Chapter 14
Effects on the Removal of Uremic Toxins

Griet L.R.L. Glorieux and Detlef H. Krieter

Abstract From the moment kidney function declines, retention of many different uremic solutes starts. Many of these solutes exert pathophysiological effects playing a role in cardiovascular damage, a major cause of morbidity and mortality in chronic kidney disease. Over the past years, middle molecules (e.g. cytokines and advanced glycation end-products (AGEs)) but especially protein-bound solutes (e.g. indoxyl sulfate and p-cresyl sulfate) have been identified as some of the main toxins associated with vascular disease affecting the major cell types involved (endothelial cells, leukocytes, platelets and/or vascular smooth muscle cells). Many of these solutes, however, are difficult to remove by standard dialysis strategies. The removal of the larger middle molecules can be obtained by increasing dialyzer pore size and by applying hemodiafiltration (HDF). The removal of protein-bound solutes, however, remains limited with all current dialysis strategies, because only the free fraction of the solute is available for, mostly diffusive, removal. For the future, alternative measures, complementing dialysis removal, will have to be developed to more effectively decrease circulating levels of the difficult-to-remove uremic toxins.

Keywords Uremic toxins • Biological effects • Removal • Hemodialysis • Hemodiafiltration

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Introduction

From the moment kidney function declines, retention of many different uremic solutes starts. The kinetics of this process remain, however, far from clear. Over the years, the list of known uremic retention solutes has become progressively longer [1, 2], which can be attributed to improvements in analytic techniques and recent advances in the area of "-omics," allowing the detection of a myriad of previously unknown compounds [3, 4]. In addition, the presence of an indefinite number of posttranslational modifications of retention solutes, with each of the structural variants possibly exerting a pathophysiologic impact that differs from the mother compound, hampers the process of mapping the uremic retention solutes even more. For the time being, uremic solutes are preferentially classified according to the physicochemical characteristics affecting their clearance during dialysis which, as of today, 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]. Recent reviews point out that removal of small water-soluble compounds is important for 'acute mortality' (e.g. related to hyperkalemia, sodium removal), but that for the chronic problems of the uremic syndrome, the protein-bound solutes and the middle molecules seem to play a more essential role [5–7]. HDF combines the advantages of hemodialysis (HD), small solute removal by diffusion, with those of hemofiltration (HF), large solute removal by convection. Besides a wider molecular weight range of solutes removed, the combination of diffusive and convective transport provides more total clearance per unit of surface area than the application of each of both processes separately [8]. Nevertheless, the combination does not result in the simple summation of clearance delivered by each of the separate elements. To estimate the relative contributions of diffusion and filtration to the clearance, the equation below can be used [9]:

$$C_{total} = C_{diff} + \left[Q_{UF} / 2\right]\left(\text{mL} / \text{min}\right)$$

where C_{total} is the total clearance, C_{diff} the diffusive clearance and Q_{UF} the ultrafiltration rate. This equation is valid for postdilution HDF and ultrafiltration rates up to 100 mL/min.

Addition of convection reduces diffusive clearance. As diffusive clearance is most pronounced for small molecules, this reduction due to convection will proportionately be more important for these solutes, or more exactly, the gain in clearance due to convection will proportionately be more important for larger "middle" molecules [8].

This chapter will focus on those compounds with convincing biological effects associated to adverse outcome, see Table 14.1. Beneficial effects of their removal by HDF will be discussed.

