SECTION 1

UREMIC TOXINS

1

UREMIC TOXINS: AN INTEGRATED OVERVIEW OF DEFINITION AND CLASSIFICATION

RICHARD J. GLASSOCK AND SHAUL G. MASSRY

1.1 INTRODUCTION

As the overall function of the kidney declines in the course of chronic kidney disease (CKD) a wide variety of solutes, normally dependent on glomerular filtration, tubular secretion or renal metabolism for elimination, gradually accumulate in the body fluid compartments. Some of these solutes have biological effects that result in the malfunction of various cell types and organ systems. When these biological effects are sufficient to evoke clinically recognizable disturbances, the "uremic syndrome" is said to be present and the offending molecules are designated "uremic toxins".[1,2] These uremic toxins exhibit a broad array of physicochemical characteristics and have very diverse pathobiological effects at the cellular level.[1,2] A complete characterization of the catalogue of uremic toxins would be very useful in the design of approaches for their removal by dialysis; for ways to enhance their removal by nondialytic methods; for creation of interventions to prevent/mitigate their formation; for synthesis of inhibitors of their adverse effects on cells and organ systems—all directed at subjects with advancing CKD or end-stage renal disease (ESRD). The analysis of the issues surrounding uremic toxicity requires a useful definition and synthesis of a classification of uremic toxins. This brief essay attempts to provide a succinct approach to classification of uremic toxins, derived form a review of the current literature on the subject.[1]

Uremic Toxins, Edited by Toshimitsu Niwa.

1.2 DEFINITION OF A UREMIC TOXIN

In order to define a uremic toxin, one must first define the syndrome of uremia itself. Almost 35 years ago, the late Jonas Bergstrom gave a definition of the uremic syndrome that is just a valid today as it was then.[3] He stated that the uremic syndrome is a "toxic syndrome caused by severe glomerular deficiency associated with disturbances in tubular and endocrine functions of the kidney. It is characterized by the *retention* of toxic metabolites, associated with changes in the volume and composition of the body fluids and an *excess or deficiency* of various hormones." This very broad definition allows the uremic syndrome to embrace the retention of solutes due to failure of renal excretion (glomerular and/or tubular insufficiency) and hormonal surfeits or deficiencies arising from the disturbances wrought by kidney disease itself, such as enhanced endogenous production or impaired degradation of potential injurious solutes.

From this description of the uremic syndrome, it is clear that uremic toxins must be defined via a connection between the putative toxic substance and one or more of the pathophysiological attributes of the uremic syndrome. Making this connection requires that a series of criteria be fulfilled. These criteria are called the Massry/Koch postulates—so-called because they are a derivative of Koch's postulates for defining a pathogenetic organism as developed by one of the coauthors of this essay (SM) about a quarter century ago.[4] The requirements for an "authentic" uremic toxin are as follows:

(i) The toxin must be identified and characterized as a unique chemical entity.
(ii) Quantitative analysis of the toxin in biological fluids must be possible.
(iii) The level of the putative toxin must be elevated in biological fluids of subjects with the uremic syndrome.
(iv) A relationship between the level of the putative toxin in biological fluids and one or more of the manifestations of the uremic syndrome must be present.
(v) A reduction in the levels (or total body burden) of the putative toxin in biological fluids must result in some measurable amelioration of uremic manifestations.
(vi) Administration of the putative toxin to achieve levels similar to that observed in the uremic syndrome must reproduce the uremic manifestations in otherwise normal animals or man (*in vitro* demonstration of cellular toxicity alone is insufficient to meet this criterion).

