs. Further improvement of renal replacement therapy, including dialysis and additional therapeutic strategies, is needed for better clearance of small and middle molecular weight molecules and PBUTs, which will lead to improved survival and quality of life for dialysis-dependent chronic kidney disease patients.

**Keywords:** Adsorption, Atherosclerosis,  $\beta_2$ -microglobulin, Chronic kidney disease, Indoxyl sulfate, Protein-bound uremic toxins

## Background

Patients with chronic kidney disease (CKD), particularly those undergoing dialysis treatment, develop various systemic complications, such as cardiovascular disease, mineral and bone disorders, and infectious disease. One of the reasons underlying the development of these complications is direct or indirect interactions between various uremic toxins and organ tissues [1–3]. Thus, renal replacement therapies must effectively remove a

sufficient amount of uremic toxins in order to improve survival and prevent CKD-related complications. With progress in dialysis therapies, removal of low and middle molecular weight water-soluble molecules has improved; however, conventional dialysis treatment is limited in its ability to remove protein-bound uremic toxins (PBUTs) owing to their binding to large molecular proteins [4]. In this article, we review characteristics of uremic toxins, particularly PBUTs, as well as recent progress and future perspective on therapeutic strategies for removal of uremic toxins.

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### Uremic toxins

Progressive kidney disease induces uremic syndrome, with the retention of various solutes that are normally excreted by the kidney. Solute with biological toxicity, direct or indirect, are called “uremic toxins” (Table 1) [5]. A literature search identified 88 uremic toxins from 621 articles, and these were classified into three groups according to molecular weight and binding properties, such as water-soluble low molecular weight, middle molecular weight, and protein-bound solutes [6].

There are 40 kinds of free water-soluble low molecular weight molecules (<0.5 kDa) that are considered uremic toxins. For example, recent findings showed that serum level of trimethylamine-*N*-oxide (TMAO), molecular weight 75, is increased in hemodialysis patients [7], and clinical research demonstrated an association between serum level of TMAO and cardiovascular disease [8]. Dietary intake of phosphatidylcholine serves as fuel for intestinal microbiota, resulting in the production of trimethylamine, which is oxidized to TMAO in the liver. TMAO accelerates atherosclerosis; a direct reaction with macrophages was demonstrated in a mouse model [9]. Thus, elevated TMAO level in dialysis patients accelerates atherosclerosis by inducing functional abnormalities of macrophages. Uremic toxins with a small molecular weight are easily removed with conventional dialysis treatment. Kt/V urea is widely used to assess the efficiency of small uremic toxin removal in dialysis patients. Previous clinical studies showed that single-pool Kt/V urea up to 1.8 correlated with improved survival in Japanese hemodialysis patients [10] while high dose hemodialysis with Kt/V urea 1.71 did not show significant benefit for mortality as compared to standard dose of dialysis with Kt/V urea 1.32 [11]. According to those clinical studies, Japanese Society of Dialysis Therapy (JSDT) recommends to keep Kt/V urea more than 1.2 for hemodialysis patients for the better survival [12]. However, evaluation of Kt/V urea alone is not adequate to determine dialysis adequacy, because (1) there are limited reports concerning toxicity of urea, (2) distribution volume of urea is different from that of other water-soluble small molecules, and (3) Kt/V urea dose did not evaluate the adequacy of removal of middle molecular

weight and protein-bound molecules [13]. Thus, clinicians should consider Kt/V as a minimum requirement parameter, and consider total dialysis adequacy to include not only Kt/V but also other middle molecular weight- and PBUT-related markers.

