Controversy

Sterile filtration of dialysate: is it really of no use?

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For many years, nephrologists accepted the idea that the small pore size of low-flux dialysis membranes prevented clinically relevant transfer of pyrogenic substances, such as endotoxins or bacteria. Even though some authors noted occasional febrile reactions on dialysis, the water quality was a neglected problem of haemodialysis treatment till the 1980s [1].

Dialysis water contamination

In 1972, Raij et al. reported the occurrence of circulating endotoxins in patients with febrile reactions at the end of dialysis sessions and, in parallel, high endotoxin levels in the dialysate [2]. Even nowadays, neither tap water nor purified water or dialysate are sterile. In more than 14 000 analysed water probes of city and village water systems in South-West Germany, 5.6% contained coliform bacteria and 3.6% contained Escherichia coli [3]. Recently, Arvanitidou et al. found E. coli in 12% of tap water samples in a study of all Greek dialysis centres [4].

Because of the more frequent use of high-flux dialysis membranes, the role of endotoxin and, in particular, endotoxin fragments in the water used for dialysis or the dialysate has been reassessed during recent years. In this context, the American Association for the Advancement of Medical Instrumentation (AAMI), and Swedish and German legislation have reduced acceptable endotoxin concentrations in pure water to the range of 0.25–1.0 EU/ml. Multicentre controls of dialysates in German dialysis centres showed that only 14–23% of samples contained less than 1.0 EU/ml, and concentrations between 100 EU/ml and > 400 EU/ml were found in about 10% of dialysate probes [5]. Similar results have been reported during the last decade, from various other countries by several authors [6–8].

Role of dialysis, machine and dialyser

In addition to bacterial contamination of water, most dialysis machines are continuously contaminated by Pseudomonas strains in the dialysate circuit. Furthermore, the temperature of 37°C within the machine enhances the growth of pathogens, including Pseudomonas, E. coli and Streptococcus. A third origin of bacterial contamination is the use of dialysate bicarbonate concentrates, in contrast to the relative protection guaranteed by acetate-concentrates [6].

Furthermore, the geometry of the dialysate may play a role. Many attempts have been made to increase the efficiency of the dialysis procedure. In this respect, the wall thickness of cellulosic membranes has been progressively reduced to minimize its resistance against low molecular solute passage. In comparison to the cellulosic membrane, a more homogeneous pore size could be achieved by synthetic membranes, which should reduce, at least theoretically, the risk of endotoxin transfer. However, the development of high-flux membranes again intensified the discussion about potential endotoxin transfer from the dialysate to the blood compartment during dialysis. According to the Hagen-Poiseuille law, flow resistance in hollow fibre dialysers increases if the luminal diameter is diminished or with increasing hollow fibre length. The increase of membrane permeability and flow resistance of hollow fibre dialysers enhances backfiltration, resulting in the transfer of several litres of dialysate into the blood compartment during a standard haemodialysis session. Since the molecular weight of many endotoxin fragments, including lipid A, is low, it has been suspected repeatedly that such compounds cross high-flux membranes during haemodialysis.

The potential transfer of pyrogenic substances across the membrane was investigated in several studies, in which different materials and methods were used, including differences in pyrogens, size and type of membrane, dialyser geometry, transmembrane pressure and flux, various solutions infused into the blood compartment, differing dialysate circuits, and multiple methods for the detection of pyrogens with variable sensitivities. As to the different endotoxins,
endotoxin fragments or exotoxins used, the procedures to produce such biogenic material were also variable.

**Evidence from in vitro studies**

Given the multiplicity and complexity of the methods used to address this issue, it is not surprising that results are difficult to compare, especially since most in vitro studies document the stimulation of only one or a few immune system reactions. Nevertheless, we should not neglect the available results but instead carefully consider their potential clinical relevance. Endotoxin and endotoxin fragment transfer across the dialysis membrane in vitro was found by several authors, using aqueous solution, in the presence or absence of up to 10% human albumin, different types of membrane and various detection methods, such as cytokine stimulation or LAL test [9–11]. Evans and Holms found a significant transfer of cytokines across the cellulose triacetate (CTA) dialyser when the dialysate was challenged with *E. coli* or *P. aeruginosa* but not with *Enterobacter cloacae* [12]. Dialysers with a modified CTA (lower hydraulic permeability), compared to the original CTA dialyser used by Evans and Holms, permit a lower passage of cytokine-inducing substances from *P. aeruginosa* contaminated dialysate compared to high-flux polysulphone membrane [13]. Different chemical composition of membranes may have influenced the endotoxin permeation, e.g. polysulphone dialysers from three different producers exhibited in vitro a very different endotoxin permeability. In the same experiment one membrane was nearly impermeable for *E. coli* endotoxin and another polysulphone membrane exhibited an approximately 1000-fold higher endotoxin permeability [11].

During in vitro dialysis with whole blood, 50 min as well as 3 h after having challenged the dialysate with a 1:100 dilution of *P. aeruginosa* filtrate, Pereira *et al.* [13] failed to detect IL-1γ production by circulating mononuclear cells harvested from blood compartment. Thus, Pereira *et al.* could not document a significant transfer of cytokine-inducing substances across CTA dialysers in whole blood experiments, using *P. aeruginosa* culture filtrate in the dialysate compartment. In contrast, Schindler *et al.* [14] observed the passage of IL-1β- and IL-1Ra-inducing substances across cuprophane but less across polysulphone or polyamide dialyzer membranes during in vitro dialysis with saline diluted whole blood and *P. aeruginosa*-contaminated dialysate. Differing flux and pressure conditions within the dialyser might have contributed to such discrepancies due to different intensities of backfiltration.

