## Classification of Uremic Toxins and Their Role in Kidney Failure

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Abstract: Advances in our understanding of uremic retention solutes, as well as improvements in hemodialysis membranes and other techniques designed to remove uremic retention solutes, offer opportunities to readdress the definition and classification of uremic toxins. A consensus conference was held to develop recommendations for an updated definition and classification scheme based upon a holistic approach that incorporates physicochemical characteristics, and, dialytic removal patterns of uremic retention solutes and their linkage to clinical symptoms and outcomes. The major focus is on removal of uremic retention solutes by hemodialysis. The identification of representative biomarkers for different classes of uremic retention solutes and their correlation to clinical symptoms and outcomes may facilitate personalized and targeted dialysis prescriptions to improve quality of life, morbidity, and mortality. Recommendations for areas of future research were also formulated, aimed at improving understanding of uremic solutes and improving outcomes in patients with chronic kidney disease.
Classification of Uremic Toxins and Their Role in Kidney Failure: An Expert Consensus Conference

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Abstract

Advances in our understanding of uremic retention solutes, as well as improvements in hemodialysis membranes and other techniques designed to remove uremic retention solutes, offer opportunities to readdress the definition and classification of uremic toxins. A consensus conference was held to develop recommendations for an updated definition and classification scheme based upon a holistic approach that incorporates physicochemical characteristics, and dialytic removal patterns of uremic retention solutes and their linkage to clinical symptoms and outcomes. The major focus is on removal of uremic retention solutes by hemodialysis. The identification of representative biomarkers for different classes of uremic retention solutes and their correlation to clinical symptoms and outcomes may facilitate personalized and targeted dialysis prescriptions to improve quality of life, morbidity, and mortality. Recommendations for areas of future research were also formulated, aimed at improving understanding of uremic solutes and improving outcomes in patients with chronic kidney disease.
Background

Uremia is a broad term that has been variably used to describe the build-up of metabolic waste products such as urea that occurs with diminished kidney function. Along with the retention of metabolic waste products, patients with advanced kidney disease typically experience a constellation of symptoms that may include nausea, vomiting, fatigue, anorexia, muscle cramps, pruritus, mental status changes, and others that lead to a reduced quality of life as well as excess morbidity and mortality. Given the retention of metabolic waste products with advanced kidney disease, there has been much interest in using dialysis techniques to remove these substances with the hope that symptoms and outcomes would also improve. However, this goal has only been partially achieved, and outcomes for patients with kidney remain suboptimal. While our knowledge of solutes that build-up with uremia has increased, there is a growing recognition that current dialysis prescriptions (both hemodialysis and peritoneal dialysis) may not be effective in their removal. Furthermore, technological advances such as the development of new hemodialysis membranes and the ability to perform high efficiency hemodiafiltration enable the removal of molecules from the body up to ~50 kDa (1). Besides, other new technologies are being developed to remove toxins that build up with abnormal kidney function (2).

In light of these developments, an expert conference was convened to identify limitations in the current definition and classification of uremic retention solutes/toxins. Experts in the field were tasked with a comprehensive review of the current definition and classification of uremic retention solutes and posed several critical questions and recommendations to define these toxins better and map future studies for improving outcomes.

Methodology

A diverse panel of clinicians and researchers representing experts in the field of uremia and uremic toxins were identified and invited by the conference chair (C.R.) to participate. In addition, a few individuals were chosen based on experience in managing consensus processes. The conference was held virtually, over three days from November 30 to December 2, 2020, with additional small group sessions over the subsequent weeks. This consensus meeting used a modified Delphi method to achieve consensus, as previously described (3). The consensus conference began with a pre-conference comprehensive literature search and appraisal of scientific evidence to identify key themes that are central to uremia and uremic toxins. Conference participants were divided into three workgroups (Supplemental Table 1) and were tasked with addressing the following themes:-Critical appraisal of limitations in the current
Redefining uremic toxins

Rationale

In 2003, the European Uremic Toxin Work Group (EUTox) proposed five criteria for an organic solute to be classified as a uremic toxin (Figure 1, left column) (4). Inorganic solutes (e.g., water, potassium, sodium, magnesium, phosphate) were excluded in these criteria given the available literature on these solutes and their divergent intradialytic removal patterns from other solutes of interest.

The current view of uremic toxicity incorporates many solutes that are retained during kidney failure and have different physiochemical characteristics and diverse adverse effects on biological systems (5). Moreover, there are differences in toxicity of solutes, depending on whether a solute is studied alone or in conjunction with other solutes that may interact in complex ways (6,7). Besides, protein-bound solutes exhibit a large variation in their binding affinities to various plasma proteins (8), and toxicity may be exerted by the free fraction or the total concentration of these solutes (10). Undisputable proof of the toxicity of a specific solute can in principle only be obtained if selective removal is linked with improved outcomes and amelioration of symptoms but such studies have been conducted only for a few uremic toxins (10); in those cases, proof of toxicity is seldom unequivocal, likely because the impact of specific toxins may be superseded by that of other solutes with overlapping biological effects and which may interact in various ways (11).

By 2012, EUTox listed 146 uremic retention solutes (4,8). New technologies enable expansion of the list, creating a more comprehensive picture of uremic toxicity than was initially appreciated (6,11,12). In this context, the question was raised whether the current definition of a uremic toxin can be maintained or requires revision. We concluded that modifications are necessary to...
accommodate new advances in the field especially with the development of newer hemodialysis techniques. Figure 1 summarizes the current definition, the terminological limitations of that definition, and the proposed update.

