CKJ REVIEW

Uraemic toxins and new methods to control their accumulation: game changers for the concept of dialysis adequacy

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Abstract

The current concept of an adequate dialysis based only on the dialysis process itself is rather limited. We now have considerable knowledge of uraemic toxicity and improved tools for limiting uraemic toxin accumulation. It is time to make use of these. A broader concept of adequacy that focusses on uraemic toxicity is required. As discussed in the present review, adequacy could be achieved by many different methods in combination with, or instead of, dialysis. These include preservation of renal function, dietary intake, reducing uraemic toxin generation rate and intestinal absorption, isolated ultrafiltration and extracorporeal adsorption of key uraemic toxins. A better measure of the quality of dialysis treatment would quantify the uraemic state in the patient using levels of a panel of key uraemic toxins. Treatment would focus on controlling uraemic toxicity while reducing harm or inconvenience to the patient. Delivering more dialysis might not be the best way to achieve this.

Key words: dialysis adequacy, new adequacy concepts, uraemic toxicity

Introduction

The current concept of dialysis adequacy focusses on urea and creatinine clearance (CrCl) by the dialysis system. This is rather simplistic and encourages providing more treatment by dialysis, ignoring other potentially fruitful strategies such as additional sessions of isolated ultrafiltration (UF), preserving renal function, reducing toxin generation rate, reducing toxin transfer from the gut, selectively adsorbing key toxins and modifying diet. These alternative methods could already be applied before dialysis starts or could be complementary to dialysis. According to the laws of mass action, biochemical effects of any toxin would be proportional to its concentration [1], which would depend as much on generation as on clearance. We now have considerable knowledge of uraemic toxicity and improved tools for limiting their accumulation. It is time to make use of these. A broader concept of adequacy that focusses on uraemic toxicity is required.

Knowledge of uraemic toxicity has grown spectacularly over the past decades (Figure 1). Although barely discussed until late in the previous century, interest has increased exponentially since then. With the founding of the European Uraemic Toxin Workgroup (EUTox; www.uremic-toxins.org), an encyclopaedic list of uraemic retention solutes with their concentrations in uraemia became available [2]. A recent update confirmed the progressive increase in the number of identified retention solutes [3]. This can be attributed to improvements in analytic techniques and in the recent advances in the area of ‘-omics’, allowing profiling of the total proteome/metabolome within a biological sample [4, 5].
Uraemic toxins are preferentially classified according to the physicochemical characteristics affecting their clearance during dialysis, which is still the main therapeutic option for their removal. Traditionally, this subdivision focuses on three types of molecules: the small water-soluble compounds [molecular weight (MW) < 500 Da], the larger ‘middle molecules’ (MW > 500 Da) and the protein-bound compounds [2]. Additionally, salt and water overload could be considered as causing uraemic toxicity. In the future, alternative classifications may be developed, based on new knowledge concerning, e.g. the generation of solutes as proposed in a recent review by Meijers et al. [6] pointing to new targets for decreasing levels of uraemic toxins (Figure 2). Ideally, nondialysis treatments to reduce uraemic toxicity could be started at earlier stages of chronic kidney disease (CKD).

**Current adequacy methods**

Current guidelines recommend quantifying dialysis by urea clearance. The evidence for this was based on the results of the national cooperative dialysis study (NCDS) [7], the first, and still one of the very few, randomized controlled trials (RCTs) designed to investigate the effect on outcome of varying dialysis dose. The NCDS randomized anuric patients dialysing thrice weekly into four groups according to target time-average blood urea nitrogen (BUN) and dialysis session length. The dialysis dose, quantified as the fractional volume cleared per dialysis (Kt/V), was prescribed for the individual patient to achieve the target BUN levels. Patients with higher urea generation rates were prescribed higher Kt/Vs to achieve their allocated BUN target. This study found a significantly reduced hospitalization rate (P < 0.0001) in the patients randomized to achieve low urea (time-averaged BUN 35 versus 75 mg/dL). Patients randomized to longer dialysis time (4.5 h) had ∼50% reduced probability of being admitted compared with those treated by shorter dialysis (3.25 h), but this difference was not significant (P = 0.06). Subjects randomized to low BUN had to be given higher dialysis dose or have lower generation rate to achieve the BUN target. Since lower urea generation would have been due to lower dietary protein intake, usually associated with worse survival, the benefit of low BUN was likely to be due to the increased dose. The study concluded that achieving lower BUN levels was more effective at improving outcome than increasing session length. Secondary analysis of the NCDS, using a urea kinetic model to separate the effect of clearance and generation, suggested that the association between urea clearance as Kt/V and outcome was present at low clearance (Kt/V < 0.9) but was insignificant at clearance levels regarded as adequate by modern standards [8].

