

CKJ REVIEW

# Clinical relevance of abstruse transport phenomena in haemodialysis

Sudhir K. Bowry<sup>1</sup>, Fatih Kircelli<sup>2</sup>, Mooppil Nandakumar<sup>3</sup>  
and Tushar J. Vachharajani<sup>4</sup>

<sup>1</sup>Dialysis-at-Crossroads (D@X) Advisory, Bad Nauheim, Germany, <sup>2</sup>Global Medical Information and Education, Fresenius Medical Care, Bad Homburg, Germany, <sup>3</sup>Fresenius Medical Care, Singapore Pte Ltd., Singapore and <sup>4</sup>Department of Hypertension and Nephrology, Glickman Urological and Kidney Institute, Cleveland Clinic Lerner College of Medicine of Case Western Reserve University, Cleveland, Ohio, USA

Correspondence to: Sudhir K. Bowry; E-mail: [sudhir.bowry@outlook.com](mailto:sudhir.bowry@outlook.com)

## ABSTRACT

Haemodialysis (HD) utilizes the bidirectional properties of semipermeable membranes to remove uraemic toxins from blood while simultaneously replenishing electrolytes and buffers to correct metabolic acidosis. However, the nonspecific size-dependent transport across membranes also means that certain useful plasma constituents may be removed from the patient (together with uraemic toxins), or toxic compounds, e.g. endotoxin fragments, may accompany electrolytes and buffers of the dialysis fluids into blood and elicit severe biological reactions. We describe the mechanisms and implications of these undesirable transport processes that are inherent to all HD therapies and propose approaches to mitigate the effects of such transport. We focus particularly on two undesirable events that are considered to adversely affect HD therapy and possibly impact patient outcomes. Firstly, we describe how loss of albumin (and other essential substances) can occur while striving to eliminate larger uraemic toxins during HD and why hypoalbuminemia is a clinical condition to contend with. Secondly, we describe the origins and mode of transport of biologically active substances (from dialysis fluids with bacterial contamination) into the blood compartment and biological reactions they elicit. Endotoxin fragments activate various proinflammatory pathways to increase the underlying inflammation associated with chronic kidney disease. Both phenomena involve the physical as well as chemical properties of membranes that must be selected judiciously to balance the benefits with potential risks patients may encounter, in both the short and long term.

**Keywords:** albumin loss, endotoxin, haemodialysis membranes, hypoalbuminemia, transport

## INTRODUCTION

Transport phenomena in haemodialysis (HD) do not distinguish between removal of unwanted toxic substances from those that are vital for body functions and need to be retained in blood [1]. Transport processes across the semipermeable membrane in HD are bidirectional and non-specific; toxic, as well as useful substances, can move in either direction across the membrane

wall [1, 2]. Thus, during every HD session uncontrolled elimination of useful substances also takes place and, under certain circumstances, substances having toxic potential can enter the patient's bloodstream [3, 4].

The dominant and enforced direction of transport is from the blood to the dialysis fluid compartment to rid the blood of substances (deemed 'toxic') that accumulate in the condition of uraemia. A crucial but lesser degree of transport in the opposite

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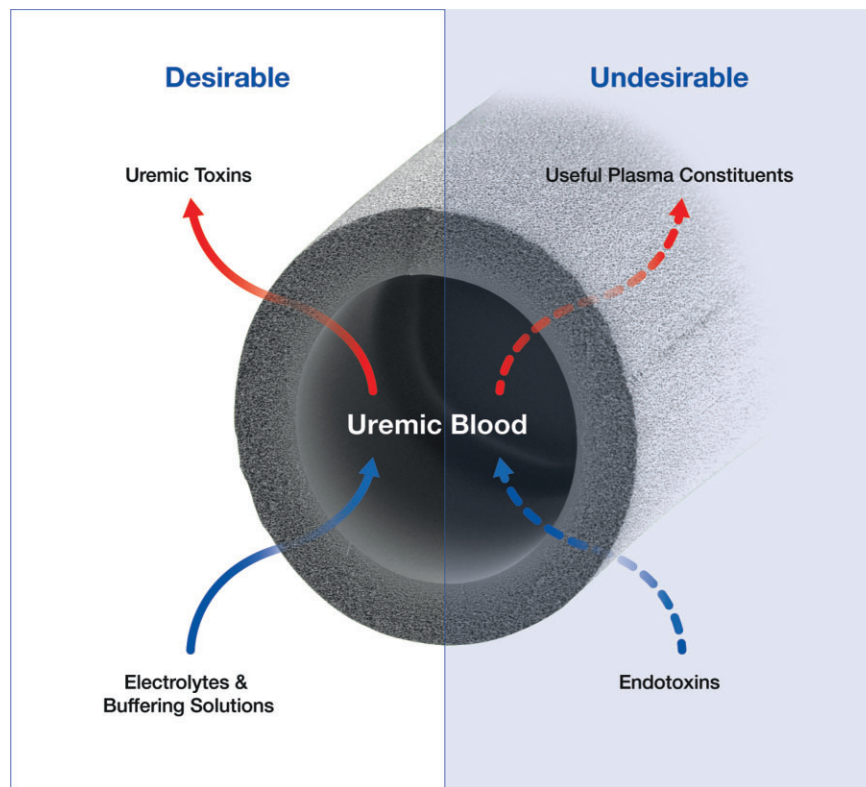


FIGURE 1: The essence of HD therapy: four phenomena, having common modes of transport across the membrane wall, may occur simultaneously during every HD session. The net effect of all the events determines the overall efficacy of treatment, affecting patient well-being as well as long-term outcomes.

direction is necessary to replenish the blood with electrolytes depleted during dialysis and to correct metabolic acidosis with buffers to control acid-base derangements. Understanding and accomplishing a higher degree of control of transport in both directions is the central objective of delivery of dialysis that determines its efficacy and tolerance, as well as outcomes [5].