A seventh criterion could be added to this list; namely, that a consistent and plausible pathobiologic mechanism should be able to explain the linkage between the putative toxin and the uremic manifestation (e.g., cellular toxicity, inhibition of signal transduction, metabolic perturbations). These postulates are difficult to apply directly to those disturbances that are part and parcel of the uremic syndrome but that emanate from surfeits or deficiencies of certain hormones or biologically active

TABLE 1.1 Factors Influencing the Toxicity of Substances Accumulating in Uremia

(1) The rapidity of changes in the levels in biological fluids
(2) Fluctuations in levels over time (time averaged vs. peak levels)
(3) Penetration into sites of action
(4) Intrinsic toxicity versus dependency of metabolism to more (or less) toxic compounds
(5) Distribution in body fluids (protein binding, lipophilicity, hydrophilicity)
(6) Presence and activity of naturally occurring inhibitors or promoters
(7) Rate of metabolism at active sites

Reproduced with permission of Ref. [1].

peptides (e.g., erythropoietin, calcitriol) consequent to the loss of renal mass in CKD. Nevertheless, these postulates are quite useful for the definition of uremic toxins resulting from retention of solutes normally excreted by the kidney and substances that arise in enhanced levels endogenously (from excessive synthesis or impaired degradation) as kidney disease progresses to symptomatic uremia (e.g., parathyroid hormone).

The demonstration of a linkage between a specific putative uremic toxin and a clinical manifestation of uremia can be a formidable task, as the symptoms and signs of "uremia" are extraordinarily diverse.[5] The ability of a specific putative toxin to elaborate a clinical manifestation is governed by a panoply of factors (see Table 1.1). These complicate enormously the task of identifying "authentic" uremic toxins as they require longitudinal in addition to cross-sectional analysis, body fluid compartmental studies, and the influence of naturally occurring inhibitors and promoters. Some toxins may also exhibit "tropism" for specific cellular types or organ systems (e.g., neurotropism) (see below).

1.3 CLASSIFICATION OF UREMIC TOXINS BY PHYSICOCHEMICAL CHARACTERISTICS

A classification of putative uremic toxins according to their physicochemical characteristics (molecular mass, polarity, protein binding, chemical structure) has been the time-honored and most popular approach.[1,2] In this schema, uremic toxins are categorized into four nonoverlapping categories; namely (i) polar, water soluble, nonprotein bound, low molecular mass (<500 Da); (ii) polar, water soluble, protein bound, low molecular mass (<500 Da); (iii) middle molecular mass (>500 and <3000–12,000 Da), nonprotein bound; (iv) high molecular mass (>3000–12,000 Da), nonprotein bound.[1,2] The work of the European Uremic Toxin Work Group (EUTox) has been invaluable in creating a uniform approach to classifying uremic toxins, and have pointed out the necessity for standardized schema for analysis of their *in vitro* effects and the enormous difficulties posed by variability in reported concentrations of putative toxins.[2] In their landmark review in 2003, the EUTox group created an encyclopedic listing of uremic retention solute (90 total),

68 of which were <500 Da molecular mass, 10 had a molecular mass of >500–12,000 Da, and 12 had molecule mass of >12,000 Da. Twenty-five of the retention solutes were protein bound, all but two had molecular mass of <500 Da. The concentration of these putative toxins in biological fluids ranged broadly from ng/L to g/L. Of all the toxins identified almost 40% were either "middle" molecules or were protein bound. The development of large-scale, rapid capillary electrophoresis-mass spectrometry analysis of body fluids has greatly enhanced the ability to identify and characterize potential uremic toxins.[6–9]

Inorganic substances (H_2O, Na^+, K^+, H^+, Mg^{2+}, PO_4^{2-}, Ca^{2+}, SO_4) and trace metals (Al, Cr, Si, Pb) can also qualify as uremic toxins. For example, retention of sodium chloride and water can evoke disastrous consequences on the cardiovascular system in CKD and ESRD and contribute markedly to organ dysfunction (left ventricular hypertrophy), morbidity (hypertension and congestive heart failure) and mortality (sudden cardiac death).[10] Also, acidosis (retention of H^+ ion) can wreak havoc in many cell and organ systems.

