assessments of glomerular density) and lower glomerular density score. Interestingly, glomeruli with segmental sclerosis, included in the glomerular density metric, were also more common in the group with lower glomerular density (2 versus 0.2%). The infrequent and highly variable presence of segmental sclerosis likely underlies the lack of statistically significant difference in sclerosis between groups with higher versus lower glomerular density. Exclusion of patients with decreased renal function at time of biopsy likely explains why this lesion was so rare. Not surprisingly, interstitial fibrosis >10% was linked to lower glomerular density than interstitial fibrosis <10%, with similar distinction when the interstitial fibrosis threshold was set at 20%. Yet, these variables of interstitial fibrosis/tubular atrophy did not predict outcome in univariate analysis, with similar distinction when the interstitial fibrosis threshold was set at 20%. Yet, these variables of interstitial fibrosis/tubular atrophy did not predict outcome in univariate or multivariate analysis nor did global sclerosis per se. The robust predictive value of the simple metric of glomerular density thus may have complex underlying pathophysiological mechanisms that may not be easy to unravel. Nonetheless, in this group of patients who were indistinguishable at the onset, this information, in conjunction with a response of proteinuria to therapeutic intervention, significantly contributed to identification of patients with poor prognosis.

Regardless of the causality of the decrease in number of glomeruli relative to cortical area, this simple technique may offer additional guidance to identify patients in whom we should target our most aggressive interventions.

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(See related article by Tsuboi et al. Low glomerular density is a risk factor for progression in idiopathic membranous nephropathy. Nephrol Dial Transplant 2011; 26: 3555–3560.)

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Rapid assessment of microbiological purity of dialysis water: the promise of solid-phase cytometry assessment and the epifluorescence microscopy method

