Patients on maintenance dialysis suffer a substantial mortality risk. Traditionally, this increased mortality is attributed to enhanced cardiovascular damage, in itself mostly linked to hastened calcification and vascular stiffness related to inflammation. Many uremic toxins have been associated with increased inflammation and cardiovascular mortality. Accordingly, much attention has been attributed to the removal of these substances during dialysis. In this issue of the Clinical Journal of the American Society of Nephrology, Madero et al. (1) described a new methodology to increase removal of protein-bound uremic toxins (PBUTs) by adding a substance, in this case ibuprofen, to the bloodstream prefiler that displaces the uremic toxins from their binding place on albumin. This resulted in a threefold increase in instantaneous clearance of p-cresyl sulfate (PCS) and indoxyl sulfate (IS), which disappeared after stopping the ibuprofen infusion. There was no effect on clearance of nonprotein-bound substances, such as urea or creatinine, adding credibility to the hypothesis that displacement of PBUTs is indeed the underlying mechanism. Whereas all of these form proof of concept that displacing PBUTs is feasible in vivo and results in enhanced clearance as expected, several major questions remain unresolved before we can jump to introducing these techniques in clinical practice.

Many different techniques are currently being explored to enhance clearance of PBUTs. Some, just as in the work by Madero et al. (1), follow the concept of displacing the PBUTS from their binding place (2,3). During passing of the patient’s blood through the dialyzer, only the free fraction of protein-bound solutes is removed, and the equilibrium is not restored immediately, resulting in an increase of the percentage of protein binding. Because most of these PBUTs are quite small in their free form, the clearance of the free fraction is effective and rapid. Displacing PBUTs from their binding place is, therefore, an attractive option to enhance the clearance of PBUTs. Not all PBUTs might, however, be equally affected by such an intervention, because different mechanisms and locations of protein binding exist. PCS and IS are relatively easily dissociated from albumin by drug displacement, whereas this is much more difficult for others (e.g., 3-carboxy-4-methyl-5-propyl-2-furan-propanoic acid). Human serum albumin contains different domains where protein binding is possible. Most compounds preferentially bind to one of the two principal binding sites commonly referred to as Sudlow binding sites I and II. Ibuprofen is the archetypical molecule binding to Sudlow site II, and PCS and IS bind to Sudlow site II as well. Therefore, ibuprofen will not displace uremic toxins, such as 3-carboxy-4-methyl-5-propyl-2-furan-propanoic acid, that bind to Sudlow site I. However, because our understanding of protein binding is unsatisfactory, other beneficial or essential substances could be removed to a higher degree by these more intensive dialysis techniques. As our understanding improves of which uremic retention products have the most causal effect on mortality and should, therefore, be targeted, improving the clearance of all PBUTs will necessitate the concomitant use of multiple displacers. These substances that are going to be used for displacement need to be cleared efficiently and rapidly, and preferentially, they should be inert. This is the most important, because combinations of these displacers will be necessary.

Next to adding substances to the serum to displace albumin (1), adding, for example, hypertonic sodium at the arterial side just before the dialyzer to increase ionic strength also can be used to enhance displacement of PBUTs. Another strategy is the use of substances that have very high affinity for PBUTs (2). These can be applied at the serum or the dialysate side depending on the carrier. For example, using a hexadecyl-immobilized cellulose bead-containing column for hemoperfusion coupled to a regular hemodialyzer, serum levels of free IS, PCS, indole acetic acid, and phenyl sulfate were reduced by about one third, but total PBUT level did not change (4). Adsorptive beads have been added to the dialysate site (5). Such a strategy resulted in increased removal of PBUTs in a closed loop setting, because it keeps the concentration gradient across the membrane maximal and thus, can be used to decrease the volume of dialysate (e.g., in a home dialysis setting). However, it is conceptually difficult to see how such a strategy would be successful in enhancing PBUT removal in a setting of regular
dialysis with high dialysate flows, where the concentration gradient is already near maximum.

