Nefrologia 46 (2026) 501558

Sociedad Española de Nefrología

nefrología

Revista de la Sociedad Española de Nefrología

journal homepage: www.revistanefrologia.com

Editorial

From convection to disruption: The next step in dialysis for protein-bound toxins

De la convección a la disrupción: el siguiente paso en la diálisis de toxinas unidas a proteínas

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We read with great interest the study by Escudero-Saiz et al. evaluating the role of post-dilutional hemodiafiltration (HDF) in the clearance of protein-bound uremic toxins (PBUTs).[1] This single center study, conducted with 137 patients undergoing HDF, evaluated the clearance of p-cresyl sulfate (pCS) and indoxyl sulfate (IS), molecules with high toxicity, high protein binding, and which have been associated with an increased risk of cardiovascular mortality in patients requiring hemodialysis (HD). This study shows the current ceiling of PBUTs removal with actual HD failed, and further technical optimization of convection yields only marginal gains. In light of these observations, alternative mechanistic strategies aimed at modulating protein binding have gained renewed attention. In particular, albumin-binding competitors such as ibuprofen have demonstrated the capacity to enhance PBUT removal in clinical studies, most notably in the single-session trial by Madero et al. and in crossover study by Escudero et al. Although these investigations provide relevant human data currently available in this field, they were limited to short-term exposure and therefore do not allow definitive conclusions regarding long-term safety in a population already burdened by elevated cardiovascular and gastrointestinal bleeding risk.

Collectively, these data suggest that meaningful progress in PBUT removal will require integrated approaches that extend beyond conventional dialysis metrics, combining optimized diffusion with innovative binding-displacement or adsorptive strategies, and ultimately linking biophysical efficacy to patient-centered outcomes. Although albumin binding is central to the pathophysiology of PBUTs, serum albumin concentrations were not reported, and no significant association with toxin reduction ratios was observed an absence that limits a more nuanced interpretation of interindividual variability in clearance and of strategies aimed at pharmacological displacement. Authors conclude that the removal of these PBUTs occurs predominantly through diffusive mechanisms, in line with previous reports, although prior studies were limited by substantially smaller sample sizes compared with the cohort analyzed by Escudero-Saiz et al.[2]

Uremic toxins are metabolic byproducts whose accumulation contributes to the development of uremic syndrome, leading to a wide range of deleterious systemic effects. These include the acceleration of renal aging, promotion of atherosclerotic plaque formation largely responsible for the elevated cardiovascular risk observed in patients with chronic kidney disease (CKD) and clinical manifestations such as pruritus.[3,4]

While small, water-soluble toxins are generally efficiently removed during HD sessions, PBUTs pose a particular challenge. Despite their low molecular weight, their strong binding to albumin markedly limits their dialytic clearance. Consequently, PBUTs are believed to play a significant role in the persistently high morbidity and mortality rates observed in patients undergoing renal replacement therapy.[5,6]

Albumin beyond oncotic pressure: implications for uremic toxicity and dialysis

Albumin, synthesized predominantly by the liver, is the most abundant plasma protein and plays a central role in systemic homeostasis. Traditionally, its clinical relevance has been largely attributed to the maintenance of plasma oncotic pressure. However, this narrow view fails to capture the multifunctional nature of albumin, whose biological roles extend well beyond volume

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https://doi.org/10.1016/j.nefro.2026.501558

Available online 16 April 2026
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regulation.[7] Accumulating evidence highlights albumin as a key modulator of oxidative stress, owing to its capacity to scavenge reactive oxygen species (ROS) and reactive nitrogen species (RNS), as well as a highly versatile carrier for a wide range of endogenous and exogenous ligands.[8]

Albumin transports not only hormones, vitamins, fatty acids, and drugs, but also metabolic waste products and uremic toxins, thereby exerting a direct influence on toxin distribution, bioavailability, and clearance. This remarkable transport capacity is underpinned by its structural flexibility and its ability to engage in both covalent and non-covalent molecular interactions. Of particular importance is the free thiol group at cysteine-34 (Cys34), which accounts for a substantial proportion of albumin's antioxidant activity and participates in redox-dependent modifications. In addition, reversible non-covalent interactions such as hydrophobic forces, electrostatic interactions, and Van der Waals forces enable albumin to bind a broad spectrum of ligands in a dynamic and competitive manner.[9]

A major advance in understanding albumin–ligand interactions was the identification of two principal drug binding regions on human serum albumin by Sudlow and colleagues in the 1970s.[10] These regions, now referred to as Sudlow sites I and II, remain highly relevant in contemporary nephrology, as they constitute key binding domains for PBUTs and represent critical determinants of their limited dialytic removal.