There are 25 types of middle molecular weight molecules, those molecular weight from 0.5 to 60 kDa, such as  $\beta_2$ -microglobulin ( $\beta_2$ -m) and  $\alpha_1$ -macroglobulin. Clearance of middle molecular weight molecules by a hemodialyzer is not as great as that of small molecules owing to their larger molecular weight; however, the use of high-flux dialyzer or hemodiafiltration results in better clearance of middle molecular weight molecules than hemodialysis with low-flux dialyzer.  $\beta_2$ -m, a representative middle molecular weight molecule (11.8 kDa), correlates with survival in dialysis patients. In the randomized hemodialysis (HEMO) study, the relationship between serum  $\beta_2$ -m levels or dialyzer  $\beta_2$ -m clearance and mortality over a period of 2.84 years was analyzed [14]. In time-dependent Cox regression models, predialysis serum  $\beta_2$ -m levels were associated with all-cause mortality in maintenance hemodialysis patients [14]. In Japan, Okuno et al. reported that all-cause and non-cardiovascular mortality in hemodialysis patients with a serum  $\beta_2$ -m level greater than 32.2 mg/L were higher than that in those with a level lower than 32.2 mg/L [15]. Although HEMO study did not demonstrate an association between serum  $\beta_2$ -m level and cardiovascular mortality [14], several studies reported the possibilities of a relationship between  $\beta_2$ -m and cardiovascular disease. A report showed that serum level of  $\beta_2$ -m in CKD patients, including those undergoing chronic dialysis, was associated with vascular calcification as well as cardiovascular events/mortality during a mean follow-up of 969 days [16]. A cross-sectional study revealed that serum  $\beta_2$ -m level correlated with heart valve calcification, which is associated with carotid intima media thickness in dialysis patients [17]. Another clinical study showed that serum  $\beta_2$ -m levels positively correlated with several cardiovascular risk factors, such as highly sensitive C-reactive protein, troponin-T, myeloperoxidase, and N-terminal pro-B-type natriuretic peptide, and inversely correlated with prealbumin and ankle-brachial index [18]. In addition to all-cause and cardiovascular mortality, mortality due to infections was also increased in dialysis patients with high serum  $\beta_2$ -m levels [19]. These reports indicate that a high serum level of  $\beta_2$ -m is associated with mortality, partially due to cardiovascular disease and infection in patients undergoing maintenance hemodialysis treatment. There is no data to show that increase of  $\beta_2$ -m removal by renal replacement therapy induces better survival in dialysis patients, and further studies are needed to understand it. With present evidences, JSDT recommends the maintenance of serum

**Table 1** Requirements for a uremic toxin

1. The toxin is a unique chemical entity.
2. Quantitative analysis of the toxin in biological fluids is possible
3. The levels of the toxin in biological fluids increase with deterioration of kidney function.
4. A positive relationship between toxin level in biological fluids and manifestations of uremic syndrome is present.
5. Administration of the toxin at concentration seen in patients with kidney disease shows toxic effects related to uremic syndrome both in vivo and in vitro.

$\beta_2$ -m at a pre-dialysis level of less than 30 mg/L [12]. In peritoneal dialysis patients, serum  $\beta_2$ -m level is conversely related with residual kidney function [20], and patients should switch their renal replacement therapy from peritoneal dialysis to hemodialysis when the serum level of  $\beta_2$ -m increases above 30 mg/L [21]. From another point of view,  $\beta_2$ -m is a precursor protein for dialysis-related amyloidosis (DRA).  $\beta_2$ -m-related amyloid fibrils are formed and deposited primarily in osteoarticular joint tissues, resulting in various osteoarticular disorders, such as carpal tunnel syndrome, destructive spondyloarthropathy, and bone cysts in dialysis patients [22]. Duration of dialysis treatment is one of the risk factors for osteoarticular disorders, and the accumulation of  $\beta_2$ -m during long-term dialysis treatment may be related to this development [23]. Conformational change of the  $\beta_2$ -m molecule is needed for amyloid fibril formation in the in vitro setting [24], and intermediate states of  $\beta_2$ -m molecules are found in hemodialysis patients [25]. Thus, the accumulation and conformational change of  $\beta_2$ -m molecules are required for the development of DRA during prolonged dialysis treatment.