The Hemodialysis (HEMO) study is the only RCT designed to investigate the effect of higher dose of dialysis and outcome [9]. It found no benefit in increasing clearance above a Kt/V of 1.2, confirming the results of the NCDS. While there was no difference in outcome between the groups randomized to high dose versus standard dose, within each group, there was an association between poor outcome and failure to achieve the target Kt/V [10].

In peritoneal dialysis (PD), there is also no RCT evidence to support any specified Kt/V. The adequacy of PD in Mexico (ADEMEX) study showed no benefit of increasing Kt/V above 1.7 in anuric patients [11].

The decision when to start dialysis is a difficult and an important one for the patient. Ideally, we would have a measure of...
of uraemic state so that we could start dialysis at the point when the advantages of dialysis outweigh the disadvantage. Estimation of glomerular filtration rate using serum creatinine (eGFR) has proven to be worse than useful as patients starting dialysis with low eGFR have a better outcome [12]. When eGFR is calculated from measurements of creatinine and urea clearance from urine collections, this association disappears [13]. It is possible that GFR measured by a more direct method would better predict outcome, but this has not yet been tested and would be difficult to apply in routine practice. Ideally, the uraemic state would be quantified by the direct measurements in plasma of one or more key uraemic toxins.

The initiation dialysis early and late (IDEAL) study prospectively investigated outcome in patients randomized to starting dialysis at a CrCl (estimated from serum creatinine) of 12 ml/min/1.73 m², whether or not the patients had symptoms of uraemia. The control group were patients starting dialysis when symptomatic or when CrCl dropped below 7. No difference was found [14]. It seems that survival is as good without dialysis but with even minimal levels of renal function compared with dialysis.

The evidence for controlling salt and water overload as a quantifiable and modifiable measure of the quality of dialysis seems at least as compelling as Kt/V [15]. Salt and water overload can be measured accurately by bioimpedance spectroscopy (BIS). Patients dialysing with longer dialysis sessions have improved outcome, compared with conventional treatment [16]. In a study comparing extended time and conventional dialysis, the worse outcome for short treatments was confined to patients who were salt and water overloaded [17]. This suggests that it is the better control of salt and water rather than the higher Kt/V, which is responsible for the improved outcome. Intensive control of fluid overload has been shown to reverse heart abnormalities.

Numerous studies have shown that residual renal function is associated with better outcome in dialysis patients. Where clearance measurements include the contribution of both dialysis and renal function, it is the contribution of the renal function, which has the dominant influence on outcome [18, 19].

So, it seems that, compared with normal renal function, a minimal dialysis providing <10% of the weekly urea clearance and much less than that for all other solutes will preserve life in the short term and avoid overt uraemic symptoms. But dialysis patients suffer from a range of long-term problems that reduce survival. Increasing the dose of dialysis, at least quantified by using standard methods, hardly improves outcome. With modern dialysis, it is easy to provide these minimal levels of clearance relatively noninvasively. Haemodialysis (HD) performed over 2 h three times weekly would deliver an adequate dose of dialysis with respect to small and middle molecule clearance, as defined in the NCDS study. However, with such short sessions, UF rates would be unacceptably high unless fluid weight gains between dialysis could be limited.

If we understood more about uraemic toxicity, we could use treatments other than dialysis to avoid or reduce toxicity. Where dialysis is required, we could fine-tune it to reduce the toxicity. This could improve the outcome for the patient or allow a less invasive and individualized treatment, specifically controlling the level of toxins causing problems for the patient, while limiting harm or inconvenience to the patient.

