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Editorial Comment

Ultrapure dialysis fluid—how pure is it and do we need it?

Ingrid Ledebo

Gambro Corporate Research, S-220 10 Lund, Sweden

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The issue

Originally a definition of water quality for the semiconductor industry, the term 'ultrapure' has been adopted by the dialysis community. The discussion has developed from basic definitions and methods for quantification to clinical benefits and recommendations for implementation. Today, ultrapure dialysis fluid is subject to the demands of evidence-based medicine or at least evidence-based cost-effectiveness. The sequence from growing awareness to widespread conviction and practical implementation of ultrapure dialysis fluid is similar to the process for biocompatible dialysis membranes some 10–15 years ago. So, where do we stand with regard to ultrapure dialysis fluid?

What is ultrapure?

The term 'ultrapure' started to appear in dialysis literature in the early 90s and it meant that the fluid was highly purified in comparison to standard procedures [1]. The fluid appeared to be free from bacteria and endotoxin when routine methodology was used for testing. One millilitre of water or dialysis fluid spread on a suitable agar plate or tested for endotoxin showed neither growth nor activity. Some people misunderstood this and reference to 'sterile dialysate' was heard, although the fluid was far from sterile according to the definitions of the pharmacopoeia [2,3]. Ultrapure was later defined as containing <0.1 colony forming unit/ml (CFU/ml) using sensitive assays and <0.03 endotoxin unit/ml (EU/ml), the latter being the sensitivity level for the simplest of the Limulus Amoebocyte Lysate (LAL) assays, the gel clot test [4]. This definition is now widely accepted and referred to in clinical guidelines [5] as well as national and international standards [6,7]. In comparison to standard quality dialysis fluid ultrapure means a reduction of at least three logarithms of the number of bacteria and at least one logarithm of the endotoxin content [8].

How pure is ultrapure?

When setting target levels for any standard, it is important to define also the methodology for quantification. With reference to bacterial numbers, there is no such thing as an absolute bacterial count. To illustrate this we can talk about 'total cell count' including both living and dead cells or 'vital cell count' referring only to cells that can take up and metabolize a certain stain. The most common method for estimating a load of living bacteria is to spread a fluid sample on an agar plate with suitable growth medium and count the colonies formed after appropriate incubation. The number of CFU reflects the number of living cells that can grow under the conditions used, i.e. medium, temperature and time. The trick is to mimic the natural growth conditions for relevant strains as closely as possible. If we are interested in pathogenic organisms we should use a rich medium, e.g. blood agar, and cultivate at 37°C. Being interested in water bacteria we should select a nutrient-poor medium and cultivate at or below room temperature for 5–7 days [4,9].

Even if all living cells in a sample grow into colonies, the CFU-number is always an underestimation of the true number of viable cells since bacteria appear in pairs, clumps and chains, each and all giving rise to one colony. To avoid fusion of colonies and provide a basis for statistical reliability, the sample volume should be adjusted to the degree of contamination so that the number of colonies on a plate is between 30 and 100. Thus, a 1000 ml sample should be used for ultrapure fluids and give <100 colonies.

Similar considerations apply to endotoxin, which strictly speaking is Gram-negative cell wall components, released mainly from dead cells. The classical endotoxin, LPS and lipid A, can be quantified with LAL tests of varying sensitivity

Correspondence and offprint requests to: Ingrid Ledebo, PhD, Gambro Corporate Research, Box 10101, S-220 10 Lund, Sweden. Email: ingrid.ledebo@gambro.com

and complexity. Peptidoglycan, a building block in Gram-positive as well as Gram-negative cell walls, requires an assay made from silk worm larvae, not widely available [10]. Other bacterial cell wall components with pyrogenic properties can today only be detected by their capacity to induce formation of cytokines using tests not generally performed by routine laboratories [11].

To conclude the question of estimating levels of bacteria and endotoxin in fluid samples, we need to realize that limitations in methodology must be considered when interpreting results and target values must be combined with defined methodology. The European Best Practice Guidelines for Hemodialysis give detailed recommendations on how to test dialysis fluid in order to validate that it is ultrapure [5]. If these instructions are followed, we will know whether our fluid can be referred to as ultrapure or not, but we will never know how pure it really is. Information on fluid purity should always be expressed in relation to a maximum level of contaminants and the assays used.

Ultrapure dialysis fluid—do we need it?