The intended, bi-directional transport necessary for the very functioning of HD therapy is accompanied by two inadvertent and largely underestimated transport phenomena that can negatively impact HD therapies. First, substances that are neither the consequence of uraemia nor known to express any toxicity are actively eliminated along with the toxic substances dialysis therapies target for removal [6]. The removal of 'useful' substances (e.g. enzymes, hormones, vitamins, nutrients, metabolites, and coagulation and immune factors) during each session has been known throughout the development of HD and acknowledged as an undesirable but unavoidable consequence of HD [7, 8]. However, it has not merited the same intensity of investigation as devoted to the search, and removal, of uraemic toxins that accumulate in uraemia. Second, toxins arising under certain conditions within the extracorporeal circuitry of HD could enter the bloodstream from the dialysis fluid compartment. Endotoxins, byproducts of bacterial growth and lysis, have been shown to trigger biological reactions of varying severity in HD patients [9]. Figure 1 portrays the two obscure transport phenomena that occur simultaneously with the desirable and more recognized and studied transport events that constitute HD. We detail the scientific background, mechanisms and clinical consequences of the two undesirable transport processes in relation to those that are essential and describe approaches to mitigate their effects.

## TRANSPORT OF ESSENTIAL SUBSTANCES INTO THE DIALYSATE COMPARTMENT IN HAEMODIALYSIS

### Equivalence of toxic and non-toxic substances in the membrane separation context

Membrane separation processes in HD are based on the criterion of size exclusion in conjunction with the specific transport mechanisms of solute removal [10–13]. What is, or is not, able to pass across the thickness of the membrane wall depends primarily on the molecular weights (size) of the plasma proteins and peptides relative to the mean size of the pores at the innermost blood-contacting region of the hollow-fibre membrane [14, 15]. Because the pores at this surface are not uniform in size and smaller and larger pores are distributed about the mean value, a broad range of substances of varying size are eliminated during HD [16]. Significantly, this size distribution of pores varies considerably along the length (~25–30 cm for most dialysers in routine clinical use today) of each fibre, resulting in variable removal of compounds from the proximal to distal end of the dialyser.

Throughout the history and development of HD the preoccupation has been, firstly, to ascribe toxicity to substances known to be retained in uraemia and search for newer uraemic toxins using sophisticated analytical technologies [17, 18]. Secondly, newer treatment modalities and technologies strive to decrease the concentrations of selected (unwanted) substances as efficiently as possible [19, 20]. The captivation of achieving these two goals has led to the neglect of another major phenomenon that takes place simultaneously during each treatment session, viz. the elimination during each HD session of an

array of substances that present no toxicity whatsoever and are in fact highly valuable for body functions [7, 8, 21]. The number of publications addressing uraemic toxicity and toxins and their removal far outweigh those that attempt to identify vital compounds removed inadvertently during HD, or to determine the physiological consequences imparted on the patient [3]. The authors are unaware of any prospective randomized controlled study that has sought, at the outset, to correlate excessive removal of different, useful non-toxic substances with patient outcome measures.

Uraemic toxins and substances having essential biochemical functions are physically indistinguishable in terms of their removability by semipermeable membranes used for dialytic therapies [15, 22]. Like the uraemic toxins targeted for removal during HD, compounds that the body cannot afford to lose have a broad molecular weight size range. As discussed elsewhere, less than 100 free (non-protein bound) compounds up to the size of albumin (MW 68 500 Da) have been found to express toxicity, most of these being below ~30 000 Da [23, 24]. Intermittent HD therapy (three sessions/week, each 4 h duration) successfully reduces their plasma concentrations to sustain life of end-stage renal disease (ESRD) patients. With several hundreds, or even thousands, of proteins and peptides known or estimated to be present in plasma and many being in the same size range as the culprit toxic compounds, their removal (together with that of uraemic toxins) during HD must occur [25–27]. Again, in HD substances are removed based on their size and not on their biological activity or potency within the body.

The indistinguishability of essential proteins and uraemic toxins in terms of their membrane separation principles is best appreciated by examining the sieving functionality of membranes [28–30]. Briefly, the sieving coefficient (SC) profile of an HD membrane describes its solute-removing capabilities in terms of the size (MW) of molecules and indicates the probability and extent to which molecules of a given size may or may not traverse the membrane wall. Manufacturers of HD membranes usually display such sieving profiles of membranes in product specification data sheets to indicate whether the membranes are 'low- or high-flux' that, although being a subjective and an arbitrary ranking, is indicative of the removing capacity of a membrane for various-sized uraemic retention solutes considered as candidate uraemic toxins [31].

### Number of plasma proteins present in spent dialysate (ultrafiltrate)

Using proteomics, Weissinger *et al.* conducted a study in 2004 to identify molecules present in ultrafiltrates from uraemic and normal plasma obtained with membranes classified as low-flux and high-flux; the authors considered 'high-flux' membranes as having 'a larger pore size' and ability to 'remove more middle molecules' than 'low-flux' membranes [32]. In ultrafiltrate from uraemic plasma, 1394 polypeptides were detected in the high-flux samples compared with a lower number of 1046 in the low-flux samples, as would be expected because of the difference in the sieving profiles of the two membrane classes. Virtually all polypeptides had a molecular weight <6000 Da (99.1% of the high-flux ultrafiltrate and 99.7% from the low-flux group). Considering as of 2018, that fewer than 100 uraemic retention solutes can be considered as uraemic toxins (i.e. for which sufficient evidence is available to demonstrate their adverse biological effects and/or affecting outcomes), the number of substances being removed from blood during HD therapies is a fraction of those going through, and that can therefore be assumed

to be 'useful' [23–25, 27]. It is important to note that neither of the two membrane types used in the study of Weissinger *et al.* are comparable to membranes in current use; both classes of membranes today are of considerably 'higher flux'. Where membranes having ultrafiltration coefficients (KUFs) of around 10–15 mL/min mmHg were considered as high-flux some 20 years ago, these are now at the lower end of the low-flux range, as today high-flux have KUFs of up to and beyond 100 mL/min mmHg. Thus, in relation to the data of Weissinger *et al.* the number of essential polypeptides and proteins removed by contemporary membranes would not only be several times more, but also compounds of considerably larger molecular weight (even beyond the size of albumin) are possibly being regularly 'lost' by the patient during each session. The proportion of known uraemic toxins is a fraction of the total plasma proteome, most of which comprises proteins serving essential body functions. Kratochwill emphasizes the significance of more selective protein removal during HD therapy, as the 'wrong proteins' being removed could represent a so far neglected risk for the patient [7].

The sieving potential of a selection of essential plasma proteins (i.e. non-toxic) not considered to contribute to the condition of uraemia by a typical modern-day high-flux membrane is displayed in Figure 2. The selection is purely arbitrary and includes common and well discussed compounds in various fields of medicine and diagnostics to demonstrate the potential losses of useful compounds occurring during every single HD treatment session.

