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The membrane perspective of uraemic toxins: which ones should, or can, be removed?

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ABSTRACT

Informed decision-making is paramount to the improvement of dialysis therapies and patient outcomes. A cornerstone of delivery of optimal dialysis therapy is to delineate which substances (uraemic retention solutes or 'uraemic toxins') contribute to the condition of uraemia in terms of deleterious biochemical effects they may exert. Thereafter, decisions can be made as to which of the accumulated compounds need to be targeted for removal and by which strategies. For haemodialysis (HD), the non-selectivity of membranes is sometimes considered a limitation. Yet, considering that dozens of substances with potential toxicity need to be eliminated, and targeting removal of individual toxins explicitly is not recommended, current dialysis membranes enable elimination of several molecules of a broad size range within a single therapy session. However, because HD solute removal is based on size-exclusion principles, i.e. the size of the substances to be removed relative to the mean size of the 'pores' of the membrane, only a limited degree of selectivity of removal is possible. Removal of unwanted substances during HD needs to be weighed against the unavoidable loss of substances that are recognized to be necessary for bodily functions and physiology. In striving to improve the efficiency of HD by increasing the porosity of membranes, there is a greater potential for the loss of substances that are of benefit. Based on this elementary trade-off and availability of recent guidance on the relative toxicity of substances retained in uraemia, we propose a new evidence-linked uraemic toxin elimination (ELUTE) approach whereby only those clusters of substances for which there is a sufficient body of evidence linking them to deleterious biological effects need to be targeted for removal. Our approach involves correlating the physical properties of retention solutes (deemed to express toxicity) with key determinants of membranes and separation processes. Our analysis revealed that in attempting to remove the relatively small number of 'larger' substances graded as having only moderate toxicity, uncontrolled (and efficient) removal of several useful compounds would take place simultaneously and may compromise the well-being or outcomes of patients. The bulk of the uraemic toxin load comprises uraemic toxins below <30 000 Da and are adequately removed by standard membranes. Further, removal of a few difficult-to-remove-by-dialysis (protein-bound) compounds that express toxicity cannot be achieved by manipulation of pore size alone. The trade-off between the benefits of effective removal of the bulk of the uraemic toxin load and risks (increased loss of useful substances) associated with targeting the removal of a few larger substances in 'high-efficiency' HD treatment strategies needs to be recognized and better understood. The removability during HD of substances, be they toxic, inert or beneficial, needs be revised to establish the pros and cons of current dialytic elimination strategies.

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SOLUTES RETAINED IN URAEMIA—THEIR IDENTITY

Uraemia is just one of the manifestations of kidney failure. Uraemia, characterized biochemically by elevated levels of urea, is associated with fluid, electrolyte and hormonal dysregulation, various metabolic derangements and a clinical syndrome often characterized by anorexia, nausea, vomiting, altered mental status and pruritus [1, 2]. The progressive loss of kidney function often includes hypertension (due to volume overload) and anaemia (lack of erythropoietin) as components of uraemia. If no transplant is available, dialysis is the only available treatment to combat uraemia (also referred to as 'uraemic syndrome') [3]. Accumulated organic waste products are successfully removed in part by dialysis therapy. Without this elimination, death ensues rapidly, while with dialysis, patients with end-stage kidney disease (ESKD) may survive several years, or even decades. However, the extracorporeal procedure is unable to satisfactorily correct various imbalances associated with renal failure, including electrolyte and pH derangements linked to the condition [2, 4]. To alleviate the symptoms of uraemia, efficient removal of unwanted substances retained in ESKD and balancing volume overload are therapeutic targets of all dialysis therapies [5]. The prerequisite for this objective is first, ascertaining the identity and biological activities of entities that need to be removed and second, selecting appropriate semipermeable membranes and treatment strategies [6, 7].

Urea and uraemia

There is the general perception that the observation of accumulation of urea in blood in kidney disease concomitantly led to the coining of the term uraemia as a disorder [2]. In fact, the term uraemia came into reluctant acceptance and regular usage decades after the chemical discovery and isolation of urea from urine [4]. The period between the recognition of retention of urea and linking it to a clinical condition (kidney failure) and description as a specific syndrome (uraemia) was one of considerable scientific dissonance. Richet's [8, 9] revealing historical essays on the early days of uraemia expound on this inauspicious initial discord around uraemia that perhaps prevails today. Since the condition was first recognized and described in the 1800s, attempts to 'explain' or 'unravel' uraemia continue [10, 11].

Urea, an end product of protein metabolism, has played a unique role not only in the defining and understanding of uraemia, but also as a tool helping to achieve therapeutic targets for dialysis therapies [12]. Being a small (60 Da) solute that is widely distributed in total body water, urea is easily measurable and dialyzable and was thus a convenient surrogate marker for other retained low molecular weight solutes as well as for assessment of efficiency of dialytic therapies [13]. However, urea has also been the source of confusion, having been suspected initially of expressing toxicity. It was then considered largely inert for well over a century, until the formative days of dialysis, and recently reverted to being classified as a toxin again [14]. Likewise, its once established reputation and usefulness for assessing dialysis dose and adequacy is today controversial and in question [15, 16]. The ongoing search beyond urea either as a surrogate toxin or as a relevant marker for adequacy of dialysis continues. The reappraisal and demise of these two established cornerstones of dialysis is evidential of the predicaments afflicting the field of uraemic toxicity and its treatment [17–19].

Uraemic retention solutes versus uraemic toxins—terminology

The most appropriate term for solutes normally cleared by the kidney but retained in kidney failure is uraemic retention solutes (URS). However, even though not all substances retained during kidney failure exhibit toxicity—in whichever way this is established or defined—the term uraemic toxins has found widespread usage and both URS and uraemic toxins are often used interchangeably. Be it nomenclature or semantics, therein lies the primary quandary affecting the contentious topic of uraemic toxicity: for any (new) substance discovered and present in abnormally elevated amounts in ESKD, there is an inclination to ascribe toxicity to it. Instances of URS incorrectly assumed to be toxic have been documented and will be discussed below.

Membrane attributes contributed to the generation of the 'middle molecules' (MM) hypothesis

The concept of MMs remains firmly embedded in the field of dialysis [20]. There is voluminous literature on the subject and even though the term is ill-defined and despite its generality, a fascination prevails. Since its dissemination, the notion of MMs has unquestionably contributed to a better appreciation of uraemia, its complexity and the interplay of a multitude of factors involved in its pathophysiology. It has also given rise to considerable confusion; researchers have struggled to understand exactly what MMs actually constitute, giving rise to misconceptions and indeed misappropriations resulting largely from the boundaries being periodically redefined [21–23]. It must be noted that long before the MM hypothesis was formulated, substances in the body fluids of uraemic patients were described that could have been considered MMs [22, 24].

The MM hypothesis was founded upon observations in the early years of dialysis that removal of certain higher molecular weight substances was more efficient with the peritoneal membrane than with HD and led to better clinical results [25]. Subsequently, in 1971, Babb *et al.* [13] observed 'molecules that because of their size are very slowly dialyzable when compared to urea', speculating that these 'middle molecules play an important role in the toxicity of uraemia' and the 'very low dialyzability is due almost entirely to a very high membrane diffusion resistance'. In this landmark paper the authors predicted, rightly, that if their 'square meter-hour hypothesis' is verified, 'it will have an enormous impact on the future of haemodialysis' but also cautioned that 'the importance of 'middle molecules' will have been proven', an endeavour that continues today. The implications of these predictions are addressed at various points in this publication and supplement.

The overture of the MM hypothesis was that despite adequate urea removal by membranes available at the time, patient outcomes were nevertheless poor, with symptoms they experienced that could only be explained by the lack of removal of other undefined toxic metabolites [13]. Further support for the conjecture was provided by a comparison with peritoneal dialysis (PD) patients, who fared better despite less efficient urea removal. Thus began the search for other compounds causing,

or explaining, uraemic symptoms, providing the impetus for the field of uraemic toxicity.