**How to evaluate uraemic toxicity?**

The evaluation of uraemic toxicity starts with identifying and quantifying the solutes that are present in uraemic biological fluids in abnormal concentrations. The biological effects of these potential uraemic toxins can be evaluated at relevant concentrations in in vitro/ex vivo and/or in vivo experiments. In addition, clinical association studies can suggest a role of specific uraemic solutes in disease. The final approach is trying to decrease the concentrations in vivo, and only when an improvement of hard outcome of CKD patients is demonstrated, a causal relation is confirmed [20].

**Analytical techniques**

Individual uraemic retention solutes are analysed using colorimetric, fluorescence and high-performance liquid chromatographic (HPLC) methods. HPLC is also used to study groups of solutes sharing physical characteristics. As soon as ‘omic’ techniques, analysing total profiles of uraemic retention solutes, became available, they were introduced into research on uraemic toxicity [4, 21–26].

In the context of uraemia, proteomics and metabolomics have been the main ‘-omic’ applications [4, 21, 22, 24–28]. Proteomics is suited for the study of peptides and proteins (middle molecules) [29], while metabolomics focusses on small molecules. ‘-Omic’ strategies are complementary and particularly useful as an approach for identifying pathways that are disturbed in a given pathology [30, 31].

Recently, proteomics have been applied in biomarker discovery, and a new proteome classifier assessing CKD and its prognosis has been proposed [32]. This study demonstrated that, although a high urinary protein excretion invariably resulted in renal failure progression, a low urinary protein excretion did not preclude death or dialysis. Even in patients without proteinuria, a low CKD273 score predicted renal failure progression within a follow-up period of 5.6 years [32]. This finding would need to be validated in independent cohorts before implementing into clinical practice [33].

Uraemic solutes identified in this way might not only be useful biomarkers but also real culprits in the progression of CKD and CKD-related cardiovascular disease (CVD).

When the concentrations of uraemic retention solutes applied in assays to evaluate their biological effects exceed those encountered in uraemia, conclusions on the solutes’ toxicity might have relatively little clinical relevance [2]. Therefore, quantification of the confidently identified metabolites of interest should be performed by targeted methods before testing of the biological activity of uraemic retention solutes becomes possible (Table 1). Assessment of the pathophysiologic role of these newly detected metabolites will enable novel key culprits for the uraemic syndrome to be pointed out as the first step to pursue their specific removal.

**Biological evaluation of toxicity of uraemic solutes**

**Small water-soluble compounds**

Urea was the first uraemic retention solute to be identified and is amongst all uraemic retention solutes the one with the highest concentrations in the blood of uraemic patients. It reflects protein intake in the stable patient and has been used to assess nutrition and dialysis efficacy in renal patients. Toxicity of urea has remained elusive, and it has been thought that the uraemic syndrome was related to associated uraemic retention solutes but not to urea per se. However, more recently indirect toxic effect, via protein/albumin carbamylation [34] a risk factor for mortality in CKD [35], as well as limited direct toxic effects have been attributed to urea. Urea was found to induce the generation of reactive oxygen species (ROS) and insulin resistance in vitro and in mice [36]. In an in vitro study, Vaziri et al. showed that urea induced disruption of the intestinal epithelial barrier function by decreasing...
The guanidines, asymmetric dimethylarginine (ADMA) and symmetric dimethylarginine (SDMA), are found in uraemia [42, 43]. Water-soluble guanidines are also responsible for the generation of other uraemic toxins like tumour necrosis factor-alpha (TNF-α), a pro-apoptotic protein [38].