Sudlow site I, located within subdomain IIA of albumin, consists of a large hydrophobic pocket and serves as the primary binding site for many drugs and for several clinically relevant PBUTs, including IS and pCS. The strong affinity of these toxins for albumin markedly restricts their free fraction in plasma, thereby limiting their clearance by both renal excretion and conventional dialysis techniques, which rely predominantly on diffusive transport[11,12] (Fig. 1).

In contrast, Sudlow site II, situated in subdomain IIIA, is characterized by a smaller binding cavity, and exhibits a higher susceptibility to competitive displacement. This site commonly binds non-steroidal anti-inflammatory drugs (NSAIDs) and shows high sensitivity to fluctuations in free fatty acid concentrations. In patients with chronic kidney disease, and particularly during dialysis, changes in ligand competition at Sudlow site II may substantially alter the unbound fraction of drugs and toxins, with important pharmacokinetic and clinical consequences[12–14] (Table 1).

These observations challenge the long-standing view of albumin as a passive determinant of oncotic pressure or a marker of nutritional status. Instead, albumin emerges as a dynamic and functionally complex protein that critically modulates uremic toxicity and the efficacy of extracorporeal clearance strategies. A deeper understanding of albumin–ligand interactions may inform the development of novel therapeutic approaches aimed at improving the removal of protein-bound solutes in kidney disease.[15,16]

The accumulation of PBUTs is a major pathophysiological feature of CKD. These molecules are not merely waste products destined for renal excretion. Rather, they exert distinct physiological functions. In this context, IS represents a paradigmatic example. This PBUT derived from the metabolism of dietary tryptophan primarily from animal proteins by gut microbiota and subsequently processed in the liver, is not merely inert retention solute.[5] It has been incorporated into the remote sensing and signaling theory (RSST), which proposes that small organic molecules mediate inter-organ and inter-organismal communication. Through this network, IS participates in the regulation of drug absorption, distribution, metabolism, and excretion (ADME). This signaling system involves a coordinated interaction between the gut, liver, brain, and kidney.[17] IS has been shown to scavenge superoxide radicals. Additionally, IS has been shown to induce microglial hyperactivation via aryl hydrocarbon receptor (AhR), altering microglial morphology and enhancing neuroinflammatory responses that may contribute to synaptic dysfunction and neurodevelopmental or neurodegenerative processes.[18]

Progressive accumulation of IS in uremic syndrome disrupts metabolic homeostasis, it also impairs inter-organ and inter-organismal remote communication. These alterations contribute to systemic dysfunction. IS has been consistently associated with the progression of CKD and vascular injury, promoting atherosclerotic lesion formation and thrombosis through activation of the AhR, leading to oxidative stress, pro-inflammatory signaling, and vascular smooth muscle cell proliferation pathological processes that are particularly evident in advanced stages of CKD.[19]

EUTox Group identified around 31 different PBUTs; the bloodstream circulates forms bound-unbound or free fractions.[20]

Multiple strategies are currently being investigated to reduce uremic toxin burden in patients with advanced CKD. These include pharmacological approaches aimed at limiting intestinal absorption, such as AST-120 and Naturen G.[21] Competitive binding at Sudlow sites, as demonstrated in the work by Escudero et al. (e.g., furosemide, ibuprofen), amino acids (e.g., tryptophan), salvianolic acid, and fatty acids introduced into the bloodstream during HD has been investigated as a strategy to increase the dissociation of PBUTs from albumin and thereby enhance their removal by the dialysis membrane. Among these compounds, *in vitro* studies lithospermic acid (LA) has been identified as one of the most effective displacers, increasing the clearance of IS and pCS by over 190%.[22] However, despite its efficacy, this approach failed to translate into routine clinical practice due to secondary adverse effects. Consequently, the search for novel binding competitors with improved safety profiles