Contrary to popular belief, the molecular weight size range originally specified for the MMs was 500–5000 Da [26]. The square-meter hypothesis was considered too restrictive and was renamed the 'middle molecule hypothesis', with the size range of MMs adjusted to 500–2000 Da [22, 27]. Since then, the constitution of MMs in terms of their size range has undergone regular readjustment. In 1994 they were 300–12 000 Da [28], and in 1997 Ringoir [29] redefined MMs to be 300–5000 Da. Shortly thereafter, the deliberations of the European Uraemic Toxin Work Group (EUTox WG) established yet another definition of MMs being from 500 to 60 000 Da (upper limit inferred, not specified) [30]. Recently, in a special issue of *Toxins* (novel issues in uraemic toxicity), MMs were redefined as ≥500–32 000 Da [31]. The issue of shifting the boundaries is far from being trivial: the entire justification for the study of retention solutes and their biological activity is centred around the argument it would lead to the development of more specific or targeted therapeutic strategies. With phrases such as small-, medium- or large- middle molecules now appearing regularly in publications, the doors are truly open for a broad and highly subjective interpretation of MMs [32–34]. Even in the 1980s, the term MM was misinterpretated because neither the upper nor the lower limits of 'middle' had been determined satisfactorily [23]. The 60 000-Da upper limit of MMs inferred by the EUTox WG is also contentious, as albumin (66 800 Da) is just beyond the MM range, yet it is widely regarded as a 'large' protein. As will be justified subsequently, the concept of MMs particularly from the membrane separation perspective, needs to be considered today more in terms of the historical sense rather than as a precise, defined scientific entity or term [22]. This does not in any way diminish the value of the MM hypothesis in triggering a better understanding of uraemia or uraemic toxicity or its position as a landmark in the annals of dialysis that contributed to the advancement of dialysis therapies [35].

Reclassification of MMs by the EUTox WG 2003

A comprehensive evaluation and classification of URS was undertaken by the EUTox WG founded in 1999 [30, 36]. The endeavour sought primarily to derive an overview of URS, considered at the time to play a role in uraemic toxicity, to facilitate a better understanding of uraemia and conditions related to it. Improving therapeutic strategies aimed at the prevention of disease and disease progression were further objectives of the WG. The focus was on HD, which is the most frequently used renal replacement therapy (RRT) modality to combat uraemia. Significantly, recognizing that HD is a highly technology-dependent therapy, the EUTox WG involved partners from industry at the outset to jointly pave the way for the development of better defined and more specific dialysis URS removal strategies.

The entire undertaking was centred around the concentration levels of retention solutes, i.e. the relative differences between uraemic and normal concentrations, as well as their changes and interindividual variability, as uraemic pathophysiology is dependent on abnormal solute levels. The first product of the EUTox WG was a publication classifying the known uraemic retention solutes, which is now regarded as a reference starting point on the topic of uraemic toxicity [30]. Essentially, a listing or inventory of all the solutes implicated in uraemia was created, underlining the complexity of uraemic solute retention as well as the challenges facing its therapeutic correction (particularly by HD). With >2800 scientific papers published by members of the EUTox WG since 1995 and >300 publications in which at least two member groups of the EUTox WG jointly contributed, the efforts of the group towards nephrology and dialysis are admirable and its opinions carry weight. Best practice guidelines, academic research projects and corporate strategies are influenced by the findings of the EUTox WG, its participants and their publications.

The biological potency of the substances listed in terms of their clinical relevance was not considered in any detail in this initial undertaking. In the EUTox classification of 2003 only a rather loose reference ('negative interaction with biological functions') was made regarding the purported toxicity of the retained solutes [30]. Abnormal concentrations and the size (i.e. molecular weight) of the retained solutes featured prominently in the classification, using the MM hypothesis as a fulcrum for the classification. As mentioned, the EUTox WG redefined the original MM (hypothesis) size range (500–2000 Da), keeping the lower limit of 500 Da, but the upper limit was not restricted and all solutes with a molecular weight >2000 Da were also classified as MM. An upper limit of 60 000 Da can be assumed, as molecules larger than this size were excluded from the deliberations of the EUTox WG on the basis that they 'are not filterable through the glomerular basement membrane'. Of the 90 compounds selected, 68 solutes were thus classified as 'small' solutes (≤500 Da), with the remaining 22 as MMs (≥500 Da). Inexplicably, 12 of these 22 MMs >12 000 Da, but they did not provide a reason for the selection of this particular size limit [presumably pertaining to the molecular weight of 11 800 Da of β_2-microglobulin (β_2-m), an arbitrary surrogate of 'larger' uraemic toxin compounds]. Inorganic compounds (e.g. water, sodium, phosphate, potassium) were excluded, although it is acknowledged that they exert significant toxicity and are major targets of removal in dialysis therapies [37]. The URS are subdivided into three categories [30, 38]: low molecular weight solutes (≤500 Da; small, free, water-soluble compounds), compounds known or likely to be protein-bound (most <500 Da, i.e. not MMs) and MMs (≥500 Da; some of which are protein bound).

The search for additional uraemic retention solutes/uraemic toxins

In 2012, another 56 retention solutes were added to the original list compiled by the EUTox WG in 2003 [39]. This compounded matters further, as an already crowded field in need of sifting became more complex towards the goal of elucidating the pathophysiology of uraemia and, in developing more specific therapy strategies. Today it is still widely acknowledged that other, probably several, URS remain unidentified [40]. It needs to be recognized, however, that the mere identification and characterization of additional 'novel' retention solutes—or their removal—even if shown to be highly toxic, are unlikely to help improve dialysis therapies *per se*. With the size exclusion-based principles of dialysis and improved dialysis membranes in use today, most of these substances may already be removed in routine dialysis—just their identity may be unknown today. For example, should another molecule (e.g. with a size of 7500 Da) retained in uraemia, non-protein-bound and exhibiting toxicity be discovered, it is already being removed during current HD procedures—together with others within this size range. Its contribution to disease (clinical relevance) and dialysis-related outcome, too, would already have been integrated and observed within outcome studies. Even with the elucidation of extraordinary *in vivo* toxicity, it is unlikely that the identification of such a compound would bring about a marked improvement in patient

well-being; uraemic toxicity, as discussed below, is the summation of 'toxicities' of several compounds and HD removes groups of substances adequately. There are abundant cases whereby novel new toxins have received undue attention as markers or surrogates of uraemic toxicity, e.g. trimethylamine-*N*-oxide (TMAO), lanthionine, *N*-methyl-2-pyridone-5-carboxamide (2PY), indoxyl sulphate, *p*-cresylsulphate and fibroblast growth factor-23 (FGF-23), as discussed in subsequent sections below.

Approaches for the characterization and identification of URS

Well before the advent of sophisticated analytical techniques, identification and quantitative analysis relating higher concentrations of various substances with specific uraemic symptoms was recognized as early as the 1980s [41]. Identification of URS relied mainly on size-exclusion techniques using membrane filtration or gel chromatography comparing uraemic plasma or serum with that of normal subjects and fractions of uraemic ultrafiltrate [22]. Later, other chromatographic techniques were used, with separation achieved according to the size, shape, polarity, electrical charge or complexing of solutes. Depending on the experimental conditions, different mixtures of molecules were observed in the chromatograms. Quantitative measurements or identification of individual solutes were difficult to achieve from the eluted peaks with a high degree of certainty [42]. Each peak or spot on the chromatogram spectra is not necessarily indicative of the retention or toxicity of the substance, as it may be a useful substance that need not be removed during dialysis.

Today, more powerful analytical tools facilitate the identification of these yet to be characterized substances compared with the painstakingly laborious methods of the past. Two approaches that are now being utilized in uraemic toxicity research are metabolomic and proteomic profiling of biological fluids, the former focusing on small molecules while the latter is suited for the study of peptides and proteins [40, 43–45]. Both approaches involve highly sophisticated software-backed analytical techniques such as nuclear magnetic resonance spectroscopy and mass spectrometry. These are usually preceded by a chromatographic separation step, e.g. gas chromatography, liquid chromatography, or capillary electrophoresis. An elaborate workflow of the various stages involved for metabolomics (proteomics being a targeted analytical approach) has been shown by Vanholder *et al.* [30]. Numerous 'peaks' or 'spots' ('hits') are discernible, depending on the origins and handling of the sample e.g. uraemic sera, urine, ultrafiltrate or haemodialysate [44]. The oft-discussed issue of detecting hitherto unidentified URS is thus a challenging and costly exercise and, as the example of *p*-cresol (described below) shows, caution needs to be exercised, as there is a compulsion to ascribe toxicity to molecules simply because of their presence in uraemic blood [6, 43, 46]. Comprehensive profiling with advanced analytical methods of URS (elevated concentrations and toxic) found in ESKD patients would help us understand the benefits:risk ratio of dialysis membranes.

