[Dialysis Water Hygiene]

Mortality among long-term haemodialysis patients, particularly from cardiovascular diseases, is significantly increased. This is ascribed to the chronic inflammatory conditions of the patients which must be seen at least partly as being the effect of microbial substances in the dialysis water. Recent studies in Japan confirm this hypothesis. In 131,000 long-term dialysis patients, they show a 28% higher mortality level with the application of dialysis fluids containing endotoxins in comparison with the mortality rate with dialysis fluids in which no endotoxins were detected using the most sensitive methods.

Bacteria in the dialysis water

How do bacteria get into the dialysis water?

The dialysis water (permeate) is generally obtained using reverse osmosis, which can be described as pressure-driven filtration of a fluid through semi-permeable membranes. These membranes have tiny "pores" that are so small that all pollutants, as far as possible, are retained and only water penetrates them. This process also effectively prevents the contamination of dialysis water by the feed water, because intact bacteria are far larger than the permeability of the membrane. Even with minor membrane failures which are not detected immediately, reverse osmosis is generally not a relevant source of bacteria in the dialysis water. It is more frequently the case that bacteria get into the ring main primarily through the dialysis machines (backflow) and when they are connected and disconnected. The ring main systems, as used normally for water distribution in dialysis, cannot therefore be considered sterile.

Are bacteria in the dialysis water dangerous?

Bacteria are a natural constituent of the world around us. They exist almost all the time, everywhere, including in the human body. Most bacteria are not interested in humans; many are useful, and only a few are harmful as pathogens. In the dialysis water, the relevant risk is not from the bacteria themselves, but from the products that they release actively or passively. Essentially, dialysis water is not germ-free (sterile).

What do bacteria do in the dialysis water?

Bacteria do not like swimming around freely in the water, where they can be easily attacked. They prefer to stay on surfaces such as the walls of the water system pipes. Here, they can live protected in communities with other bacteria or micro-organisms in what we call biofilm. If we need to find out about the quantity of bacteria in the water system, merely determining the bacteria freely available in the water is inappropriate, since most bacteria are settled in the biofilm on the system walls.

Biofilm

What do bacteria do in the biofilm?

Generally speaking, the bacteria in the biofilm have quite different characteristics from those of mobile planktonic cells. They have adapted to suit the environment in which they live. This has many different consequences, although the characteristics with which we are familiar are generally based on studies of planktonic cells, since this cultivation method is easier to handle in the laboratory. It is particularly problematic that bacteria released from the biofilm often cannot be cultivated with standard

methods in the laboratory but are nonetheless viable (VBNC stage). This status is reversible, which means that the bacteria in question regain their potentially harmful properties. This effect leads to the microbiological contamination being underestimated.

Why is the biofilm problematic?

The bacteria in the biofilm have a metabolism and can reproduce or die off. In all cases, substances are excreted in the fluid around them. In this way, substances get into the dialysis water which can, under certain circumstances, penetrate the dialysis membrane and trigger serious reactions in the blood. The bacteria themselves are far too large to be able to pass through the dialysis membrane or the intact membranes of sterile filters.
If bacteria have the chance to reproduce, they do it with remarkable speed. If a massive biofilm exists in the dialysis water system, a sudden strong presence of bacteria in the liquid can sometimes occur. This goes hand in hand with the formation of large quantities of extracellular polymers which, as slime, can block all the filters in the system. A temporary shutdown of the dialysis device in question is then inevitable.

The formation of a manifest biofilm in the dialysis water system must be prevented at all costs.

Where are biofilms found?

Biofilms are found on the interfaces of fluids and the surfaces of water-bearing systems. They are not evenly distributed and prefer certain materials such as the rubber on seals or the plastic material of pipes and hoses. As the normal means of supplying water to the dialysis machines, the latter are therefore a particular problem zone. The result of the uneven distribution is that the composition of the fluid is not homogeneous either. This effect is strengthened by the circumstance the pieces/flakes of the biofilm are constantly breaking away and carried in the fluid. If a biofilm flake of this type gets into the sample, this produces unrealistically high measured values which cannot generally be confirmed in repeated sample testing. In practice, these peculiarities lead to confusion and misinterpretation on a large scale. Generally, any reasonable analysis is not possible because of the lack of homogeneity of the fluid if a biofilm has formed.

Why are bacterial products in the dialysis water a problem?

