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The quality of dialysis water

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Abstract

Introduction. Every week, haemodialysis patients are exposed to ~400 l of water used for the production of dialysis fluids which, albeit with the interposition of a semi-permeable artificial membrane, come into direct contact with the bloodstream. It is therefore clearly important to know and monitor the chemical and microbiological purity of dialysis water.

Methods. In this review, we analyse the sources of chemical and microbiological water contamination, and the problems involved in water purification systems and modalities. We also analyse the compliance of dialysis units with the microbiological standards established by the most widely accepted guidelines relating to the quality of dialysis fluids.

Results. The risk of chemical contamination is due mainly to the primary pollution of municipal water, whereas the most important microbiological problem is the control of bacterial growth in the water treatment and distribution system. Dialysis water treatment implies various levels of pre-treatment, a final purification module (which, in many cases, is reverse osmosis: RO) and a hydraulic circuit for the distribution of the purified water. RO-based treatment systems produce water of optimal chemical and microbial quality, and so dialysis units need to concentrate on maintaining this quality level in the long term by means of effective maintenance and disinfection strategies. The most widely accepted standards for water purity are those recommended by the Association for the Advancement of Medical Instrumentation and the European Pharmacopea, which respectively allow bacterial growth of <200 and <100 c.f.u./ml, and an endotoxin concentration of <2 and <0.25 IU/ml. However, a number of multicentre studies have reported that 7–35% of water samples have bacterial growth of >200 c.f.u./ml, and up to 44% have endotoxin levels of >5 IU/ml.

Conclusions. The results of multicentre studies indicate that the microbial quality of dialysis fluids is still a too often neglected problem, particularly as there is evidence of a possible relationship between dialysis fluid contamination and long-term morbidity. The time has now come to take advantage of innovations in water treatment processes and improvements in dialysis machines in order to modify clinical practices and start improvement processes aimed at decreasing the risk of microbial contamination to the minimum, as it has already been successfully done in the case of chemical contamination.

Keywords: dialysis fluids; end-stage renal disease; haemodialysis; on-line fluids; quality assurance; water

Introduction

Dialysis fluids are solutions that are produced by proportional dilution devices incorporated in the dialysis machine (on-line production) or separate from them (off-line production) by mixing concentrated electrolytic solutions or powdered salts with appropriately pre-treated municipal water.

To be considered safe for drinking, municipal water must satisfy precise standards of turbidity and contamination, based on the assumption of a limited daily intake with food of ~2 l per person. However, haemodialysis patients are not only exposed to municipal drinking water, but also to a considerable amount (~400 l per week) of water used for the production of dialysis fluids. Furthermore, whereas drinking water reaches the bloodstream only after passing through the highly selective barrier of the gastrointestinal mucosa, dialysis fluids come into direct contact with the bloodstream with the only interposition of a semi-permeable artificial membrane. Finally, it must be remembered that, in some dialysis units, water is not only used to produce dialysis fluids, but may also be used for other purposes, such as dialyser rinsing and reuse. It is therefore clearly important to know and

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monitor the chemical and microbiological purity of dialysis water.

Contamination of dialysis water

Water is one of the best known solvents. It is therefore not surprising that a wide variety of chemical and microbial contaminants can be found in municipal water, albeit within the limits considered acceptable for human consumption. Some of these substances, such as mineral salts, suspended particles (sand and clay) and products of organic origin (lignin, tannin), may be naturally present in the hydric reserves from which the water is obtained; others, such as copper, zinc and lead, may be released by the pipes forming the water distribution system. It is also possible that water, which is usually collected from surface-fed reservoirs or underground wells, may be contaminated by substances used in agriculture (fertilizers, pesticides, nitrates), residues or rejects of industrial processes (hydrocarbons, fluoride, alogenates) and municipal dumps. The health authorities require the addition of various substances to municipal water in order to make it safe for drinking: in areas where turbidity exceeds the allowed limits, water can be treated with flocculating agents such as aluminium sulfate and iron salts, whereas chlorine and chloramines are commonly used to keep microbial contamination under control. Finally, chemical agents may be added to municipal water for other public health reasons, e.g. fluoride is added to the water in some areas for the prophylaxis of dental caries.