Middle molecules

As mentioned earlier, the gradual increase of cytokines in CKD is, in addition to the reduced renal clearance, partly attributed to an increased generation in response to uraemic toxins [55, 56]. In clinical studies in CKD, pro-inflammatory cytokines are used as a hallmark of micro-inflammation [57]. The pathophysiological role of cytokines at concentrations as occurring in CKD is often neglected. It was recently demonstrated that, among several pro-inflammatory cytokines, TNF-α alone was pro-oxidative but only at high-range uraemic concentrations. The increase in ROS production could be blocked by aludimunab, although blocking had no effect on the oxidative stress in whole blood from HD patients, suggesting that other uraemic toxins than TNF-α are more crucial in this process [58].

protein-bound compounds

Protein binding in CKD has been considered for some time, e.g. in the context of competition for drug binding [59]. It recently gained new interest as new dialysis techniques might have the potential to improve clearance of protein-bound toxins [60].

Table 1. Key uraemic retention solutes

<table>
<thead>
<tr>
<th>Uraemic retention solutes</th>
<th>MW (Da)</th>
<th>Normal concentration, mean (SD or range)</th>
<th>Uraemic concentration, mean (SD or range)</th>
<th>Ratio</th>
<th>U/N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Small water-soluble</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urea (g/L)</td>
<td>60</td>
<td>&lt;0.4</td>
<td>2.3 (1.1)</td>
<td>5.7</td>
<td></td>
</tr>
<tr>
<td>ADMA (µg/L)</td>
<td>202</td>
<td>&lt;0.6</td>
<td>878.7 (38.4)</td>
<td>14.5</td>
<td></td>
</tr>
<tr>
<td>SDMA (µg/L)</td>
<td>202</td>
<td>76.1 (21.0)</td>
<td>664.6 (606.0)</td>
<td>8.5</td>
<td></td>
</tr>
<tr>
<td>Middle molecules</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>jβm (mg/L)</td>
<td>11 818</td>
<td>1.9 (1.6)</td>
<td>43.1 (18)</td>
<td>22.7</td>
<td></td>
</tr>
<tr>
<td>IL-6 (ng/L)</td>
<td>24 500</td>
<td>4.0</td>
<td>8.6 (3.7)</td>
<td>2.1</td>
<td></td>
</tr>
<tr>
<td>TNF-α (ng/L)</td>
<td>26 000</td>
<td>7.0</td>
<td>57.8 (10.8)</td>
<td>8.2</td>
<td></td>
</tr>
<tr>
<td>Protein-bound</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pCS (mg/L)</td>
<td>188</td>
<td>1.9 (1.3)</td>
<td>41 (13.3)</td>
<td>21.6</td>
<td></td>
</tr>
<tr>
<td>IS (mg/L)</td>
<td>212</td>
<td>0.53 (0.29)</td>
<td>44.5 (15.3)</td>
<td>84.0</td>
<td></td>
</tr>
<tr>
<td>IAA (mg/L)</td>
<td>175</td>
<td>0.5 (0.3)</td>
<td>2.4 (2.2)</td>
<td>4.8</td>
<td></td>
</tr>
<tr>
<td>HA (mg/L)</td>
<td>179</td>
<td>3.0 (2.0)</td>
<td>87.2 (61.7)</td>
<td>29.1</td>
<td></td>
</tr>
<tr>
<td>p-OHHA (mg/L)</td>
<td>195</td>
<td>NA</td>
<td>18.3 (6.6)</td>
<td>–</td>
<td></td>
</tr>
</tbody>
</table>

Extracted from [2, 3].

NA, not available; ADMA, asymmetric dimethylarginine; SDMA, symmetric dimethylarginine; jβm, beta 2 microglobulin; IL-6, interleukin-6; TNF-α, tumour necrosis factor-alpha; pCS, para-cresyl sulfate; IS, indoxyl sulfate; IAA, indole acetic acid; HA, hippuric acid; p-OHHA, para-hydroxyhippuric acid.

the expression of the tight junction proteins [Zona Occludens-1 (ZO-1), Claudin-1 and Occludin] [37]. Trecherel et al. explored regulatory proteins of apoptosis and showed an upregulation of Bcl2-associated death promoter (BAD), a pro-apoptotic protein [38].

