Why and how to monitor bacterial contamination of dialysate?

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Introduction

The microbiological contamination of water concentrates and dialysate fluid is a problem which dates back to the distant past, but has recently gained again great actuality. Published data document that the bacteriological quality of the dialysate fluid plays a role in the ‘biocompatibility’ of haemodialysis (HD) [1,2]. Endotoxins in the dialysate fluid may enter the blood compartment and potently activate monocytes to produce pro-inflammatory cytokines [2]. Increased production of cytokines, mainly of IL-1, IL-6 and TNF-alpha, is thought to be causally related to several
distinct acute and chronic problems of HD patients (Table 1) [3–5]. These cytokines trigger a series of acute phase reactants, and consequently, these patients exhibit a state of chronic microinflammation (Table 1) [6,7]. Some studies have found a relation between these acute phase reactants and mortality of patients on HD [6].

The above situation is potentially aggravated by the fact that currently popular HD modalities, e.g. use of bicarbonate dialysis and high flux dialysers, predispose to bacterial contamination of the dialysate fluid and to transfer of endotoxin from dialysis fluid to blood causing more frequent pyrogen reactions [3,8, 9]. Further factors which increase the risk comprise re-utilization of dialysers and centralized dialysate production, specifically production of bicarbonate-containing dialysate [3,5].

**Factors influencing the risk of microbiological contamination of dialysis fluid**

Water used for HD is frequently contaminated by microbes [10–14]. The risk is less when modern methods of water treatment are used, i.e. double reverse osmosis or deionizer and reverse osmosis [14]. Contamination of treated water is more frequently found in haemodialysis centres and water treatment systems which had been built some years ago [12,14].

The following parts in the water treatment system are most prone to bacterial infection:

(i) the parts downstream from an activated carbon filter which eliminates the chlorine,
(ii) all types of resin and porous elements (softeners, resinous deionizers), filters made of activated carbon and other filters,
(iii) storage and sedimentation tanks
(iv) segments in the water distribution system where water is stagnant [6,11].

Microbiological contamination can be avoided if certain elements of the water treatment system are improved. The distribution system could be made from tubes of inert material, ideally of stainless steel. However, because of its low cost, PVC is usually used. High grade stainless steel is the only material resistant to biological corrosion. In the design of the system dead spaces should be avoided, as these predispose to bacterial growth and biofilm formation which are difficult to eliminate. The tubes distributing water from a circulating system to the machines should be as short as possible. The lumen of the tubes should be as small as possible. This will guarantee a high velocity of circulation without excessive decrease in the driving head of pressure. Continuous electrical self-regenerating deionizers are relatively safe with respect to bacterial contamination. Whenever possible, it is preferable to avoid a storage tank. If a tank is used at all, water should enter from above as in a shower, it should wet the anterior surface in its entirety and it should exit from the inferior part. One must avoid that residues remain and that certain parts are not moistened by water. The storage tank should be opaque to avoid the growth of algae [15]. Water for haemodialysis should have a specific resistivity \( \geq 1 \text{ M}\Omega/\text{cm} \) at 25°C or its equivalent \( \leq 1 \mu\text{S} \) of conductivity.

Several systems have been developed to eliminate, or at least diminish, bacterial contamination of treated water; (i) infusion of chlorine after the activated carbon filter and prior to reverse osmosis, (ii) submicronic filters with pores of less than 0.1 \( \mu \text{m} \) diameter, (iii) ultraviolet irradiation, (iv) disinfection with ozone, (v) pressurized circuits able to withstand sterilization in an autoclave. However, none of these preceding measures can currently replace either periodic preemptive disinfection of the water treatment system or disinfection when bacterial contamination is detected [15].

Concentrates with low pH and high solute concentration are not easily contaminated with microorganisms. However, bicarbonate-containing concentrates and bicarbonate in powder form are frequently contaminated. The degree of contamination is not equal in the different commercial concentrates it depends to a high degree on the type of the preparation and the way it is handled [8]. Some bicarbonate concentrates have been treated by gamma irradiation. The circuit within the HD machine is very susceptible to contamination and predisposes to formation of bacterial biofilms. The major factors which render the dialysis fluid within the dialysis machine susceptible to bacterial contamination include: (i) its electrolyte and glucose content, (ii) the temperature of the dialysate and (iii) the presence of stagnant zones in the circuit of certain machines. Machines with continuous flow constitute a great advance over those with closed circuits. New machines have incorporated several recommended features: (i) low volume of hydraulic circuit, (ii) high dialysate rates, (iii) global disinfection or autolavage and (iv) non stagnant zones. The above considerations justify frequent disinfection of the dialysis machines. Usually there is no correlation between the colony count in cultured samples taken from (i) the water