PBUTs are strongly protein-bound and are difficult to remove with conventional hemodialysis treatment. For example, indoxyl sulfate (IS) and p-cresyl sulfate (PCS) are 97.7 and 95.1 % protein-bound, respectively, and reduction rates of IS and PCS by standard hemodialysis are only 31.8 and 29.1 %, respectively [26] (Table 2).

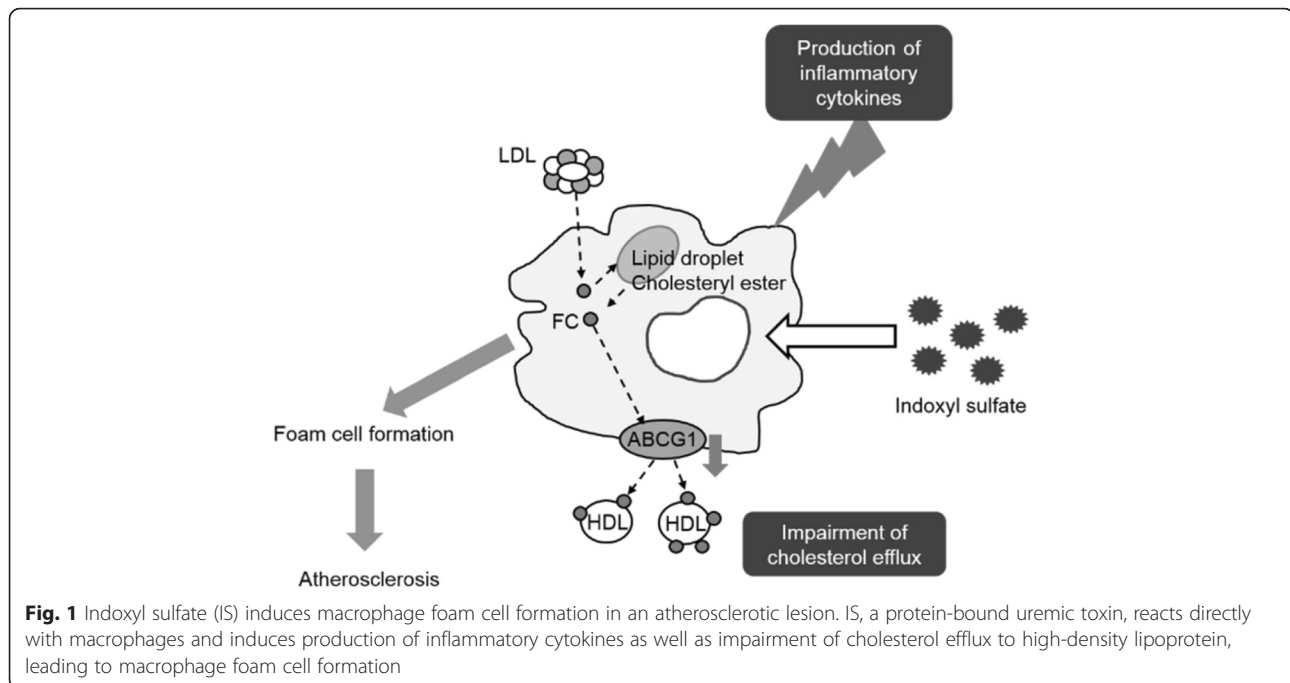
Recently, the relationships between several PBUTs and mortality in CKD patients were reported. Serum level of IS increased with the progression of CKD, particularly in patients undergoing dialysis treatment. IS was associated with increased cardiovascular mortality in CKD patients as well as aortic calcification and pulse wave velocity [2]. Indoleacetic acid (IAA) showed trends similar to IS, and