**Recommendations:**

1. We suggest that the current definition of uremic toxins should be adapted in terminology to account for the growth in knowledge in the field (Figure 1).
2. We suggest that the scope of the definition should remain limited to organic solutes.

**Physicochemical classification of uremic toxins**

**Rationale**

In 2003, EUTox categorized uremic toxins according to their physicochemical characteristics that affect clearance during hemodialysis. This classification was essentially inspired by the need to simplify and organize uremic toxicity concepts within a framework of therapeutic removal approaches, mainly by hemodialysis. These classes include small water-soluble compounds with low molecular weight (<500 Da), protein-bound solutes, and so-called middle molecules (>500 Da) (13). Of note, the term middle molecule is a misnomer as it refers to low molecular weight peptides. The term was likely partially inspired by the removal pattern of hemodialysis membranes used at the time of formulation of the middle molecule hypothesis (14).

**Statement 1 - The current physicochemical classification of uremic toxins does not adequately address or reflect how current/modern hemodialysis technologies (mechanisms of adsorption, convection, and diffusion) remove toxins.**

The current physiochemical subdivision can be considered artificial as there is a continuum in the molecular weight of uremic solutes, and any cut-off based on molecular weight is arbitrary (10). Besides, the degree of protein binding for these uremic solutes is variable and complicates any schema-based solely on molecular weight. Nevertheless, as hemodialysis remains the most frequently applied therapeutic strategy to reduce the concentration of uremic toxins in advanced chronic kidney disease (CKD), the most practical classification approach is based upon the principles of removal patterns by hemodialysis, noting that it only applies to conventional hemodialysis and not to peritoneal dialysis or hemodialysis timeframes deviating from typical 4-h
thrice-weekly sessions (15), (16). Also of note, the original classification does not account for the compartmental partitioning behavior of solutes within the body (17) or alternative strategies to decrease uremic toxin concentration [e.g., preservation of residual kidney function (18,19), adsorptive techniques (20), or strategies aimed at decreasing solute generation (21,22,23)]. Finally, it should be acknowledged that any classification based upon dialysis strategies does not take into account that uremic signs and symptoms in advanced kidney disease may be present before the initiation of dialysis.

The mechanism of adsorption to hemodialysis membranes plays a role in removing uremic toxins, albeit membranes with truly enhanced adsorptive properties are still in the pipeline (20,24-29). Concerning the clinically available membranes, a marked reduction in the sieving coefficient for solutes with molecular weight >12 kDa demonstrates the adsorptive phenomenon of membrane caking derived from the deposition of plasma proteins (albumin-bound or soluble uremic toxins included) obstructing some pores, causing a time-dependent loss of efficiency during the hemodialysis session (30).

Newer hemodialysis membranes are likely to change the ability to remove higher molecular weight solutes that may be toxic. The ability to remove larger uremic toxins relies largely on convection. The high-flux-dialyzer, when applied in the hemodialysis modality, has a molecular weight cut-off of 25 kDa (31), being boosted up to 30 kDa when in hemodiafiltration mode (32). A new class of membranes is the medium cut-off membrane with a cut-off of 56 kDa, a mean pore radius of 5 nm, and a fiber inner diameter of 180 µm (33). As a comparison, the high-flux membrane has a mean pore radius of 3.9 nm and an inner diameter of ~200 µm (1,31,33,34). Clearance is more efficient for larger molecules (25–58 kDa) with medium cut-off membranes than high-flux membranes. Clinical trials have consistently demonstrated increased clearance of larger molecular weight molecules such as complement factor D, free κ light chains, TNF-α, and β2-microglobulin (35,36). We believe the classification of middle molecules should include the impact of different hemodialysis membranes on their clearance, ultimately allowing the personalization of therapies. We recognize that this approach is limited in that it is focused solely on hemodialysis versus other forms of kidney replacement therapy such as peritoneal dialysis and transplantation.

**Recommendation**

1. We suggest that the current definition of uremic toxins should be based on hemodialysis strategies, membranes, and removal patterns acknowledging that any classification based on
cut-off values and/or molecular spatial configuration or charge would be arbitrary and likely will need to be changed as technological development changes solute removal patterns.

Classification based on toxicity

Rationale

Uremic toxicity negatively affects multiple organ systems and metabolic pathways (Figure 2); cardiovascular damage (37), increased susceptibility to infection (38) and neurologic manifestations (39) are major factors affecting mortality and quality of life of patients with CKD. However, the current physicochemical classification of uremic toxins provides no insight into where benefit may come from increased clearance of a class of uremic toxins, nor where problems may lie by inadequate clearance of a class of uremic toxins.

Statement 2 The current physicochemical classification of uremic toxins does not adequately reflect the biological consequences of the toxins and is not able to identify which toxins possess the most clinical relevance.

Wolley and colleagues (40) reviewed the breadth of impact for one group of uremic toxins, a subgroup of middle molecules with molecular weights greater than 15 kDa. The authors demonstrated how these molecules are involved in chronic inflammation, cardiovascular disease, secondary immunodeficiency, and symptom burden. Their review emphasizes that a physicochemical classification of uremic toxins does not aid clinicians in addressing a specific complication of kidney failure. For example, in a patient at high risk of cardiovascular diseases, there will be involvement of uremic toxins from small water-soluble, middle-molecules, and protein-bound groups. Likewise, for the clinicians trying to improve the outcomes for a patient with recurrent infections, they will have to target uremic toxins from all three groups (water-soluble, middle-molecules, and protein-bound). There may therefore be a logic to look at a re-classification of uremic toxins based on clinical consequences.