<table>
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<th>Table 1. Effects of the activation of proinflammatory cytokines</th>
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<td><strong>Short term</strong></td>
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<td>Pyrogen reaction: fever, chills, nausea, vomiting, hypotension,</td>
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<tr>
<td>headache, myalgia</td>
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<td>Postdialysis syndrome</td>
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<td><strong>Long term</strong></td>
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<td>Impairment of the immune response</td>
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<td>Dialysis-associated ( \beta )-amyloidosis?</td>
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<td>Diminished response to erythropoietin</td>
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<td>Atherosclerosis</td>
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<td>Reduced muscular force</td>
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<td>Bone loss?</td>
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<td>Higher mortality?</td>
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<td><strong>Acute phase reactions</strong></td>
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<tr>
<td>Positive correlation: C-reactive protein, serum amyloid, fibrinogen</td>
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<tr>
<td><strong>Negative correlation:</strong> albumin, prealbumin, transferrin</td>
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supply, (ii) the bicarbonate concentrate or (iii) the dialysate fluid. This finding as well as the variability of the species identified in the three types of samples suggest that there is an environmental source of contamination [8]. Bacterial contamination may be the result of inadequate aseptic technique in the operation of the machine or the presence of disinfection-resistant biofilms [16].

On the other hand, if the dialyser membrane is tight, bacteria are not able to pass from the dialysate compartment into the blood compartment. However, endotoxins, i.e. biologically active bacterial products, can permeate. Such microbiological products are produced wherever sites of bacterial contamination exist. Furthermore, these substances are not inactivated with the routine disinfection procedures, and concentrations increase further upon lysis of bacteria. Endotoxins are bacterial products able to activate monocytes. They may permeate into the blood via back-filtration and, in the case of low molecular weight products via back-diffusion. Filtration of the dialysate with endotoxin absorbing membranes, such as polysulfone or polyamide membranes, are useful to deal with this problem [2,11].

**Relationship between bacterial contamination of the dialysate, endotoxin and cytokines**

In the blood compartment endotoxins are able to activate monocytes and induce the formation of cytokines. Monocyte activation is not a straight-forward process, but is determined by several factors which increase or decrease the production of cytokines. This multifactorial process is influenced by (i) quantity and type of endotoxin, (ii) type of membrane, (iii) plasma factors and (iv) the state of alternate systems causing monocyte activation and inactivation [9]. The presence of proteins or whole blood is a factor which potentiates activation of monocytes. At least two proteins are known to be necessary, one of which is a transporter protein and the other a permeation factor [17]. Additional stimuli or signals such as complement activation are very important for cytokine production by monocytes. To complicate matters further, some counter-regulatory cytokines, such as IL-10, also play a role [18]. Consequently, one should not be surprised that the state of nutrition as well as the reactivity of the immune system are of considerable importance. Some endotoxins are able to induce formation of IL-1 at concentrations of no more than 0.05 ng/ml.

**How can one adequately handle this problem?**

Despite a clear causal relation, there is little statistical relationship between (i) the average degree of bacterial contamination in terms of colony-forming units per ml (CFU/ml), (ii) the concentrations of endotoxin detectable by the Limulus amoebocyte lysate (LAL) test and (iii) the production of cytokines by monocytes of patients exposed to such dialysate [3,13, 14]. Bacterial contamination of the dialysate is the cause of this phenomenon. The endotoxins are the contaminants which are transferred to the patient, but what we are most concerned about and what we try to avoid is monocyte activation. Which controls are necessary and which treatment strategies are advisable? From a practical point of view the clinician has the obligation to control bacterial contamination of the dialysate in order to avoid the entire pathogenetic sequence. This does not imply that it is not important to control other aspects of this pathogenetic sequence as well including endotoxins, cytokines, acute phase reactants, and clinical signs and symptoms, e.g. pyrogenic reactions (Table 1).

Sampling of water for bacteria should be performed on a weekly basis. If this is not feasible, it should be done at least on a monthly basis. A very basic issue is how and when to take bacteriological samples and how to handle them. One should aim at maximal sensitivity. To this end it is necessary to take large volumes with meticulous sampling techniques and good conditions of transportation. The samples must be processed immediately using culture media which are poor in nutrients. They must be incubated at environmental temperatures and at 38°C and for prolonged periods [3,8,11]. Some microbes are perfectly adapted to such hostile media as water and dialysis fluid which contain hardly any nutrients. Rapid growth under nutrient-poor conditions is characteristic for this type of microbes as is their capacity to colonize stagnant flow areas. The analysis of these peculiar microbes must take these properties into account. The type of culture medium and the conditions of incubation have considerable influence on the results [8,19]. The best results are obtained with R2A (Reasoner’s 2-agar) or SMA (Standard Methods agar) for water cultures and R2A or TSA (Trypticase soya agar) for culturing dialysate [8,19]. Incubation at both 22°C for water and 37°C for dialysate is necessary to obtain maximum recovery of bacteria. Others have proposed the use of TGS-agar (Tryptone glucose extract agar) [3].