Sieving profiles of membranes are, unfortunately, interpreted in absolute terms. While a  $SC = 1$  (on the flat part of the curve) implies that a molecule passes unimpeded through the membrane and  $SC = 0$  (lower end of curve) means it is totally retained in the blood, such a relationship erroneously assumes that all pores are identical, circular and of a defined size. This usually implies that the mean pore size of the membrane is such that the largest molecule that needs to be removed (for all intents and purposes, molecules just below the size of the albumin molecule) can pass through the membrane unhindered. Such an 'ideal' dialysis membrane neither exists nor is likely given that hundreds of millions of kilometres of hollow fibres need to be produced cheaply each year. As a proportion of the pores are larger or smaller than the estimated mean pore size, the extent to which any molecule can be fully eliminated can only be approximately estimated. Thus, say a protein of MW 20 000 Da shows an SC value of 0.4 from the sieving profile, the literal interpretation would imply that its plasma concentration is reduced by 40%; in reality, because of the non-uniformity of membrane pore sizes and their distribution, considerable variation in its removal occurs in practice. This point is particularly relevant at the lower end of the SC profile where, misleadingly, a SC close to zero is regularly cited for albumin to indicate its limited elimination during HD sessions. Given its abundance in plasma, an  $SC_{\text{Albumin}}$  as low as 0.01 can still equate to substantial losses of albumin (and other proteins) during HD, depending on the overall quality of the membrane [33, 34].

### Albumin: the benchmark of essential substances not to be lost during haemodialysis

Approximately 55% of the total protein mass in plasma is made up by albumin alone [35]. This singular fact elicits appreciation of the myriad of physiologic roles albumin has in numerous body functions in normal health as well as in diseased states, including chronic kidney disease (CKD) [36–40]. In CKD, albumin has multiple roles and low serum albumin concentration is



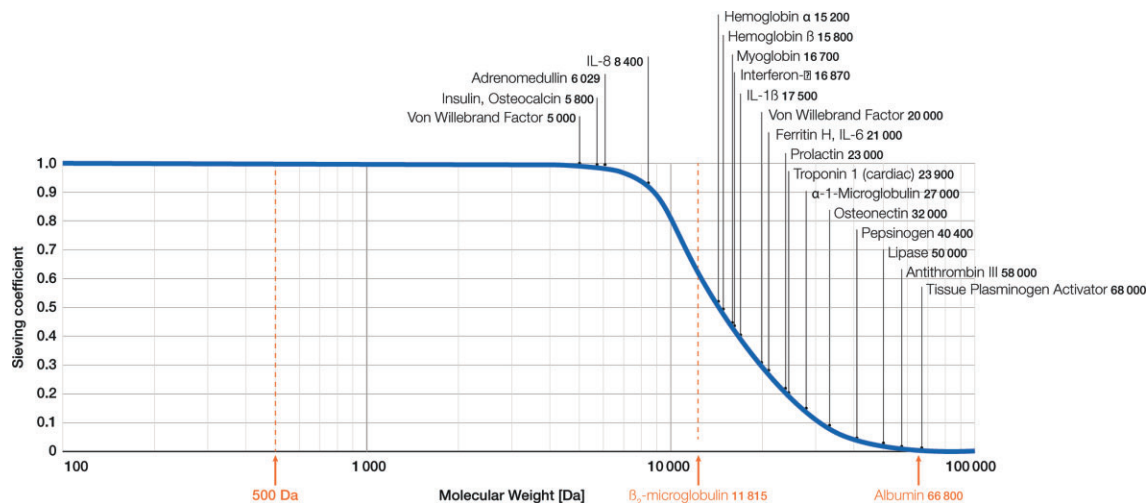


FIGURE 2: The removability by standard 'high-flux' membranes during HD of non-uraemic toxins, i.e. compounds that do not contribute to the uraemic syndrome. The selection of the substances is arbitrary, and includes proteins having not only plasma-based functionality, but also proteins released into the blood from tissues and cells due to physiological events, and not necessarily involved in, or the result of, specific disease processes [59]. These 'essential' proteins that are common and well discussed compounds in various fields of medicine and diagnostics are in a similar size range as the known uraemic compounds targeted for removal in HD.

considered as a powerful predictor of mortality and associated with increased morbidity [41–44]. In patients with chronic diseases such as CKD, hypoalbuminemia is mainly attributed to the combined effects inflammation and malnutrition [41, 45–47].

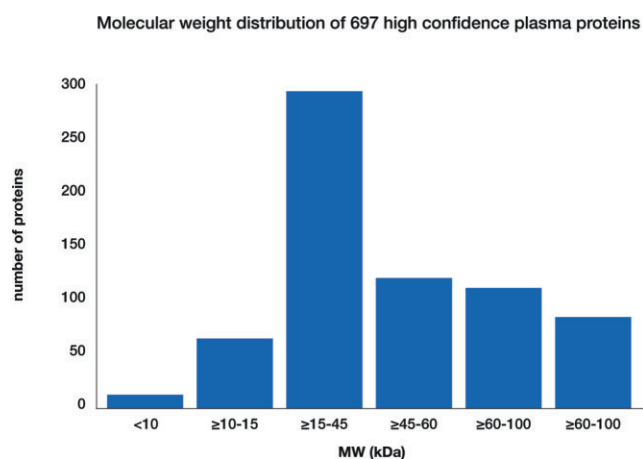
Regular fluctuation or long-term depletion of albumin levels resulting from dialysis therapies is understandably cause for some concern, particularly as the inflammation– nutrition axis, amongst other factors, is decisive towards determining patient outcomes and well-being [41, 47, 48]. In striving to increase the efficiency of removal of substances with toxic potential, an increasing number of large-molecular weight substances are today being implicated for removal during dialysis. Some, like the free light chains  $\kappa$  (kappa) and  $\lambda$  (lambda), hitherto not considered by the EUTOX Working Group and not established in the analysis of Vanholder *et al.* as belonging to the uraemic toxins category or having relevance in end-stage kidney failure, are being promoted to justify the application of highly porous membranes that simultaneously result in increased albumin losses [23, 49]. With such industry-driven strategies, the higher losses of albumin during HD are justified along two lines of argumentation. Firstly, it is asserted that patients on peritoneal dialysis (PD), known to regularly lose considerably higher amounts of albumin per treatment session compared with patients on HD, are not at a disadvantage [50, 51]. This claim is contentious as the generally inferior long-term outcomes of PD patients compared with HD patients have been linked to the high albumin losses of 6–8 g per day [51–53]. Secondly, for membranes that leak proteins, higher albumin losses are portrayed as being advantageous as a few uraemic toxins bound to albumin may be eliminated. Again, the argument is flimsy, as of all the uraemic retention solutes for which there is reasonable evidence for toxicity, only six are bound to proteins, presumably to albumin [23]. It is widely acknowledged that HD strategies involving either highly porous protein-leaking membranes or even convective therapies are largely ineffective in reducing the concentrations of uraemic toxins bound to proteins, with negligible removal observed [50, 54–57]. Not all HD membranes classified as 'high-flux' are comparable in their ability to specifically and efficiently remove middle molecules, or curtail the unwanted excessive leakage of essential proteins from the patient's blood [58].