Although many potential uremic toxins have elevated plasma concentrations due to impairment of renal excretion, many are also associated with increased synthesis or impaired degradation of normal substances produced endogenously (e.g., parathyroid hormone). It must be emphasized that the plasma concentrations of putative uremic retention solutes are very poorly correlated with the prevailing level of glomerular filtration rate (GFR),[11] and the plasma levels of each specific solute may have a unique association with the level of GFR.[11] These observations add emphasis to a neglected phenomenon well recognized in the aglomerular kidney of marine teleosts (anglerfish),[12] specifically that the tubules represent an important site for elimination of putative toxic by-products of metabolisms. This phenomenon was pointed out in an elegant essay by Jerome Lowenstein in 2011.[12]

Thus, residual activity of transport systems in tubules of diseased kidneys (specifically the organic anion transporters [OAT] in the proximal tubule) may have important influence on the concentration of toxins at low levels of GFR. This phenomenon gives rise to the notion that enhanced expression of the OAT might be able to limit the accumulation of uremic toxins even with advanced loss of GFR.[13]

The low molecular mass solutes (<500 Da) have attracted a great deal of attention over many years. Urea (a low molecular mass, nonprotein-bound solute) has been used as a "surrogate" for authentic uremic toxins, although its intrinsic toxicity is greatly limited to very, very high plasma concentrations seldom achieved even in advanced uremia.[14] The evidence that urea *per se* functions as an authentic uremic toxin is very weak.[14] Nevertheless, its spontaneous degradation to isocyanate can lead to the "carbamylation" of serum and tissue proteins, such as albumin or hemoglobin.

Protein-bound uremic toxins are of great theoretical and practical importance.[15–18] Such protein binding may greatly limit the ability of diffusive or convective dialysis therapies to remove the compound efficiently, and this explain the limitations of extracorporeal therapies using membranes of low molecular mass "cutoff" for the treatment of uremia. Displacement of the uremic toxin from its protein-binding site might be a very attractive way of enhancing uremic solute removal by dialysis.[15–18] The most

well studied of the protein-bound uremic solutes include *p*-cresyl sulfate and indoxyl sulfate.[19–24] Both of these uremic solutes originate in the colon from the action of resident bacteria—thus, there is an important contribution of the colon to the uremic state,[25] leading to the potential for treatment of uremia by oral adsorbents.[26] Other protein-bound uremic solutes include asymmetric dimethylarginine (ADMA), homocysteine, pentosidine, deoxyglucosone, derivatives of nucleosides, and advanced glycation end products.[27,28] ADMA appears to accumulate in uremia more as a result of disturbed renal metabolism that from impaired renal excretion.[27,28] It is noteworthy that the R^2 values of ADMA levels in relationship to eGFR is only 0.167 (the R^2 value to creatinine is 0.737).[11] Uric acid and other nucleotide derivatives are emerging as important candidates for low molecular mass uremic toxins.[29–31]

Middle molecules (>500–12,000 Da) have been regarded as important in the uremic syndrome and its response to dialysis treatment, ever since the seminal observations leading up to the "middle molecule hypothesis" were made by Babb and Scribner 40 years ago.[32] As noted above, EUTox identified about 10 such middle molecule uremic toxins in their survey.[2] These compounds are often glucuronide conjugates, polypeptides (such as β2-microglobulin), carbohydrate derivatives, advanced glycation or oxidation end products, or polypeptide hormones (such as parathyroid hormone or its fragments).[2,33–37] These compounds may exert their toxic effects via engagement of other intermediary processes. The high molecular mass (>12,000 Da) nondialyzable toxins have been less well characterized, but include cytokines, chemokines, Ig light chains, complement factors, advanced glycation or oxidation end products, inhibitor proteins, chemotaxis-inhibiting peptides.[2]

1.4 CLASSIFICATION OF UREMIC TOXINS ACCORDING TO PATHOBIOLOGICAL PROCESSES UNDERLYING ACCUMULATION

The uremic toxins classified by their intrinsic physicochemical properties can accumulate in body fluid compartments though a number of distinct mechanisms. A *Type I* mechanism represents the accumulation in body fluids of toxic substances normally produced *endogenously* by metabolic processes largely as a result of reduced renal excretory capacity. A *Type II* mechanism is a surfeit of toxic substances in body fluids as a result of excess *endogenous* production or impaired degradation (or both) but not necessarily due to reduced renal excretory capacity. A *Type III* mechanism is the accumulation of toxic substances in biological fluids from *exogenous* sources by virtue of reduced renal excretory capacity often combined with continued dietary consumption. A *Type IV* mechanism is a deficiency or reduced activity of substances normally produced *endogenously* as a result of decreased synthesis, enhanced degradation, or biological inhibition. Combinations of more than one pathobiological process are possible. For example, urea is a uremic toxin that arises because of a combination of *Type I* and *Type III* processes—excessive accumulation due to impaired renal excretion and continued production due to exogenous (dietary) consumption of protein as a precursor of urea. It is helpful

to keep this classification of the processes underlying accumulation of uremic toxins when approaching a patient with the uremic syndrome.