In 2018, a scoring system for uremic retention solutes was developed to classify solutes according to experimental and clinical evidence of their toxicity (10). This unique classification was based on objective and reproducible criteria and considered most uremic solutes then known (Table 1) despite limitations (e.g., it is a scoring system based on the number of conclusive studies). Thus solutes that are studied most frequently have a higher likelihood to reach a high score; the classification lacks systematic literature analysis), it provides a framework for defining target
molecules for future uremic toxicity analyses and removal studies. The expert group considered other classification systems but felt that this was the most evidence-based approach available.

**Recommendation**

1. We suggest using the 2018 classification system (10) that reflects the degree of known toxicity based upon published peer-reviewed literature to define the pathophysiologic impact of each uremic retention solute. Periodic updates will be required as new evidence of the toxicity of solutes becomes available, and new solutes are identified.

2. We suggest that the pathophysiologic impact of each uremic toxins (e.g., inflammatory, cardiovascular) and solute origin (e.g., intestinal generation, posttranslational modification) should be stated wherever available.

3. We suggest focusing on a limited number of key body system effects that are the most prominent in uremia, such as cardiovascular damage, susceptibility to infection, and neurologic manifestations for pathophysiologic classification.

**Classification based upon patient outcomes**

**Rationale**

In addition to the high morbidity and mortality associated with kidney failure, patients have a high symptom burden. Studies have demonstrated that reducing the symptom burden is as, if not more, important to many patients than an extended survival. Therefore, there has been much interest in recent years in developing robust, reproducible methods (41-44) to measure the patient experience. Additionally, there are now coordinated international research programs (45) targeting methods for improving what patients with kidney failure experience. However, the current classification of uremic toxins does not include patient experience or outcomes. The current uremic toxins classification does not help clinicians prescribe a dialysis regime for a patient with restless leg syndrome, fatigue, or prolonged recovery time after a dialysis session. Therefore, it would now be logical to look at the classification of uremic toxins in light of the symptoms and patient outcomes they cause. A classification such as this could then allow dialysis prescriptions to be specific to individual patient complaints such as pruritus or restless leg syndrome.

**Statement 3. The current physicochemical classification of uremic toxins does not adequately address patient experience or outcomes and does not reflect personal patient characteristics by**
which the dialysis prescription should be made (e.g., targeting the prevention of cardiovascular disease, loss of residual kidney function, deterioration of vascular access, or quality of life).

Since the original classification of uremic retention solutes, significant advances have been made to understand their clinical impact in uremia. For example, urea, once considered biologically inert, has been associated with insulin release (46), free radical production (47), apoptosis (48), and disruption of the intestinal barrier (49). Similarly, molecules like β2-microglobulin, complement factor D, immunoglobulin free light chains, endothelin, fibroblast growth factor-23 have been shown to have significant effects on the cardiovascular system, inflammation, and fibrosis (13,50-53). On the other hand, studies demonstrating adverse effects of molecules like adiponectin, interleukin-10, leptin, resistin, or visfatin are lacking (54). The HEMO (55) and the MPO (56) studies suggested that high-flux hemodialysis membranes compared to low-flux hemodialysis membranes are associated with lower risk of mortality in certain sub-groups of patients with long dialysis vintage, diabetes, and serum albumin of ≤4 gm/dL. Though not conclusive, these results indicate a potential advantage of increasing the spectrum of hemodialytic removal of uremic toxins to include larger molecules. It should be noted that the retention of inorganic solutes, which as per definition are not considered as uremic toxins, may offset or supersede any beneficial effects derived from the removal of organic solutes given their undisputed link to cardiovascular morbidity and mortality. The current classification of uremic solutes does not express their clinical relevance, nor identifies candidate molecules whose dialysis clearance and blood levels may be monitored to assure dialysis adequacy and improvement in clinical outcomes (10,13). Therefore, future classification attempts must aim to map patient profile or phenotype to a single or panel of biomarkers and suggest reduction or removal techniques that can be best utilized to decrease levels.

**Recommendations**

1. Future studies should focus on correlating solute concentrations or the effect of interventions on solute concentrations with clinically relevant outcomes and outcomes of importance to patients.

2. Ideally, dialysis prescriptions would be tailored to improve these symptoms and quality of life based upon removal patterns of uremic solutes linked to symptoms and outcomes.

**Assessment of toxin measurement and removal capacity**

**Rationale**
A marker of solute removal should be linked to its toxicity (and improvement of symptoms with removal) and be representative of other toxins with comparable characteristics. Given the unpredictable impact of kinetics on removing various uremic toxins in intermittent dialysis strategies like maintenance hemodialysis (16,57,58), we suggest that (pre-hemodialysis) concentration after a sufficiently long equilibration is a better measure of toxin removal than clearance or pre to post-removal ratio calculations. Depending on the efficiency of removal, multi-compartmental solutes will need different equilibration times (Figure 3). An equilibration time of 4 weeks allows most solutes (except those with very low dialytic concentration reduction ratios, which are observed when the volume of distribution is large relative to the dialytic clearance) to reach equilibrium while minimizing the occurrence of confounders (e.g., loss of residual kidney function, need for antibiotics, changes in dialytic prescription, changes in dietary intake).

**Recommendations**

1. For assessment of toxin removal by extracorporeal treatment, we recommend measuring the pre-hemodialysis concentration of a toxin after a period of equilibration (≥4 weeks).
2. For comparability reasons, we suggest using the same equilibration time (4 weeks) to study any other strategy than extracorporeal removal to decrease toxin concentration (e.g., medication, dietary intervention, xenobiotics, and others).