Summing up these in vitro studies, one cannot exclude the occurrence of a significant transfer of bacterial or endotoxin fragments across the dialysis membrane. This is the reason why we have to look carefully for any reactions in our patients which might be related to the lack of dialysate sterility.

**Evidence from clinical studies**

Several clinical observations indicate that bacterial and endotoxin contamination of the dialysate are relevant for haemodialysis patients. In this context, it is not correct to exclude or underestimate studies in which AAMI standards or other legislations are not fulfilled. Bacterial counts of 10^5 to 10^6 CFU/ml, as criticized by Nube, are just above the previous AAMI limit which allowed 2x10^5 CFU/ml bacteria in the dialysate. Similarly, endotoxin levels of 0.4 to 1.45 ng/ml, as reported by Olmer *et al.* [15], were not exceptional in the USA, Sweden or Germany [5–7]. The negative results obtained by Nube, Grooteman and their group do not exclude the risk of pyrogen transfer across dialysis membranes since they used dialysates with very low endotoxin concentration (0.05–1.13 EU/ml; mean 0.18 EU/ml). Filtration of their dialysate reduced endotoxin content to a median value of 0.06 EU/ml (range 0.05–0.19 EU/ml) [16]. Furthermore, in their study only seven of eleven patients have been dialysed with sterile filtered dialysate. In an earlier study of this group, mean endotoxin concentrations in the dialysate were <5 (<5–14.5) and 24.3 (<5–132) pg/ml [17]. The failure to observe a significant beneficial effect of sterile filtration under such conditions does not exclude a risk of endotoxin and bacterial contamination of the dialysate in general.

In contrast to the results by Nube *et al.*, the post/pre-ratio of intercellular IL-1Ra and IL-1β concentration of peripheral blood leucocytes increased significantly (P<0.02) in a multi-centre study during haemodiafiltration plus backfiltration, compared with haemodiafiltration without backfiltration [18]. In good agreement with these data, Quellhorst reported an increase of serum CRP during haemodialysis with non-sterile, filtered dialysate from 0.3 to 1.2 ng/ml, i.e. remaining within the normal range [19]. However, when these patients were dialysed with sterile filtered dialysate for three and six months, C-reactive protein (CRP) increased only from 0.3 to 0.4 ng/ml, i.e. a difference that was no more significant [19]. In the same in vivo study, when patients were switched to sterile filtered dialysate, pre-dialysis β2m serum levels decreased by a further 13%. Even though the serum β2m levels are not significantly correlated with β2m amyloidosis, it is remarkable that Baz *et al.* [20] reported a much lower incidence of carpal tunnel syndrome in patients who dialysed with sterile filtered dialysate than in patients with no sterile filtration. Similar results have been reported by Koda *et al.* [21]. Although no prospective controlled study is available, the data published by Kleophas *et al.* [22] support these observations. Using the Genius system with a highly sterile dialysate, these authors reported an incidence of the carpal tunnel syndrome which was comparable to the patients in Berland’s group who had been treated with sterile filtered dialysate.

In all annual national surveillances of dialysis associated diseases in the USA during 1990–1995, both dialyser reuse and high-flux dialyser use were
associated with pyrogenic reactions after adjustment for confounding factors [23].

A potential transfer of pyrogenic substances from the dialysate during routine dialysis has been suggested by Pegues et al. [6] also. When standard dialysors or high-efficiency dialysers were used, sterile filtration did not reduce the incidence of pyrogenic reactions. However, when patients were dialysed with high-flux membranes the incidence of pyrogenic reactions decreased from 1.2/1000 dialysis sessions using non-filtered dialysate to 0.4/1000 dialysis sessions using sterile filtered dialysate.

Conclusions

Even though the immune system of our patients is stimulated repeatedly during each dialysis session by a variety of factors, the role of endotoxin and bacterial contamination of the dialysate must not be neglected. Several clinical reports suggest that the bacteriological contamination of the dialysate must not be neglected. A variety of factors, the role of endotoxin and bacterial stimulation repeatedly during each dialysis session by a high-flux membrane. However, when patients were dialysed with high-flux membranes the incidence of pyrogenic reactions decreased from 1.2/1000 dialysis sessions using non-filtered dialysate to 0.4/1000 dialysis sessions using sterile filtered dialysate.

It is true that dialysis patients in general survive numerous toxic influences and, we hope, will continue to survive well despite the continuous exposure to such toxins. Most of the side effects caused by the dialysis procedure and their potential clinical significance have been difficult to define. Many of these have only been understood recently, such as the numerous reactions originating from contact with foreign material, complement activation, cytokine stimulation, oxidative stress, and worsening of β2m amyloidosis, as well as ethylene oxide hypersensitivity and silicon particle storage in various organs. Immune stimulation by foreign material is a continuing and complex problem in haemodialysis. In this context, bacteriological or endotoxin contamination of the dialysate does play a role. Filtered dialysate would at least not increase, but decrease the risk.

References


18. Special Issue of the JASN on dialysate and dialysis fluid. JASN 1997; 8: 1745–1754

Editor’s note
See also Controversy by Nubø et al. (pp. 1986–1991).