The molecular and cellular consequences of microbial contaminants reaching the blood stream across a dialysis membrane were postulated 20 years ago in the interleukin hypothesis [12]. This has been confirmed by *in vitro* and *in vivo* experiments and today there are an increasing number of clinical studies showing the impact of fluid quality on important patient-related parameters. The major clinical effects of improved fluid quality are seen in a number of inflammation-related parameters. Levels of interleukin-6 and C-reactive protein fall significantly within a couple of months, serum albumin concentration and other nutrition-related parameters improve, anaemia management becomes less drug-dependent and plasma concentrations of β_2-microglobulin and advanced glycation end products are reduced. There are even signs that residual kidney function is better preserved (reviewed in [13–15]).

As a component of the MIA syndrome and a strong inducer of cardiovascular complications, inflammation is considered one of the most urgent problems in dialysis management today and anything that can improve the inflammatory status of dialysis patients should be seen as highly valuable [16]. The critical question is whether the benefits connected with ultrapure fluid are clinically relevant and can be expected to contribute to improved outcome for dialysis patients? The dialysis society is disillusioned by lack of improved outcome in response to the perceived upgrades of therapy—technological as well as pharmacological—that have been introduced over the past two decades. If we could not see a benefit from high-flux over low-flux membranes or from high dose over standard dose of therapy, how could we expect improved quality of dialysis fluid to have any effect? Without going into the shortcomings of the HEMO study in detail, neither the flux nor the dose difference is likely to have had any significant impact on inflammatory parameters. Fluid quality was not addressed and the mix of reused cellulosic and synthetic membranes probably obscured any membrane-related protection against contaminated fluid. The response to the question above is that we need every tool accessible to us that can reduce or prevent inflammation in dialysis patients and ultrapure dialysis fluid is turning out to be such an instrument.

Ultrapure dialysis fluid—can we afford it?

The relevant question about ultrapure dialysis fluid may not be whether we need it, but rather how it could be implemented and whether we can afford it? We can use two basic approaches for cleaning up the fluid—removal of bacterial products or prevention of bacterial growth. Starting from standard quality dialysis fluid and subjecting it to one filtration step with an ultrafilter placed on the dialysis machines close to the dialyser should provide ultrapure fluid according to our definitions. Depending on the conditions, the ultrafilter could be used for several months and the cost per treatment is, therefore, small.

The other approach is to prevent bacterial growth in the fluid system and thus eliminate the risk of metabolites and debris reaching the patient. For this we need to start with high-quality water and preserve this quality throughout the entire flow path. Biofilm formation must be blocked by frequent, prophylactic disinfection of the entire system, and only concentrates of high microbiological quality should be used [8,13,17]. As an extra precaution we may still want to insert an ultrafilter before the fluid reaches the dialyser. With this approach we are protecting the patients from all known and measurable bacterial products and hopefully also from the so far unknown [18]. This procedure requires equipment that can disinfect and be disinfected and for the rest it is mainly a question of staff education and relevant operational routines.

How far should we go—is ultrapure enough?

Having seen the shortcomings of present quantification methods for bacteria and endotoxin, some people may wonder if ultrapure fluid according to present definitions and methodology is sufficiently clean, or we should reduce the probability of contamination even further e.g. by using sterile fluid. Let me respond by referring to systems for on-line convective therapy where ultrapure dialysis fluid is made sterile by one additional step of controlled ultrafiltration and this fluid is used in large volumes for substitution [19]. The safety is proven by sensitive immunological tests and by the millions of treatments performed [20,21].

A parallel process takes place in haemodialysis when ultrapure dialysis fluid is backfiltered into the blood across the dialysis membrane. So, ultrapure should be sufficient for haemodialysis, as long as the dialyser functions as an ultrafilter.

Conclusion

It is unlikely that there will ever be a randomized, controlled study giving survival data for patients treated with ultrapure *vs* standard quality dialysis fluid, so we must decide whether it is important without such evidence. The European Best Practice Guidelines for Haemodialysis have made this decision and recommend the use of ultrapure dialysis fluid as a goal for all patients and all modalities [5]. In other parts of the world it is still a debated issue but the real controversy appears to be economic rather than scientific [14,22]. Recommendations for ultrapure dialysis fluid according to present definitions should be included in all clinical practice guidelines, thus bringing it to the attention of dialysis practitioners and health care authorities not only in Europe but also in the rest of the world.

Conflict of interest statement. None declared.

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