THE PURPORTED TOXICITY OF URS

How is a solute retained in uraemia classified as a uraemic toxin?

Classical toxins are generally exogenous compounds that enter the body, while uraemic toxins are endogenously produced substances from metabolic processes or microbiota present in the body [47]. The purported toxicity of URS is related to their increased concentration in kidney failure due to reduced clearance as compared with solutes removed by the healthy kidney [5, 47]. This primary premise of uraemic toxicity is as valid today as it was in earlier definitions, e.g. in 1997 Ringoir [29] laid down the stepwise stages required for the demonstration of toxicity of a URS: (i) increased presence in uraemia, (ii) identification by means of a well-defined methodology, (iii) toxicity at a cellular or metabolic level upon its administration and (iv) cure of the intoxication through removal of the 'toxin' by dialysis or specific antidotes.

Assessment of the biological activity of URS has proceeded simultaneously with the exercise of identification, characterization and classification of new solutes suspected of playing a role in uraemia. Again, in deciphering their relevance to uraemia in terms of their clinical symptomology, there is the compulsion to assign some form of toxicity, i.e. harmful effect, to a URS because it is present at abnormally high levels in uraemic patients. Several issues arise with this supposition, most significant of which is how a solute is deemed a causative agent playing a specific role in a symptom of uraemia. The initial classification of the EUTox WG only loosely referred to this issue, with URS considered as uraemic toxins 'when they interact negatively with biologic functions' or have an 'impact on one or a few biological systems' or have 'some form of (strong) or (substantial) biologic activity' to exert toxicity.

It is difficult to imagine substances present in blood as not having some form of biologic activity. And, if biologic activity is expressed or demonstrated, what criteria construe the interaction to be negative or deleterious? In 1985, Brunner and Mann [22] listed several categories of uraemic disorders and disturbances that could be related to the biologic activities of MM fractions derived from chromatographic methodologies. Without having pure substances or knowledge of the precise identity of solutes and constrained by the techniques available then, the paper nevertheless presents an impressive review of the literature showing how early investigators strove to address the toxicity of retained substances. Today, with more sophisticated methodologies, the biological effects of isolated uraemic solutes can be evaluated at relevant concentrations in *in vitro*/*ex vivo* and/or *in vivo* experiments [5, 48]. Glorieux and Tattersall [48] state that the final approach in demonstrating toxic effects of a solute is to try to decrease the concentrations *in vivo* and only when improvement of hard outcomes of chronic kidney disease (CKD) patients is demonstrated can a causal relation be confirmed. Establishing such a specific relationship would require randomized controlled trials (RCTs); such studies are challenging because to check their toxicity would require a method to selectively decrease their concentration. The toxicity of solutes retained in uraemia is presently based on clinical studies suggesting an associative rather than a causal relationship. Laboratory experimental set-ups are often highly subjective and depend on the preferences, expertise and tools available to the researcher; bias being a risk of scientific research [49]. Most studies focus on establishing negative biological effects for URS; few prospective research studies set out to establish the non-toxicity or beneficial effects of URS present in abnormal levels in uraemia [50].

The dilemma of defining, or assigning, toxicity for URS present in abnormal concentrations is best exemplified by considering the fate of two solutes. One is urea, whose reputation has fluctuated from being initially a suspect, then benign and, more than a century later, being revived as a culprit toxin [51]. The other, *p*-cresol, a protein-bound retention solute, was

initially shown to express toxicity but eventually fell out of favour following methodological inconsistencies.

Urea—is it just a marker or a uraemic toxin?

In the early years of the 19th century it was first suggested that urea might be a toxin and if not separated from the blood, those excess amounts might lead to specific disorders; the concept was considered totally unacceptable [8–10]. During the middle of that century, the concept of urea having toxicity became more agreeable after evidence was presented relating elevated blood urea concentrations in certain patients with observed deleterious effects. Thereafter, a reversal of opinion began when studies experimenting with animals having intact kidneys and injected with urea showed no toxicity. Since then and until recently, urea has essentially been considered an inherently biologically inert molecule that does not exhibit toxicity, even though its levels correlate with the severity and progression of CKD. Today, measurements of urea are a surrogate marker of uraemic retention characterizing CKD and of adequacy of overall solute removal during dialysis [52, 53].

Lau and Vaziri [14] presented a case for the direct and indirect toxicities of urea by assessing its *in vitro* and *in vivo* toxicity at the cellular and systemic levels, correlating urea concentration increases with CKD progression. They reviewed the accumulating evidence indicating a negative impact of elevated urea, including its potential role in the pathogenesis of cardiovascular disease (CVD), a major cause of mortality in dialysis patients. Studies indicating urea-induced inflammation, oxidative stress, apoptosis of vascular smooth muscle cells and endothelial dysfunction—all directly promoting CVD—were presented in the review. There are widespread indirect effects of elevated urea as a result of the carbamylation reaction, where isocyanic acid (a product of urea catabolism) alters the structure and function of proteins in the body. Carbamylation has been linked with renal fibrosis, atherosclerosis and anaemia [14]. Evidence showing the direct toxicity of urea in terms of biochemical modifications induced by urea in *in vitro* and animal studies have been reviewed by Vanholder *et al.* [16]. At least five studies showed a direct biochemical effect of urea at the elevated concentrations seen in CKD interfering with a host of biochemical and organ functions. Urea is now being viewed more as a toxin than merely a marker of uraemic retention in CKD and of the adequacy of intradialytic solute removal [48]. That, over the course of two centuries, the position of urea in uraemia has remained elusive is indicative of the complexity and the investigative difficulties associated with its thorough understanding [51]. Likewise, other candidate toxins have been resurrected, e.g. symmetric dimethylarginine, previously considered inert but now appearing to exert toxicity [54].

The artefact of *p*-cresol as a uraemic toxin

Following the identification and classification of URS, a period of intense—and at times highly competitive—research activity followed to not only assign toxicity, but to lead the way in describing the toxic potential of solutes and explain mechanisms involved in uraemia. Several solutes seen as potential game changers emerged and many publications resulted about a handful of substances [48]. Protein-bound molecules, especially those linked to cardiovascular pathways, were of particular interest, with various research groups working simultaneously on their purported toxicity [55]. Indoxyl sulphate and *p*-cresol/*p*-cresylsulphate were among the most studied in this category and several investigations demonstrated their pathophysiological effects. Just over a decade after the EUTox WG publication, a systematic review was published including no fewer than 27 studies involving the two solutes [56].

Elevated levels of *p*-cresol (108 Da), measurable in serum samples of uraemic patients with a gradual increase in concentration as renal failure progresses, led to it being considered a major toxin [57, 58]. In these early studies, most laboratory determinations involved an acidification step for deproteinization. However, using methanol deproteinization (i.e. without acidification), no *p*-cresol could be detected in serum from normal or uraemic patients, yet substantial amounts of its conjugate, *p*-cresylsulphate were found, suggesting the acidification step promoted the conversion of *p*-cresylsulphate to *p*-cresol. Hence it is not the parent compound *p*-cresol, but its conjugates like *p*-cresylsulphate that prevail in the body and the renal retention solute [59].

The examples of urea and *p*-cresol/*p*-cresylsulphate raise the issue of erroneous laboratory methodology and perhaps bias that complicates the designation of a toxic label to URS [49]. With lessons learned from these cases and fast-evolving laboratory technologies, we should expect a lesser likelihood of misidentification of toxins in future studies.