### Potential consequences of loss of other useful compounds during haemodialysis

Fewer than 100 compounds retained in uraemia can today be declared 'uraemic toxins' [23, 24]. These are solutes that accumulate in end-stage kidney failure and for which there is evidence showing negative *in vitro* and *in vivo* biological activity and whose plasma concentration reduction by HD potentially leads to better patient outcomes and well-being. This status quo is the product of some exacting effort over decades to address the fundamental question of toxicity ever since the uraemic condition began to be recognized: which accumulated substances contribute to the condition and need to be eliminated from blood to alleviate symptoms associated with ESRD?

The  $\leq 100$  uraemic toxins (the majority being peptides and proteins covering a broad molecular weight range) are a small part of a much larger pool of plasma constituents. Estimates of the number of peptides and proteins present in plasma varies. The Human Proteome Organization (HUPO) attempts to obtain a reliable blood plasma protein list for their diagnostic potential and to characterize specific disease processes or tumours by their plasma 'signatures' [59]. The plethora of proteins in blood—every human protein has the potential to be present in plasma—includes those residing in blood and having plasma-based functionalities as well as proteins picked up by blood circulating through tissues and released into the plasma from physiological events. In 2008, Schenk *et al.* [59] primarily identified 1193 distinct proteins, reducing the number to 697 as the 'stringently validated high confidence protein set'. As Figure 3 shows, most of these are well below 100 kDa, and most in the size range of the 79 uraemic retention solutes deemed toxic by Vanholder *et al.* [23].

With the development and availability of highly advanced analytical tools, the sequencing of the human genome and subsequent identification of protein-coding genes resulted in a catalogue of  $\sim 20\,000$  proteins, of which a few thousand have been detected in plasma [60]. A total of 6130 different proteins were identified in five blood proteomes, from which 1682 proteins were in platelet-rich plasma and 912 in platelet-free plasma [26]. The recently curated plasma peptide atlas compiles 178 mass



**FIGURE 3:** Histogram depicting the molecular weight distribution of 697 observed plasma proteins analysed by Schenk et al. [59]. Most of the proteins are under 60 000 Da, i.e. below the size range of the albumin molecule, removal of which during dialysis in large amounts is generally considered undesirable as it negatively impacts patient outcomes. As most of the known uraemic toxins are well below this size range and targeted for removal in HD, several essential proteins (i.e., non-toxins) could inadvertently be eliminated during dialysis with highly porous membranes.

spectrometry-based methods (pooled from different recent proteomics databases) to describe a total of 3509 proteins in plasma [35]. In 2002, a list of 289 proteins detected in plasma or serum was documented [27]; since then, with sophisticated automated analytical techniques that have evolved, the list of clearly identified compounds would be expected to be considerably higher.

The objective of this exercise of determining the number of substances present in plasma is straightforward: in targeting the removal in HD of a small number (<100) of uraemic toxins, any of the numerous plasma proteins—known or those yet to be identified—that are neither toxic nor involved in any way with uraemia would also be removed during HD. With the molecular weight of most known uraemic toxins being below that of albumin (66 800 Da), any ‘useful’ (non-toxic) molecule approaching this size, or even slightly above, would be eliminated from the patient’s blood. Bearing in mind the determinants of HD membranes that govern their sieving function, the smaller the size (MW) of the solute, the easier its elimination; conversely, decreasing amounts of solute removal will always occur as the MW approaches that of the albumin molecule.

The sustained loss from plasma during each HD session of undefined amounts of innumerable substances that can be assumed to have biological significance for vital body functions is a neglected area of nephrology. Proteins in the circulatory system mirror an individual’s physiology [25]. Measurement and diagnostic analyses of levels of biomarkers of disease in blood, plasma or serum are the cornerstones of routine clinical practice in medicine. Alteration of concentrations of inorganic or organic compounds beyond normal ranges are usually indicative of perturbations or abnormalities requiring therapeutic intervention. Periodic, lifelong reduction during HD in levels of unspecified proteins that otherwise have no toxicity but have essential biochemical functions cannot be in the interests of a patient’s wellbeing. Patients on HD are thus unique in that their therapy causes a weekly reduction in plasma concentrations of numerous inorganic or organic compounds that have little or no relevance to the core clinical condition (CKD) they are being treated for. It is not inconceivable that this unintended depletion

results in a lowering of concentrations that would otherwise constitute a pathological condition or diseased state [47]. Nevertheless, given that they survive so long and have acceptable quality of life means the benefits of HD still outweigh the disadvantages purely from an overall mass removal perspective.