1.5 THE RELATIONSHIPS OF UREMIC TOXINS TO THE PATHOBIOLOGY OF UREMIA

In recent years, a new concept has emerged that the uremic syndrome is strongly associated with a state of "chronic inflammation" and enhanced "oxidative stress" manifested by an increase in "positive" acute phase reactant proteins (such as CRP, IL-6, fibrinogen, ferritin, and serum amyloid A protein) and a reduction in "negative" acute phase reactant proteins (albumin, transferrin, prealbumin).[38,39] The proposed origins of this inflammatory state include (1) an imbalance between pro- and anti-inflammatory factors; (2) underlying organ-based chronic inflammation (occult infection [periodontal disease, infected vascular access, vulnerable atherosclerotic plaques], kidney inflammation associated with basic disease); (3) exposure to inflammatory promoters (endotoxin-contaminated dialysate, bioincompatible membranes). No doubt in individual patients, multiple factors explain the presence of an inflammatory state.

Certain candidate uremic toxins, such as uric acid or ADMA, may be potent promoters of inflammation, and in turn inflammation can lead to the generation of uremic toxins, such as advanced oxidation products via the generation and inadequate scavenging of toxic oxygen radicals.[40,41] Indoxyl sulfate, a putative uremic toxin, can also promote further progression of renal disease by activating harmful mediators such as transforming growth factor-β (TGF-β).[42] Thus, the accumulation of uremic toxins may also exert a positively reenforcing action on the basic process of tissue and organ damage, in addition to their effect on manifestations of the uremic syndrome *per se*.[42]

The "toxicity" of ADMA has also emerged as an important element in new concepts of the pathobiology of uremic toxicity.[40,41] This methylated amino acid is highly protein bound, and its concentration in plasma is elevated in uremia. The elevation is predominantly caused by the inhibition of its major kidney-derived metabolizing enzyme (dimethylarginine dimethylaminohydrolase-1; DDAH-1) rather than by markedly decreased renal excretion. ADMA, along with uric acid, is a potent inhibitor of endothelial cell nitric oxide synthase (eNOS).[40,41] Impaired eNOS and reduced nitric oxide production by endothelial cells may lead to vasoconstriction, elevated blood pressure, and vascular damage. Oxidative stress associated with uremia may also impair the effectiveness of DDAH-1, proving a link between endothelial cell dysfunction and inflammation in uremia. DNA methylation and repair may also be adversely affected by putative uremic toxins.[43,44] These some retention uremic solutes (such as homocysteine and its metabolites) could have profound effects on gene expression and epigenetics.[43,44]

Thus, the pathobiology of uremic toxicity needs to be viewed as a complex, dynamic, interacting system of effector, promoter, and inhibitory molecules occurring in a situation of reduced renal excretory capacity, impaired defensive ability, and

superimposed deficiency states. The cumulative adverse effects on cellular and organ system function will depend on the balance of these factors.

1.6 CLINICAL MANIFESTATIONS OF UREMIA AND THE ROLE OF TROPISMS

The clinical manifestations of uremic toxicity are broad and diverse. As pointed out previously every organ systems in the body can be affected. Each individual uremic toxin may have its own unique profile of "tropisms." That is, each toxin may have a preferential action on only one system (monotropic) or act on only a few systems (oligotropic). Most uremic toxins studied so far have effects on multiple systems (pleiotropic), perhaps by interference with very fundamental common pathways of cellular behavior (elevated cytosolic calcium, nitric oxide synthesis, DNA methylation and repair, defense against oxidative stress), such as exemplified by parathyroid hormone, uric acid, and other derivatives of purine nucleotides and ADMA. However, some toxins (such as guanidino compounds) may exhibit relative specificity for certain organ systems (hematopoiesis, neuronal function, bone metabolism, endothelial cell integrity).[45] Elucidation of the "tropic" behavior of individual toxins is an important element in their full characterization and classification.