Guatidines have been considered as uraemic toxins since the 1970s [39]. Guanidines are neurotoxins [40, 41]. They may also have cardiovascular toxicity since several guanidines are, based on leucocyte activation, pro-inflammatory at concentrations found in uraemia [42, 43]. Water-soluble guanidines are also responsible for the generation of other uraemic toxins like tumour necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6), two middle molecules [42, 44]. The guanidines, asymmetric dimethylarginine (ADMA) and symmetric dimethylarginine (SDMA), are released from proteins that have been post-translationally methylated and subsequently hydrolysed. ADMA has for a long time been recognized as an inhibitor of nitric oxide synthase [45], a propensity that affects both the general and the uraemic population [46-48]. Infusion of ADMA in healthy volunteers, achieving a concentration as in uraemia, resulted in a decrease in cardiac output and a rise in vascular resistance [49]. SDMA, a structural analogue of ADMA, has long been considered inert [45, 50]. Its biologic activity was at first suggested by Bode-Boger et al. [51], showing a dose-dependent inhibition of NO synthesis mainly attributed to limiting the L-arginine supply to endothelial NOS. SDMA plays a role in leucocyte activation by enhancing generation of ROS, which is attributed to increased calcium influx via store-operated Ca²⁺ channels [52] and activation of nuclear factor (NF)κB resulting in cytokine production [44]. Inhibition of NF-κB activation by N-acetylcysteine (NAC) and ROS production with SKF96365 and captopril prevented leucocyte activation [44, 52]. Recently, Speer et al. [53] demonstrated that SDMA accumulates in high-density lipoprotein (HDL) particles from patients with CKD. This complex of HDL and SDMA is recognized by endothelial Toll-like receptor-2, leading to enhanced nicotinamide adenine dinucleotide phosphate-oxidase-dependent ROS production and thereby reducing endothelial NO bioavailability in vitro and increasing arterial blood pressure in vivo. Hence, SDMA may be involved directly or indirectly in the pathogenesis of CVD via accumulation in HDL and seems neither to be inert nor to be a simple marker of renal function or CVD. However, the sole increase of SDMA by exogenous infusion in otherwise healthy mice affected neither renal function nor blood pressure or cardiac function [54].

Middle molecules

Protein binding in CKD has been considered for some time, e.g. in the context of competition for drug binding [59]. It recently gained new interest as new dialysis techniques might have the potential to improve clearance of protein-bound toxins [60].

Protein-bound uraemic retention solutes have been studied extensively over the past decades with focus on their role in the increased susceptibility to infection and cardiovascular complications.

The biological effects of the prototype protein-bound solute, indoxyl sulfate (IS), have been studied the most. A recent systematic review [61] including 27 studies demonstrating pathophysiological effects of IS and/or p-cresyl sulfate (pCS) described their interference with several key metabolic processes involved in the uraemic syndrome. These included inflammation, oxidative stress, endothelial dysfunction, epithelial-to-mesenchymal transition, cardiac cell proliferation and renal tubular cell senescence. Since then, additional reports supporting the above evidence were published, covering increased crosstalk between leukocytes and endothelium, glycolcylx degradation and vascular leakage [62]; apoptosis of osteoblasts [63]; inhibition of drug metabolism [64]; induction of tubular endothelial growth factor receptor leading to tissue remodelling [65] and inhibition of breakdown of angiotensin II [66].

Similar effects were also described for other protein-bound toxins [67]. Indole acetic acid (IAA) was shown to inhibit endothelial progenitor cell production opposing their beneficial effect on vessel repair and neovascularization [68]. IAA induces endothelial inflammation and oxidative stress and activates an inflammatory AhR/p38MAPK/NF-κB pathway [69]. Recently, the ability of IAA to induce tissue factor production was associated with increased pro-coagulant activity [70, 71]. The induction of tissue factor occurred via the aryl hydrocarbon receptor pathway [71].