The danger caused by bacteria in the dialysis water does not lie primarily in the organisms themselves and the possibility of patient infection, but rather in the effect of the substances that are released by the bacteria. These substances can at worst be toxins, i.e. poisons, and/or have a pyrogenic (fever-inducing) effect. The best known representatives of this group are the bacterial endotoxins (BET) which are released from the capsules of gram-negative bacteria when they die. Fragments of nucleic acids such as DNA plus proteins such as enzymes also belong to this group, also known as CIS (cytokine-inducing substances). These substances cause fever and other reactions which can be dangerous for the patient, especially if they occur repeatedly or continuously over an extensive time. Chronic inflammatory conditions to be observed in dialysis patients, which may manifest as continuing microinflammation with fatal consequences, must be regarded at least partly as the effect of microbial substances in the dialysis water and consequently in the dialysate.

Avoiding biofilms

How can biofilms be avoided?

It is essential to avoid the formation of biofilms in the dialysis water system. This should be done through regular preventive disinfection which, in the current state of technology, can only be carried out routinely, practicably and safely using heat. The disinfection strategy (frequency, duration, etc.) should be matched to the complexity and size of the dialysis water system and validated as specified, for example, in the relevant DIN EN ISO standard 23500. A biofilm that is already established can only be removed using drastic chemical and/or physical methods. On the other hand, the formation of a biofilm can be prevented relatively simply by following an appropriate disinfection plan. Several disinfection cycles (at least two) per week have been found to be appropriate; monthly or half-yearly intervals are too long at any event, since as a result of the effects described above, bacteria can keep getting into the water system, and these can and will form biofilms in a surprisingly short time. In addition, a supplementary immediate inactivation or removal of the bacteria, for example through inline filters in the ring main, makes good sense if they occur in the dialysis machines or their connection due to the known backflow effect.

Is it possible to rehabilitate old ring main systems with a heavy build-up of biofilm?

In practice, it is not possible to remove an established biofilm using heat cleaning alone, and chemical cleaning methods do not produce the desired result in most cases either. In our experience, the best success is achieved by the alternating repeated use of peracetic acid based substances and agents containing chlorine. It should also be borne in mind that fixed deposits can frequently build up in old ring mains in connection with biofilms, which can easily form the basis for the establishment of a new biofilm. The complete replacement of the pipework system by hot-cleanable pipes with suitable surfaces is strongly advised, and preventive heat disinfection must then be carried out to prevent biofilm formation.

What are endotoxins?

The commonest types of pyrogenic (fever-inducing) substances are bacterial endotoxins, which are released from the capsules of gram-negative bacteria when they die. The fragments, which consist of lipopolysaccharides, are described using the overall term endotoxins, even though they may differ considerably in size and composition. Like other pyrogens, endotoxins are not toxic themselves in small doses, but in the blood, even in the lowest concentrations, they will trigger violent reactions in the immune system, during which particular messenger substances (cytokines) such as interleukins are released. Like the associated bacteria, endotoxins are also natural constituents of our environment. In blood, they help detect bacteria and cause the immune system to start fighting them.

Why should endotoxins be measured, or how can the existence of a biofilm be proved?

Endotoxins are important indicators of the presence of bacteria, also and particularly in biofilm. They can be measured extremely sensitively if the limulus test (limulus = horseshoe crab) is applied, or the recombinant Factor C assay (rFC assay) based on it is used. The use of photometric or fluorimetric test methods allows a sensitivity down to below 0.005 EU/ml (1 EU corresponds to approx. 0.1 ng endotoxin), which is required for the detection of biofilms. Nystrand calculated that 430,000 cells of Pseudomonas aeruginosa in suspension are needed to produce 1 EU/ml (Rolf Nystrand. Microbiology of Water and Fluids for Hemodialysis. J Chin Med Assoc, May 2008, Vol 71 No 5). Highly sensitive endotoxin test methods measure as low as 0,005 EU/ml or even 0.001 EU/ml, which would correspond to approx. 2,000 cells or approx. 400 cells. With population densities of typical biofilms of > 100,000 CFU per cm^2 surface area, highly sensitive endotoxin measurement seems sufficient for the detection of biofilm. Since the endotoxins of P. aeruginosa essentially react

weakly in the LAL test, compared with most other endotoxins, an even higher sensitivity must be assumed in practice. Our own many years of experience have shown, additionally, that stable endotoxin concentrations of less than 0.01 EU/ml are only measured in water systems without harmful biofilm formation. **The highly sensitive measurements of the endotoxin concentration in the dialysis water is currently the only practicable solution for the detection of biofilms in dialysis water systems!**

What is the clinical relevance of the quality of the dialysis water?