Water for bacteriological analysis should be sampled at distinct points in the water distribution system: (i) main water supply, (ii) water distal to the decalcification column, (iii) water distal to the activated charcoal cartridge, (iv) water distal to the reverse osmosis, (v) water of some of the lines supplying the dialysing machines, selected on a rotating basis, and (vi) samples of the dialysis fluid proximal to the dialyser taken from all dialysis machines. The samples must be collected meticulously: a site of direct access to the circuit must be selected; the site from where the sample is taken must be disinfected. The sample must be taken while water is running freely and after the first millilitre has been rejected. The volume should be at least 1 ml. It should be collected in a recipient vessel which is sterile and free of pyrogens. The sample should be kept at 4°C and be cultured within less than 24 h. The culture plates should be read at 48–72 h and after 5–7 days.
Bacterial growth in the cultures of samples taken at different points and times can give quite variable results. Bacterial contamination is a biological (and not a physical) process which is subject to great variability. In view of this it has been suggested that endotoxin testing, specifically of the dialysis fluid, should be part of the regular quality control in dialysis. Endotoxin determination of dialysis fluid is obligatory in some countries and is recommended by the European Pharmacopoeia [20].

Currently different methods for detection of endotoxins are available allowing the demonstration of endotoxin passage through dialysis membranes. These methods comprise: (i) the LAL test, (ii) cytokine production by monocytes, (iii) activation of neutrophils, (iv) isotopic labelling, (v) determination of antibodies against endotoxin, and (vi) the silk-worm larva test. The most frequently used method is the LAL test. Two types of LAL-assay-kits are available: the simple gel-clot method and the chromogenic test. The latter is most specific, sensitive and a quantitative method. The lipopolysaccharides (LPS) are the best known endotoxin. They are pyrogenic and can be detected using this methodology. Nevertheless one should not forget that endotoxins which are detectable by LAL assay represent only part of the pyrogenic products. They are the ones with the greatest molecular weight. LAL-negative pyrogens may be of particular importance, because pyrogenic reactions during hemodialysis correlate with the amount of bacterial growth in the dialysis fluid, but not necessarily with endotoxin levels as detected by the LAL test [1]. Although the LAL assay is not adequate to monitor all dialysis fluid pyrogens, it is the most reliable test available.

The most useful method for determination of endotoxins in HD would be the measurement of the biological effects in patients monitoring cytokine production by monocytes [9]. This method, however, is laborious, expensive and not widely accessible. A simpler method has been proposed to monitor the biological effects of endotoxins [3]. Acute phase reactants in plasma, basically C-reactive protein (CRP), are tightly related to the concentration of IL-6 and to complications in dialysis patients as listed in Table 1. Possibly in the future these measurements will be monitored routinely, but currently its usefulness has not been documented.

Acute symptoms and signs, i.e. pyrogenic reactions, are infrequent and other manifestations may easily be confounded with several acute complications of HD (see Table 1). Nevertheless one should stay alert in order not to miss and misinterpret them.

Necessity to standardize control measures for detection of bacterial contamination of the dialysate

The existence of different norms of controls indicates that available evidence is limited that the type of analysis and selection of sampling sites determine outcome. Ideally intervention should start before manifesterations of overt bacterial contamination occur, i.e. at 100 CFU/ml. To this end one should look for the source of contamination and this source must include some of the systems installed to reduce bacterial contamination as mentioned above. In this case one should go ahead and disinfect. The norm that necessitates corrective action is 100 CFU/ml in the water and 1000 CFU/ml in the dialysis fluid [20]. The LAL test detects cases of endotoxin contamination with negative bacteriologcal results. The alarm level in calling for corrective action should be less than 0.5 U/ml. We believe current methods to determine cytokine production by monocytes of patients are not sufficiently standardized. When pyrogenic reactions occur immediate search for bacterial contamination endotoxins is indicated. There is a need for complete guidelines regarding dialysate fluid quality. Standardization of these parameters in different countries, at least in Europe, would allow validation of the results at an international level [8].

As a final comment, disinfection of the water supply system and of the monitors should be carried out with products that are not only disinfectants, but also detergents which are able to remove incrustations. In the majority of cases, more than one product is necessary or one must use one of the commercially available mixtures, always keeping in mind that they must reach sufficient concentrations and for the sufficient time, and that they may cause corrosion. Their complete elimination must be verified by appropriate analysis [15].

Conclusions

The contamination of dialysate by bacteria and pyrogens is currently a serious problem of HD. Ideally a dialysis fluid of pharmacological quality should be used, similar to that of infusion fluids. For the time being, it must be assured that the dialysis fluid is free of bacterial contamination, so as to guarantee the absence of endotoxin. A good and modern water treatment plant is indispensable as are concentrates of high quality and machines which are easy to disinfect and equipped with filters for sterile filtration of dialysis fluid. Periodic disinfection of all the elements in the water circuit is necessary to maintain this level of quality. There is need for complete guidelines regarding dialysate fluid quality.

Acknowledgements. We thank Professor E. Ritz for translating the text from Spanish to English.

References