Recent metabolome studies repeatedly demonstrate increased levels of hippurates. Boelaert et al. demonstrated an increase, already from CKD Stage 3 on, of the known hippuric acid (HA) and 2-,3-,4-hydroxyhippuric acid. They also identified increased levels of an unknown aminohydroxyhippuric acid and of the sulphate and glucuronide conjugates of hydroxyhippuric acid [4]. HA was first isolated from horse urine, hence its name, and is a microbial co-metabolite. In general, literature on toxic effects of
hippurate is fairly old; somewhere along the way, interest in HA got lost. Satoh et al. demonstrated that subtotally nephrecto-
mized rats given HA in their drinking water showed a decrease in
inulin clearance, pointing to glomerular dysfunction. This
was supported by the significant increase in whole kidney scler-
osis index. In addition, N-acetyl-glucosaminidase (NAG) excre-
tion rate, an indicator of proximal tubular injury, was higher in
the uraemic toxin-overloaded rats compared with the control
rats [72]. More recently, HA was shown to inhibit the transport
of two important efflux pumps expressed on human tubular
cells [73]. Next to hippurate, hydroxyhippurates were increased
in plasma from CKD patients. p-Hydroxyhippuric acid (p-OHHA)
inhibits Ca\(^{2+}\) ATPases, needed for restoring intracellular Ca\(^{2+}\)
homeostasis after cell activation. Increased intracellular Ca\(^{2+}\) mod-
ulates various polymorphonuclear leukocyte (PMNL) functions
such as oxidative burst and degranulation as well as apoptosis as
demonstrated by Cohen by the decrease in caspase activity in
PMNL in the presence of p-OHHA [74].

### Uraemic toxins and outcome

Several uraemic toxins have been linked to outcome in CKD
patients or patients on dialysis [75].

ADMA concentration was correlated to intima media thick-
ness, an index of vascular damage, in a dialysis population [46].
ADMA levels were found to be associated with high risk of
death and cardiovascular events in predialysis patients [76–78]
and dialysis patients [47]. A clinical study in 142 patients with
different stages of CKD demonstrated a correlation of SDMA with
TNF-\(\alpha\) and IL-6 [44], which was markedly more significant than
for ADMA [44]. Similarly, in a cohort of 288 dialysis patients,
serum SDMA was a risk factor for death, in contrast to serum
ADMA [79].

- Elevated levels of cytokines and other inflammation markers
  have been related to all-cause and cardiovascular mortality in HD
  patients [80, 81]. In a population with advanced CKD, already hav-
ing increased TNF-\(\alpha\) concentrations, but not yet affected by pos-
sible negative effects of dialysis therapy, the concentration of
TNF-\(\alpha\) was not associated to adverse outcome [58], as was also
shown for earlier stages of CKD [82]. This is in contrast to soluble
TNF receptor 1 (sTNFR1) and sTNFR2, which are independently
associated to all-cause mortality or an increased risk for cardio-
vascular events in advanced CKD irrespective of the cause of
kidney disease [83], and IL-6, which has repeatedly been shown
as a strong predictor for outcome in CKD/dialysis [82, 84, 85].

- For the protein-bound solutes, IS and pCS, highly significant
  associations between concentration and hard end points such as
  cardiovascular events, progression of renal failure and mortal-
  ity have been demonstrated [86–91]. Serum IAA is an independ-
  ent predictor of mortality and cardiovascular events in patients
  with CKD [69].

Finally, numerous studies have now linked the control of salt
and water overload to outcome [15, 17, 92]. Intensive removal of
excess fluid improves left ventricular hypertrophy. Most or all of
the benefits of longer or more frequent dialysis sessions may
be due to improved control of salt and water overload.

### How to decrease concentrations/prevent accumulation of uraemic toxins?

#### Dietary modification

In anuric patients, fluid intake is usually driven by the need to
dilute dietary salt. One litre is required for every 8 g of sodium
chloride ingested [93]. Dietary sodium restriction would help
avoid salt and water overload and/or the need for UF. Similarly,
restrictions in dietary potassium and phosphate are often
recommended.

A very low-protein diet plus ketoacids (VLPD+) has been used
to reduce urea generation and may delay or reduce the need for
dialysis [94]. VLPD+ has also been shown to reduce the generation
rate of IS, a known uraemic toxin [95].