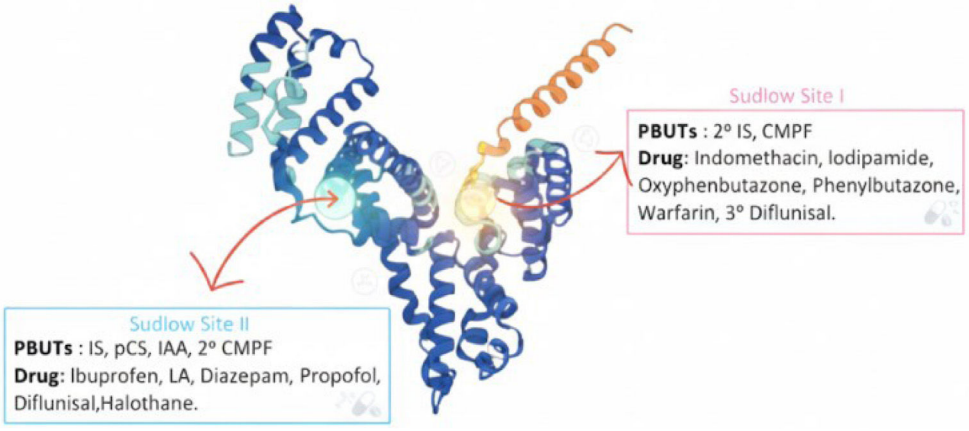

Fig. 1. Albumin structure and Sudlow sites. IS, indoxil sulfate; pCS, p-cresyl sulfate; IAA, indole-3-acetic acid; CMPF, 3-carboxy-4-methyl-5-propyl-2 furanpropanoic acid; LA, lithospermic acid; PBUTs, protein-bound uremic toxins.

Table 1
Albumin binding sites.[13,14,16,18,31,32]

Feature	Sudlow site I	Sudlow site II
Albumin subdomain	IIA	IIIA
Ligand preference	Bulky, heterocyclic, aromatic, predominantly anionic compounds	Smaller, hydrophobic, aromatic compounds
Primary binding forces	Hydrophobic interactions, hydrogen bonding	Hydrophobic interactions
Representative drugs	Warfarin, phenylbutazone, indomethacin, furosemide, ceftriaxone, sulfonylureas, naproxen	Diazepam, ibuprofen, diclofenac, ketoprofen, flurbiprofen, valproate, phenytoin, clofibrate
Hormones and endogenous ligands	Bilirubin, thyroxine (T4), triiodothyronine (T3), bile acids	Cortisol, aldosterone, progesterone, long-chain fatty acids
Protein-bound uremic toxins (PBUTs)	3-Carboxy-4-methyl-5-propyl-2 furanpropanoic acid (CMPF), *p*-cresyl sulfate and indoxyl sulfate	Indole-3-acetic acid, non-sulfated indole derivatives, *p*-cresyl sulfate and indoxyl sulfate
Clinical relevance	Major site for drug–drug and drug–toxin interactions; central to PBUT competition in chronic kidney disease	Sensitive to free fatty acid concentrations; displacement effects during inflammation or uremia
Dialysis implications	Strong protein binding limits diffusive and convective clearance of PBUTs	Altered binding may modify free drug fractions during dialysis

has intensified. In a study by Wang et al., multiple rounds of structure-based screening identified five candidate molecules that could competitively displace IS and pCS from albumin. These molecules exhibited binding affinities comparable to or greater than LA.[23]

Several strategies have been explored to increase the free fraction of PBUTs without affecting albumin. These approaches focus on reducing protein–toxin binding to enhance the toxin's free fraction in plasma, thereby improving its removal during hemodialysis by increasing the concentration gradient across the membrane. This can be achieved either by adding competing displacers that release toxins from albumin-binding sites, or by modulating protein–toxin interactions through changes in ionic strength, pH, or electromagnetic fields.[24]