**Proposal for a new classification system of uremic solutes**

**Rationale**

It should be emphasized that decreased uremic toxin clearance due to low glomerular filtration rate is not the sole reason for toxin accumulation in kidney failure. For example, excessive production of cytokines and soluble receptors due to local tissue inflammation is a major contributor to middle molecule accumulation (54). Besides, gut dysbiosis generates a broad spectrum of uremic toxins (57). Thus, a broader view of uremic solutes that goes beyond simply retention with poor glomerular filtration rate is needed. Recent data regarding the origin of uremic toxins and the new development of hemodialysis methods and new membranes with the ability to clear uremic toxins with specific characteristics, or by using drugs/molecules to facilitate the shift from bound fraction to free fraction (58), lead us to propose a new classification beyond the classic physicochemical classification.
Statement 4. New measurement tools for uremic toxins are needed in each class that goes beyond physicochemical classification.

As the currently available tests (limited to a few relevant molecules such as phosphate, urea, serum creatinine) are not sufficient for clinical needs, new validated biomarkers are needed. For example, the accumulation of toxins in the uremic milieu nurtures an intermediate inflamed phenotype related to oxidative stress, fibrosis, senescence, mitochondrial dysfunction, and tissue hypoxia that promote premature aging (59) by vascular calcification, left ventricular hypertrophy, osteoporosis, sarcopenia, frailty, and cognitive dysfunction. Thus, to better target the intermediate inflammatory phenotype, we suggest considering the kinetics of a wide range of uremic toxins in addition to the urea kinetics. The ideal biomarker should be inexpensive, easy to measure, globally available, correlate with severity of disease, and be sensitive to early subclinical disease, recovery, and response to therapy. We believe that the new classification is clinically more relevant.

Recommendation
1. The new classification schema must link uremic solutes to traditional clinical outcomes and quality of life measures, including pruritus, restless legs syndrome, and recovery time after dialysis (60,61).

We propose a panel of biomarkers representing each uremic toxin class (Figure 4). For small (<500 Da) water-soluble molecules, and urea (60 Da) correspond to the criteria mentioned above and could be included in the biomarker panel. Creatinine (113 Da) could also be considered a biomarker of small water-soluble toxins, but only if factors that are known to confound its concentration, such as age, muscle mass, Kt/V, and normalized protein catabolic rate are accounted for (62). However, it should be noted that there is little evidence linking creatinine directly with uremic symptoms or outcomes. For evaluation of small-middle molecular weight (0.5−15kDa) clearance, we recommend using parathyroid hormone (9.5 kDa) and β2-microglobulin (11.8 kDa). For estimation of medium-middle (>15−25 kDa) and large-middle (>25−58 kDa)-molecular weight clearance, we recommend analyses of κ (22.5 kDa) and λ (45 kDa) free light chains, respectively. Until validation of a more widely available estimate of protein-bound solutes, clearance of protein-bound solutes is best estimated by analyses of indoxyl sulfate and paracresyl sulfate. It should be noted that residual kidney function can significantly contribute to the removal of solutes for which protein binding limits clearance by hemodialysis. Finally, it is important to recognize that the evidence base for use
of some biomarkers is immature and requires additional study. Importantly, studies linking removal of these biomarkers to clinical outcomes are required.

**Recommendation**

1. Candidate biomarkers representing different types of uremic retention solutes should be identified and used as proxies to study various dialytic and non-dialytic removal strategies.

**Statement 5. Available and newer dialysis technology (including membranes) must be measured for its effective removal of uremic toxins in each class.**

In recent years, the clearance profiles of the latest generation of hemodialyzer membranes have improved remarkably. Several characteristics should be considered for the evaluation of new membranes. These include new permeability indices, the hydrophilic or hydrophobic nature of membranes, adsorption capacity, and electrical potential (63). Furthermore, molecular weight retention onset, molecular weight cut-off, and the mass transfer area coefficient should be measured (64). Some studies support the choice of high volume post-dilution hemodiafiltration over the current dialysis techniques (65,66). Beyond diffusion and convection, the removal pattern of the uremic toxins by hemodialysis methods could be enhanced by adsorption techniques (58) or by using drugs/molecules to facilitate the shift from bound fraction to free fraction (67). Consideration of uremic toxin characteristics has an impact on treatment choice. Therefore, clinicians should consider molecular radius, electrical charges, protein binding solute characteristics, high vs. low molecular weight, hydrophilic vs. hydrophobic, endogenous vs. exogenous, secretion by kidney tubules, and different volumes of distribution (68).

**Statement 6. Prototype uremic biomarkers should be validated as new measurement tools of uremic toxicity**

Identifying prototype biomarkers that could be used to optimize the management of kidney failure is essential. Current methodologies for the evaluation of the adequacy of dialysis such as Kt/V should not be abandoned until high quality clinical studies support the use of novel biomarkers. These biomarkers need to be linked to improving clinical outcomes, i.e., they are directly or indirectly linked to uremic toxicity processes *in-vivo*. These biomarkers need to predict uremic manifestations, provide information about mechanisms and prognosis, improve the safety of interventions to address uremia, or be used as a surrogate marker of a uremic toxin or clinical
outcome. The relationship between the accumulation of uremic toxins, intervention, and outcome should be considered. Although the role of various uremic toxins in pathophysiological processes that drive morbidity has been widely studied, the extent of the effect following intervention is less clear. Moreover, the effect between a change in biomarkers and aspects of quality of life is virtually unexplored. Novel treatments should establish whether a change in uremic toxin biomarkers affects traditional clinical outcomes and whether it improves quality of life. In addition, the cost of using novel biomarkers must be assessed and be sensitive to resource-constrained environments to ensure wide-spread uptake.