multivariate analysis showed that IAA, but not IS and PCS, remained a significant predictor of mortality and cardiovascular events [27]. Direct interactions of uremic toxins with blood vessel cells are thought to be a major pathophysiological mechanism for the development of cardiovascular disease in CKD patients [1]. When various types of PBUTs at the concentration found in dialysis patients, with or without albumin, were exposed to human umbilical vein endothelial cells (HUVEC), production of reactive oxygen species (ROS) from cells increased most intensely with IS, followed by indole glucuronide, hippuric acid, and PCS, in the absence of albumin. Even in the presence of albumin, IS and 3-carboxy-4-methyl-5-propyl-2-furanpropionic increased ROS production from HUVEC in vitro [26]. When macrophages differentiated from THP-1 cells were exposed to IS in vitro, IS decreased cell viability but promoted macrophage inflammatory cytokine production as well as ROS production. IS also reduced macrophage cholesterol efflux and decreased ATP-binding cassette transporters G1 expression [28]. These results indicate that direct interactions of IS with macrophages may be a major cause of atherosclerosis acceleration in patients with CKD (Fig. 1). In an animal study, atherosclerotic lesions in apolipoprotein E deficient mice were significantly accelerated by uninephrectomy or subtotal nephrectomy as compared to those with normal kidney function [29–31]. When the mice were treated with an oral charcoal adsorbent AST-120 after renal ablation, AST-120 treatment dramatically ameliorated the kidney damage-induced atherosclerosis [29]. These mice had less aortic deposition of IS, as well as reduced aortic expression of inflammatory cytokines. These results suggest that PBUTs, particularly IS, accelerate atherosclerosis as a result of direct interaction with macrophages and endothelial cells

**Table 2** Characteristics of protein-bound uremic toxins

| Uremic toxins                                     | Pre-dialysis concentrations (mg/dL) | Protein binding (%) | Reduction rate with hemodialysis (%) |
|---|-------------------------------------|---------------------|--------------------------------------|
| Indoxyl sulfate                                   | 2.99 ± 0.18                         | 97.7 ± 0.2          | 31.8 ± 1.4                           |
| Indoxyl glucuronide                               | 0.25 ± 0.03                         | 59.6 ± 1.5          | 79.6 ± 0.9                           |
| Indoleacetic acid                                 | 0.12 ± 0.01                         | 94.1 ± 0.6          | 44.3 ± 1.5                           |
| P-Cresyl sulfate                                  | 3.71 ± 0.28                         | 95.1 ± 0.6          | 29.1 ± 1.7                           |
| P-Cresyl glucuronide                              | 0.58 ± 0.09                         | 32.3 ± 2.6          | 80.7 ± 1.1                           |
| Phenyl sulfate                                    | 1.35 ± 0.15                         | 90.7 ± 0.9          | 65.2 ± 1.4                           |
| Phenyl glucuronide                                | 0.06 ± 0.01                         | 76.1 ± 1.3          | 69.2 ± 8.6                           |
| Phenylacetic acid                                 | 0.05 ± 0.01                         | 60.5 ± 2.4          | 35.0 ± 7.0                           |
| Phenylacetyl glutamine                            | 4.15 ± 0.37                         | 44.9 ± 2.1          | 75.7 ± 1.0                           |
| Hippuric acid                                     | 4.43 ± 0.53                         | 48.3 ± 2.5          | 68.9 ± 1.6                           |
| 4-Ethylphenyl sulfate                             | 0.0242 ± 0.0044                     | 99.3 ± 0.1          | 5.5 ± 2.5                            |
| 3-Carboxy-4-methyl-5-propyl-2-furanpropionic acid | 2.11 ± 0.13                         | 99.7 ± 0.1          | -30.5 ± 3.1                          |