Table 14.1 Overview on uremic toxins relevant for removal by HDF

Uremic retention solute	Normal concentration	Uremic concentration Mean (SD or range)	Ratio U/N Mean (SD or range)	Max. RR (%) in HDF
Small water-soluble				
Phosphate (mg/dL)	2.6–4.5	>5	2	<60[a]
Middle molecules				
β2-microglobulin (mg/L)	1.9 (1.6)	43.1 (18)	22.7	80
Interleukin-6 (ng/L)	4.0	8.6 (3.7)	2.1	NA
Tumor necrosis factor- α (ng/L)	7.0	57.8 (10.8)	8.2	NA
Fibroblast growth factor-23 (ng/L)	26.3 (0.8)	149.6 (102.8)	5.7	NA
Complement factor D (mg/L)	1.9 (0.5)	20.6 (13.0)	10.8	NA
Protein-bound				
p-cresyl sulfate (mg/L)	1.9 (1.3)	41 (13.3)	21.6	<50
Indoxyl sulfate (mg/L)	0.53 (0.29)	44.5 (15.3)	84.0	<50
Indole acetic acid (mg/L)	0.5 (0.3)	2.4 (2.2)	4.8	<50
Hippuric acid (mg/L)	3.0 (2.0)	87.2 (61.7)	29.1	75
Advanced glycation end-products				
N-carboxymethyl-lysine (mg/L)	0.35 (0.13)	18.5 (5.0)	5.3	NA
Pentosidine (μg/L)	51.6 (18.8)	579.5 (299.3)	11.2	

Based on data from Duranton et al. [1]
NA not available
[a]Reduction Ratio (RR) not appropriate because of phosphate refilling in case of falling below the individually different threshold level

Which Solutes Should Be Removed?

Small Water-Soluble Compounds

Phosphate (95 Da) is one of the smallest solutes qualified as uremic toxin [2, 10]. It accumulates in chronic kidney disease (CKD) and leads to adverse effects on biological systems. Hyperphosphatemia contributes to metabolic disturbances such as hyperparathyroidism, vitamin D resistance, and hypocalcemia. It leads to organ damage particularly of the parathyroid glands, bones, and most importantly the cardiovascular system. Elevated phosphorus levels are associated with arterial and valvular calcification, arteriosclerosis, and an increased risk of cardiovascular death [11, 12]. For further reading, see Chap. 11.

Middle Molecules

The group of middle molecules is mainly composed of small peptides. Many of these are implied in cardiovascular disease, by causing inflammation, endothelial damage, smooth muscle cell proliferation, activation of coagulation or by interfering with calcium/phosphorus household [13]. There is thus a pathophysiologic rationale for optimizing their removal. However, their effect on relevant cell mechanisms at the concentrations occurring in uremia has barely been studied. Data on the association of middle molecule concentrations with clinical outcome parameters are more elaborate.

β2-Microglobulin

The most widely-used surrogate marker for middle molecule retention and removal is β2-microglobulin (β2M; 11.8 kDa). It is the β-chain of the major histocompatibility complex and is expressed on most nucleated cells. Free β2M circulates in the blood as a result of shedding from cell surfaces or intracellular release. In general, this molecule is, however, considered inert. Nevertheless, Wilson et al. [14] identified by proteomic analysis β2-microglobulin as the most adequate marker of severity of peripheral vascular disease in a population with no or moderate CKD. In addition, β2-microglobulin has been associated with arterial stiffness in the general population [15] and bone remodeling in non-CKD postmenopausal women [16]. With regard to outcome studies, in two secondary analyses of the HEMO study conducted in HD patients, β2-microglobulin was related to overall and infectious mortality [17, 18]. Higher β2M levels correlate with various cardiovascular risk factors and inflammation markers, such as C-reactive protein (CRP), interleukin-6 (IL-6), and tumor necrosis factor-α (TNF-α) [19], and are associated independently with cardiovascular mortality and cardiovascular events [20]. However, when the oxidative burst of leukocytes was investigated, β2M did not show proinflammatory properties and therefore may not by itself be a causative factor of vascular damage [21]. The serum β2M level is also a new predictor of diabetes-related mortality in diabetic patients irrespective of renal function [22] and is associated positively with insulin resistance [23].

Cytokines

The concentration of cytokines gradually increases in CKD [24], which is thought to be mainly attributed to an increased generation in response to uremic toxins [25–27] and reduced renal clearance [28, 29]. According to the former, cytokines can be considered as secondary uremic retention solutes. Among several pro-inflammatory cytokines, only TNFα revealed to exert pro-oxidative effects on leukocytes at high range uremic concentrations [30]. In a population at different stages of CKD, IL-6 (24.5 kDa) was related to mortality, whereas there was no association for TNF-α (26 kDa). The latter was confirmed in a selected CKD stage 4–5 group [30, 31].