URS widely recognized as being uraemic toxins

It is not the objective of this article to establish criteria or examine the evidence that designates an individual retention solute as a toxins. Recently, several articles have detailed the toxicities of uraemic substances known to play a pathophysiological role in the genesis of renal damage in CKD [5, 31, 39, 40, 48]. For various reasons, some substances have merited more interest than others from researchers seeking to explain the uraemic condition and its manifestations. Some hard clinical outcomes are easier to study than others, which impacts the design of clinical trials. Substances seen as culprits in the progression of CKD and related cardiovascular complications prevalent in uraemic patients have received particular attention [48].

Two articles present the most comprehensive and systematically reviewed evidential compilation of uraemic toxicity [60, 61]. They include molecules long known to have toxic effects together with re-evaluation of newly detected biological effects, as well as recently detected molecules identified with advanced analytical techniques. The effect on 11 biological systems and organs was considered, with a focus on cardiovascular damage, inflammation and fibrosis. A list of 79 URS considered to impact functions that contribute to uraemia and associated complications (based on their biochemical and clinical role), leading to increased morbidity and mortality in CKD, was produced [61]. With the systematic methodology applied by Vanholder *et al.* [61], for the first time it is possible to obtain unanimity regarding the relative toxicity of compounds that are considered to define the key pathophysiologic processes of uraemia and its manifestations. Hitherto, the task of navigating through the field of uraemic toxicity, particularly for newcomers to the field, was compounded by an ever-increasing number of substances being implicated in the condition of uraemia, with an overemphasis on the benefits of removing individual URS. Periodically, newer substances emerged, sometimes with exaggerated claims of their novelty as biomarkers or surrogates of uraemic toxicity. Industry was always quick to focus on new ‘magic bullets’ to try to gain a competitive advantage. The authors of this seminal paper made it abundantly clear that combatting uraemic toxicity needed to be achieved by decreasing the overall concentration of URS rather than target removal of individual solutes.

HD procedure itself as a contributor to toxicity

HD combats the symptoms of uraemia by removing uraemic toxins already present in the blood of ESKD patients. The HD procedure itself is a potential source of substances that could result in unwanted biological effects to the patient, i.e. exogenous substances either generated or present as contaminants during the dialysis procedure. Preventing this source of inadvertent 'toxicity' is essential, as such periodic insults are believed to affect long-term patient outcomes. Effects of endotoxins present in dialysis fluids with bacterial contamination have been well studied and are discussed in a separate section of this supplement. Another recognized form of procedure-related toxicity is cytotoxicity due to substances leaching from components of artificial surfaces that affect body tissues other than blood components. Exposing mouse fibroblast cells to extracts derived by incubating various dialysers with culture medium showed variable degrees of cytotoxicity as assessed by the impact on metabolic activity and cell growth [62]. Both the membrane polymer type and the method of dialyser sterilization affected the two measures of cellular activity.

EVIDENCE-LINKED URAEMIC TOXIN ELIMINATION: A REVISED APPROACH TO COUNTERACT URAEMIC TOXICITY IN HD

Which uraemic toxins need to be removed in HD

Throughout the history of dialysis, developers and providers of HD therapies have struggled to identify the substances whose concentrations need lowering. The ensuing nephrologist-industry battle hinged (aside from cost considerations) on the lack of adequate guidance as to which substances need elimination from blood via extracorporeal techniques. Now, through the assiduous efforts of the world's researchers, clusters of key toxicity-exhibiting retention solutes playing a role in uraemia have been collated using a weighted ranking system after sifting through evidence from hundreds of publications [60, 61]. This scoring approach evaluates the credibility of evidence supporting whether a substance should be classified as toxic but does not review the relative toxicity of the substance. Further, in terms of weighting, the scoring system does not differentiate evidence generated between laboratory and clinical studies; apparently evidence from clinical outcomes and a clinical causality link has preference over experimental results. Nevertheless, with this more authoritative guidance on the deleterious effects of the spectrum of URS, the reappraisal of existing dialysis strategies can be approached with renewed assurance. Significantly, the authors' explicit emphasis was on the need to decrease concentrations of groups of solutes, stating that the removal of individual solutes to improve outcomes was misguided [60, 61].

Using a weighted scoring system to classify compounds according to the experimental evidence of their toxicity (number of biological systems affected) and overall clinical and experimental evidence, the final selection was narrowed down to 12 substances with the highest scores for overall evidence level (Table 1A) [60]. Also, a similar shortlist of 11 compounds published 3 years earlier is presented (Table 1B) [48].

Comparing the two lists, some interesting observations can be made that illustrate both the dilemma of assessing the uraemic toxin labyrinth and how the topic of uraemic toxicity is evolving based on newer evidence. There is some overlap (only five substances appear in both lists), but since 2015, compounds like TMAO, ghrelin and kynurenines, previously not the focus of widespread attention and much less discussed as toxins than many other compounds, have emerged as frontrunners having higher clinical relevance in terms of uraemic toxicity. The example of TMAO, although known since 1997, requires mention, as it came to prominence in 2011 via profiling using the metabolomics approach [63, 64]. Subsequently it has become recognized as an agent with 'major toxic potential' and identified as a strong predictor of cardio-vascular risk and linked to hard outcomes in the CKD population' [60]. Yet, although these credentials merit its inclusion on the shortlist as one of the solutes with the highest scores, it remains debated whether this compound is 'a culprit or just a marker, in view of several paradoxical observations' [65]. Herein, within a single volume of a journal, we have expert pronouncements that are far from providing an unambiguous interpretation on the toxic potential of a compound. Another example is that of FGF-23, an MM (molecular weight = 22.5 kDa) proclaimed over a relatively short period of time to be an important cardiovascular toxin with evidence of its involvement in an array of pathological mechanisms in CKD [66]. As the association between FGF-23 and clinical events is yet to be established as causal or casual, doubts remain whether it unequivocally is a uraemic toxin or simply a biomarker [67, 68].

Furthermore, from the two lists, one could reach the conclusion that interleukin- and tumour necrosis factor-α (Table 1B) are now regarded as lesser uraemic toxins, or at least the evidence is not strong enough to be included in the more recent (2018) listing shown in Table 1A. Whether a compound can be judged as having high or low toxicity is not only based on the emergence of new data, but also a matter of individual perception. Thus, for those not totally familiar with the field of uraemic toxicity and who have been unable to follow it over long periods, following the fluctuating fates of toxins is an arduous exercise. For example, parathyroid hormone, makes an appearance in the more recent list but not that of 3 years earlier. This despite having been ardently regarded by many as an established uraemic toxin for more than half a century, having multiple undesired biological effects [69, 70].

In summary, although we know more about several players involved in uraemic toxicity, consensus on selecting the more important substances has been, and may remain, difficult to achieve. Nonetheless, applying the ideals of evidence-based medicine, i.e. utilization of the best possible evidence for informed decision-making in clinical practice, the systematic exposition of Vanholder *et al*. needs to be heeded [71, 72]. The collective removal of as many as possible of 71 compounds (free or bound to proteins) would make clinical sense if this is balanced with the simultaneous and inevitable loss of essential compounds and, according to the guidance, would most likely result in improved outcomes [52, 73].

Which uraemic toxins can be removed by HD?