### Mitigating loss of albumin and other useful substances during dialysis therapy

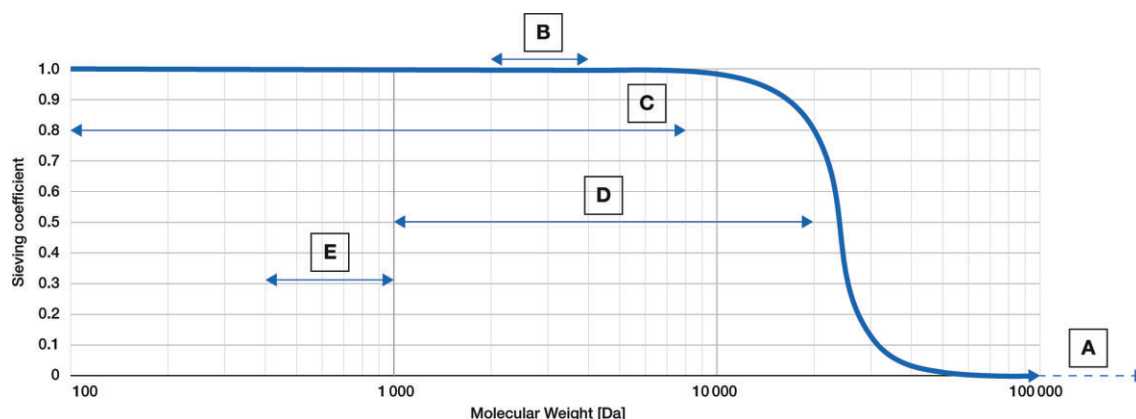
The most effective approach to curtail the loss of essential, non-toxic compounds from blood during HD is to refrain from using excessively porous (‘more open’) membranes—the larger the mean pore size, the greater the risk of loss of useful substances. As discussed in other parts of this Supplement, the concept of ‘flux’ itself is poorly defined and understood; in transport phenomena terms, the KUF is the pertinent measure of the flux of a membrane [2]. High-flux membranes are a heterogeneous class of membranes, with their KUF specifications today ranging from around 15 to well over 100 mL/min mmHg, thereby varying in the degree to which they remove uraemic toxins, albumin and other essential proteins. Current knowledge of uraemic toxicity shows that most uraemic toxins are below ~30 000 Da; targeting removal of compounds beyond this size with membranes having excessively large pore sizes is unnecessary and is linked to avoidable patient disadvantages.

## TRANSPORT OF TOXIC SUBSTANCES INTO THE BLOOD COMPARTMENT DURING HAEMODIALYSIS

Dialysis patients are exposed to large volumes of fluid throughout the period they are treated. Considering a typical dialysis session of 4 h and a dialysis fluid flow rate of 500 mL/min and 800 mL/min for high-flux modalities, for a thrice weekly treatment schedule an exposure of 360 and 576 L per week or ~18 720 or 29 952 L per year, respectively, results. The volumes of fluid patients are exposed to can be considerably higher for convective treatment modalities whereby replacement fluids are prepared ‘online’ from the available dialysis fluid [61, 62]. Considered as a determinant of adequacy of dialysis, water for HD thus needs to be of high purity, both chemical and microbiological [63]. Elaborate multi-component water treatment systems within each dialysis centre ensure high purity in terms of chemical contaminants in line with established international standards and requirements. Ensuring appropriate microbiological purity for dialysis fluids (contaminated with bacterial by-products) is more complex, as the heterogeneity of the contaminating biological entities demands variable approaches to counteract their effects.

### Endotoxins: source, chemical characteristics and biological potency

Endotoxins originate from the outer cell envelope of all Gram-negative bacteria, irrespective of whether the organisms are pathogenic or not. As the name suggests, they are part of the wall cell and are an integral part of the intact organism performing critical roles of interacting and reacting to the environment. Endotoxins are shed during cell growth and upon cell death (lysis) disruption of the cellular membranes exposes and releases fragments of various sizes and biological reactivity into the surrounding environment [64] (Figure 4).



**FIGURE 4:** The size range of bacterium-derived substances that could prevail in dialysis fluids should they be contaminated by Gram-negative bacteria (from Jofré et al. [64]). Both endotoxins and exotoxins of the size range of most of the uraemic toxins targeted for removal in HD are present. Because of the bidirectionality of membranes and separation processes being size based, these substances have the potential to enter the patients' bloodstream to induce pro-inflammatory reactions. A = LPS (>100 000 Da); B = lipid A (2000–4000 Da); C = other LPS fragments (<8000 Da); D = peptidoglycans (1000 to 20 000 Da); E = muramyl peptides (400–1000 Da). Secreted bacterial toxins such as exotoxin A (71 000 Da) or its fragment (<1000 Da) and other exotoxins (20 000 to 50 000 Da) may also be present, but their role in HD is less well defined. Other cellular components (bacterial DNA) of variable sizes have also been shown to pass into the blood stream during dialysis.

Chemically, endotoxins are lipopolysaccharides (LPS) to which the biological activity is associated. The cell envelope of the Gram-negative bacterium comprises the inner plasma membrane, which is separated by the periplasmic space composed of the peptidoglycan sheet from the outer membrane. LPS is asymmetrically distributed in the outer leaflet of this outer membrane wall, which also contains phospholipids and various outer membrane proteins (e.g. lipoproteins and porins that sometimes play a role in virulence but are not considered endotoxins) imbedded amongst the LPS molecules. The inner leaflet of the outer membrane is composed of phospholipid, the same as the plasma membrane [65]. Unlike bacteria, endotoxins are heat stable and boiling for 30 min does not destabilize endotoxin but certain powerful oxidizing agents such as superoxide, peroxide and hypochlorite have been reported to neutralize them.

LPS has two components, lipid A and polysaccharide, composed of three distinct regions, lipid A, a short core oligosaccharide, and the O-antigen polysaccharide [66]. The intensity of the biological activity of LPS molecules depends on the composition of these units, especially the nature of the lipid A domain, which is responsible for the major bioactivity. The detailed structure of lipopolysaccharide differs from one bacterium to another and production of diverse lipid A structures and structural modification are related to the virulence of bacteria [67, 68]. To alter properties of the outer membrane or evade the host immune response, Gram-negative bacteria employ a wide variety of chemical modifications to alter LPS. The structural heterogeneity of LPS is reflected by the diversity and intensity of their biological reactivity and LPS of Gram-negative bacteria are particularly inflammatory and are amongst the most potent bacterial inducers of cytokines. During infectious processes, the production of inflammatory cytokines including tumour necrosis factor (TNF), interleukin-1 $\beta$  (IL-1 $\beta$ ), gamma interferon (IFN- $\gamma$ ) or chemokines orchestrates the anti-infectious innate immune response [69]. LPS signalling, leading up to a cytokine storm, can be deleterious and contributes to mortality consecutive to sepsis or toxic shock syndrome accompanied by a wide spectrum of pathophysiological reactions that incorporate activation of the coagulation and complement cascades [70].