Dialysis filters with enhanced adsorptive capacity have also been developed. In this way, emerging technologies are being explored and include mixed matrix membranes, zirconium-based metal organic frameworks, and filters based on nanoporous activated carbon monoliths. In *ex vivo* studies, these systems have demonstrated the ability to remove IS and pCS.[25]

Beyond the aforementioned approaches, the toxin albumin interaction can be conceptualized as a thermodynamic system residing in a local minimum of free energy. Binding affinity is quantitatively governed by the Gibbs free energy of association (ΔG bind), whereby increasingly negative values denote a stable and spontaneous complex.[26] Within this framework, the persistence of protein-bound uremic toxins reflects their position within an energy landscape characterized by deep local minima, which confers both thermodynamic stability and kinetic persistence.[27] In contrast to competitive displacement by albumin binding drugs such as ibuprofen which relies on site occupancy and pharmacological competition an alternative strategy is to perturb the energy landscape itself. Perturbation of this landscape through the introduction of external energy may shift the complex from a stable minimum toward a metastable state, increasing the likelihood of transient dissociation. Crucially, such energy input does not directly disrupt covalent structure but rather modulates the balance of non-covalent forces, effectively lowering the kinetic barrier to unbinding. For this strategy to be clinically meaningful in the dialysis setting, the lifetime of the dissociated state must exceed the reassociation time constant, thereby permitting extracorporeal removal of the liberated fraction before rebinding occurs.

In this context, Jakowski patented the use of electromagnetic fields applied to dialysis circuits.[28] This approach is intended to disrupt non-covalent interactions between albumin and PBUTs, thereby increasing the free toxin fraction available for dialysis clearance. A similar principle underlies the MI-TRAM device, which uses radiofrequency to induce albumin toxin dissociation.[29] This technology has been proposed both as an adjunct to dialysis and as part of artificial kidney development, supported by the Kidney X initiative. However, to date, no studies have demonstrated its clinical efficacy.

Within this framework, our group conducted studies to explore physical strategies for disrupting binding albumin-toxin using ex vivo uremic plasma. We applied an external electric field to the dialysis circuit via copper plates. Clearance of pCS and IS was evaluated; no significant differences were observed compared to standard conditions (unpublished data).

Smith et al. elegantly quantified toxin protein energy interactions using computational simulations, revealing that IS exhibits the highest average binding energy to albumin when compared with pCS, indole-3-acetic acid (IAA), and hippuric acid (HA).[14] These findings reinforce the notion that the strong affinity of IS for albumin constitutes a critical barrier to its extracorporeal removal. Building on this mechanistic insight, we are currently developing a proof-of-concept strategy that applies controlled external physical forces to promote reversible albumin toxin dissociation, thereby increasing the fraction of dialyzable free toxin without relying on pharmacological displacement. This innovation initiative, termed *Hemoshake*, aims to determine the ability of mechanical energy to increase the free fraction of protein-bound uremic toxins and enhance their extracorporeal removal. However, the translational relevance of this approach remains to be established.

As a strategy to reduce PBUTs, preservation of residual kidney function (RKF) in dialysis patients remains clinically relevant. Even at minimal levels, RKF was associated with significantly lower concentrations of seven of eight measured non-urea solutes compared with patients without RKF, with reductions ranging from 24% (hippurate) to 3.7% (asymmetric dimethylarginine). These findings indicate that very low RKF continues to provide meaningful non-urea solute clearance and should not be considered negligible. Accordingly, clinical management should incorporate strategies to preserve RKF, and dedicated trials in incident hemodialysis patients are warranted.[30]

Moving beyond empiric displacers toward rationally designed molecules, it reopens a field that had been tempered by translational failures. Together, these emerging strategies suggest that the future of PBUT removal may lie not in adding stronger competitors, but in learning how to disrupt albumin binding transiently and safely itself shifting dialysis from a purely diffusive process toward a mechanistically informed intervention. Future studies integrating dynamic dialysis models, comprehensive safety assessments, and multi-toxin displacement profiles will be essential to determine whether this concept can finally be translated into clinical benefit.

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