Knowing which substances need to be removed (from a clinical view point) and how such removal is technically realized (i.e. selection of the appropriate membrane and treatment modality) are altogether separate considerations. This article is concerned with URS (i.e. uraemic toxins) from a removability or dialyzability (i.e. essentially membrane) perspective. As far as the physical separation by HD is concerned, the abnormal concentrations in disease states, the precise biological potency (i.e. toxicity or deleterious effects), or any arbitrary classifications (e.g. categorization as small or middle molecules) of individual entities is

Table 1. Changing focus on key URS deemed to express toxicity based on availability of new evidence. The upper panel (A) shows solutes with the highest ranking scores for overall evidence level derived from references published in 2018 [60, 61]. The lower panel (B) shows a 2015 list of key solutes selected by Glorieux and Tattersall [48]

Panel A URS with the highest scores for overall evidence levels*	Molecular weight (Da)
Small water-soluble	
Asymmetric dimethylarginine	202
Trimethylamine-*N*-oxide	75
Uric acid	168
Middle molecules	
β_2-microglobulin	11 818
Ghrelin	3370
Parathyroid hormone	3334
Protein-bound compounds	
Advanced glycation end products	5–20 kDa
p-cresylsulphate	188
Indoxyl sulphate	212
Indole acetic acid (IAA)	175
Kynurenines	208
Phenyl acetic acid	136

Panel B URS exhibiting toxicity	Molecular weight (Da)
Small water-soluble	
Asymmetric dimethylarginine	202
Symmetric dimethylarginine	202
Uric acid	60
Middle molecules	
β_2-microglobulin	11 818
Interleukin-6	24 500
Tumour necrosis factor-α	26 000
Protein-bound compounds	
Advanced glycation end products	5–20 kDa
p-cresylsulphate	188
Indoxyl sulphate	212
Indole acetic acid	175
HA acetic acid	179
P-OHHA	195

perhaps less relevant. Viewed solely as a separation boundary, the dialysis membrane is unable to discriminate between molecules based on their nomenclature, biological reactivity or level of toxicity. Emphasis of this viewpoint may appear paradoxical considering that the bulk of this publication is devoted to addressing solute biological toxicity, whose importance may appear to be lessened. Contrarily, these deliberations are essential prerequisites: before determining what can be removed, one needs to establish what should or needs to be removed, and that must be based on toxicity and clinical considerations.

From the membrane perspective, the collective removal of all 79 solutes with toxic potential identified by Vanholder *et al.* [60, 61] is technically feasible. In plasma separation procedures for instance, membranes enable separation of all cellular components of blood from the plasma component. As discussed in other articles in this supplement, membrane manufacturing technology is highly versatile and production of membranes can be tailored to have a broad range of porosities, from 'tight' to more 'open' structures. The molecular weight of the 71 compounds with expressed toxicity ranges from small (<50 Da; ammonia, mono-methylamine, cyanate, dimethylamine) to large (64 000 Da; complement factor Ba). Several protein-bound substances have an effective size larger than this upper limit; as most substances in this category are bound to albumin, for simplicity we thus assume they are >66 800 Da.

However, from the membrane separation perspective, only the following sequence is relevant in terms of size of the 71 solutes: small → large → protein bound. Moreover, for all dialysis procedures the upper limit of 66 800 Da is crucial: it sets the physiochemical and clinical restraints of all conventional HD treatment modalities. Clinically it is generally accepted that regular loss of albumin during each session over long periods of time is detrimental to patient well-being [74–78]. The issue is contentious though, from two points of view. First, in striving for more efficient removal of 'larger' uraemic toxins, membrane manufacturers create increasingly more open membranes to justify increased elimination of larger toxins. Second, as many small uraemic toxins are bound to proteins (predominantly albumin), an additional advantage (reducing the risk of either all-cause mortality or cardiovascular mortality) is not observed by using more efficient convective therapies or membranes leaking albumin [79].

Linking uraemic toxins with membrane separation criteria: solute size versus membrane pore size. To determine the most appropriate strategy allowing removal of the 79 solutes (the list

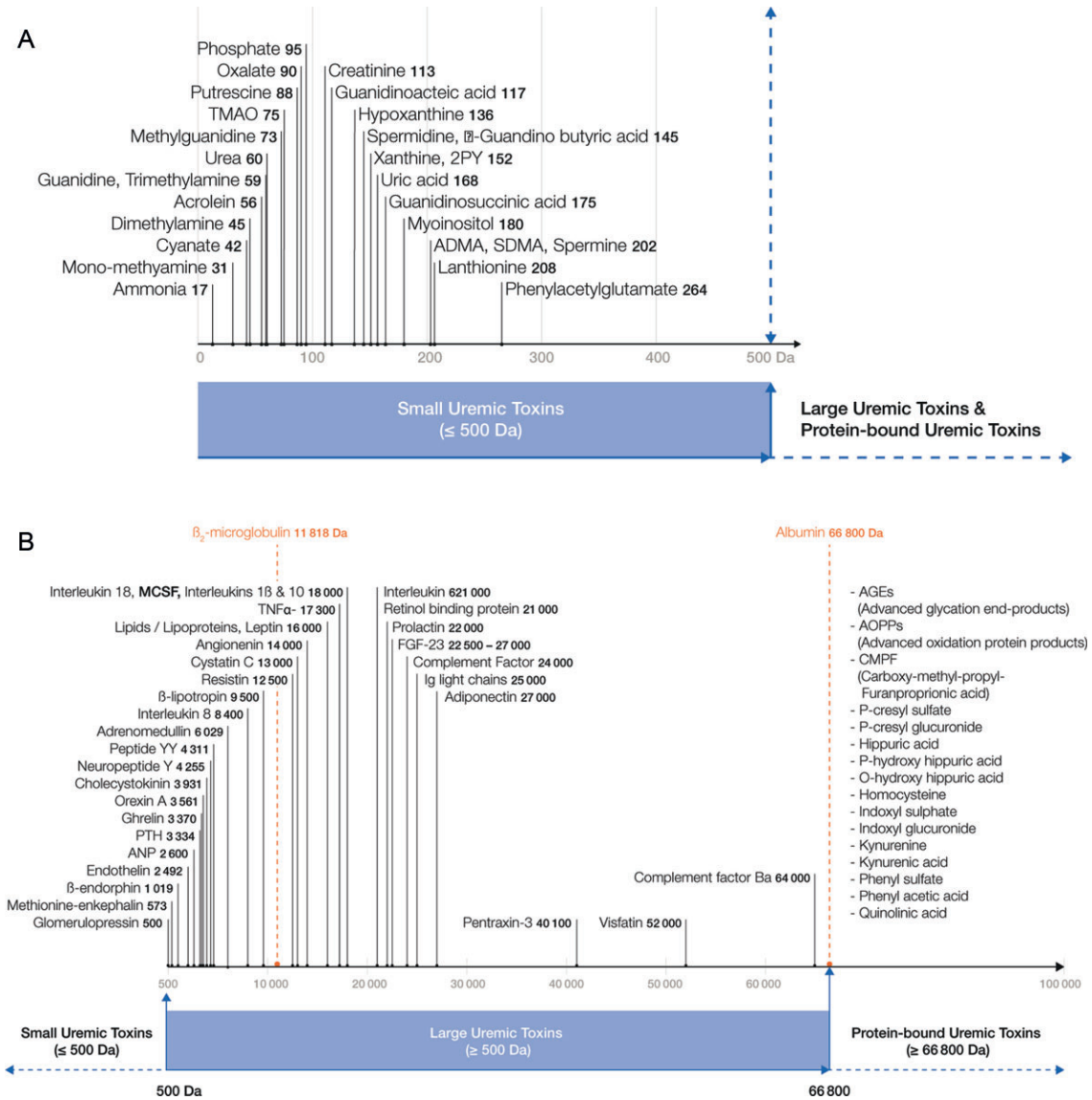

FIGURE 1: The size-range distribution of all URS for which there is a body of evidence indicating their toxic potential according to the assessment of Vanholder *et al.* [60, 61]. (**A**) 'Small molecules' <500 Da. (**B**) 'Middle molecules', larger solutes up to the size of albumin and those that are protein-bound (assumed mainly to be albumin) and thus shown on the right side of the albumin molecule.

decreases to 71 as, some of these were grouped together) for which there is evidence of toxicity, the first objective is to visualize their size relative to that of albumin, the removal of which needs to be minimized (Figure 1). For membrane separation purposes, only two categories are important, free and protein-bound toxins, i.e. up to the molecular weight (66 800 Da) of the former and beyond for the latter. Because of this broad size range and for clarity, the position of small uraemic toxins (≤500 Da) is displayed separately in Figure 1A from the remainder of the larger solutes (≥500 Da) relative to the albumin molecule as shown in Figure 1B.

Fifty-nine of the 79 individual uraemic toxins, i.e. 74.7%—the bulk of the uraemic toxin load reviewed and defined by Vanholder *et al.* [60, 61]—have a molecular weight >30 000 Da. Of the remaining 20 compounds, only 3 (3.8%) are between 30 000 and 66 800 Da (the molecular weight of albumin). The rest [17 (21.5%)] are protein-bound, hence their effective size is .66 800 Da. These are generally recognized as the solutes that are 'difficult to remove' by current haemodialytic strategies.