1.7 CONCLUSION

An exposition of uremic toxicity requires an integrative analysis of the physicochemical properties of putative toxins (molecular size, protein binding), an understanding of the pathobiological processes responsible for their formation and accumulation, and a mechanistic view of how they alter fundamental cellular and organ behavior. A consideration of both glomerular filtration and tubular secretion is essential for the proper understanding of levels of putative uremic toxins in the body fluids in CKD and ESRD. An explanation of how individual or groups of toxins lead to clinical manifestations of uremia requires a consideration of tropism (monotropic, oligotropic, and pleiotropic toxins). This "multidimensional" integration allows for a better understanding of the complexity and the potential for mapping of the important elements of uremic toxicity. The long-term importance of better understanding of the chemical basis of uremia is to aid the development of better and more rational methods of treatment including ablation of organ sources of putative toxins, or the medical suppression of the activity of such organs, reduction of exogenous sources of toxic precursors, reduction in (colonic) absorption of putative toxins, enhancement of extra-renal removal of toxins (intra- or extra-corporeal), supplementation for replacement of deficiencies, suppression of toxic effects at the cellular level, replacement of renal tissue or its products.[46,47] Dialytic therapy of uremic toxicity is just one small part of the overall picture of uremia.

REFERENCES

1. Glassock RJ: Uremic toxins: What are they? An integrated overview of pathobiology and classification. *J Ren Nut* 18:2–6, 2008.
2. Vanholder R, De Smet R, Glorieux G, Argiles A, Baurmeister U, Brunet P, Clark W, Cohen G, De Deyn PP, Deppisch R, Descamps-Latscha B, Henle T, Jorres A, Lemke HD, Massy Z, Passlick-Deetjen J, Rodriguez M, Stegmayr B, Stenvinkel P, Teta C, Wanner C, Zidek W for the European Uremic Toxin Work Group (EUTox): Review on uremic toxins. Classification, concentration and inter-individual variability. *Kidney Int* 63:1934–1943, 2003.
3. Bergstrom J, Furst P: Uremic toxins. *Kidney Int Suppl* S9–S12, 1978.
4. Massry SG: Parathyroid hormone: A uremic toxin. *Adv Exp Med Biol* 223:1–17, 1987.
5. Merrill JP, Hampers CL: *Uremia. Progress in Pathophysiology and Treatment*. New York, Grune and Stratton, 1971.
6. Niwa T: Recent progress in the analysis of uremic toxins by mass spectrometry. *J Chromatogr B Anal Technol Biomed Life Sci* 877:2600–2606, 2009.
7. Rhee EP, Souza A, Farrell L, Pollak MR, Lewis GD, Steele DJ, Thadhani R, Clish CB, Greka A, Gerszten RE: Metabolite profiling identifies markers of uremia. *J Am Soc Nephrol* 6:1041–1051, 2010.
8. Niwa T: Update of uremic toxin research by mass spectrometry. *Mass Spectrom Rev* 3:510–521, 2011.
9. Schiffer E, Mischak H, Vanholder RC: Exploring the uremic toxins using proteomic technologies. *Contrib Nephrol* 160:159–171, 2008.
10. Glassock RJ, Pecoits-Filho R, Barberato SH: Left ventricular mass in chronic kidney disease and ESRD. *Clin J Am Soc Nephrol* 1:S79–S91, 2009.
11. Eloot S, Schepers E, Barreto DV, Barreto FC, Liabeuf S, Van Biesen W, Verbeke F, Glorieux G, Choukroun G, Massy Z, Vanholder R: Estimated glomerular filtration rate is a poor predictor of concentration for a broad range of uremic toxins. *Clin J Am Soc Nephrol* 6:1266–1273, 2011.
12. Lowenstein J: The anglerfish and uremic toxins. *FASEB J* 25:1781–1785, 2011.
13. Toyohara T, Suzuki T, Morimoto R, Akiyama Y, Souma T, Shiwaku HO, Takeuchi Y, Mishima E, Abe M, Tanemoto M, Masuda S, Kawano H, Maemura K, Nakayama M, Sato H, Mikkaichi T, Yamaguchi H, Fukui S, Fukumoto Y, Shimokawa H, Inui K, Terasaki T, Goto J, Ito S, Hishinuma T, Rubera I, Tauc M, Fujii-Kuriyama Y, Yabuuchi H, Moriyama Y, Soga T, Abe T: SLCO4C1 transporter eliminates uremic toxins and attenuates hypertension and renal inflammation. *J Am Soc Nephrol* 12:2546–2555, 2009.
14. Linthorst GE, Avis HJ, Levi M: Uremic thrombocytopathy is not about urea. *J Am Soc Nephrol* 5:753–755, 2010.
15. Jourde-Chiche N, Dou L, Cerini C, Dignat-George F, Vanholder R, Brunet P: Protein-bound toxins—Update 2009. *Semin Dial* 4:334–339, 2009.
16. Vanholder R, Baurmeister U, Brunet P, Cohen G, Glorieux G, Jankowski J; European Uremic Toxin Work Group: A bench to bedside view of uremic toxins. *J Am Soc Nephrol* 5:863–870, 2008.
17. Raff AC, Meyer TW, Hostetter TH: New insights into uremic toxicity. *Curr Opin Nephrol Hypertens* 6:560–565, 2008.