### Transport mechanisms of passage of endotoxins into bloodstream

**Physical characteristics of endotoxins relevant to the dialysis situation.** There is a wide disparity in estimates of molecular weight of endotoxins from Gram-negative bacteria in the literature. Depending on the bacterial source and strains, isolation methodology and characterization techniques, the molecular mass has been variously estimated to be >100 kDa >1000 kDa and even beyond if various subunit associations are considered [70–73]. The exact conformation of isolated, non-bacterially attached, LPS is not easy to determine and the large LPS and large amphiphilic molecules form either monomers, micelles or even vesicles [74]. However, the typical molecular weight of monomeric form LPS of *E. coli* is reported to be between 11.8 and 18 kDa [74]. Such is the breadth of the size ranges specified, it is perhaps more pertinent to examine the smallest entity LPS entity that expresses biological activity. Although lipid A structures widely vary amongst different bacterial species, it is responsible for most of the physiological activities, i.e. toxicity of LPS [65, 71, 74, 75]. With a MW ranging from about 1300 to 2200 Da and relevance to the dialysis situation, the molecular weight of endotoxins or their biologically active components ranges from as low as around 1300 Da to several magnitudes higher, as cited above [76–78].

**The phenomenon of backtransport in dialysis.** The bidirectionality of HD membranes described above can result in the unwanted transport of endotoxins into the blood stream. Should dialysis fluids be contaminated with strains of Gram-negative bacteria, there is the potential of LPS of various molecular weights and biological potency to traverse the semipermeable barrier. The widespread utilization of bicarbonate for buffering of dialysis fluids provides a source of carbon to promote microbial growth [79–81]. With highly variable quality of drinking water supplied to clinics from local municipal plants to produce dialysis fluids, proliferation of bacteria in dialysis water supply systems is a worldwide issue [82]. Contamination of dialysis water and fluids afflicts even countries with advanced healthcare systems, with high levels of contamination with several bacterial strains in most clinics [83]. Over the years, the increasing



usage of more porous membranes and convective modalities aimed at better elimination larger uraemic toxins has compelled providers and administrators to address the water quality issues at their HD [78].

Backtransport, in HD, implies the transport of water and substances in it that occurs from the dialysate to the blood compartment, and is the sum of two components [84], as follows.

**Backdiffusion.** Like the diffusion-based movement of solutes from the blood to the dialysis fluid compartment along a concentration gradient, a similar difference in concentrations of certain compounds can cause transport in the reverse direction; any LPS molecules present in the dialysis fluids (higher concentration region) then enters the blood compartment lacking endotoxins.

**Backfiltration.** This is a convective mechanism that is coupled to the phenomenon of internal filtration and occurs within dialysers containing high permeability membranes to enhance removal of larger solutes not easily removed by diffusion [85]. The total amount of water flowing from blood to dialysate (determined by the ultrafiltration rate) within the closed blood and dialysate compartments of a dialyser is termed internal filtration rate [86]. The net filtration rate, however, is the internal filtration rate minus the amount of water flowing from the dialysate to blood compartment, i.e. the backfiltration rate [87]. The pressure profiles along the length of a dialyser contribute to both the internal filtration and backfiltration; whenever the transmembrane pressure (TMP) is positive, the hydraulic flow is from the blood to the dialysate side (internal filtration) [88]. However, if at any point the TMP is negative (towards the distal end of dialyser), transport in the reverse direction occurs from dialysate to the blood side. According to the Hagen–Poiseuille formula describing flux, fibre geometry (internal diameter and fibre length) determines the hydrostatic pressure drop and thus the relative extent of internal filtration/backfiltration along the filter, increasing with decreasing fibre diameter and decreasing with length of fibres [89].

The biochemical reactions triggered by endotoxins entering blood by the combined effects of backdiffusion and backfiltration during HD has been recognized for some time [90–92]. Repeated exposure of high-flux HD patients to backtransport of dialysate contaminants aggravates uraemia-associated inflammatory response syndrome and contributes to long-term morbidity [64, 93, 94]. ESRD itself is essentially an inflammatory condition with multiple potential causes, and additional insults by any aspect of the dialysis procedure worsens the prognosis of dialysis patients in terms of cardiovascular disease (CVD) and mortality [95, 96]. Such is the significance of purity of dialysis fluids, it is now widely considered as a mandatory requirement towards the delivery of HD [4, 63, 97, 98]. However, LPS is not the only and maybe not the most important product in dialysate that induces generation of cytokines; short bacterial-derived DNA fragments are also present in dialysis fluids [99]. These fragments are of sufficiently small size to pass through dialyser membranes. Bacterial DNA fragments may be an overlooked factor contributing to inflammation in HD patients [100].

### Mitigating transfer of endotoxins into the bloodstream during dialysis

Several strategies are available to minimize the risk of entry of endotoxins into the bloodstream during HD. The combined effect of the following approaches virtually eliminates the prob-

ability of backtransport of LPS fragments across the membrane wall.

**Ensuring high microbial water quality.** In addition to regularly educating the entire staff at each HD centre of the background, principles and consequences of endotoxin impurities on patient outcomes, implementation of strict hygienic rules particularly around the patient and the machine goes a long way towards affording microbiological safety of HD fluids. Disinfection procedures need to be carried out regularly according to defined protocols, especially during changes of ultrafilters (described below). Many countries have established validation and maintenance processes to ensure consistent HD fluid quality throughout the entire fluid ‘tap to patient’ pathway [101, 102]. It must be emphasized that the highest possible dialysis fluid quality is recommended for conventional as well as convective HD modalities involving high permeability membranes [103].

Regular testing of water and fluid for bacterial or endotoxin contamination is now considered mandatory in most countries and an integral part of contemporary dialysis practices advocated by various guidelines. The two facets crucial for microbiological monitoring are firstly, use of appropriately sensitive assays that can detect low levels of LPS and, secondly, the need to meet recognized standards. The assay used most is the limulus amoebocyte lysate (LAL) test, either manual (gel-clot) or based on kinetic turbidimetric principles using chromogenic substrates. For either, it is crucial for proper sterile sample collection procedures to be followed and testing carried out rapidly (within the specified time interval) by laboratories well-versed with microbial testing methodologies [104].

Water, and dialysis fluids made from it, need to conform to quality standards established by different regulatory bodies to ensure patient safety [97, 105]. It is not the intent of this paper to compare the recommended limits of the various standardization authorities and the reader is referred to Ward *et al.* for the details of the guidance of different authorities [101]. Table 1 illustrates the limits cited and widely accepted in the more recent publications.