Figure 1A and B show, using a linear scale, the size distribution of all 79 substances for which there is evidence of toxicity. To better assess their removability during HD, recognized membrane separation principles need to be applied. Selective separation in HD is achieved almost wholly on the basis of differences in size, shape or chemical structure, i.e. the physical–chemical properties of solutes relative to those of the membrane [80]. This size-based relationship is described by the sieving properties of the membrane, i.e. its morphology. The mean size of the pores and their distribution at the innermost separating region of the membrane determines which substances can traverse the membrane wall and which remain in the blood [76]. It is important to fully appreciate the scope and limitations of the sieving coefficient (SC) concept, as it is widely misinterpreted and even misappropriated.

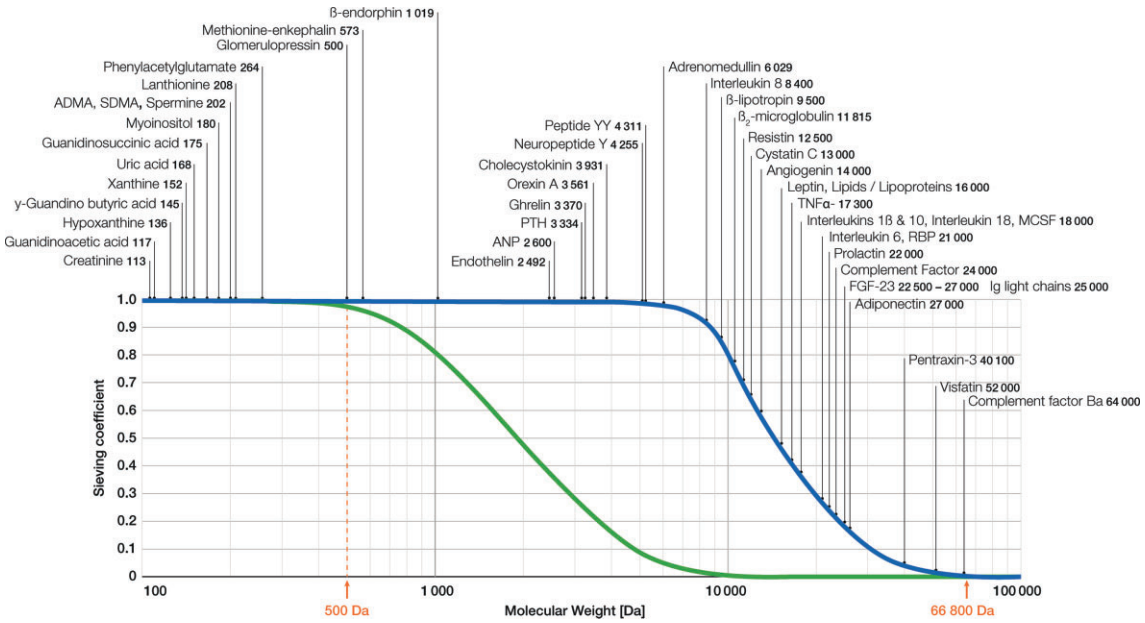

FIGURE 2: ELUTE concept, showing the removability of 46 of the 79 uraemic toxins for which there is evidence of toxicity according to weighted score ranking methodology of Vanholder *et al.* [45] by a 'typical' high-flux membrane (blue line). The SC (*y*-axis) is linked to the mean pore size and their distribution in the membrane; the molecular weight (*x*-axis) is also related to the size of the molecules (i.e. Stokes radius based on globular proteins). The higher the SC (*y*-axis) for a specific substance and any membrane, the higher the removability. An additional 11 compounds from the list having molecular weights <100 Da are not shown, for reasons of diagrammatic clarity; they can be identified from Figure 1A. The protein-bound uraemic toxins for which there is evidence of toxicity (15 compounds) are not shown and are assumed to have a molecular weight >66 800 Da and thus lie to the right of this point; these can be identified from Figure 1B. This figure shows typical sieving coefficient profiles of two membranes, one with a smaller mean pore size (green line, termed 'low-flux') and the other with a larger mean pore size (blue line, termed 'high-flux').

The SC for a specific solute is the ratio of its concentration in the ultrafiltrate (C_f, removed by convective transport only) divided by its concentration in plasma water (C_p):

$$SC = C_f / C_p.$$

SC profiles for each membrane are derived in the laboratory and SC plotted (*y*-axis) against molecular weight (*x*-axis, logarithmic scale). A value of 1 (100%) for a given solute implies that it is smaller than the mean size of the pores and thus passes through the membrane unimpeded; an SC value of 0 (0%) indicates its size is too large compared with the mean pore size and so it cannot go across the membrane wall. Depending on the membrane morphology (pore size at the inner separating region), molecules lying on the flat upper part of the curve (i.e. SC = 1) can pass freely across the membrane.

Figure 2 shows typical SC profiles for two membranes, one with a smaller mean pore size (green line, termed 'low-flux') and the other with a larger mean pore size (blue line, termed 'high-flux'). The former allows reduced passage of β_2-m (a recognized surrogate of 'larger' MMs), while the latter, having an SC for β_2-m of 0.8, is indicative of higher removability of 'larger' uraemic toxins. The figure shows the position of all free (i.e. excluding protein-bound) solutes from the uraemic toxin list of Vanholder *et al.* [60, 61] with a molecular weight >100 Da; 11 substances (<100 Da) are not shown for clarity of presentation, as the *x*-axis is on a logarithmic scale. Protein-bound substances, all assumed to be >66 800 Da (molecular weight of albumin) are also not shown, as explained above.

We see that 35 of the 79 (44.3%) uraemic toxin compounds (small and middle molecules) lie on the flat part of the SC curve and are thus entirely (100%) eliminated during dialysis with a standard 'high-flux' membrane. Further, four compounds have an SC between 1 and 0.8 (including β_2-m), meaning that they are removed up to 80%. Between an SC of 0.8 (β_2-m) and 0.5 (50% removal), four additional substances are removed and up to an SC of 0.3, another three. Only three compounds (pentraxin 3, visfatin and complement factor Ba) are removed to a much lesser degree (SC <0.1 up to SC = 0), with minimal removal of the largest, complement factor Ba (molecular weight 64 000 Da). Hence a membrane designed to remove a negligible amount of albumin (molecular weight 66 800 Da) removes all toxins below this upper limit, albeit to increasing extent (as their size decreases) towards the left of the position of albumin. Thus, other than the 17 protein-bound substances, all toxins are removed to a certain extent, with only three compounds having minimal removal.

Figure 2 indicates the removability during dialysis of the entire toxin load, excluding those ≤500 Da, which are all removed and those that are protein-bound, i.e. compounds deemed to have some toxic potential, regardless of the intensity of their toxicity. Each of these compounds exhibits varying degree of intensity of toxicity ('negative biological activity') that depends on the particular body systems they are involved in or impact.