Others are exploring the use of bioengineered tubular cells capable of active uremic toxin secretion. The idea builds on the fact that renal excretion of PBUTs depends largely on active tubular secretion through a range of transporters that cooperate in basolateral uptake and luminal excretion. Successful production of a model using a three-dimensional cell monolayer formation of human conditionally immortalized proximal tubule epithelial cells seeded within hollow fibers has been reported (6). Such a system was able to secrete IS in an in vitro perfusion system, showing the proof of concept. However, many additional hurdles will need to be overcome before such a system can safely be used routinely in clinical practice, and costs and logistics might prove to be substantial.

An alternative strategy to improving removal would be to reduce production of PBUTs. Because these substances are mostly formed by fermentation in the gut, modification of the diet by reduction of protein intake and increase in fiber compounds could reduce PCS and IS levels in patients with CKD (7). However, results of trials testing this hypothesis have been disappointing. In addition, such a diet might be cumbersome or even undesirable, because protein restriction might enhance malnourishment and intake of fruits and vegetables to increase fiber intake might result in hyperkalemia. Therefore, current administration of probiotics, prebiotics, and synbiotics is being tested as an alternative to diet to reduce intestinal generation and/or absorption of PBUTs. Intestinal adsorption of PBUTs using, for example, AST-120 reduced serum levels of PCS and IS in animal models. In a 4-week crossover study with patients who were anuric undergoing hemodialysis, 6 g of AST-120 daily substantially reduced serum levels of total and free IS and PCS, but a randomized, controlled trial in patients with CKD failed to confirm a difference between AST-120 and placebo in levels of PBUTs (8).

All of these make clear that more interdisciplinary research in the area of protein binding and removal strategies is needed before these strategies to enhance removal or reduce generation of PBUTs can be more successfully adopted in clinical practice.

Moreover, some more fundamental questions still remain. First, increasing instantaneous clearance of a toxin does not necessarily result in an equivalent or clinically relevant decrease in its time-averaged serum concentration (TAC), because the limiting factor for removal often is the exchange between the different body compartments of the patient and its generation (9). On the basis of mathematical modeling, intercompartment clearance between extraplasmatic and plasma compartment was more than fivefold lower for strongly protein-bound proteins than for urea (10). When looking at supplemental figures 1 and 2 in the paper by Madero et al. (1), the decrease in serum concentration of PCS and IS already started before the ibuprofen infusion was started, and the rate of decline did not substantially alter afterward. Because a true mass balance in the patient over the complete session is lacking, it is impossible to say whether using this technique actually results in lower TACs of PBUTs and if this is meaningful from the clinical perspective. It might be that techniques enhancing the displacement of PBUTs from their ligand will also still need long extended dialysis hours to allow for meaningful reduction of TACs of these PBUTs.

Second, the whole idea of the benefit of enhancing removal of PBUTs starts from observational data relating them with cardiovascular morbidity and mortality. Although some in vitro and animal studies support such a causal relationship, it is unclear whether their removal will actually improve outcomes. Some middle molecules have been associated with cardiovascular morbidity and inflammation. However, whereas high-volume hemodiafiltration results in better removal of middle molecules, clinical studies provide rather disappointing results in terms of improved outcomes with this technique. Many explanations for these negative results have been forwarded, but it has never been suggested that the underlying premise itself might not be completely right. Cardiovascular comorbidity has already been accumulating for years in patients with CKD by the time that they start dialysis. Small changes in additional burden after they start dialysis might, therefore, be completely irrelevant, because all damage has already been done. If we seriously believe that some middle molecules or PBUTs do matter in a causal way for cardiovascular disease, we need to tackle the problem long before patients start dialysis. In addition, it is often underemphasized in the literature on uremic toxicity that the major drivers of death in patients on dialysis are fluid overload and infection. Any intervention that has even a minor negative effect on these aspects might result in worse, not improved, outcomes in clinical practice. Small benefits of removing a small incremental amount of toxins are potentially over-ruled by insults related to the technique that is being used. When translating these techniques to enhance PBUT removal from proof of concept to the bedside of the patient, we need thus to ensure they can be administered in a safe and logistically feasible manner so that errors in fluid balance or risks of infection by the need for many additional steps in building up the dialysis circuit are as minimal as possible.

In conclusion, enhanced removal of PBUTs by using displacers is maybe promising and certainly interesting, but the road to success is still long and winding.

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