Serum samples were obtained from patients undergoing maintenance hemodialysis whose Kt/V and protein-catabolic rate were 1.40 ± 0.03 and 0.97 ± 0.02 g/kg/day, respectively



in the aorta. AST-120 also modulated CKD-induced cardiac damage, with decreased serum/urine levels of IS and oxidative stress markers, such as 8-hydroxy-2'-deoxyguanosine and acrolein, in a rat model [32]. Concerning bone disease, thyroparathyroidectomy and progressive partial nephrectomy in rats induced an increase of the mineral/matrix ratio and carbonate substitution as well as decreased storage modulus and crystallinity as compared to thyroparathyroidectomy alone. AST-120 abolished kidney damage-induced bone abnormalities and decreased serum IS concentration [33]. These findings suggest that uremic toxins react with various organs, directly or indirectly, and removal of these toxins with AST-120 will result in improved organ function. Clinical studies have not shown clearly that AST-120 had beneficial effect on CKD-related complications as compared to rodent studies, probably owing to the dose of AST-120. Many basic and clinical research studies suggest the importance of PBUTs in various CKD-related complications; however, there is still no recommendation concerning adequate concentration or removal of PBUTs for improved survival, and commercial measurements of these toxins are not clinically available.

#### Removal of uremic toxins by renal replacement therapies

Current progress in renal replacement therapies has improved the removal of various uremic toxins in dialysis patients, but current methods are insufficient to prevent CKD-related systemic complications.

#### 1) Hemodialysis

Removal of uremic toxins, particularly middle molecular weight molecules, has been improved with hemodialysis using the high-flux dialyzer membrane [34]. A large randomized, controlled trial showed that high dose hemodialysis increased Kt/V urea, but not clearance of  $\beta_2$ -m, and use of a high-flux dialyzer increased clearance of  $\beta_2$ -m, but not Kt/V urea [11]. The removal of small and middle molecular weight molecules by hemodialysis is dependent on treatment time, even with slow flow of both blood and dialysate [35]. Compared with hemodialysis for 4 h with 350 ml/min blood and dialysate flow rate, 8-h dialysis sessions with 190 ml/min blood and dialysate flow rate increased the removal of urea and  $\beta_2$ -m by 22.6 and 39.2 %, respectively. Reduction rate of IS and PCS are 31.8 and 29.1 %, respectively, with regular hemodialysis treatment [26], and there was a trend towards the increased removal of these molecules with 8-h hemodialysis as compared to routine 4-h treatment [35]. Another clinical study showed that daily hemodialysis maintained lower serum levels of IS and IAA as well as lower levels of non-protein-bound solutes [36]. Recent finding showed that the time extension for hemodialysis resulted in improved removal of not only small and middle molecular weight molecules but also some PBUTs as to standard hemodialysis when patients used the same high-flux dialyzer, dialysis flow, and blood flow [37]. Dialyzers also affect the efficiency of PBUT extraction. Large

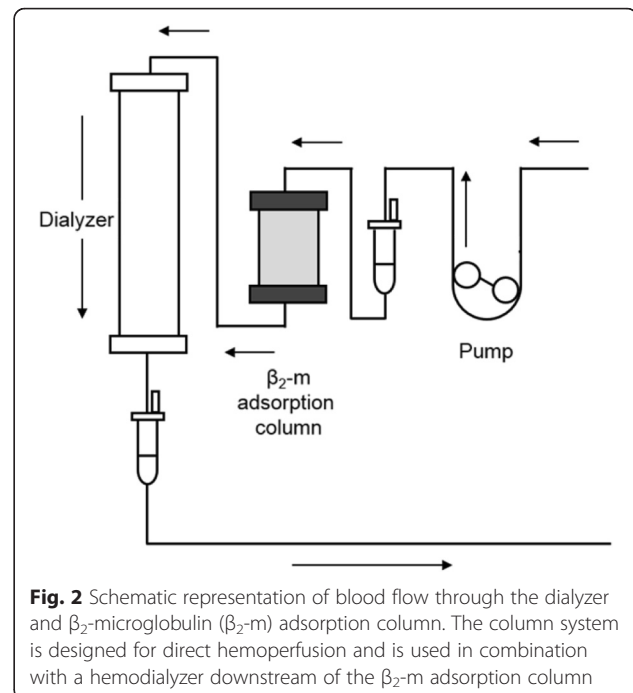
pore super-flux cellulose triacetate membranes (SF) showed much removal of IS, IAA, and hippuric acid than low-flux cellulose triacetate membranes (LF) (removal rate of IS; SF  $32.5 \pm 8.5\%$  vs LF  $24.8 \pm 6.5\%$ ) [38]. These studies suggest that prescriptions for hemodialysis, such as the type of dialysis membrane, treatment time, and blood/dialysate flow rates, affect the clearance of not only small and middle molecular weight molecules but also some PBUTs; however, these changes remain insufficient to prevent CKD-related complications.