Vanholder *et al.* [60, 61] further listed 24 uraemic toxins with the highest toxicity score. Figure 3 shows the removability, by a typical high-flux membrane, of these highest-ranking molecules with regards to their toxicity: 8 of them are removed completely (i.e. SC = 1), 9 to varying extent and 7 are protein-bound substances (or groups) that are not effectively removed by conventional dialytic procedures used in routine clinical practice. Of these 24 compounds with the highest toxicity score, a further 12 solutes were ranked as having the highest scores

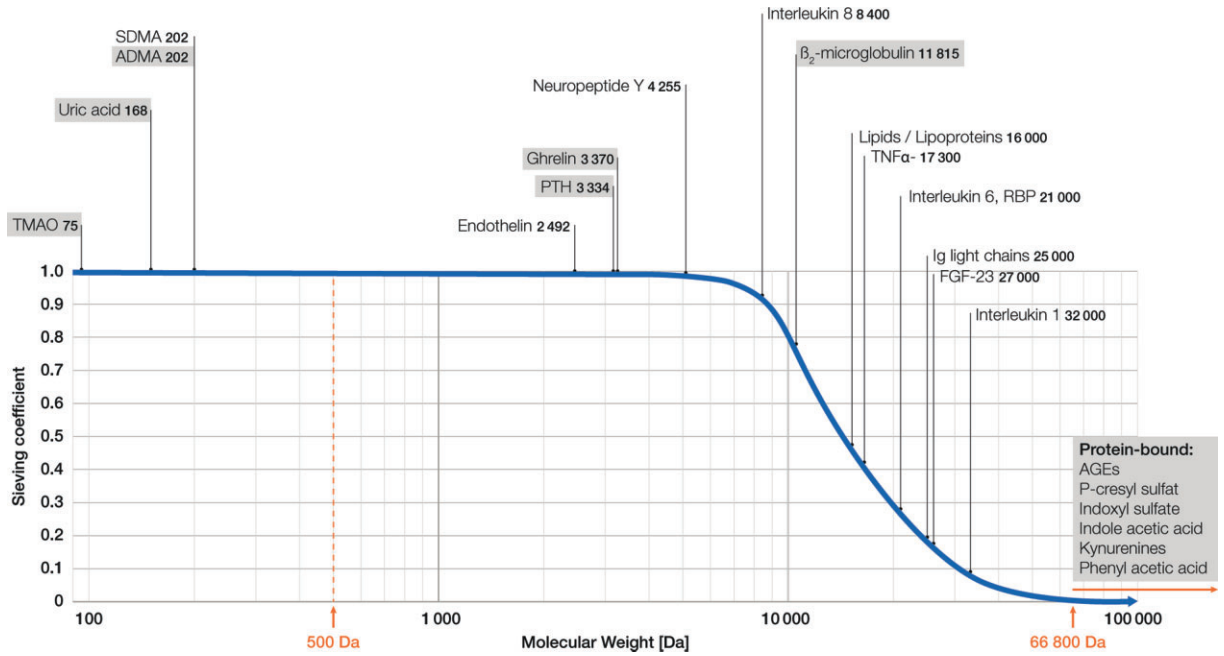

FIGURE 3: Removability of the 22 uraemic toxins with the highest toxicity score. Those lying on the flat part of the curve are removed totally, while those on the sloping part of curve are all removed at decreasing levels as molecular weight increases. The 12 molecules that are shaded were shown by Vanhoder *et al.* [60, 61] to be the highest ranking molecules in terms of toxicity; only 6 of these (along the sieving curve) are removed by dialysis, while those (protein-bound uraemic toxins) in the panel to the right of the 66 800 Da albumin molecule cannot be removed by dialysis, as their sieving coefficient is 0.0.

for overall evidence level (overall evidence score ≥3; maximum possible, 4) and experimental score (≥5; maximum possible, 7) and are highlighted by the grey shading in Figure 3, with the largest non-protein-bound uraemic toxin (PBUT) compound, β_2-m removed by 80%. Five of the six of these compounds exhibiting the highest overall evidence level are removed totally (100%, flat part of curve). The elimination of the remaining six, being protein-bound, are not impacted by standard high-flux membranes.

Removing PBUTs. Several substances (Figure 1B), mostly small molecules, expressing toxicity are protein-bound rather than free solutes [6, 31, 81, 82]. Even though some of the PBUTs have shown the highest scores in terms of the robustness of experimental data for their toxicity by affecting several host systems, Vanholder *et al.* [60, 61] emphasize the dearth of clinical data for many of the substances in this category. In the normally functioning kidney, they are removed through the active transporter-mediated process of tubular secretion that dialysis procedures are unable to replicate and are hence designated non-dialyzable or difficult to remove toxins, even by convective therapies [83, 84]. Albumin, indispensable to several body functions, is the major protein to which these substances are bound [85, 86]. Thus, as the combined effective size of the PBUTs is larger than that of free albumin (66 800 Da), all HD therapies strive not to induce the loss of substances beyond this size. While manufacturers of membranes that are intrinsically leaky to albumin may justify this as an advantage in terms of detoxification, the minor advantage accrued in its removal is far outweighed by the long-term detriment albumin loss causes to the well-being of the patient [86]. Several approaches, including some novel but untested, ones have been proposed, but none seem to have found enthusiasm for commercialization for routine clinical application [87–89]. Adsorption-based extracorporeal techniques are not specific to individual substances, and even if successful, would incur substantial increases in cost to achieve diminution of a fraction of the uraemic toxin load in ESKD [90, 91].

Another approach to deal with this class of toxins is to consider preventing their generation or remove them before they enter the blood stream. In fact, four (indoxyl sulphate, p-cresylsulphate, indole acetic acid, phenyl acetic acid) of the six PBUTs with the highest overall evidence level (Figure 3) originate from colonic microbial metabolism [92]. Strategies to remove such uraemic toxins originating in the gut from the microbiota by ingestion of adsorbers appear to be simpler and less radical than the ones suggested for removal by cumbersome (and costly) additional steps added to standard HD procedures. The unavailability of acceptable strategies to counteract the adverse effects of PBUTs, renders their impact as highly questionable currently [93]; thus the emphasis in HD modalities has to be on the removal of all other compounds, without overemphasis on PBUTs.

Reducing the overall uraemic toxin load during HD therapies. The sieving curve only indicates what can or cannot traverse the membrane, a technical aspect that needs to be coupled with the biochemical considerations of uraemic toxicity. To date, these two intrinsically related aspects have been considered separately. Apart from the physical–chemical attributes of the membrane in relation to those of the solute, the other determinant of dialyzability of solutes is related to clinical considerations involving physiological and patient criteria. Solute mass transfer (kinetics) between multiple interconnected compartments within the body and in the extracorporeal circuitry occurs by both active and passive transport mechanisms [45]. The extent to which an individual solute is removed depends on its equilibration and redistribution between different

body compartments during the RRT procedure and influenced by several treatment-related factors. These include the transport mechanism involved (therapy modality and conditions selected according to the patient criteria), membrane thickness, hollow fibre diameter, dialyzer surface area, secondary membrane formation, frequency and duration of treatment as well as blood and dialysis fluid flow rates. While insightful, kinetic modelling is complex, and variable interactions (e.g. protein binding) are difficult to incorporate in theoretical models [94]. Further, information derived on single molecules from these models cannot always be extrapolated to other molecules. For example, phosphate is a small molecule, but its removal kinetics are complicated and resemble those of MMs and vary according to the dialysis conditions [95, 96]. Likewise, although clearance of β_2-m is greater with haemodiafiltration (HDF), with its higher convective transport than with high-flux dialysis, β_2-m concentrations after long-term HDF are only slightly lower than those obtained with high-flux dialysis. Resistance to β_2-m transfer between body compartments is one explanation, suggesting that to decrease plasma levels of this solute, increased treatment times or frequency of treatment are required [97].

How much of the uraemic toxin load should be removed?

Each of the 79 solutes deemed uraemic toxins has its own solute mass transfer characteristics or profile, i.e. separate kinetic models would be required to estimate the extent to which concentrations of each solute are lowered during HD. With a multitude of uraemic toxins with varying degrees of potency to choose from, estimation of the reduction of the overall 'toxic load' is usually performed based on characteristics of a single selected surrogate solute. The results are extrapolated to reflect the lowering concentrations of the entire spectrum of substances considered to possess toxic potential. Clearly such a modelling approach, necessary as it is in guiding clinical practice, is not only based on several assumptions, but also highly contentious for its generality.

Estimating the dose or adequacy of dialysis is traditionally expressed based on the removal of urea, which lends itself as a candidate for many reasons [12]. A marker of protein metabolism and whose concentration correlates with the progression and severity of CKD, it is small, water soluble, non-protein-bound and freely diffusible across compartments. Its laboratory measurement is neither cumbersome, nor costly. The parameter Kt/V_{urea} (dialyzer clearance of urea multiplied by dialysis time and normalized for urea distribution volume) has been established as a measure for estimating the dose or adequacy of HD [17–19]. To address the issue of frequency of dialysis, introduction of the standard Kt/V_{urea} to express the weekly dose of dialysis has established itself in recommendations devised worldwide for therapy practices, quality control measures as well as reimbursement purposes. With increasing emphasis on removing solutes other than urea, more general expressions of dialysis doses e.g. the reduction ratio, enable an estimation of the total body clearance of a solute.