In contrast, in a HD population, TNF-α was a stronger predictor of mortality than IL-6 [32].

In view of improving removal of cytokines, also anti-inflammatory cytokines like interleukin 10 (IL-10, 18 kDa) of which their generation is increased in response to low-grade inflammation in CKD, will be removed. IL-10 is capable of effective down-regulation of proinflammatory cytokines, chemotactic factors and adhesion molecules [33]. A possible beneficial role of IL-10 in CKD has been proposed by Girndt et al. [34], showing that genetic polymorphisms leading to low IL-10 concentration are associated with increased cardiovascular risk in dialysis patients. So, beneficial effects are to be expected only when an equilibrium between pro- and anti-inflammatory factors is restored.

Fibroblast Growth Factor-23

Elevated levels of fibroblast growth factor-23 (FGF-23, 32 kDa), a molecule essentially linked to bone mineral homeostasis, has been associated with progression of kidney failure [35], cardiac dysfunction [36] and overall mortality [37, 38]. Although merely seen as a marker, a recent study in animals showed a direct hypertrophic effect on the heart after chronic injection [39]. These data thus suggest that middle molecule removal could favor outcome, see also Chap. 11.

Complement Factor D

Complement factor D (24 kD) is involved in the regulation of the alternative complement pathway. Due to accumulation in the intravascular compartment [40], serum concentrations are increased in CKD [41]. Elevated complement factor D concentrations enhance the activity of the alternative complement pathway [42] and inhibit neutrophil degranulation [43].

Protein-Bound Compounds

The toxicity of retained protein-bound solutes, which are largely intestinally generated, remains a matter of debate, as in many experimental studies excessively high free concentrations resulted in an overestimation of their potential toxicity [44].

Indoxyl Sulfate and p-Cresyl Sulfate

A recent systematic review unraveled 27 studies where adequate free concentrations of two prototypic protein-bound solutes, p-cresyl sulfate (pCS; 188 Da) and indoxyl sulfate (IS; 212 Da), had been applied [45]. Interference was shown with several key

metabolic processes involved in the uremic syndrome, such as inflammation, oxidative stress, endothelial dysfunction, leukocyte-endothelial interaction, epithelial-to-mesenchymal transition, cardiac cell proliferation and renal tubular cell senescence. These data refer to cardio-vascular morbidity and mortality and to the progression of renal failure. Together with observational studies showing a highly significant association between concentrations of protein-bound toxins and hard endpoints, such as cardio-vascular events, progression of renal failure and mortality [46–51], these data offer strong arguments in favor of a key role of IS and pCS in the uremic syndrome. Since then, additional reports supporting the above evidence were published, covering: increased cross-talk between leukocytes and endothelium, glycocalyx degradation and vascular leakage [52]; apoptosis of osteoblasts [53]; inhibition of drug metabolism [54]; induction of tubular endothelial growth factor receptor leading to tissue remodeling [55]; and inhibition of breakdown of angiotensin II [56].

Indole Acetic Acid

Similar effects were also described for other protein-bound toxins [57]. Indole acetic acid (IAA; 175 Da) was shown to inhibit endothelial progenitor cell production opposing their beneficial effect on vessel repair and neovascularization [58]. IAA induces endothelial inflammation and oxidative stress and activates an inflammatory AhR/p38MAPK/NF-ƙB pathway [59]. Recently, the ability of IAA to induce tissue factor production that was associated with increased pro-coagulant activity was revealed [60, 61]. The induction of tissue factor occurred via the aryl hydrocarbon–receptor pathway [61] In addition, serum IAA is an independent predictor of mortality and cardiovascular events in patients with CKD [59].