When dialysis was first introduced, water was either untreated or simply underwent some rudimentary softening processes, which meant that complications related to chemical contamination, such as the hard water syndrome, were not unusual. It is now well known that many of the chemical substances in municipal water are potentially dangerous for dialysis patients, some of which (calcium, sodium, aluminium, chloramines, fluoride, copper, zinc, sulfates, nitrates) are able to lead to well-defined acute or chronic poisoning syndromes. The majority of dialysis units are therefore equipped with water purification systems based on reverse osmosis (RO) which, together with sound pre-treatment processes, is capable of almost completely removing chemical contaminants. However, although this has significantly reduced the clinical occurrence of this type of contamination, the problem should never be underestimated because serious accidents due to the chemical contamination of dialysis water have been reported even in the last few years [1].

The microbial contaminants most frequently found in dialysis water are bacteria and their degradation products, such as endotoxins and peptidoglycans; however, fungi, viruses and protozoa can also be encountered occasionally. In a study of almost 17 000 tap water samples performed in Germany in 1983, $<8\%$ had a bacterial growth content of >100 c.f.u./ml [2]. As some guidelines [3] allow bacterial contamination of up to 200 c.f.u./ml for dialysis water, this means that the microbial quality of municipal water is relatively adequate. In fact, the main problems relating to the microbial quality of dialysis water are less frequently due to the bacterial contamination of municipal water, and more frequently due to water purification techniques, and to the characteristics and maintenance of water treatment and distribution systems.

Microbial contamination of dialysis fluids can cause acute intradialytic complications (pyrogenic reactions, cardiovascular instability, headache, nausea, cramps) and, by maintaining a state of chronic micro-inflammation, it may be involved in the pathogenesis of a number of chronic complications typical of the uraemic state, such as amyloidosis, atherosclerosis and malnutrition [4–7].

Water treatment systems

From what has been said above, it seems clear that, before being used for the production of dialysis fluids, municipal water needs additional purification treatment in order to ensure appropriate characteristics of chemical and microbial purity. However, as there is no single type of purification treatment capable of doing this in a constant and long-lasting way, dialysis water treatment systems combine various purification modalities depending on the nature of the contamination, the water flow needed and the desired purity level. These systems usually involve the pre-treatment of municipal water, a final purification process (RO in many cases) and a hydraulic circuit for the distribution of the purified water.

The central aims of the pre-treatment process are to remove suspended particles and to decrease the hardness of water in order to protect the membranes of the RO system from plugging and scaling. This is done by using filters of different porosity placed at crucial points of the system and pre-treatment elements containing sodium exchange cationic resins (water softeners) which are able to remove calcium and magnesium from water. Chlorine and chloramines can pass through the majority of elements of the purification system (including RO systems) and cause severe haemolytic reactions when they come into contact with the bloodstream. In order to avoid this catastrophic event, dialysis water is pre-treated with activated carbon (AC) filters that can remove chlorine and its derivatives, as well as other organic contaminants. The pre-treatment of municipal water solves some of the critical problems of chemical contamination, but also increases microbial problems because filters, softener resins and AC offer a large surface areas on which bacteria and other microorganisms can easily grow.

RO is based on a tangential flow filtration process in which water is pushed by high pressure through semi-permeable membranes that can reject the majority of contaminants: up to 95–98% of dissolved salts, and up to 99% of bacteria, endotoxins and substances with a molecular weight of >200 Da are removed this way.

To obtain an even higher level of purity, water treatment systems are often equipped with two RO systems in series. Another water purification technique is deionization (DI), which is based on ion-exchange resins. This effectively removes cations and anions from water, but cannot remove bacteria and pyrogens, and may actually increase bacterial contamination. DI is often used together with RO, but it can also be used alone as the final water purification system. However, given the growing contamination of municipal water and increasingly restrictive quality standards, it still seems to be less capable of guaranteeing the required purity levels for dialysis water.

Hydraulic distribution is an important part of the dialysis water treatment system and its large surface area makes it one of the most critical points in terms of microbial quality. To decrease the risk of microbial contamination, reserve purified water tanks must be avoided and the distribution system must consist of a loop of low calibre pipes in which water can continuously circulate 24 h a day, and be free of dead spaces and stagnation zones. Polyvinyl chloride (PVC) was the most widely used material in the past, but other materials, such as stainless steel, polyvinylidene fluorine (PVDF) and cross-linked polyethylene (PEX), are currently preferred because they can be disinfected more easily, avoid the release of particles or chemical contaminants, and have a very smooth surface that makes bacterial adhesion more difficult.