18. Lin CJ, Wu CJ, Pan CF, Chen YC, Sun FJ, Chen HH: Serum protein-bound uraemic toxins and clinical outcomes in haemodialysis patients. *Nephrol Dial Transplant* 11:3693–3700, 2010.
19. Niwa T: Indoxyl Sulfate. In: *Textbook of Nephrology*, 4th ed., edited by SG Massry, RJ Glassock, Philadelphia, Lippincott, Williams and Wilkins, 2001, pp 1269–1271.
20. Yu M, Kim YJ, Kang DH: Indoxyl sulfate-induced endothelial dysfunction in patients with chronic kidney disease via an induction of oxidative stress. *Clin J Am Soc Nephrol* 1:30–39, 2011.
21. Glorieux G, Vanholder R: New uremic toxins—Which solutes should be removed? *Contrib Nephrol* 168:117–128, 2011.
22. Liabeuf S, Barreto DV, Barreto FC, Meert N, Glorieux G, Schepers E, Temmar M, Choukroun G, Vanholder R, Massy ZA;European Uraemic Toxin Work Group (EUTox): Free *p*-cresyl sulphate is a predictor of mortality in patients at different stages of chronic kidney disease. *Nephrol Dial Transplant* 4:1183–1191, 2010.
23. Vanholder R, Bammens B, de Loor H, Glorieux G, Meijers B, Schepers E, Massy Z, Evenepoel P: Warning: The unfortunate end of *p*-cresol as a uraemic toxin. *Nephrol Dial Transplant* 26:1464–1467, 2011.
24. Schepers E, Meert N, Gloriex G, Goeman J, Van der Eycken J, Vanholder R: *p*-Cresyl sulphate, the main *in vivo* metabolite of *p*-cresol, activates leucocyte free radical production. *Nephrol Dial Transplant* 22:592–596, 2007.
25. Aronov PA, Luo FJ, Plummer NS, Quan Z, Holmes S, Hostetter TH, Meyer TW: Colonic contribution to uremic solutes. *J Am Soc Nephrol* 9:1769–1776, 2011.
26. Goto S, Yoshiya K, Kita T, Fujii H, Fukagawa M: Uremic toxins and oral adsorbents. *Ther Apher Dial* 2:132–134, 2011.
27. Leiper J, Nandi M, Torondel B, Murray-Rust J, Malaki M, O'Hara B, Rossiter S, Anthony S, Madhani M, Selwood D, Smith C, Wojciak-Stothard B, Rudiger A, Stidwill R, McDonald NQ, Vallance P: Disruption of methylarginine metabolism impairs vascular homeostasis. *Nat Med* 13:198–203, 2007.
28. Kielstein JT, Fliser D, Veldink H: Asymmetric dimethylarginine and symmetric dimethylarginine: Axis of evil or useful alliance? *Semin Dial* 4:346–350, 2009.
29. Arese M, Strasly M, Ruva C, Costamagna C, Ghigo D, MacAllister R, Verzetti G, Tetta C, Bosia A, Bussolino F: Regulation of nitric oxide synthesis in uraemia. *Nephrol Dial Transplant* 8:1386–1397, 1995.
30. Heinig M, Johnson RJ: Uric acid in hypertension, renal disease and metabolic syndrome. *Cleve Clin J Med* 73:1059–1064, 2006.
31. Rutkowski B, Swierczynski J, Slominska E, Szolkiewicz M, Smolenski RT, Marlewski M, Butto B, Rutkowski P: Disturbances of purine nucleotide metabolism in uremia. *Semin Nephrol* 24:479–483, 2004.
32. Scribner BH, Babb AL: Evidence for toxins of "middle" molecular weight. *Kidney Int Suppl* S349–S3511975.
33. Vanholder R, Van Laecke S, Glorieux G: The middle-molecule hypothesis 30 years after: Lost and rediscovered in the universe of uremic toxicity? *J Nephrol* 2:146–160, 2008.
34. Rodriguez M, Lorenzo V: Parathyroid hormone, a uremic toxin. *Semin Dial* 4:363–368, 2009.
35. Geara AS, Castellanos MR, Bassil C, Schuller-Levis G, Park E, Smith M, Goldman M, Elsayegh S: Effects of parathyroid hormone on immune function. *Clin Dev Immunol* pii:418695, 2010.