Biomarkers need to be sensitive to subclinical toxicities and respond to extracorporeal or enhanced endogenous toxin removal. A multidimensional understanding of disease biology using –omics technologies (genomics, transcriptomics, proteomics, cytometric, and metabolomics) and "big data" methodologies is necessary for understanding the complex pathophysiology of uremia (Supplement Figure 1). After the relationship between uremia pathophysiology and target biomarkers is understood, a biomarker discovery phase should follow by testing candidate biomarkers in CKD patients to identify biomarkers with the highest performance. The highest performers should be validated in a larger, diverse group of patients. In the next phase, studies need to assess the impact of biomarker-guided protocols on clinical outcomes. Finally, test platform development with rapid turnaround time, low cost, and high accuracy should be completed before implementation in clinical practice.

**Research recommendations**

Given the many unknowns in the field of uremic toxins, the consensus group felt strongly that continued research was critical. A research agenda was identified and listed in Supplemental Table 2. This agenda links with the above statements and further enhances the move away from the classification of uremic solutes based solely on physiochemistry and removal patterns based upon prior dialytic techniques and membranes. Adherence to the research agenda is likely to yield substantial increases in our knowledge base regarding the uremic syndrome and ultimately improve patients' outcomes.

**Summary**

Advances in our understanding of uremic toxins and the availability of new hemodialysis membranes and techniques have led to a reappraisal of the definition and classification of uremic toxins. We recommend a more holistic classification that includes physicochemical characteristics and correlation to clinical symptoms and outcomes. Besides, the identification of representative
biomarkers that correlate with removal patterns and are clinically relevant in terms of toxicity may
lead to more personalized and targeted dialysis prescriptions and facilitate the search for non-
dialysis strategies that have the opportunity of improving the quality of life and outcomes for
patients with advanced kidney disease. Validation of the novel classification will require big data
methodologies, validation in external cohorts and experimental evidence of toxicity. Of note, new
data on uremic toxins and removal techniques are continuously being published and these
recommendations may therefore require modifications as new results become available.
Disclosures

P.J. Blankestijn reports receiving consulting fees from Baxter and Medtronic; receiving research funding from Ablative Solutions, European Commission, and Recor; receiving honoraria from Medtronic and Baxter, fees payed to institution; and serving on the Editorial Board of *Nephrology Dialysis Transplantation*.

M. Cozzolino reports receiving research funding from Abbvie, Baxter, Keryx, and Shire; receiving honoraria from Abbvie, Amgen, Baxter, Shire, and Vifor-Fresenius; serving as a scientific advisor or member of Abbvie, Amgen, Keryx, Shire, and Vifor; and speakers bureau for Abbvie, Amgen, Keryx, Shire, and Vifor.

C. Hutchison reports consultancy agreements with, receiving reserach funding from, and receiving honoaria from Baxter.

L. Juillard reports consultancy agreements with Amgen, Astellas, Baxter, Fresenius, Hemotech, Leo, Novartis, Otsuka, Sanofi, and Vifor; receiving research funding from Amgen, Baxter, and Sanofi; receiving honoraria from Amgen, Astellas, Baxter, Fresenius, Hemotech, Leo, Novartis, Otsuka, Sanofi, and Vifor; and serving as a scientific advisor or member of Amgen, Astellas, Baxter, Fresenius, Hemotech, Leo, Novartis, and Vifor.

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M. Kaushik reports receiving honoraria from Baxter Healthcare and Fresenius Medical Care; speakers bureau for Baxter Healthcare and Fresenius Medical Care; and serving as a member of ARA-EDTA, European Society of Intensive Care Medicine, International Society of Nephrology, NKF, Singapore Society of Nephrology, and Society of Transplantation Singapore.

H. Kawanishi reports receiving honoraria from Kyowa-Kirin Co. Ltd. H. Kawanishi reports serving as a scientific advisor or member of PDOPPS Steering Committee; serving on the Editorial Boards of *Blood Purification, Peritoneal Dialysis International, and The Journal of Vascular Access*; and serving as president of International Society of Blood Purification (ISBP).

Z. Massy reports receiving research funding from Amgen, Baxter, the French government, Fresenius Medical Care, Genzyme-Sanofi, GlaxoSmithKline, Lilly, Merck Sharp and Dohme-Chibret, and Otsuka and government support for CKD REIN PROJECT AND EXPERIMENTAL PROJECTS. Z. Massy reports receiving honoraria on consultation fees to charities or for travel from AstraZeneca, Baxter, and Genzyme-Sanofi; and serving as a scientific advisor or member of *Journal of Nephrology, Journal of Renal Nutrition, Kidney International, Nephrology Dialysis Transplantation, and Toxins.*
T. Reis reports employment with CDRB - Clinica de Doencas Renais de Brasilia; consultancy agreements with AstraZeneca, Baxter, Contatti Medical (CytoSorbents), and Eurofarma; receiving honoraria from AstraZeneca, Baxter, B.Braun, Contatti Medical (CytoSorbents), Eurofarma, and Jafron; and speakers bureau for AstraZeneca, Baxter, B.Braun, Contatti Medical (CytoSorbents), Eurofarma, and Jafron.