The water produced by osmosis has an optimal chemical and microbial quality: the problem is how to maintain this level of quality. This is less difficult in the case of chemical contamination (chemical contaminants do not develop after they have been removed) than in the case of microbial contamination. The only way to keep bacterial growth under control is to undertake preventive disinfection so often that it prevents microorganisms from growing. Consequently, RO membranes must undergo periodic disinfection and cleaning in order to avoid the risk of contamination on their clean side. Furthermore, disinfection must involve all the pipes in the distribution system, including the inlet lines to the dialysis machines. If these operations are not performed, the contamination extends to the inner surface of the pipes, thus leading to the development of a polymeric organic matrix (or biofilm) in which bacteria can easily grow. Once it has developed, the biofilm is a constant source of bacterial contamination that is very difficult to completely remove even after drastic attempts of disinfection.

For disinfection purposes, it is possible to use various chemical agents (such as hypochlorite and peracetic acid) or heat, and ozone is also currently used. All of the chemical disinfection procedures have the same problems: they are time-consuming and rather complex; it is difficult to achieve the right concentration at all points of the system; and care must be taken to identify possible toxic residues. Heat has the advantage that it is easily dosable, it reaches all the surfaces of the system and it does not leave residues. It is often suggested that UV lamps can help to solve the problem of the microbial contamination of treatment systems, but they are not very effective because they only act on a single spot of water, whereas bacterial growth develops on the surface of pipes and can extend to the whole system.

In order to guarantee the quality of the feeding water and dialysate, many dialysis machines are equipped with a final filtration system made of microfilters (with a porosity ranging from 0.1 to 2 μm) and/or ultrafilters (with a rejection capacity ranging from 1000 Da to 0.1 μm). These systems can remove bacteria and endotoxins by means of sieving and absorption processes which, in addition to filtering characteristics, also depend on the conditions of use (exposure time, disinfection) and on the contamination levels of the treated fluid. However, the existence of these barrier systems should not make us forget that dialysate is the result of a chain of production processes and that, in order to guarantee the highest quality, the best strategy is to counteract contamination at each phase of the process, starting from the purification and distribution of the water that constitutes ~95% of the dialysate itself.

Standards for water purity

A number of standards for chemical and microbial quality have been proposed, the most widely accepted of which are the recommendations of the Association for the Advancement of Medical Instrumentation (AAMI) and those of the European Pharmacopea (EP) [3,8]. Both establish very restrictive maximum levels of chemical contamination, yet they are substantially different in terms of microbial contamination. The AAMI recommendations allow an upper limit of 200 c.f.u./ml for microbial contamination and 2 IU/ml for endotoxin contamination of water, and establish a level of 50 c.f.u./ml for bacterial growth and 1 IU/ml for endotoxin concentrations above which corrective actions, such as disinfection or re-testing, should be performed. The EP recommendations establish more restrictive limits: bacterial growth < 100 c.f.u./ml and endotoxins < 0.25 IU/ml.

More recently, the concept of ultrapure water has been introduced, defined as microbial contamination of < 0.1 c.f.u./ml and endotoxin contamination of < 0.03 IU/ml. However, the need to minimize the levels of bacterial contamination still remains a matter of wide debate and some uncertainty [9]. This uncertainty is due to the fact that, although there is some evidence that microbial contamination of dialysate may be related to some long-term complications of uraemia, the benefit of improving the purity of dialysis fluids to these levels has not yet been demonstrated.

The purity level of dialysis fluids also depends on the dialytic modality for which they are destined. The recent European Best Practice Guidelines on dialysis fluid purity suggest the minimal objective of following

the recommendations of the EP, and consider ultrapure water as being highly desirable for all treatment modalities, but particularly for high-flux dialysis. In relation to the techniques characterized by the on-line production of the reinfusion solution, the European Best Practice Guidelines consider ultrapure water a basic prerequisite [10]. Only a few countries, such as France [11], have developed specific legislation concerning the methods of on-line dialysis fluid production. In the absence of specific national recommendations, the legislative reference for these techniques in Europe is Community Directive 93/42/EEC on Medical Devices. According to this directive, the safety of dialytic treatments using expressly designed and certified (CE-mark) machines for the on-line production of reinfusion solutions is guaranteed by the use, maintenance and control of the machines in accordance with the manufacturer's instructions. As a consequence, it is particularly important that the dialysis units are organized in such a way as to guarantee that the quality of dialysis water is maintained within the contamination limits established by the manufacturer [12].