36. Glorieux G, Cohen G, Jankowski J, Vanholder R: Platelet/leukocyte activation, inflammation, and uremia. *Semin Dial* 4:423–427, 2009.
37. Thomas G, Jaber BL: Convective therapies for removal of middle molecular weight uremic toxins in end-stage renal disease: A review of the evidence. *Semin Dial* 22:610–614, 2009.
38. Stenvinkel P: Inflammation in end-stage renal disease: The hidden enemy. *Nephrology* 11:36–41, 2006.
39. Palm F, Nangaku M, Fasching A, Tanaka T, Nordquist L, Hansell P, Kawakami T, Nishijima F, Fujita T: Uremia induces abnormal oxygen consumption in tubules and aggravates chronic hypoxia of the kidney via oxidative stress. *Am J Physiol Renal Physiol* 2:F380–F386, 2010.
40. Kielstein JT, Zocalli C: Asymmetric dimethylarginine: A cardiovascular risk factor and a uremic toxin coming of age. *Am J Kidney Dis* 46:186–202, 2005.
41. Cardounel AJ, Cui H, Samouilov A, Johnson W, Kearns P, Tsai AL, Berka V, Zweier JL: Evidence for the pathophysiological role of endogenous methylarginines in regulation of endothelial NO production and vascular function. *J Biol Chem* 12:879–887, 2007.
42. Niwa T: Role of indoxyl sulfate in the progression of chronic kidney disease and cardiovascular disease: Experimental and clinical effects of oral sorbent AST-120. *Ther Apher Dial* 2:120–124, 2011.
43. Perna AF, Ingrosso D, Violetti E, Luciano MG, Sepe I, Lanza D, Capasso R, Ascione E, Raiola I, Lombardi C, Steinvinkel P, Massy Z, De Santo NG: Hyperhomocysteinemia in uremia—A red flag in a disrupted circuit. *Semin Dial* 4:351–356, 2009.
44. Stenvinkel P, Ekstrom TJ: Does the uremic milieu affect the epigenotype? *J Ren Nutr* 1:82–85, 2009.
45. De Deyn PP, Vanholder R, Eloot S, Glorieux G: Guanidino compounds as uremic (neuro) toxins. *Semin Dial* 4:340–345, 2009.
46. Kinugasa E: Markers and possible uremic toxins: Japanese experiences. *Contrib Nephrol* 168:134–138, 2011.
47. Baurmeister U, Vienken J, Ward RA: Should dialysis modalities be designed to remove specific uremic toxins? *Semin Dial* 4:454–457, 2009.