C. Ronco reports consultancy agreements with Asahi, Astute, Baxter, B.Braun, Biomerieux, Biporto, Cytosorbents, GE, Jafron, Medtronic, OCD, and Toray; receiving honoraria from Astute, Baxter, B.Braun, Estor, Fresenius, GE, Jafron, Medtronic, and Toray; and serving as the Editor-in-Chief of Blood Purification and Contributions to Nephrology and Cardiorenal Medicine and as an Associate Editor of Nephrology Dialysis and Transplantation.

M.H. Rosner reports consultancy agreements with Baxter; receiving research funding from Kadmon and National Institutes of Health; receiving honoraria from American Society of Nephrology and Baxter; serving as an Editor-at-Large of CJASN; and serving as a scientific advisor or member of American Society of Nephrology and on the Data Safety Monitoring Boards of clinical trials sponsored by AstraZeneca, Reata, and Travere.

P. Stenvinkel received consultancy fees, research grants, speaker's honoraria from Amgen, Astellas, AstraZeneca, Baxter Healthcare, Bayer, Fresenius Medical Care, Pfizer, Reata, and Vifor.

R. Vanholder reports consultancy agreements with Baxter Healthcare, BBraun, Fresenius Medical Care, Jafron, Kibow, and Nextkidney Project; has received travel support and honoraria from Baxter Healthcare and B. Braun Avitum AG; serving as an advisor to B. Braun Avitum AG, Baxter Healthcare, Debiotech, Fresenius Medical Care, Jafron, Kibow, and the Dutch Kidney Foundation; and serving as a scientific advisor or member of European Kidney Health Alliance, International Scientific Advisory Board Dutch Kidney Foundation, JASN, Nature Reviews Nephrology, and Nephrology Dialysis Transplantation.

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Because Dr. Mitchell H. Rosner is an Editor-at-Large of CJASN, he was not involved in the peer review process for this manuscript. Another editor oversaw the peer review and decision-making process for this manuscript.
References:


Sulfate and P-cresol Sulfate in Hemodialysis Patients: Results from an In Vitro Study and an In Vivo Pilot Trial (xuanro4-Nature 3.2). Toxins 12: 170, 2020


Clinical Journal of the American Society of Nephrology


### Table 1. Uremic toxins with the highest toxicity evidence score.

<table>
<thead>
<tr>
<th>Highest evidence score (4)</th>
<th>Second highest evidence score (3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>p-cresyl sulfate</td>
<td>Advanced glycation end products</td>
</tr>
<tr>
<td>β₂-microglobulin</td>
<td>Indoxyl sulfate</td>
</tr>
<tr>
<td>Asymmetric dimethyl arginine</td>
<td>Uric acid</td>
</tr>
<tr>
<td>Kynurenines</td>
<td>Ghrelin</td>
</tr>
<tr>
<td>Carbamylated compounds</td>
<td>Indole acetic acid</td>
</tr>
<tr>
<td>Fibroblast growth factor-23</td>
<td>Parathyroid hormone</td>
</tr>
<tr>
<td>Interleukin-6</td>
<td>Phenyl acetic acid</td>
</tr>
<tr>
<td>Tumor necrosis factor-α</td>
<td>Trimethyl methylamine-N-oxide</td>
</tr>
<tr>
<td>Symmetric dimethyl arginine</td>
<td>Retinol binding protein</td>
</tr>
<tr>
<td></td>
<td>Endothelin</td>
</tr>
<tr>
<td></td>
<td>Immunoglobulin light chains</td>
</tr>
<tr>
<td></td>
<td>Interleukin-1β</td>
</tr>
<tr>
<td></td>
<td>Interleukin-8</td>
</tr>
<tr>
<td></td>
<td>Neuropeptide Y</td>
</tr>
<tr>
<td></td>
<td>Lipids and lipoprotein*</td>
</tr>
</tbody>
</table>

Adapted from Vanholder et al. (10). The ranking was based on the number of experimental and clinical studies showing toxicity with a downgrade if 25% of the retrieved studies showed no effect or a benefit. A score between 4 and 0 was possible, with only the toxins scoring 4 or 3 displayed in this table. Per score the toxins are ranked top to bottom according to the proven number of affected organ systems.

*: posttranscriptional modifications.
**Figure 1. Definition of uremic toxins**

<table>
<thead>
<tr>
<th>Current (Vanholder R et al. KI Suppl 2003)</th>
<th>Terminology limitations</th>
<th>Suggested update</th>
</tr>
</thead>
<tbody>
<tr>
<td>Such a compound should be chemically identified, and accurate quantitative analysis in biological fluids should be possible</td>
<td>The terms “chemically identified” and “biological fluids” are overly broad and nonspecific</td>
<td>Solute identification and accurate quantitative analysis in plasma, serum, or blood should be possible</td>
</tr>
<tr>
<td>The total body and plasma levels should be higher in uremic than in nonuremic subjects</td>
<td>Unclear whether total body levels of a solute can be measured accurately</td>
<td>Plasma, serum, or blood levels should be higher in CKD than in subjects with normal kidney function</td>
</tr>
<tr>
<td>High concentrations should be related to specific uremic dysfunctions and/or symptoms that decrease or disappear when the concentration is reduced</td>
<td>Reduction of solute concentrations may or may not translate to clinical improvement</td>
<td>Negative effects, conforming with or contributing to biological or clinical changes in CKD, should be proven in vivo, ex vivo, or in vitro</td>
</tr>
<tr>
<td>Biological activity, conforming to clinical changes observed in conjunction with the uremic syndrome, should be proven in vivo, ex vivo, or in vitro studies</td>
<td>Concentrations may refer to either the free or bound fraction of protein-bound solutes</td>
<td>Biologically active concentrations in these studies should conform to those found in plasma, serum, or blood of CKD patients</td>
</tr>
<tr>
<td>Concentrations in these studies should conform to those found in body fluids or tissue of uremic patients</td>
<td>Body fluids or tissue is nonspecific</td>
<td></td>
</tr>
<tr>
<td>Uremic is a nonspecific term</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The left panel represents the current definition of uremic toxins, with the blue text indicating terminology that we identified as needing an update. The middle panel elaborates on the limitations associated with the identified terms. The right panel shows the newly proposed definition of uremic toxins.