**Usage of special endotoxin-adsorbing filter systems.** The increased application of HD membranes with high permeability and convective treatment modalities led to technological innovations to negate the potential risk of backtransport of cytokine inducing LPS fragments. It is now customary for manufacturers to incorporate specially designed membrane filters that have the capacity to adsorb LPS onto extracorporeal circuit [106]. These ‘ultrafilters’ are usually installed at the back of each machine and final dialysis fluid (after mixing of acid concentrate, bicarbonate and water) pass through such filters before entering the dialyser.

The mechanism by which LPS adsorbs to the membrane surface of polysulfone-based ultrafilters has been described as hydrophobic–hydrophobic interactions [4]. Separation of LPS by hydrophobic interaction using a number of matrices is a common chromatographic approach used in several applications [107, 108]. These processes utilize the highly hydrophobic property of the lipid A part of LPS to interact with other hydrophobic molecules (Figure 5) [4, 109]. The hydrophobic domains of the synthetic polymers (e.g. polysulfone membranes) provide the binding sites for lipid A (responsible for the major bioactivity of endotoxins) containing bacterial products [68, 72, 91, 110].

**Protection from endotoxins by dialysers during each treatment session.** The same mechanism (hydrophobic–hydrophobic

**Table 1. Microbiological purity requirements for water and dialysis fluids, assessed either in terms of the endotoxin units or as bacterial colony forming units. A variety of standards and requirements are available in the literature; here some of the more recently published and accepted levels are shown for different fluid designations [105]**

	Water	Standard dialysate	Ultrapure dialysate <sup>a</sup>	Sterile dialysate
<b>Endotoxin levels, EU/mL<sup>b</sup></b>				
Canaud and Lertdumrongluk [97]	<0.25	<0.25	<0.03	<0.03
Ward et al. [101]	<0.25	<0.5	<0.03	Non-pyrogenic <sup>d</sup>
Bolasco [104]	<0.25	–	–	<0.01
<b>Bacterial limits, CFU/mL<sup>c</sup></b>				
Canaud and Lertdumrongluk [97]	<100–200	<100–200	<0.1	<10 <sup>-6</sup>
Ward et al. [101]	<100	<100	<0.1	Sterile <sup>e</sup>
Bolasco [104]	<100	–	–	0

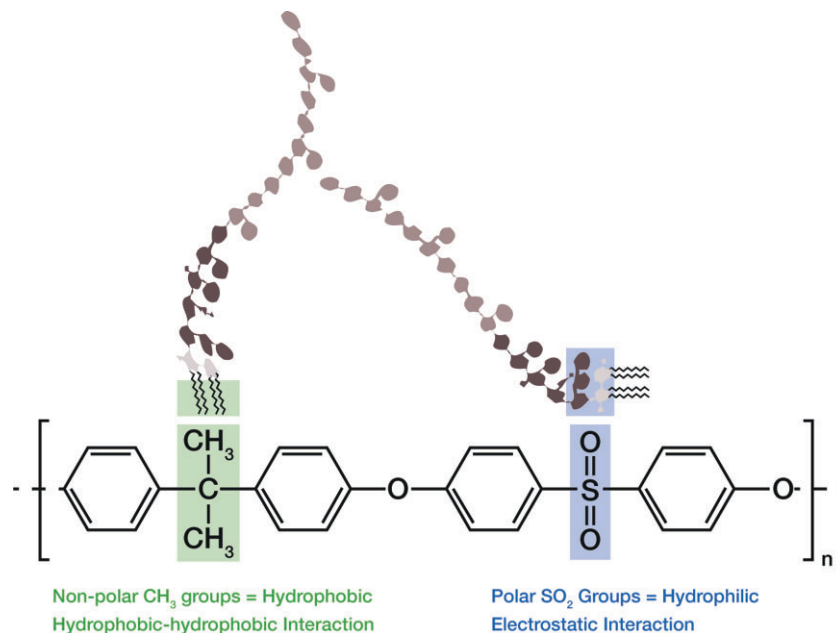
<sup>a</sup>Ultrapure dialysis fluid defined as containing <0.1 colony forming unit/mL (CFU/mL) using sensitive microbiological methods.

<sup>b</sup><0.03 endotoxins unit/mL (EU/mL) using the LAL assay [98].

<sup>c</sup>Threshold detection limit of LAL assay is 0.03 EU/mL.

<sup>d</sup>Microbiological technique-dependent (e.g. poor media TGEA, R2A, 17–23°C, 7 days).

<sup>e</sup>Defined as being ‘free from viable microorganisms’ and non-pyrogenic as ‘not eliciting a pyrogen [febrile] reaction’ [101].



**FIGURE 5:** The mechanism by which endotoxin fragments interact with certain regions of the membrane polymer. Hydrophobic regions (acyl chains) of the lipid A part of the LPS molecule adsorb to hydrophobic domains (–CH<sub>3</sub> groups) of the polysulfone membrane polymer, while electrostatic interactions occur between the –SO<sub>2</sub> groups of polysulfone and the sugar residues, core and O-antigen part of the lipid A molecule. Such interactions thus offer a degree of safety to the patient each time this type of dialysers are used for dialysis treatment. Further, such interactions have been used to develop special ultrafilters used in the dialysis fluid circuit as endotoxin adsorbers to assure high microbiological purity of dialysis fluids.

interaction) that enables LPS to be retained on the membrane surface of ultrafilters is also utilized to ensure further safety for the patient each time a highly-endotoxin adsorbing dialyser is used for the dialysis therapy. Although there are several polysulfone-based dialysers, not all offer the same degree of protection from endotoxins because of the variable hydrophobic–hydrophilic balance of each membrane during production processes. The unique polysulphone (hydrophobic polymer) and polyvinylpyrrolidone (hydrophilic copolymer) combination of different dialysis membranes results in membrane chemistries that are totally different. Thus, even for membranes from the same polymer family, highly variable endotoxin retention capacities are observed [106, 111–113].

## CONCLUSIONS

Systemic and persistent inflammation and malnutrition are prevalent in HD patients and are major contributors to CVD, which is rampant in patients with CKD and on dialysis therapy [114, 115]. Cardiovascular morbidity and mortality in patients with CKD are high, and the presence of CVD worsens outcomes of CKD patients [116]. The malnutrition–inflammation–atherosclerosis (MIA) axis, already present in advanced stages of kidney failure before the start of dialysis, can be amplified by several factors during dialysis, in both in PD and HD [117, 118]. Curtailing dialysis-related CVD, inflammation and malnutrition has been recognized as a focal point for the management of CKD [95, 119].