As bacterial growth can be significantly influenced by factors such as the medium, incubation time and temperature [13], the application of the above-mentioned standards is effective only if it is associated with appropriate microbiological techniques. The AAMI recommendations for microbial contamination are based on techniques using tryptic soy agar as the medium and incubations at 37°C for 48 h; the EP does not specify the microbiological technique to be used. The techniques based on nutrient-poor media, such as Reasoner's 2 agar (R2A) and tryptone glucose extract agar (TGEA), and prolonged incubations (5–7 days) at room temperature (20–23°C) have proved to be more sensitive than the standard techniques in showing the microbial contamination of dialysis water [14,15]. These microbiological techniques and the analysis of an adequate volume of water (100–1000 ml to be filtered through a 0.22 μm bacterial filter and incubated on an appropriate agar plate) are essential when the objective is to verify whether the microbial quality is within the range of ultrapure water (<0.1 c.f.u./ml).

Improving the quality of water

Over the last 12 years, a number of multicentre studies aimed at evaluating the microbial quality of dialysis water and dialysate have been performed in the USA [16], Canada [17] and Europe [18,19]. In these studies, between 7.4 and 35.3% of the water samples did not comply with the standards proposed by the AAMI, and the percentage of water samples with endotoxin levels >5 IU/ml even reached 44% in one study [18]. It must be stressed that these studies probably underestimated the microbial contamination of water, because only one of them used a microbiological technique with nutrient-poor medium (R2A) and long incubation times [16]. A multicentre study performed in 2001 analysed the contamination of dialysis water produced by 25 systems in 24 dialysis units in Lombardy (Italy) using R2A at room temperature for 7 days as the medium: of the 75 analysed water samples, 15% had a bacterial count of $\geq$100 and 11% a bacterial count of $\geq$200 c.f.u./ml [20]. Although apparently satisfactory when compared with those of previous multicentre studies, these results can be improved further as the technical characteristics and maintenance of the systems were certainly not optimal: DI was the final stage of purification in one dialysis unit, 48% of the distribution systems were made of PVC, 8% had a non-loop configuration, and only 28% performed disinfection procedures at least monthly. Taken together, these results suggest that the microbial quality of water is still a too often neglected problem and that, although current technology makes it possible to achieve results that were unimaginable in the past, a considerable proportion of dialysis units still have much to do to ensure a high level of microbial purity.

The best approach in planning a water treatment process is to consider it in terms of quality assurance and continuous quality improvement, which means that it is first necessary to establish the chemical and microbial quality standards to be achieved. After having made a detailed analysis of the starting situation (previous controls of the chemical and bacterial contamination of municipal water and dialysis fluids) and the specific needs of the dialysis unit (water flows, the number and modalities of treatment), the technical characteristics of the system that best correspond to these requirements can be determined in relation to the available financial resources. Once the system has been developed or updated accordingly, it is possible to plan its maintenance (the regeneration of softeners, the replacement of filters, resins and AC, the frequency and method of disinfection) and a monitoring system (periodic analyses of chemical and microbial contamination, the monitoring of chloramine removal and resistivity and hardness of water) designed to verify and document that the pre-established levels of chemical and microbial quality have been achieved and are maintained over time. Finally, the corrective actions to be taken in the cases where the results of the chemical and microbial controls do not satisfy the pre-established standards must be defined in advance.

Conclusions

The quality of dialysis fluids depends on their components (water and concentrate) and how they are prepared, i.e. on the dialysis machine. As dialysis fluids are mainly comprised of water, it is obvious that water plays a very important role in their chemical and microbial quality and, consequently, the choice of the dialysis water treatment system is crucial. However, it would be a big mistake to believe that making an optimal choice of the treatment system means that all of the problems relating to water quality have been solved, because it also significantly depends on the maintenance and monitoring of the system, and the operators' awareness of the issues related to the

contamination of dialysis fluids and the management of the system itself. It must not be forgotten that concentrated solutions are produced in an industrial context and guaranteed by the manufacturer, whereas dialysis fluids are prepared extemporaneously without any chance of quality control before use. The quality of the dialysis fluids is certainly the direct responsibility of the people managing the dialysis unit.