Hippurates

Metabolome studies repeatedly pointed to accumulation of hippurates. Boelaert et al. demonstrated an increase, already from CKD stage 3 on, of the known hippuric acid, 2-,3-,4-hydroxyhippuric acid and the unknown aminohydroxyhippuric acid and sulfate and glucuronide conjugates of hydroxyhippuric acid [3]. Hippuric acid (HA; 179 Da) was first isolated from horse urine, hence its name, and is a microbial co-metabolite. It originates from polyphenolic compounds in the diet such as fruit vegetables, tea and coffee, metabolised to form benzoic acid which is at the site of the liver and renal cortex conjugated to glycine to form hippuric acid [62]. 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 sub-totally nephrectomized 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 sclerosis index. In addition, NAG (N acetyl glucoseaminidase) excretion rate, an indicator of proximal tubular injury, was higher in the uremic toxin overloaded rats compared to the control rats [63]. More recently, HA was shown to inhibit the transport of two important efflux pumps

expressed on human tubular cells [64]. 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 $[Ca^{2+}]_i$ modulates various polymorphonuclear leukocyte 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 [65].

Advanced Glycation End Products

The link between the accumulation of AGEs and inflammation has been emphasized before [66]. Nutritional AGEs contribute to this effect since AGE-rich food administered to diabetics increased endothelial free radical production and decreased arterial responsiveness to vasodilatory stimuli [67]. The main representatives of the AGE group are N-carboxymethyl-lysine, pentosidine, and methylglyoxal. Plasma pentosidine is associated with inflammation and malnutrition in ESRD patients starting dialysis therapy [68]. The receptor for AGEs has been shown to exert direct effects on nuclear factor-κB activation in dialysis patients [69], in its turn leading to activation of the inflammatory cascade. The receptor for AGE ligand S100A12 (also known as EN-RAGE) contributes to inflammation and the development of atherosclerosis, and has shown an association with mortality risk in HD patients [70]. As markers of oxidative stress, AGEs also contribute to the activity of inflammatory processes, and are believed to participate in atherosclerosis progression, mainly through modification of matrix proteins, platelet aggregation, defective vascular relaxation, and abnormal lipoprotein metabolism [71].

Influence of HDF on Uremic Toxin Removal

Removal of Small Solutes

By adding of a diffusive component, low small solute removal, the major drawback of purely convective HF, has been overcome in HDF. Depending on the flow settings, small solute clearance in online postdilution HDF is even superior compared to low- and high-flux HD, although this effect is rather modest [72–75]. However, the site of the infusion is crucial because in predilution HDF, small solute clearance is not improved and can be even worsened [76, 77].

The removal of phosphate by dialysis differs from a typical small solute such as urea; it rather resembles that of a middle molecule. This is explained by the electric charge of the phosphate molecule, resulting in the attachment of surrounding water molecules and, consequently, a behaviour like a larger solute. Due to its complex transport kinetics deriving from a four compartmental distribution in the body, the elimination of phosphate during 4–5 h lasting dialysis sessions is limited [78]. Also a consequence of the complex kinetics, the determination of reduction ratios must be regarded as an inade-

quate measure for phosphate removal. Despite continued elimination of phosphate, refilling from the third and fourth compartment to maintaining an individually different minimum serum phosphate level prevents results exceeding 60 % [78].

Nevertheless, a number of studies have proven a superior clearance of phosphate during postdilution HDF compared to standard HD [79–81]. Improvements of the instantaneous plasma clearance between 10 % and 15 % have been determined [73]. This beneficial effect from single sessions may also result in a better control of the phosphate level over longer terms [80]. The large Dutch CONTRAST study demonstrated a decrease of the phosphate level from 5.18 to 4.87 mg/dL and an increase of the proportion of patients reaching phosphate treatment targets from 64 to 74 % over a 6-month period in patients randomized to postdilution HDF. No such changes were observed in patients on low-flux HD [82]. Similar results over 12 months were obtained by a smaller British study comparing postdilution HDF to high-flux HD [83], but several other trials failed to show an advantage of online HDF [84, 85], underlining the need for a control of the dietary phosphorus intake, use of oral phosphate binders, and residual renal function in investigations on phosphorous homeostasis [86].