In long-term haemodialysis patients, mortality, particularly due to cardio-vascular diseases, is clearly higher, in the range of 10 percent according to the literature. This is ascribed to the chronic inflammatory conditions of the patients, which must be seen at least partly as the effect of microbial substances in the dialysis water and consequently in the subsequent dialysate. Recent studies in Japan confirm this hypothesis. In 131,000 long-term dialysis patients, they show a 28% higher mortality level with the application of dialysis fluids containing endotoxins in comparison with the mortality rate with dialysis fluids in which no endotoxins were detected using the most sensitive methods (Dialysis Fluid Endotoxin Level and Mortality in Maintenance Hemodialysis: A Nationwide Cohort Study. Takeshi Hasegawa, MD, PhD, MPH et al. Am J Kidney Dis. 2015; 65(6): 899-904). For this reason, a direct correlation between the mortality rate in long-term dialysis patients and the quality of the water / dialysate must be assumed.

The poorer the microbial water quality, the higher the mortality rate.

Are ultrafilters/sterile filters important in dialysis?

Ultrafilters in haemodialysis are intended to keep bacteria and undesirable substances away from the actual dialysis location and thus from the patient's blood. In fact, because of their size, bacteria cannot penetrate intact sterile filters and endotoxins are also held back effectively through adsorption. Apart from protecting against bacteria and endotoxins, the ultrafilters are also intended if possible to keep at bay other substances that act as CIS (cytokine-inducing substances). However, many of these substances can nonetheless penetrate currently used ultrafilters and dialysis machine membranes (Schindler R, Beck W, Deppisch R et al. Short bacterial DNA fragments: Detection in dialysate and induction of cytokines. J Am Soc Nephrol 2004; 15: 3207–3214). This remains initially unnoticed since, after filtration, the endotoxins are not there as indicators of the CIS. "Sterile filters" before the dialysis machine or for the on-line production of substituate are therefore not an effective protection against harmful pyrogens from the biofilm.

At this point, it is all the more important to consider prevention, i.e. it is essential to prevent the formation of a biofilm with the inevitable production and excretion of undesirable substances into the dialysis water. Ultrafilters should therefore not only be used as a backup before the dialysis site; they are also necessary as "inline filters" in the ring main system to be able to remove bacteria from the fluid phase immediately. Bacterial infestations of this type occur regularly when dialysis machines are attached and detached (retrograde contamination). Immediate removal of the bacteria from the fluid prevents the formation of dangerous biofilms (see above).

Monitoring the dialysis water

What is the maximum quantity of bacteria and endotoxins that dialysis water should contain?

Under the standard regulations, bacterial concentrations of 100 bacteria per ml are permitted in the dialysis water. This figure dates back to Robert Koch, who defined a maximum concentration of this level in 1883 in connection with the great cholera epidemic in Hamburg. He postulated that, under these circumstances, there was no danger of an epidemic as a result of the drinking water. Today, the definition of 100 bacteria per ml seems rather random, particularly because, with dialysis water, we are working with quite different culture media and cholera epidemics are not the problem. Since, according to what has been stated above, it is the biofilm that is the problem, rather than the bacteria, this should be avoided. From our experience, a bacterial concentration of an average of 5 bacterial per ml dialysis water should not be regularly exceeded and not exceeded at all at any machine connection in order to be able to sustainably prevent the formation of any biofilm.

The situation is similar for the endotoxin concentration. The value of 0.25 EU/ml required under the official regulations and standards is random and unhealthy, since at this high concentration, the immune system is already overstimulated, with the fatal consequences described above. Endotoxins are the indicators of the cytokine-inducing substances which should not occur if possible in the dialysis water. Endotoxins should not be detected using today's most sensitive detection methods anywhere, particularly at any machine connection. In terms of measurement, a concentration of 0.01 EU/ml should not be exceeded. In our experience, biofilm formation can then be sustainably avoided. It is not compliance with maximum values that counts – it is the proven absence of harmful biofilm in the entire dialysis water system that is important.

Are pathogens in the dialysis water tolerable?