Uraemic toxicity is inextricably linked to increased inflammation, malnutrition and atherosclerosis [120]. Many of the uraemic toxins have been shown to play a significant role in multiple processes leading to uraemic atherogenesis [23, 121]. The compounds have been the subject of extensive experimental investigation, validation in clinical trials and reporting in the scientific literature [54, 122–126]. Uraemic toxins constitute the uraemia-related risk factors contributing to CVD and, together with traditional risk factors for CVD (including advanced age, male gender and smoking, as well as one or multiple comorbid conditions, e.g. hypertension, diabetes, dyslipidemia), increase further the cardiovascular burden of HD patients [113, 127]. Any further amplification of this burden of CVD by the HD procedure (by bioincompatibility, cytokine-inducing substances) makes a bad situation worse [115, 128].

Endotoxins from dialysis fluids with bacterial contamination contribute to one of the two main sources of inflammation from HD (the other being membrane bioincompatibility). We have also described the approaches to mitigate the potential threat of endotoxin permeability during dialysis therapy using specially designed, membrane-based and validated ultrafiltration systems that efficiently capture LPS fragments to prevent their entry into the blood. Good hygiene practices coupled with periodic testing of dialysis fluids for endotoxins and bacteria are prerequisites to the overall strategy of not adding to the inflammatory load dialysis patients already have [129]. A final line of defence from endotoxins during each dialysis session is offered by certain dialyser types containing membrane material that negates the effects of endotoxins by hydrophobic–hydrophobic adsorption [130].

Hypoalbuminemia, a powerful predictor of mortality in patients with chronic renal failure, is the result of the combined effects of inflammation and inadequate protein and caloric intake in patients or increased excretion (urinary albuminuria) [41, 42, 47, 127]. Inflammation and malnutrition both reduce albumin concentration by decreasing its rate of synthesis, while inflammation alone is associated with a greater fractional catabolic rate (FCR) and increased transfer of albumin out of the vascular compartment. Malnutrition may worsen patient outcome by aggravating existing inflammation, accelerating atherosclerosis. All mechanisms leading to hypoalbuminemia signify increased cardiovascular risk through increased vascular permeability and vascular dysfunction. Considering that hypoalbuminemia is now known to be associated with advanced fluid overload—achieving normovolemia is a major goal in contemporary dialysis and abnormalities in fluid status are associated with increased mortality—any exacerbation, however small, of the hypoalbumenic state is detrimental to the HD patient [46, 131].

The debate regarding the tolerable amount of albumin lost per treatment is highly contentious, instigated more by the interests of industry, which seeks to pursue acceptance of increased albumin loss to justify more porous membranes to eliminate increased levels of uraemic toxins. Hitherto, the consensus has been that a loss of 2–3 g/treatment may be tolerable and that an albumin loss ~2.5 g/treatment does not affect serum albumin levels [50]. Higher albumin losses (up to ~5 g/treatment) are justified for the removal of protein-bound uraemic toxins. However, recent evidence has shown that from the overall number of substances for which today there is evidence of toxicity, only a handful are bound to proteins and dialysis strategies are ineffective in lowering the concentrations of this class of uraemic toxins. Substantial quantities (6–12 g) in a session) of amino acids are lost during each HD session with standard membranes and more porous membranes (leaking up to 23 g albumin/treatment) could

be expected to cause the loss of clinically significant amounts of amino acids [3, 132]. The long-term repercussions of increased albumin (and amino acid) losses could be considerable, but as yet the acceptable upper limit of dialysis-related albumin is still unknown and remains to be determined [2, 57]. Whether the advantages of enhanced removal of larger uraemic toxins by highly permeable membranes or convective therapies outweigh the adverse effects of increased albumin loss on patient outcomes is far from being resolved [133]. Until the issue is settled through sound clinical investigation, increased albumin and simultaneous loss of amino acids has to be regarded as detrimental to the patient: the nutritional status is being compromised and amplification of the MIA syndrome presents a disadvantage to patient well-being [47].

The primary focus in this communication has been on the unwanted bidirectional transport of essential plasma components (albumin, peptides) from the blood into the dialysate and of pyrogenic substances in the opposite direction from the dialysis fluids into the bloodstream. There are however other compounds that may be removed during HD but are desirable to the patient at a given time. HD patients receive several medications to treat a variety of comorbid conditions or to combat or correct imbalances or perturbations created directly by uraemia or the dialysis procedure itself [134]. The daily pill burden (mean of 19) in dialysis patients is one of the highest in any chronic disease state and is a major contributing factor for the poor health-related quality of life [135]. Most of these pharmacological agents are small-sized molecules and in their free form easily cleared from blood during each HD therapy session, thereby decreasing their efficacy [136, 137]. In addition, the uncontrolled loss of medications during HD has economic implications considering that costs of pharmacological agents are a major factor contributing to the high overall costs of dialysis therapies [138]. Other than assessing the loss of heparin or antibiotics during HD, the undesirable consequences of loss of medications, particularly by the more efficient high flux or haemodiafiltration modalities, the issue has not been evaluated in detail [139]. Dialysis regimens with higher degree of safety and efficacy than is possible by empirical means only need to be devised [137, 139].

## SYNOPSIS

- (i) Bidirectional transport of solutes across semi-permeable membranes is inevitable in HD; in both directions, desirable and unwanted transport processes occur simultaneously during each HD session.
- (ii) The overall benefits acquired from striving to achieve efficient removal of uraemic toxins needs to be balanced by considering the equally efficient removal from blood of several (unknown) substances that are vital for diverse body functions.
- (iii) HD reduces the concentration of uraemic toxins that accumulate in blood in kidney failure, but the procedure may result in the undesirable entry from the dialysis fluid compartment into blood of endotoxins that have potent biological reactivity, e.g. inducing inflammation.
- (iv) Furthermore, the desired efficacy of several medications that are prescribed to HD patients may be compromised as, by virtue of their small size, they are easily removable during HD therapies.

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## CONFLICT OF INTEREST STATEMENT

S.K.B. received consultancy fees towards the writing of this article. He is a former employee of Fresenius Medical Care, Germany. F.K. is an employee of Fresenius Medical Care, Germany. M.N. is an employee of Fresenius Medical Care, Singapore.

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