Removal of Middle Molecules

Convection is the driving force for the removal of large solutes, which pass the dialysis membrane almost exclusively by solute drag effectuated by the transmembrane ultrafiltration of plasma water [87]. Clinical studies investigating the treatment efficacy of convective therapy procedures mostly measured the elimination of β2M as the surrogate parameter for middle molecule removal, not least due to historical reasons: Years before an association of the predialysis β2M level with mortality became evident [17, 20], β2M was shown to play a major role in dialysis-associated amyloidosis, which may be retarded by efficient β2M removal [88].

As indicated above, β2M elimination during a single session correlates with convective volume [89, 90]. Numerous studies have demonstrated a considerably increased β2M removal in online HDF versus HD [74, 91, 92]. At optimum settings of the flow rates, a reduction of the pretreatment β2M level of up to 80 % during a 4 h HDF session is achievable. Compared to high-flux HD, a recent trial investigating last generation high-flux dialysis membranes found an improvement of instantaneous plasma clearance and reduction ratio, established efficacy parameters for β2M removal, in online postdilution HDF of 60 % and 15 %, respectively [73]. This considerable difference was even more pronounced for larger marker molecules, such as cystatin C (13.3 Da) and myoglobin (17.6 kDA), although, treatment settings in HD and high-efficiency HDF were kept identical, except for the substitution flow rate [73].

A beneficial effect of improved single session β2M removal on pretreatment β2M levels over the long-term has been shown, when online HDF was compared to

low-flux HD, a dialysis form not eliminating middle molecules [92]. This effect was particularly obvious in patients without residual renal function [93]. Compared to high-flux HD, lower predialysis β2M levels over longer periods of time after a switch to online HDF were not consistently observed. Some observational studies were able to show such a difference [83, 89], while other randomized trials did not [75, 85]. Again, this finding underlines the importance of the residual renal function for predialysis β2M because differences in the levels between high-flux HD and HDF can be found in patients without any urine production [94].

The removal of other potentially relevant large solutes had been examined in only very few, mostly single center studies. Thus, their results must be interpreted with some caution. Together with lower pretreatment concentrations over a 12 month period, a significant better elimination of complement factor D in postdilution HDF compared to high-flux HD was demonstrated by Ward et al. [75]. Serum levels of TNF-α, a cytokine linked to the inflammatory response, were shown to increase during low-flux HD, while they decreased during high-flux HD and even more during online HDF [95]. This effect may well result from more intense elimination, but biocompatibility effects triggering the inflammatory response to the different dialysis procedures cannot be completely ruled out. However, a small scale study randomizing septic patients with acute renal failure to either high-flux HD or online HDF demonstrated higher reduction ratios of several plasma cytokine levels, including vascular endothelial growth factor, interleukin 6, 8, and 10 as well as TNF-α in HDF [96].

Compared to high-flux HD, online HDF also better removes the relatively large molecule FGF-23, achieving reduction ratios of 56 % versus 36 % [97]. This potentially favorable effect is particularly pronounced when the treatment length is extended to 8 h [79].

Removal of Protein-Bound Solutes

Protein-bound toxins are difficult to remove by extracorporeal renal replacement therapies. The protein-bound fraction is retained and only the free, mostly low-molecular solute can pass the dialysis membrane without differences between low- and high-flux HD [98]. The high ratio of distribution volume to clearance further affects the elimination of these substances [99], allowing only inadequate removal with current dialysis strategies. Compared with diffusive measures, i.e., higher dialysate flow rate and larger dialyzer surface area [100], the effect of convection on protein-bound solute removal is poor. Thus, increasing the ultrafiltration flow rate is little effective to improve the clearance [101]. Accordingly, clinical studies comparing the efficacy of protein-bound solute removal during online HDF with HD have shown only marginal or no differences. Most of these studies focused on the removal of the small compounds p-cresol or its main in vivo metabolite pCS, others additionally measured the also highly protein-bound IS as surrogate markers for protein-bound toxin removal.

