Assessment of the quality of dialysate

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Key words: dialysate; on-line haemodiafiltration; bacteria; pyrogens

Introduction

According to the latest National Surveillance of dialysis-associated diseases in the US, more than one pyrogenic reaction in the absence of septicaemia was reported by 22% of 2449 dialysis centres in 1994 [1]. Centres reporting pyrogenic reactions in the absence of septicaemia were also more likely to report dialysis-related sepsis (36% of 528 centres with pyrogenic reactions; 10% of 1918 centres without pyrogenic reactions) [1]. These numbers have not decreased since 1989. The occurrence of pyrogenic reactions was associated with the practice of re-use of dialysers as well as with the use of high-flux dialysers [1]. However, when re-use is not practised, pyrogenic reactions still occur in 13% of dialysis centres using high-flux dialysers and in 15% of centres using only low-flux dialysers [2]. If re-use is not practised, the most plausible explanation for dialysis-related pyrogenic reactions is bacterial contamination of the dialysate combined with the penetration of pyrogenic bacteria-derived substances across intact dialyser membranes. Indeed, several in vitro studies provide evidence that low-flux as well as high-flux dialyser membranes are permeable to pyrogens derived from contaminated dialysate [3]. The appearance of pyrogenic substances in the patients' blood depends on the degree of dialysate contamination, the composition of bacterial products in the dialysate and the ability of dialyser membranes to prevent pyrogen penetration by adsorption [4]. A recent in vivo study demonstrated that even low grade contamination of dialysate (> 200 c.f.u./ml) compared with ultrapure dialysate (0 c.f.u./ml) is associated with increased cytokine production in chronic haemodialysis patients [5]. These data support the in vitro studies demonstrating pyrogen permeability of dialyser membranes.

Determination of bacterial growth in dialysate

There are standards for the bacteriological quality of water and dialysate used in haemodialysis. According to the recommendations of the American Association of Medical Instrumentation (AAMI), bacterial growth should not exceed 200 c.f.u./ml in water and 2000 c.f.u./ml in dialysate. Several multi-centre studies in the US as well as in Europe demonstrated, that 17.8–35.3% of water samples and 11.7–19% of dialysate samples compared with ultrapure dialysate (0 c.f.u./ml) are not compliant with the AAMI standards [7,8]. According to these data, bacterial contamination of water and dialysate is an important problem in today's haemodialysis therapy, and Gram-negative microorganisms such as Pseudomonas species are predominant. Quantitative determination of bacterial growth in water and dialysate samples is influenced by the agar and the culture conditions used [9]. Unfortunately, there are no...
world-wide accepted and recommended guidelines concerning time and frequency of water and dialysate sampling, or culture agars and culture conditions to test for bacterial growth.

In our opinion, sampling and culture conditions need to be standardized in order to compare bacterial contamination within the same unit as well as between different centres. It is known that microorganisms which are able to grow in salt-rich but nutrition-poor dialysate grow better on nutrition-poor agars (e.g. tryptone glucose extract = TGE agar; or R2A agar) at room temperature during prolonged incubation times of up to 4–6 days [9]. We suggest the use of TGE agars. The agar has to be dissolved in pyrogen-free water to a concentration of 24 g/l, and should be autoclaved. After cooling down to ~60°C, the agar can be poured into pyrogen-free Petri dishes. Condensed water should be evaporated under a laminar flow hood to prevent accidental contamination of the agar plates. Covered agar plates can be stored at 4°C and should be used within 3 weeks.

We propose use of the sampling procedure outlined in Figure 1. Because bacteria may grow in the dialysate compartment of the dialyser during haemodialysis, dialysate samples should be taken at the end of a dialysis session. To take the sample, the dialysate line from the dialysate outlet port of the dialyser should be disconnected under sterile conditions. From the free-flowing dialysate, a 20 ml sample should be taken using a pyrogen-free syringe with a 19 gauge needle without touching the housing of the dialyser. The dialysate sample can be diluted in pyrogen-free water (1:5, 1:10, 1:100) using a laminar flow hood, and 1 ml aliquots of the undiluted and diluted dialysate samples should be added to agar plates. Petri dishes should be covered and incubated at room temperature for 1 h in an upright position to allow the sample to become adsorbed by the agar. The dishes should then be turned upside down and incubation be continued for 4–6 days at room temperature. Following incubation, bacterial colonies should be counted and bacterial growth expressed in colony-forming units per millilitre (c.f.u./ml). All this work can be done in the dialysis unit. In addition, agar plates can be passed to a microbiologist in order to identify outgrown microorganisms.

**Determination of pyrogens in dialysate**

Bicarbonate dialysate is contaminated by Gram-negative bacteria, and *Pseudomonas* species are predominant [7,8]. These microorganisms release several cell wall components such as endotoxins or lipopolysaccharide (LPS) and peptidoglycans, as well as actively secreted proteins such as exotoxin A. These substances vary with regard to molecular weight, release of small molecular size fragments and toxicity. Only LPS can be measured by a specific and quantitative assay (LAL assay). Some LPS fragments and all other bacteria-derived substances are negative in the LAL assay but biologically active in that they stimulate circulating PBMCs to produce cytokines such as interleukin-1 (IL-1) and tumour necrosis factor-α (TNF-α). Proinflammatory cytokines express multiple biological activities including the induction of fever, cardiovascular instability, the elevation of acute-phase proteins and the mediation of chronic inflammatory processes. PBMCs can be isolated from donor blood and used as an in vitro assay for the detection of cytokine-inducing bacterial products. The induced cytokines should be measured in PBMC culture supernatants by specific and quantitative immunoassays. Recent studies showed that incubation of whole blood instead of separated PBMCs may provide an easy and sensitive system to detect cytokine-inducing substances in contaminated dialysate [6].