Today, after some 30 years of use, the index Kt/V_{urea} is considered to have outlived its usefulness [16, 98]. There is a lengthy list of the flaws of Kt/V, starting with a lack of proof of concept in three randomized controlled hard outcome trials and continuing with a series of conditions where the concept of Kt/V was shown to be flawed. The dialysis patient might benefit more if the nephrology community concentrated in the future on pursuing the optimal dialysis dose conforming with adequate quality of life and factors that are likely to affect clinical outcomes more than Kt/V. These include residual renal function, volume status, dialysis length, ultrafiltration rate, the number of intradialytic hypotensive episodes, interdialytic blood pressure, serum potassium, serum phosphate, serum albumin and C-reactive protein.

CONCLUSIONS

The much-exalted MM hypothesis was proposed based on the 'very low dialyzability' of (unknown) 'larger molecules' by the membranes available at the time [13, 20, 25–27]. Since then, captivation with the hypothesis has led to assigning toxicity to known URS or to search for even more unidentified compounds that may play a role in uraemia. As correctly predicted by the originators of the hypothesis, the impact of the MM theorem has been enormous, triggering research towards a better understanding of uraemia, its manifestations and approaches to counteract it. However, the relationship of these toxic entities with the central component of HD—the membrane that facilitates their elimination extracorporeally—has been less clearly delineated.

The non-specificity of solute removal by dialysis (membranes) has even been described as a 'handicap' in limiting the chances of providing proof of concept that a given solute or group of solutes has a definite biological impact [45]. However, in terms of ridding uraemic blood of scores of substances now shown to demonstrate toxicity, the same non-specificity of HD membranes is in fact a boon. Considering that focus on the removal of individual toxins is explicitly discouraged by experts, and without knowing which compound has precedence over another in terms of its toxic intensity, the collective removal of as many toxins as possible within a single procedure is both logical and cost-effective. It is difficult, with the knowledge available today, to envision how the overall toxicity of several heterogeneous substances would be counteracted without the availability of current membranes that allow elimination of a broad spectrum of substances in a single step.

The recent treatise on uraemic toxicity by Vanholder *et al.* provides much-needed guidance regarding the compounds that should be targeted for removal in HD. The evidence-based ranking of the toxic potential of key culprits of uraemia allows a reassessment of current HD strategies that many believe need changing, as overall patient outcomes remain poor. Even with the application of more efficient treatment modalities and strategies, primary outcome measures of several RCTs over the last 2 decades have failed to show significantly improved survival rates for HD patients [99, 100].

Our ELUTE analysis approach leads us to conclude that the preoccupation of the dialysis community with increasingly 'more open' dialysis membranes to remove 'larger' uraemic toxins may not be justified for two reasons [6]. First, the bulk of the uraemic toxin load is adequately diminished by membranes in current use and second, more open membranes increase the probability of causing the loss of substances that are vital for body functions [74]. From the evidence-based denotation of toxicity for the most studied uraemic retention solutes, we see that most of the uraemic toxins listed by Vanholder *et al.* [60, 61] are 'small' and effectively removed by standard dialysis therapies; only a very small proportion of free compounds are large and express toxicity. Here, there is a need to qualify 'small' or 'large' uraemic toxins, an assignment that has always challenged the dialysis community. The EuTox WG's segregation is not always easy to comprehend, with small molecules being $\leq$500 Da and anything above this size being MMs, comprising small peptide

molecules to large proteins with molecular weights of up to ∼60 000 Da. We therefore deduce that, from the membrane separation (removability) perspective only two categories of URS or uraemic toxins are relevant: free, no-nprotein-bound (i.e. removable by dialysis) and protein-bound ('difficult to remove' by HD). For each, distinctly separate removal strategies are necessary. Thus we consider the MM categorization—important as it may have been historically for the field of uraemic toxicity—to be a redundant concept in modern dialysis separation terms. To improve dialysis strategies and outcomes, the predominant question is first, to address which toxin(s) needs to be targeted for removal and second, by which appropriate technique (choice of membrane and modality selected). For both questions there are now more qualified answers; the analysis by Vanholder *et al.* now adequately addresses the former and the latter by its derivative, the ELUTE analysis presented herein. In our ELUTE approach, we have demonstrated that the 30 000-Da boundary is a valuable indicator of not just what can, but also cannot, be removed within the confines of currently available dialysis membranes to diminish the overall toxic load for ESKD patients. Unlike the MM hypothesis, no distinction is made between 'small', 'medium' or 'large' molecules; such imprecise and subjective terminology continues to give rise to considerable confusion in the field of uraemic toxicity.

Several large RCTs that set out to establish a survival advantage for more efficient treatment modalities have failed to show a benefit for high-flux membranes as their primary outcome measures defined at the outset of the trials [99, 100]. The HEMO and MPO studies were unable to unequivocally demonstrate that (more efficient) removal of larger URS reduces mortality rates. By reiterating the point that HD membranes allow passage of both unwanted (uraemic toxins) as well as unknown and known useful substances (such as a host of medications dialysis patients need to take regularly), our analysis suggests that one possible reason for the inability of these studies to demonstrate a benefit for the more efficient detoxification effect of high-flux-based therapies may be that these modalities inadvertently remove an array of compounds that are required for physiological functions and metabolism of the body. HD is successful as ESKD patients are unable to survive long without it, but it is feasible that persistent elimination of unknown amounts of essential and protective substances (including prescribed pharmacological agents) during high-efficiency procedures may negate, in part, the beneficial attributes of dialysis. Perhaps there is no discernible or demonstrable difference between presently defined low- and high-flux modalities and that striving to remove more of the larger toxins has been an erroneous supposition.

The six toxic compounds that are protein-bound for which there is evidence of toxicity (although insufficient data) are only contenders for HD-based elimination if clinicians are prepared to tolerate excessive amounts of albumin loss and other useful substances (using membranes with higher 'cut-offs') that may be to the detriment of the patient [101–103]. It is worth noting that even standard high-flux membranes can regularly incur albumin losses up to ∼6 g/treatment session, as an albumin SC of 0.01 (at the tail end of the SC profile). To curtail albumin loss, $SC_{albumin}$ needs to be $\leq$0.001 and by creating more open membranes to target PBUTs the $SC_{albumin}$ approaches $\geq$0.1, making membranes considerably leakier to proteins.

The proponents of the MM concept stated 'it must be remembered that in the early 1960s, membranes at least 300 µm thick were used that would have a very poor clearance of MMs' [27]. Today, the wall thickness of most membranes in use is several times smaller (usually 25–40 µm), resulting in significantly lower resistance to diffusion, the primary transport mechanism of dialysis. One is tempted to surmise that had thinner membranes (wall thickness ∼30 µm) or more 'open' membranes like the peritoneum (comparison to which spurred the MM hypothesis) been available at the time, larger molecular substances would have been removed and the MM hypothesis would probably not have been proposed. Instigated by the MM hypothesis, the compulsion is to remove more and more of the 'larger' substances, disregarding the constraints associated with membrane separation principles. Ironically, more of the useful substances are being simultaneously removed, and with higher efficiency [104]. Our observations suggest that most of the known uraemic toxins are removed satisfactorily by standard high-flux membranes and in straining to deal with the remainder of the (smaller) toxic load, the benefit acquired is perhaps being negated.

SYNOPSIS

(i) Adverse biological reactivity (toxicity) and size designation MMs or molecular weight) of URS alone are insufficient criteria to determine which substances need to be targeted for removal in HD.
(ii) Dialysis membrane determinants (e.g. sieving properties related to pore size) need to be considered to ascertain the extent of removal of individual uraemic toxins.
(iii) The concept of MMs is highly confounding, with multiple specifications of its boundaries in the literature today compared with the original definition; it is of immense historical significance, but redundant in terms of modern dialysis membrane technology.

The ELUTE concept (incorporating uraemic toxicity of substances in conjunction with their removability) reveals that the bulk of the uraemic toxin load comprises substances $<\sim$30 000 Da and is adequately reduced by standard dialysis membranes in current use.

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CONFLICT OF INTEREST STATEMENT

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