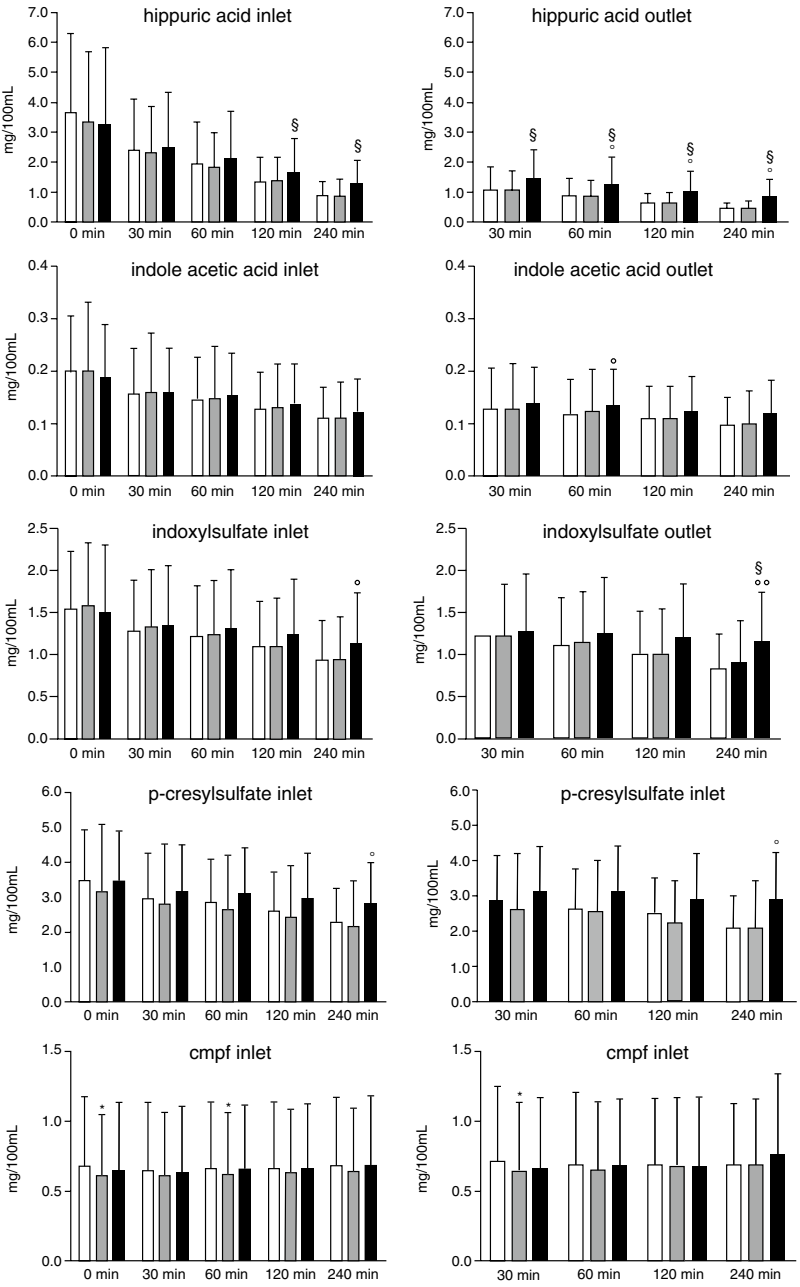

Fig. 14.1 Evolution of the inlet and outlet concentration of protein-bound compounds at different time points (0, 30, 60, 120 and 240 min). Post-dilution haemodiafiltration data are illustrated by *white bars*, pre-dilution haemodiafiltration data by *grey bars* and pre-dilution haemofiltration by *black bars*. *Pre-dilution haemodiafiltration versus post-dilution haemodiafiltration, °pre-dilution haemofiltration versus post-dilution haemodiafiltration, §pre-dilution haemofiltration versus pre-dilution haemodiafiltration; 1 symbol: $P<0.017$, 2 symbols: $P<0.001$ (Reprinted from Meert et al. [103]. With permission from Oxford University Press)

Compared with high-flux HD, an enhanced removal of p-cresol particularly in postdilution HDF was reported by Bammens and colleagues. In predilution HDF at high convective volumes, this beneficial effect was offset, probably because of impaired diffusion due to dilution of the blood entering the dialyzer [102]. Another study comparing postdilution HDF with predilution HDF at equivalent convective volume (i.e., 1:2), was unable to find a difference between the two treatment modes [103]. They determined reduction ratios of the protein-bound solutes pCS, IS, and IAA not exceeding 50 %, while HA was much more decreased by about 74 %, which must be attributed to lower protein-binding (Fig. 14.1). A more recent trial did also not confirm differences in protein-bound toxin removal between high-flux HD and postdilution HDF, demonstrating rather high reduction ratios of free and total pCS and IS hardly passing 50 % [73].