CKD, chronic kidney disease.
Figure 2. Uremic toxins and related systemic disorders

The pathophysiologic impact of uremic toxins on organ systems and associated disorders linked with outcomes. Many organ systems influence each other and contribute to kidney damage and cardiovascular morbidity.

CKD, chronic kidney disease.
The modeled effect demonstrates that solutes may be classified according to time to reach steady state. Each panel illustrates the time required for solute concentration to reach equilibrium following an increase in dialytic clearance with 4-h thrice-weekly treatment. Modeling was performed for four hypothetical solutes with varying dialytic RRs (0% for CMPF (A), 25% for β2-microglobulin (B), 50% for hippurate (C), and 75% for urea (D), respectively). Dialytic clearance was increased twofold for solutes with RR 25%, 50%, and 75% and was increased from 0 mL/min to 1 mL/min for the solute with RR 0%. Intercompartmental clearances were assumed to be higher than the dialytic clearance such that the accessible compartment refills rapidly from non-accessible compartments during dialysis. The RR can therefore refer to blood, plasma, or serum concentrations. Constant generation and absence of nondialytic clearance of each solute were also assumed. Solute concentrations are presented without any unit on the y axis, with the weeks following increase in dialytic clearance on the x axis. The arrow indicates the time at which dialytic clearance is increased. The asterisk indicates the time at which concentrations are within 1% of equilibrium for each solute during each week of dialysis from then on. The dashed blue line represents the average solute concentration over each week.

CMPF, 3-Carboxy-4-methyl-5-propyl-2-furanpropionic acid; RR, reduction ratio.
The third column from right to left subdivides molecules according to their protein affinity and is followed by a column that describes their molecular weight. On the top of each box of the molecular weight column, each colored dialyzer represents a dialysis modality and its expected capacity to remove the substances with molecular mass within the range represented in the box underneath. Although all dialyzer types remove small water-soluble compounds and protein-bound compounds, removal of protein-bound compounds is less pronounced. The black broken line indicates that many compounds with protein binding ≥80% are intestinally generated; the blue broken line indicates that some small water-soluble compounds may be intestinally generated.

ADMA, asymmetric dimethylarginine; AGEs, advanced glycosylation end products; CML, carboxymethyl lysine; CXCL12, C-X-C motif chemokine 12; CX3CL1, chemokine (C-X3-C motif) ligand 1; DMA, dimethylamine; FGF, fibroblast growth factor; FLC, free light chain; HCO, high cut-off; Hcy, homocysteine, HD, hemodialysis; HDF, hemodiafiltration; HDx, expanded hemodialysis; IGF-1, insulin like growth factor-1; IL, interleukin; IS, indoxyl sulfate; MCO, medium cut-off; MMA, monomethylamine; PAG, phenylacetylglutamine; pCS, para-cresyl sulfate; SMDA, symmetric dimethylarginine; sTNFR, soluble tumor necrosis factor receptor; TMA, trimethylamine; TMAO, trimethylamine-N-oxide; TNF, tumor necrosis factor; YKL-40, chitinase-3-like protein 1.
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Supplemental Table 2. Research recommendations for improving our understanding of uremic solutes, their dialytic removal, and their impact on clinical outcomes

Supplemental Figure 1. Big data-driven discovery and validation of candidate uremic retention solutes.

References
SUPPLEMENTAL MATERIAL

Classification of Uremic Toxins and Their Role in Kidney Failure:
An Expert Consensus Conference

Mitchell H. Rosner, Thiago Reis, Faeq Husain-Syed, Raymond Vanholder, Colin Hutchison,
Peter Stenvinkel, Peter J. Blankestijn, Mario Cozzolino, Laurent Juillard, Kianoush Kashani,
Manish Kaushik, Hideki Kawanishi, Ziad Massy, Tammy Lisa Sirich, Li Zuo, Claudio Ronco

This supplementary material has been provided by the authors to give readers additional information about their work.
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Supplemental Methods

The modified Delphi methodology (1) comprises four stages: i) Systematic search for evidence in the available literature; ii) Establishment of clinical and physiological outcomes, as well as measures to be used for comparison of different treatments; iii) Description of current practice and rationale for using current techniques and; iv) Identifying areas where evidence is lacking and therefore research is required. Overall, the consensus process relies on evidence where available, if no evidence is available, expert consensus opinion is relied on.

The consensus conference began with a pre-conference comprehensive literature search and appraisal of scientific evidence to identify key themes that are central to uremia and uremic toxins. Conference participants were divided into three workgroups (Supplemental Table 1) and were tasked with addressing the following themes.: Critical appraisal of limitations in the current definition/classification of uremic retention solutes; Rationale for updating definition and classification of uremic retention solutes and molecules of interest in the field of maintenance hemodialysis and; Proposal of a new classification of solutes of interest in uremia and hemodialysis. Participants were chosen by the conference chair (C.R.) based on their contribution in the field in the last 5 years. In addition, a few individuals were chosen based on experience in managing consensus process. A good representation of the different continents was another criterium and the final selection was based on the availability of the invited experts.