The concept of dialysis biocompatibility has rapidly become established and has found wide-ranging practical application in the development of materials for filters, dialysis lines and other accessories. The same attention has not been given to dialysis water, although there is no reason for considering it any differently, particularly given the possible association between the contamination of dialysis fluids and long-term morbidity in uraemic patients on dialysis. Innovations in water treatment processes and improvements in dialysis machines now make it possible to produce fluids with a high level of chemical and microbiological quality at only slightly increased costs, and so it is time to modify clinical practice and introduce improvement processes that decrease the risk of microbial contamination to a minimum, as it has already been satisfactorily done in the case of chemical contamination.

References

1. Ward DM. Water disasters: pitfalls and precautions. *Contemp Dial Nephrol* 1999; 12: 24–8
2. Bommer J, Ritz E. Water quality. A neglected problem in hemodialysis. *Nephron* 1987; 46: 1–6
3. AAMI Standard and Recommendation Practices. AAMI/DS-1 RD62. Water treatment equipment for hemodialysis applications. Association for the Advancement of Medical Instrumentation. Arlington, VA, USA; 2000: 2–32
4. Lonnemann G. The quality of dialysate: an integrated approach. *Kidney Int* 2000; 58 [Suppl 76]: S112–S119
5. Baz M, Durand C, Ragon A *et al*. Using ultrapure water in hemodialysis delays carpal syndrome. *Int J Artif Organs* 1991; 14: 681–5
6. Schwalbe S, Holzhauer M, Shaeffer J, Galanski M, Koch KM, Floege J. Beta 2-microglobulin associated amyloidosis; a vanishing complication of long-term hemodialysis? *Kidney Int* 1997; 52: 1077–1083
7. Zimmermann J, Herrlinger S, Pruy A, Metzger T, Wanner C. Inflammation enhances cardiovascular risk and mortality in hemodialysis patients. *Kidney Int* 1999; 55: 648–658
8. *European Phamacopeia*, 3rd edn, Supplement 2001: Monograph 1997: 1167 corrected 2000. Haemodialysis solutions, concentrated, water for diluting
9. Tielemans C, Hoenich NA, Levin NW, Lonnemann G, Favero MS, Schiffl H. Are standards for dialysate purity in hemodialysis insufficiently strict? *Semin Dial* 2001; 14: 328–336
10. The EBPG Expert Group on Haemodilaysis. European Best Practice Guidelines for Haemodialysis (Part 1); Section IV, Dialysis fluid purity. *Nephrol Dial Transplant* 2002; 17 [Suppl 7]: 45–62
11. Ministère de l'Emploi et de la Solidarieté. Circulaire relative aux spécifications techniques et a la securité de pratique de l'hémofiltration et de l'hemodiafiltration en ligne dans établissements de santé. Republique Française, Circulaire DGS/DH/AFSSAPS No 311, June 7, 2000
12. Pirovano D. Regulatory issues for on-line haemodiafiltration. *Nephrol Dial Transplant* 1998; 13 [Suppl 5]: 21–23
13. Andrulli S, Pontoriero G, Viganò E *et al*. Influence of cultural technique on evaluation of microbial contamination of dialysis fluids. *Nephrol Dial Transplant* 2002; 17 [Suppl 1]: A268
14. Van der Linde K, Lim BT, Rondeel JMM, Antonissen LPMT, De Jong GMT. Improved bacteriological surveillance of haemodialysis fluids: a comparison between tryptic soy agar and Reasoner's 2A media. *Nephrol Dial Transplant* 1999; 14: 2433–2437
15. Ledebo I, Nystrand R. Defining the microbiological quality of dialysis fluid. *Artif Organs* 1999; 23: 37–43
16. Klein E, Pass T, Harding GB, Wright R, Million C. Microbial and endotoxin in water and dialysate in the central United States. *Artif Organs* 1990; 14: 85–94
17. Laurence RA, Lapierre ST. Quality of hemodialysis water: a 7-year multicenter study. *Am J Kidney Dis* 1995; 25: 738–750
18. Bambauer R, Schauer M, Jung WK, Daum V, Vienken J. Contamination of dialysis water and dialysate. A survey of 30 centers. *ASAIO J* 1994; 40: 1012–1016
19. Arvanitidou M, Spaia S, Katsinas C *et al*. Microbiological quality of water and dialysate in all haemodialysis centres of Greece. *Nephrol Dial Transplant* 1998; 13: 949–954
20. Pontoriero G, Andrulli S, Conte F *et al*. Microbial contamination in water and dialysate: a prospective multicenter study. *Nephrol Dial Transplant* 2002; 17 [Suppl 1]: A152