There is a pragmatic answer to this: Under the standard official regulations, up to 100,000 bacteria per litre of dialysis water are allowed. Since no distinction is made in the water system between good and bad bacteria, these are naturally bound to contain pathogens now and again such as Pseudomonas aeruginosa, which is also a typical water bacterium. If one wanted to ban so-called pathogens completely, one would have to work in a sterile environment. Because the dialysis water system is not a sterile area, however, all sorts of bacteria must be expected. The internationally binding standard ISO 23500 therefore only requires the limitation of the total bacterial concentration (number of all bacteria provable under the specified methods) in the fluid. This makes sense, because the danger caused by bacteria in the dialysis water does not lie primarily in the infectious nature of these bacteria, but rather in their ability to form biofilms and in this way release harmful substances into the water.

What parameters should be determined in the dialysis water?

On the basis of what has been stated above, it is of primary importance to determine any possible biofilm in the dialysis water system, rather than the actual bacteria. The aim is to detect and avoid the possibility of establishment of a biofilm at an early stage. For this, the total bacterial concentration of the bacteria present in the fluid must be determined using processes optimized for water bacteria along with the concentration of endotoxins using the most sensitive methods. The determination of bacteria alone, which is frequently carried out, is pointless and not permitted under the relevant regulations. The measurement of endotoxin concentrations using insensitive test methods such as the frequently used limulus clotting test is useless, since it cannot exclude the existence of a biofilm.
Marked fluctuations in the microbial measurement results are clear indications of the

presence of biofilm. Individual measurements are only meaningful if they prove the existence of a biofilm through high bacterial and/or endotoxin concentrations. Conversely, an individual measurement showing low concentrations means nothing alone.
While the concentration of bacteria during the dialysis period can vary considerably locally because of the attachment and removal of the dialysis machines and the associated entry of microorganisms, the measured endotoxin concentration should always be very low or undetectable (< 0.01 EU/ml).

The endotoxin content is thus a better indicator of the long-term hygienic quality of the water system than the bacterial content. However, this should also not exceed on average a maximum value (5 CFU/ml). Only the integration of the dialysis machines with all water-bearing parts and the supply hoses into a regular preventive hot cleaning regime can minimize the described effect and prevent the entry of bacteria as a result of backflow effects. Because the dialysis water is not directly connected to the surface water, the determination of faecal bacteria such as E. coli is superfluous and a waste of money. It also makes little sense to detect Pseudomonas aeruginosa at the moment (see VBNC problems). In addition, the nature of the bacteria in the dialysis water generally only plays a subordinate role.

How frequently should the dialysis water be checked?

Because biofilms can form very quickly, the dialysis water system should actually be permanently monitored. Unfortunately, monitoring systems of this type are currently not yet available. The relevant DIN EN ISO standard 23500 recommends a month as the maximum interval for regular testing. If longer sampling intervals are required, it must be made clear (= validated) that the desired water quality is maintained in the long term when the system is in normal use. Under the regulations, tests must be carried out at least half-yearly, or preferably quarterly. In the case of interventions or modifications to the water system or dialysis incidents, additional measures to determine the water quality will be required.

Where should the sample be taken from the dialysis water?

The distribution of biofilms in water systems varies considerably; they tend to prefer certain surfaces such as the rubber on seals (see above), which are common in the water system couplings. Samples should therefore be taken only at connections that are in regular use and mounted as closely as possible to the ring main. Areas with dead volumes where there is no flow must be reported. It therefore makes sense to take the sample at machine connections, and thus close to the patient.
The sample should be taken with the machine running. This is technically possible without any problems. The number of sampling locations per investigation should depend on the size and complexity of the pipe system. At least three samples per ring in the machine (close to intake, middle, outlet) are recommended, and over time, samples should be taken from all the machine connections in turn.

Summary

The mortality and the quality of life of long-term dialysis patients depends directly on the microbial quality of the dialysis water. In order to achieve a suitably high quality, the dialysis water system must be free from harmful biofilm.

This is the case when average bacterial concentrations of below 5 CFU/ml and low, preferably undetectable, endotoxin concentrations (below 0.01 EU/ml in any event) are found over long periods in the fluid at all sampling locations in the machine.

In practice, this situation can only be achieved through regular frequent heat disinfection of the entire water-bearing system.

Marked fluctuations in the microbial measured values are clear indicators of the presence of biofilm. The microbial quality of the dialysis water must be regularly monitored during and after a validation phase.

So-called sterile filters before the dialysis machine or for the on-line production of substituate do not provide effective protection against harmful pyrogens if biofilm is present. Only the absence of biofilms throughout the water system can provide certainty here.

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