Each workgroup identified relevant studies through the National Institutes of Health PubMed platform, bibliographies of review articles and other files provided by participants. Article searches was generally limited to English language articles. Efforts were made to include mainly evidence from randomized controlled trials, however, other articles were also permissible to incorporate the best available evidence.

One group member served as the group facilitator. The conference chair served as moderator for the virtual sessions. During the sessions, summary/consensus statements were developed, requiring each work group to identify key issues, and classify current state of consensus. The findings of each workgroup were then presented to the entire group in the three plenary sessions for debate, discussion, suggested revisions, where each statement was revised until a final version was agreed upon. After each plenary session, the workgroups revised its findings based on the consensus reached by the whole group. To develop directives for future research, participants were asked to: i) Identify deficiencies in current literature; ii) Determine, where more evidence is necessary and; iii) Articulate research questions for areas, where evidence is lacking. The final product was then assessed and aggregated in a videoconference session attended by all attendees, who approved the consensus recommendations.
After the conference, a writing committee collected and edited the individual conference reports from each workgroup. Those final reports were then summarized by the writing committee into a final conference report, which was mailed to each participant for comment and revision. After approval by each member the final conference document was submitted for publication.
## Supplemental Table 1. Information regarding workgroups and work product

<table>
<thead>
<tr>
<th>Conference Chair</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Claudio Ronco (Vicenza, Italy)</td>
<td><strong>Critical appraisal of limitations in the current definition/classification of uremic retention solutes</strong></td>
<td><strong>Rationale for updating definition and classification of uremic retention solutes and molecules of interest in the field of maintenance hemodialysis</strong></td>
<td><strong>Proposal of a new classification of solutes of interest in uremia and hemodialysis</strong></td>
</tr>
<tr>
<td>Facilitators</td>
<td>Raymond Vanholder (Gent, Belgium)</td>
<td>Colin Hutchison (Herston, Australia)</td>
<td>Peter J. Blankestijn (Utrecht, The Netherlands)</td>
</tr>
<tr>
<td></td>
<td>Mitchell H. Rosner (Virginia, USA)</td>
<td>Laurent Juillard (Villeurbanne, France)</td>
<td>Mario Cozzolino (Milan, Italy)</td>
</tr>
<tr>
<td></td>
<td>Faeq Husain-Syed (Giessen, Germany)</td>
<td>Li Zuo (Beijing, China)</td>
<td>Ziad Massy (Villejuif, France)</td>
</tr>
<tr>
<td></td>
<td>Hideki Kawanishi (Hiroshima, Japan)</td>
<td>Thiago Reis (Brasilia, Brazil)</td>
<td>Kianoush Kashani (Rochester, USA)</td>
</tr>
<tr>
<td></td>
<td>Tammy Lisa Sirich (California, USA)</td>
<td>Manish Kaushik (Singapore, Singapore)</td>
<td>Peter J. Blankestijn (Utrecht, The Netherlands)</td>
</tr>
</tbody>
</table>
Supplemental Table 2. Research recommendations for improving our understanding of uremic solutes, their dialytic removal, and their impact on clinical outcomes

**Clinical Outcomes**

1. Development of large proteomic/metabolomic databases linked to patient outcomes, quality of life, and uremic symptoms.
2. Identification of a panel of clinical parameters to define adequate dialysis (to replace Kt/V).
4. Association of uremic toxin levels with outcomes in samples from randomized controlled trials (e.g., HEMO (55)).
5. Studies on outcomes using medium cut-off hemodialysis vs. high-flux hemodialysis (superiority studies) or high-flux hemodialfiltration (non-inferiority studies).
6. Understanding the role of uremic toxins in driving senescence (and surrogate markers of senescence such as epigenetic markers).

**Mechanisms of Toxicity**

1. Experimental toxicity studies focused on uremic solutes identified in observational studies with hard outcomes (e.g. phenylacetylglutamine).
2. Assessment of the interactions between different uremic toxins.
3. Assessment of the mechanisms of intestinal uremic toxin generation.
4. Development of in vitro assays that correlate with "uremic" toxicity.
5. For protein-bound uremic toxins, define whether the free concentration, the protein-bound concentration, or the total concentration determines biological effects.

**Development of efficient and sustainable uremic toxin removal**

1. Development of novel strategies to increase solute removal or decrease solute concentration.
2. Identification of "marker" uremic solutes that can serve as models for a larger group of toxic solutes (based upon molecular weight, protein binding, and so on).
3. Development of dialysis strategies with evidenced uremic toxin removal that are more compact, more resilient, and more cost-effective.
Supplemental Figure 1. Big data-driven discovery and validation of candidate uremic retention solutes.

Non-omics and omics analyses may enable the discovery of novel biomarkers and facilitate a multidimensional understanding of disease biology of uremic toxicity. Subsequent big data methodologies, validation in external cohorts and experimental evidence of toxicity can be simultaneously performed. Uremic retention solutes studied in experimental disease models could be assessed clinically with the use of non-dialysis and dialysis techniques to identify effective strategies for their removal. Biomarkers should then be validated in a larger, diverse group of patients with advanced kidney disease. In the next phase, studies need to assess the impact of biomarker-guided protocols on clinical outcomes. Finally, test platform development with rapid turnaround time, low cost, and high accuracy should be completed before implementation in clinical practice. It should be noted that within the omics domain, there are still challenges related to the standard of data quality and data quantity needed to capitalize on the full potential of these methods for discovery.
References