The method is described in Figure 2. Dialysate samples from the dialysate outlet port of the dialyser at the end of HD session should be disconnected under sterile conditions. From the free-flowing dialysate, a 20 ml sample should be taken using a pyrogen-free syringe with a 19 gauge needle without touching the housing of the dialyser. The dialysate sample can be diluted in pyrogen-free water (1:5, 1:10, 1:100) using a laminar flow hood, and 1 ml aliquots of the undiluted and diluted dialysate samples should be added to agar plates. Petri dishes should be covered and incubated at room temperature for 1 h in an upright position to allow the sample to become adsorbed by the agar. The dishes should then be turned upside down and incubation be continued for 4–6 days at room temperature. Following incubation, bacterial colonies should be counted and bacterial growth expressed in colony-forming units per millilitre (c.f.u./ml). All this work can be done in the dialysis unit. In addition, agar plates can be passed to a microbiologist in order to identify outgrown microorganisms.

**Fig. 1.** Determination of bacterial growth in dialysate.
samples should be taken following the protocol described above. The samples should be filtered through 0.45 μm filters to remove microorganisms. One ml of the filtered sample should be added to 1 ml of human donor blood in pyrogen-free polypropylene tubes. Incubation should be performed for 18 h at 37°C. After incubation, tubes should be centrifuged and whole blood culture supernatants separated to measure cytokines such as IL-1β, IL-1Ra or TNF-α by specific immunoassays. There is a donor-dependent difference in the sensitivity of the whole blood assay. In general, endotoxin concentrations as low as 10–50 pg/ml induce significant amounts of cytokines in whole blood.

We used the whole blood assay to measure the IL-1β-inducing activity in bicarbonate dialysate samples (Figure 3). Dialysate samples were taken from the dialysate outlet port of the dialyser at the end of a 4 h haemodialysis session. Figure 3 compares the bacterial growth on TGE agar during a 5 day culture at room temperature and the IL-1β-inducing activity in whole donor blood of 12 independent samples. All samples were tested on the same donor blood. IL-1β concentrations in control incubations of whole blood with pyrogen-free saline were at the detection limit of the radioimmunoassay. Although bacterial growth in the dialysate samples was low (3–125 c.f.u./ml), there was significant IL-1β-inducing activity (twice the background of 20 pg/ml in control incubations) in eight out of 12 samples. According to these data, the whole blood assay is a sensitive method to detect cytokine-inducing activity even in moderately contaminated dialysate.

There is no correlation between bacterial growth and cytokine-inducing activity in the whole blood incubation. These data confirm findings from previous studies which did not find a correlation between bacterial contamination and endotoxin concentrations measured by the LAL assay in dialysate [7,8]. For example, high bacterial growth can be associated with low endotoxin concentrations and cytokine-inducing activity. On the other hand, because LPS is released from debris of microorganisms, low c.f.u./ml are often associated with high endotoxin values. In contrast to
cell debris, growing bacteria may release cytokine-inducing as well as cytokine-suppressing bacterial products. The net pyrogenic effect of contaminated dialysate may be best described in the whole blood cytokine-inducing assay. We therefore suggest using the incubation of whole donor blood to test the pyrogenic activity of dialysate in addition to the culture of dialysate samples under optimized conditions to determine bacterial growth, and the measurement of LPS by the LAL assay.

**Conclusions and recommendations**

It is generally accepted that intact dialyser membranes are permeable to cytokine-inducing, pyrogenic bacterial products derived from contaminated dialysate under *in vitro* dialysis conditions using highly contaminated dialysate [3]. There are also recent *in vivo* data demonstrating that even moderately contaminated dialysate (<200 c.f.u./ml) clearly within the recommended AAMI standards is associated with increased cytokine production in haemodialysis patients [5]. Dialysate-dependent activation of cytokines may contribute to chronic inflammatory processes such as fibrosis, protein catabolism, β2-microglobulin amyloidosis or cardiovascular diseases seen in these patients. Our data support the concept that the use of ultrapure dialysate prevents dialysis-dependent cytokine induction. Further studies are necessary to prove that prevention of cytokine induction is beneficial with respect to progression of chronic inflammatory processes associated with long-term haemodialysis therapy.

We recommend more frequent monitoring of the dialysate quality (at least twice a month) using improved culture conditions to determine bacterial growth. In addition, a cytokine induction assay, for example the whole donor blood incubation, could be performed to measure all cytokine-inducing pyrogens in dialysate samples. In order to reduce the risk of haemodialysis-related pyrogenic reactions and dialysis-dependent cytokine induction, the dialysate quality should be improved to very pure (<100 c.f.u./ml) standards. In the case of haemodiafiltration with on-line production of infusion fluid, ultrapure, i.e. sterile and pyrogen-free, dialysate has to be obligatory. This high quality of dialysate can be achieved by ultrafiltration through high-flux membranes with high pyrogen-adsorbing capacity with a sufficiently high margin of safety.

**References**