## 2) Hemodiafiltration and hemofiltration

Hemodiafiltration (HDF) and hemofiltration (HF) are more effective to remove water-soluble middle molecules than hemodialysis due to the increase of convection, but the effect is small for PBUTs. A crossover trial clinical to examine the removal of uremic toxins by hemodialysis or post-dilution HDF was conducted [39]. Post-dilution HDF increased the instantaneous plasma clearance of urea and  $\beta_2$ -m as compared to hemodialysis; however, clearance of both total and free IS and PCS did not increase with the change from hemodialysis to HDF [39]. In the comparison of different convective strategies, removal of small molecules, such as urea and creatinine, was more effective with post-dilution HDF than pre-dilution HDF and HF [40]. In the case of middle molecular weight molecules, post-dilution HDF is superior to pre-dilution HDF for the removal of  $\beta_2$ -m. For some PBUTs, including hippuric acid, IS, and PCS, both pre- and post-dilution HDF are superior to post-dilution HF [40]. It is difficult to compare the properties to remove uremic toxins between HDF and HD using SF; however, these findings suggest that physicians should choose the types of convection based upon the type of uremic toxins to be removed most efficaciously for each dialysis patient.

## 3) $\beta_2$ -microglobulin adsorption column

The Lixelle® column was developed for direct  $\beta_2$ -m adsorption from circulating blood and is used primarily for hemodialysis patients with progressive DRA. Lixelle contains a porous cellulose bead filling with a sodium citrate buffer. The porous cellulose beads were designed to selectively adsorb  $\beta_2$ -m, having the appropriate pore size and hydrophobic interaction. This adsorption column system is designed for direct hemoperfusion and is used in combination with a dialyzer that is the downstream of the Lixelle column (Fig. 2). There are several clinical trials demonstrating the effect of Lixelle on the reduction of  $\beta_2$ -m in patients undergoing hemodialysis [41–46]. The Lixelle column induces a 60.0–78.9 % reduction in serum  $\beta_2$ -m with 157–



**Fig. 2** Schematic representation of blood flow through the dialyzer and  $\beta_2$ -microglobulin ( $\beta_2$ -m) adsorption column. The column system is designed for direct hemoperfusion and is used in combination with a hemodialyzer downstream of the  $\beta_2$ -m adsorption column

300 mg removal; the serum level of  $\beta_2$ -m decreased from 27.1–29.0 to 6.8–13.5 mg/L [41, 42, 46]. According to a prospective multicenter study, a Lixelle column (S-35) placed in series with a polysulfone dialyzer increased serum  $\beta_2$ -m reduction in patients undergoing hemodialysis as compared to hemodialysis treatment without Lixelle (reduction rate:  $74.1 \pm 6.1\%$  vs  $60.1 \pm 6.3\%$ ) [46]. These clinical studies also showed improvements in DRA-related symptoms, such as joint pain and activities of daily living [41, 43–48]. Gejyo et al. surveyed the clinical effect of Lixelle in hemodialysis patients with DRA. Of 345 patients with DRA, 56.2 % patients treated with Lixelle for  $3.5 \pm 2.7$  years reported improvement in their DRA-related symptoms [47]. Some clinical study showed shrinkage in the size of bone cysts owing to amyloidosis when evaluated by X-ray [41, 49]; thus, this direct hemoperfusion treatment may decrease the deposition of  $\beta_2$ -m-related amyloid. In addition, the Lixelle column adsorbs not only  $\beta_2$ -m but also other molecules, including inflammatory cytokines, with molecular sizes ranging from 4000 to 20,000 Da [50]. This may be another explanation for the improvement in DRA-related clinical symptoms.