### Reducing absorption from the gut

Agents that bind phosphate or exchange phosphate for other so-
lutes are used to limit phosphate accumulation in the majority
of dialysis patients. Similarly, ion-exchange resins for potassium
are occasionally used. Patiromer, an oral but nonadsorbed potas-
sium binder, is effective in clinical trials [96].

Oral active charcoal, a nonspecific binder of organic toxins,
is routinely used to treat poisoning. It has also been used success-
fully to control uraemia in patients who have refused dialysis
[97] and to improve the abnormalities in gut barrier function in
uraemia [98].

Recently, medicines have become available to limit absorp-
tion of specific classes of compound from the gut. These include
olistat for limiting fat absorption and lipoglyptin for limiting
carbohydrate absorption. It is possible that, in the future, the lim-
iting absorption of other toxins, more relevant to uraemia, will
become available.

### Reducing generation in the gut

A substantial part of the uraemic solutes is generated in the in-
testine as revealed by several studies, comparing the metabo-
lome of germ-free mice versus mice with normal microbiota
[99] and from HD patients with or without intact colon [100].
More recently, Holler et al. demonstrated the effect of prophylac-
tic antibiotics on urinary IS in stem cell transplant recipients
[101]. In spite of its importance, the intestinal microbiota is rarely
taken into account in the context of uraemic toxicity and/or in
the development/optimization of therapies. However, based on
very few targeted studies, significant differences in the microbial
composition in patients treated with HD [102] and PD [103] when
compared with healthy controls have been reported. A recent un-
targeted study confirmed that uraemia alters the composition of
the gut microbiome [104]. However, the effect of the altered mi-
crobial species composition on the metabolic activities linked
to levels of protein-bound uraemic toxins in CKD is not known
and needs further investigation, revealing whether the intestinal
microbiota could be a possible future target even at earlier stages
of CKD preventing generation rather than improving removal.

### Preservation of kidney function

Even a severely damaged kidney may be capable of producing
sufficient urine volume to prevent salt and water overload and
avoid the need for UF. The urine volume may be increased, if re-
quired, by high-dose loop diuretics.

Residual renal function helps to control phosphate, beta2-
microglobulin (β2m) [105] and potassium [106]. In HD patients,
the removal of protein-bound toxins may be entirely dependent
on residual renal function. Survival is significantly associated
with residual renal function in dialysis patients [19]. Multiple in-
terventions can help preserve residual renal function. These in-
clude controlling blood sugar and blood pressure, avoiding
nephrotoxic drugs and avoiding dehydration.

Influencing renal tubular handling of uraemic toxins may be
another alternative and novel therapeutic approach to reduce
their serum concentrations [107]. Transport of uraemic toxins
across the tubular cell membrane is facilitated by specific influx and efflux transporters. Changes in expression and/or function of influx transporters could decrease local toxicity to renal tubular cells [108, 109] and might also affect circulating concentrations if combined with effective efflux transport. Several urea toxins like indole-3-lactate, kynurenic and phenylsulfate are substrate to these transporters [99]. Drugs interfering with the function of these transporters, e.g. probenecid, inhibit the influx of uremic toxins like IS, increasing viability of proximal tubular cells [110]. However, inhibition of these influx transporters will eventually contribute to further accumulation of uremic toxins. In addition, expression of the organic anion transporters (OAT) 1, OAT3 and OAT polypeptide 4C1 (SCL04C1) is shown to be decreased in CKD [111, 112]. Interestingly, Toyo-hara et al. demonstrated that the transcription of SCL04C1 can be upregulated by statins, which leads to a higher expression on the cell membrane resulting in a decreased ureaemic toxin concentration [112]. Mutsaers et al. recently reported that ureaemic toxins inhibit substrate-specific uptake by both multidrug-resistance-associated protein (MRP4) and breast cancer-resistance protein (BCRP), two important renal efflux pumps [73]. This might again contribute to intracellular accumulation and toxicity. So, influx and efflux transporters might be an interesting target for trying to preserve tubular function, which is indispensable for the clearance of specifically protein-bound ureaemic toxins.