AGEs differ from the above mentioned small protein-bound toxins because of their heterogeneous molecular weight, covering a wide range. Accordingly, convective strategies seem to have a favourable effect on AGE removal, which is illustrated by 50 % and 300 % higher reduction ratios in online HDF (61.5 %) compared to high-flux HD (40.4 %) and low-flux HD (20.5 %), respectively [104].

The reduction ratios of various compounds are summarized in Table 14.1.

Influence of Modified Online HDF on Uremic Toxin Removal

Besides widely practiced predilution and postdilution HDF techniques, several modifications of HDF exist, which have shown to even further improve middle molecule removal, to achieve excellent middle molecule removal at reduced albumin loss or to allow safer operation conditions.

Mid-dilution HDF using a specific single cartridge dialyzer allows removal of middle molecules even exceeding that of postdilution HDF [105]. In the standard configuration, excessive blood inlet pressures were frequently observed, which led to the wide use in reverse mode without significant impairment of the treatment efficacy [106]. With regard to protein-bound toxin removal, mid-dilution HDF has no further advantage compared to postdilution HDF [107].

Online mixed-dilution HDF has shown to better preserve the dialyzer integrity than postdilution HDF by avoiding excessive hemoconcentration and dangerous pressure gradients [108]. At optimized flow rates controlled via TMP-ultrafiltration feedback, the clearance of β2M is improved, while the loss of albumin is reduced [109].

Other existing modifications of HDF do not qualify as online techniques because the substitution fluid is not infused in a controlled manner after ultrapure filtration. These techniques use the backfiltration of dialysate through the dialysis membrane as replacement fluid after excess forward-filtration of plasma water, thereby skipping redundancy as a safety feature in the ultrapure filtration process. Nevertheless, both push/pull HDF and double high-flux HDF achieve remarkably high middle molecule removal [110, 111].

Technical Limitations of Uremic Toxin Removal with Online HDF

The solute removal in convective dialysis techniques is linearly proportional to the substitution flow rate. Or in other words, best toxin removal is achieved at the highest feasible substitution flow rate. For predilution HDF, this increase is less steep and, hence, with respect to the substitution volume applied, less effective compared to postdilution HDF because of dilution of the blood and, thus, the solute concentrations entering the dialyzer. For equivalent clearance, the ultrafiltration rate needs to be at least two times greater for pre-dilution HDF compared with post-dilution [112]. In postdilution HDF, a critically high filtration fraction as a function of the convective flow rate (i.e., the sum of the substitution flow rate and the ultrafiltration flow rate set to achieve the patient's dry weight) exists. This filtration fraction puts the dialysis membrane at risk for clogging and may lead to an uncontrolled breakthrough of albumin, which passes the dialysis membrane in a controlled manner at lower flow rates [113, 114].

Despite being an essential protein associated with malnutrition, the maximum leakage of albumin during dialysis procedures is currently not defined [115]. In Japan, a limit of 4 g per session is recommended. This issue highlights the importance of choosing an appropriate dialyzer for HDF, which should represent a trade-off between maximum permeability for middle molecules and low leakage for albumin [115].

Teaching Points

- Uremic retention solutes are traditionally divided into small water-soluble compounds, middle molecules (MM) and protein-bound compounds
- Whereas small water-soluble compounds are removed by diffusion, MM are best removed by convection
- In online post-dilution HDF, diffusion of small water-soluble compounds is comparable to (high-flux) HD, while the removal of MM is markedly enhanced
- Removal of protein-bound uremic retention compounds, however, is not improved by HDF
- Intradialyzer clotting and albumin leakage may be rate limiting in high volume HDF

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