## 4) Other treatments

The removal of water-soluble small and middle molecular weight uremic toxins has been improved with hemodialysis, HDF/HF, or direct hemoperfusion; however, the efficacy of these dialysis treatments in the removal of PBUTs is small. Even in the case of



CKD patients undergoing peritoneal dialysis, residual kidney function is more important to decrease PBUTs than peritoneal clearance [51, 52]. Thus, alternative or additional therapeutic strategies will be needed for future blood purification therapies to prevent PBUT-induced dialysis-related complications, including cardiovascular disease.

The oral charcoal adsorbent AST-120 is known to reduce serum levels of IS through the adsorption of indole, which is converted from dietary tryptophan in the gastrointestinal tract in non-dialysis CKD patients [53]. Although randomized, controlled trials did not clearly demonstrate inhibited progression of renal disease in non-dialysis CKD patients with the use of AST-120 [54, 55], a clinical study showed that the treatment in pre-dialysis may affect the prognosis after initiation of dialysis treatment [56]. Thus, AST-120, the adsorption treatment, may show more benefit for prognosis in dialysis patients because this population has much higher levels of circulating PBUTs [2, 26]. When anuric patients undergoing maintenance hemodialysis treatment used AST-120 6 g/day for 2 weeks, total and free IS levels in the predialysis session decreased significantly with AST-120 treatment from 3.06 to 1.63 mg/dL and from 0.098 to 0.023 mg/dL, respectively [57]. The use of AST-120 also reduced the PCS (total and free) and phenyl sulfate (free). We also evaluated changes in the oxidative stress markers with AST-120 treatment in dialysis patients and found that the use of AST-120 induced significant reduction in oxidized albumin content as well as 8-isoprostane [57]. Another study investigated the effect of a mixed matrix membrane (MMM) for the removal of protein-bound uremic toxins [58]. The MMM hollow fiber consists of a porous macrovoid free polymeric inner membrane layer strongly attached to the activated carbon-containing outer MMM layer; it absorbed 2.27 mg PCS/g membrane and 3.58 mg PCS/g membrane in diffusion as well as convection for 4 h from human plasma [58]. The addition of charcoal to the dialysate increased the clearance of indican (12 vs 5 ml/min without sorbent), PCS (9 vs 4 ml/min without sorbent), and p-cresol (35 vs 14 ml/min without sorbent) in artificial plasma [59]. Taken together, these adsorption treatments have the potential to remove significantly more PBUTs when added to conventional dialysis treatment, and removal with adsorption may be a key strategy in next generation renal replacement therapy.

## Conclusions

Recent progress in dialysis treatment has increased the removal of uremic toxins, but these improvements are insufficient to prevent CKD-related complications and improve mortality, particularly concerning levels of PBUTs. Further improvements in renal replacement therapy including

hemodialysis or additional therapeutic strategies will be needed for better clearance of small and middle molecular weight molecules as well as PBUTs.

## Abbreviations

CKD, chronic kidney disease; DRA, dialysis-related amyloidosis; HDF, hemodiafiltration; HF, hemofiltration; HUVEC, human umbilical vein endothelial cells; IAA, indoleacetic acid; IS, indoxyl sulfate; JSDT, Japanese Society of Dialysis Therapy; MMM, mixed matrix membrane; PBUTs, protein-bound uremic toxins; PCS, p-cresyl sulfate; ROS, reactive oxygen species; TMAO, trimethylamine-N-oxide;  $\beta_2$ -m,  $\beta_2$ -microglobulin.

## Authors' contributions

SY, JK, and IN made substantial contributions to the conception and design for this review article. SY involved in drafting the manuscript. TW, YT, YK, and SG revised the manuscript critically for important intellectual content. All authors read and approved the final manuscript.

## Competing interests

The authors declare that they have no competing interests.

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