**Dialysis**

Using knowledge of the principles of diffusion, clearance of any solute by any artificial dialysis system can be predicted [113, 114]. Existing dialysis systems, or their feasible enhancements, could be optimized to achieve target clearance for any ureamic toxin or group of toxin.

Low-molecular-weight toxins are easily cleared by HD. Levels of toxins similar to that found in patients with normal renal function could be achieved by daily 8-h sessions of high-efficiency dialysis. Higher-molecular-weight toxins can also be removed effectively by dialysis, as long as they are not bound to protein and the molecules are small enough to pass through the dialysate membrane’s pores. Membranes that have a pore radius just smaller than that of albumin are available. Due to the lower rate of diffusion of these larger toxins, efficient clearance rates require larger membrane surface area and are helped by convection or fluid flow across the membrane. Haemodialfiltration, in which up to 100 mL/min of plasma water is filtered across the membrane, could reduce the levels of larger solutes to close to normal levels with daily 8-h treatments.

For protein-bound toxins, only the unbound fraction can be removed by HD or filtration. Clearance of bound toxin requires removal and replacement of the plasma-binding protein (usually albumin), using a membrane that is porous to albumin [115]. The plasma protein can be stripped of the bound toxin by contact with a competitive binding agent, before re-infusion of the plasma proteins into the patient. Systems capable of removing bound toxin are currently available but expensive. Current dialyser membranes bind certain toxins (e.g. β2m). Dialysers could be modified to include a matrix that would adsorb specific uremic toxins. Since the matrix would be in direct contact with plasma proteins, these could adsorb bound toxin. A carbon-based matrix has been shown to reduce the levels of protein-bound toxins IS and pCS in vitro [116, 117].

Excess salt and water overload can be removed by UF. Rapid UF causes ischaemia by increasing blood viscosity and reducing blood pressure [118]. Equipment to remove fluid by UF without dialysis is much simpler, cheaper and portable compared with dialysis. Isolated UF can be powered by the patient’s arterial blood pressure and needs no water or chemical supplies. Salt and water overload could be more easily avoided by more frequent or continuous UF. Harmful effects of rapid UF can be avoided by longer or continuous UF. Longer or more frequent treatments may be more acceptable to the patient using a portable or even implantable UF device [119].

**Potential new adequacy concepts**

Kt/V is useful to calibrate the dialysis process, to verify that a dialysis has been delivered as prescribed and as a measure of dialysis dose, but the achievement of a universally specified Kt/V value should not be an objective in itself. Kt/V does not predict levels of any ureamic toxin [120]. It does not even predict levels of urea. We need ways to quantify the ureamic state, so we can abandon Kt/V as a measure of dialysis adequacy.

Since toxicity should be proportional to concentration of the toxin, the quality of dialysis would be assessed on the concentrations of toxins in the patient. Adequately low levels could be achieved by limiting its generation, preserving or enhancing renal clearance as well as or instead of dialysis. Excess salt and water would be considered as a key ‘toxin’, and an adequate dialysis would limit this without excessive UF rate.

Concentrations of toxins may be predicted using knowledge of the toxin’s generation rate and clearance. Manufacturers of dialysers would provide sufficient data to allow clearance of key toxins to be predicted.

To some extent, this concept of adequacy has already been implemented for PD, where renal clearance and ability to control fluid overload are known to be crucial and small solute clearance by dialysis relatively unimportant.

**Conclusion**

The current concept of an adequate dialysis based only on the dialysis process itself is rather limited. It would be better to include factors within the patient such as dietary intake, generation and renal function. Adequacy could be achieved by many different methods in combination with, or instead of, dialysis. These include preservation of renal function, isolated UF, extracorporeal adsorption of key toxins, modifying diet, reducing intestinal absorption and toxin generation rate.

A better measure of the quality of end-stage renal disease treatment would quantify the ureamic state in the patient using levels of a panel of key toxins. Treatment would focus on controlling ureamic toxicity while reducing harm or inconvenience to the patient. Delivering more dialysis might not be the best way to achieve this.

**Conflict of interest statement**

None declared.

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