Microbiological survey of dialysate: Vantage of use of sterile bag concentrate

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Summary

Haemodialysed patients are exposed to nearly 400 litres of dialysis water weekly. The bacterial contamination of treated dialysate and water induces acute pyrogenic reactions or chronic damage and cytokine activation.

The aim of this study was to value the microbiological parameters of dialysis water and dialysate of our monitors by bacterial culture (measured as colony forming units [CFU]) of water samples at 37 °C after 48 hours, at 22 °C after 72 hours and after seven days, and by measuring endotoxin levels (endotoxin units [EU]).

In our centre, there are 16 monitors (6 monitors use sterile dialysate fluid and 10 monitors use non sterile dialysate fluid). The chemicals used for disinfection are chlorine and paracetic acid. Water samples were taken under sterile procedures every three months for a year.

No bacteria were found in the samples of water of the dialysis ring; EU were lower than the limit value of 0.25 EU/ml fixed by the European Pharmacopoeia.

The concentration of CFU and EU of the dialysate, taken from monitors with a sterile bag, were lower than those of other monitors ($p < 0.05$ t Student test). However, the levels of CFU/ml and EU/ml of dialysate samples, taken from monitors with a non-sterile bag, were lower than the guideline value of the European Pharmacopoeia (v.n. CFU < 50 CFU/ml and EU < 0,05 EU/ml).

Frequent examination of CFU and EU is essential to reduce the damage caused by the use of contaminated water, therefore the goal of future dialytic techniques will be the use of "sterile dialysate".

Key words

- Dialysis water
- Endotoxin units
- Colony forming units

HAEMODIALYSED PATIENTS are exposed to nearly 400 litres of dialysis water weekly. Therefore, the microbiological quality of dialysis water is very important for the biocompatibility of the haemodialytic treatment. The bacterial contamination of treated water and dialysate causes acute pyrogenic reactions or chronic damage and cytokine activation (1, 2).

Several in vitro studies show that intact dialyser membranes are permeable to pyrogenic bacterial substances derived from contaminated dialysate. The size of the pores of the dialyser membrane is less important than the thickness and the capacity of the membrane material to absorb bacterial products (3, 4). The cellulose low-flux membranes do not have absorption capacity and are more permeable to bacterial products than synthetic high-flux membranes (5). On the contrary, synthetic membranes present high absorption capacity due to hydrophobic interactions but have a more elevated risk of pyrogen transfer due to back-filtration; furthermore, the absorption capacity is exhausted if the dialysate is heavily contaminated (6, 7). Bacterial products transferred across the membrane may produce an acute pyrogenic reaction (fever, nausea, vomiting, hypotension, shivering) (1). In the absence of acute clinical episodes, small amounts of pyrogenic substances can activate circulating lympho-monocyte cells, which produce cytokines such as IL-1β and TNFa. These cytokines can cause acute effects: induction of acute-phase proteins, suppression of appetite and sleepiness (8). Moreover, chronic stimulation of cytokines causes more serious complications such as 2microglobulin amyloidosis, hypercatabolism, malnutrition, immunodepression and endothelial damage (9). Activation of endothelial cells by cytokines can produce a down-regulation of nitric oxide and cycloxygenase-I syntheses of endothelial cells inducing vasospasm, thrombosis and athermatous plaque formation (10).

Aim of study

The aim of our study was to value the microbiological parameters of dialysis water and dialysate of our 16 monitors. We used the dialysate concentrate of 10 monitors in plastic tanks, sterile at the beginning, but contaminated by air after opening (conventional concentrate); while in the other 6 monitors the dialysate concentrate was contained in sterile bags sealed during the haemodialytic treatment (sterile concentrate).

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Materials and methods

We performed physical, chemical and microbiological analysis of the raw water according to European standards.

The disinfection procedures were performed in our dialysis unit using the chemicals paracetic acid and chlorine. The disinfection intervals were daily after each haemodialysis. All the dialysis monitors had an ultrafilter at the treated water inflow, and only one of these was equipped with an ultrafilter at the dialysate outflow.

The dialysate samples for CFU and EU concentrations were taken at the water outlets of the dialysis monitor during "water flushing mode" immediately before the dialytic treatment. All water samples were obtained after rising and disinfecting the taps.

The bacterial culture of dialysate samples was performed every three months for one year and the amount of EU were noted (four samples for each monitor and four samples for the dialysis water ring).

The bacteriological analysis was performed by plate count with 1 ml and 0.1 ml samples; the incubation times were 72 h and seven days at 22 °C, and 48 h at 37 °C (Bioburden Vitek Card Biomerieux) (n.v. CFU/ml < 50 in dialysate water).

The concentration of endotoxins was measured using a quantitative chromogenic limulus amoebocite lysate assay (LAL test biologik -A- Italy). The test system is calibrated in endotoxin units (EU/ml) and its sensibility was 0.003 EU/ml.

The results were expressed as the means ± SD (Table 1).

Table 1

	CFU/ml	LAL EU/ml
DIALYSATE-Monitors with sterile bags	1.46 ± 0.94	0.08 ± 0.05*
DIALYSATE-Monitors with non sterile bags	4.76 ± 7.53	0.45 ± 0.42*
WATER of the ring	2	0.14

* $p < 0.05$ EU non-sterile vs. sterile bag (t Student test)

Results

No bacteria were found in the dialysis water ring; the EU concentration was lower than the limit value of 0.25 EU/ml according to the European Pharmacopoeia.

The CFU and the EU of the dialysate taken from monitors with sterile bags, were lower than the concentrations of other monitors ($p < 0.05$ t Student test). However, the media levels of CFU/ml and EU/ml of dialysate samples taken from monitors with conventional concentrate, were lower than the guideline value of the European Pharmacopoeia (v.n. CFU < 50 CFU/ml and EU < 0.05 EU/ml for dialysate water).

Discussion and conclusion

Several studies have shown the transmembrane crossing of bacterial substances from dialysate to blood compartment either using cellulose low-flux membranes or synthetic high-flux membranes (4, 5). Many authors have demonstrated acute pyrogenic reactions and chronic effects when using contaminated dialysate: the bacterial count and the LAL assay performed in water and dialysate are important to avoid risk due to bacterial contamination in the dialysis water (11-13).

The water used for dialysis in our centre was not polluted; whereas the CFU/ml and EU/ml concentration were lower in dialysate taken from monitors with sterile bags. There is a minor risk of acute reaction or chronic damage using these monitors.

Therefore, the difference of cost between the "sterile bag concentrate" and "conventional concentrate" isn't very significant (Lit. 18.000 vs. 15.000 respectively). In addition to the cost, we have to consider the transport, the packaging and storage charges for conventional dialysate contained into plastic tanks.

In conclusion, frequent examination of CFU and EU is essential to reduce potential water contamination; but the goal of future techniques will be a "sterile dialysate" to assure a more physiologic dialysis.

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