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Over the last decade, microbiological contamination of water and dialysis fluid has become a major concern for haemodialysis (HD) patients being implicated in bioincompatibility and long-term side effects of HD. In the past, gross contamination of dialysis fluid was implicated in pyrogenic reactions particularly with the extensive use of bicarbonate buffered dialysate and high-flux dialysers [1]. Nowadays, even minor levels of microbial contamination of dialysis fluid are capable of triggering inflammation by activating monocyte–macrophage cells and releasing pro-inflammatory cytokines. Chronic microinflammation, a common feature of dialysis patients, represents the strongest amplifier of most common pathophysiologic pathways in kidney disease patients associated with malnutrition, accelerated atherosclerosis, B2M amyloidosis and erythropoiesis-stimulating agent (ESA) resistance [2–4]. This is the strongest argument for using ultrapure dialysis fluids (water and dialysate) in all dialysis modalities but particularly in high-flux HD and on-line haemodiafiltration (HDF) [5]. Based on these considerations, the status of dialysis fluid has changed over recent years in the view of nephrologists, being more and more perceived as a ‘pharmaceutical drug’ rather than a ‘medical device’ [6]. Tremendous efforts have been made by water and dialysis industry to improve the microbiological purity of
Hygienic rules of maintenance concerning both the nation and biofilm formation within the dialysate circuit (chemical, thermal, mixed) contribute to prevent contamination performed after each dialysis session by various ways the dialyser. In addition, disinfection procedures usually final cold sterilization of the end product flowing through the dialysis machines are optionally equipped with captive permanent water circulation and by ensuring optimal dis-
equately engineered water distribution piping network, by water is now ensured until the dialysis machine by ad-
vices and depyrogenating filters [7]. Quality of polished siss modules (single or double in series), microfiltration de-
and non-pyrogenic water, by implementing reverse osmo-
design to produce ultrapure water, a surrogate of sterile dialysis fluids. Water treatment systems (WTS) have been
tination assay using THP-1 cell line [18]. Whatever the temperature (22°C) for 3–7 days, mimicking natural envi-
ment of these microbial agents [13–15]. HPC denotes the
ual number of cells forming colonies on the plate medium
urce of contaminant particles. This original
Endotoxin content of the water and the dialysis fluid
bioassays for detecting virtually all microbial bioactive-derived products in a research clinical setting the complexity and cost of these tests preclude their use in clinic as routine.
Indeed as recommended by international guidelines, the regular and standard assessment of microbial contamination of water and dialysis fluid relies both on specified bacteriological analysis based on colony count expressed in CFU/millilitre (or litres) and on endotoxin content determination established by kinetic LAL assay. Water and dialysis fluid purity are classified according to these criteria based on CFU and endotoxin content. Interestingly, the same level of microbial purity is now applicable both on water and dialysis fluid: pure water and dialysis fluid (CFU < 100/mL, EU < 0.25/mL); ultrapure water and dialysis fluid (CFU < 0.1/mL, EU < 0.03/mL) [19].
In addition, it is important to underline limitations associated with conventional microbiological assessment of water and dialysis fluid: firstly, no significant correlation is observed between bacterial contamination and level of endotoxin content; secondly, HPC detects only viable and planktonic bacteria missing dead or non-viable bacteria; thirdly, mycobacteria, yeast and fungi do not produce endotoxins and are not easily detected by standard plate count culture or LAL assay; fourthly, long time requirement (3–7 days) for growth culturing and relative inadequateness of LAL test to assess microbial burden contamination and fifthly, cost issue of microbial and endotoxin testing.
In their manuscript, Riepl et al. [20] report a new and promising method for rapidly assessing microbiological quality of dialysis water based on solid phase cytometry and epifluorescence microscopy. The authors took advantage of the availability of solid phase cytometry in the environmental water treatment control system, to develop a rapid technique for assessing microbiological contamination of dialysis water. In brief, the method involves filtration of water sample through a membrane, cultured on a Petri dish for 30 min, followed by staining cells retained by fluorescent dye then followed by an automated counting of fluorescent cells by laser scanning (solid phase cytometry). Results are subsequently validated by epifluorescence microscopy directly connected to the cytometer permitting a precise exclusion of contaminant particles. This original method provides a precise assessment of water contamination levels in an automated way in <3 h. Interestingly, this method also provides a mean number of total cells cultured (total direct count, TDC) and differentiating viable cells (total viable count, TVC) from dead or non-viable cells (TDC and TVC difference).
Solid phase cytometry epifluorescence microscopy method was assessed in 13 dialysis facilities from Germany. In each facility, water sampling was performed at four points invento-
torying the complete WTS: tap water, softened water, reverse osmosis water before entering the distribution circuit and water ending the distribution loop. Of the 113 water samples analysed overall, 59 were from drinking water and 54 were from dialysis water. The solid phase cytometric method was compared to conventional methods using agar HPC and LAL assay for endotoxin content. TVCs and TDCs were identified according to the solid phase cytometric method for further analysis. Autoclaved MilliQ water (double reverse osmosis
water used in the laboratory) was used as control sample or blank water in each case.

Specificity and sensitivity of the solid phase cytometry method was compared to standard plate count culture method. Overall, TDCs obtained with solid phase cytometry were 1–2 logs higher compared to plate count method in the different water samples tested indicating that a large number of cells were dead or non-viable. In addition, viable cell counts detected in tap water (drinking water) were ~10 times higher than colony counts obtained from the plate count method. Interestingly, the difference noted in viable cell counts between these two methods tends to reduce along with dialysis treated water. No significant difference in viable cell counts was observed anymore in dialysis water after being polisher by reverse osmosis. Highly significant positive correlations between the three methods (TDC, TVC and HPC) were observed on pure or ultrapure water. Based on these results, two remarks may be made: on one side, the co-efficients of correlation tend to be lower with highly purified water suggesting that non-viable cells were effectively removed by reverse osmosis module; on the other side, highly significant correlation observed between TVC and HPC in osmosed water accredits the fact that both methods are consistently assessing viable cells. Very interestingly, the solid phase cytometry method provides similar results than the conventional plate count method in <3 h when assessing reverse osmosis treated water. On a clinical perspective, this method appears to be a very appealing and promising method for rapid screening, following-up and validating water and dialysis fluid systems designed to produce ultrapure dialysis fluid for high-flux HD and on-line HDF.

This study has, however, some limitations that must be underlined and addressed in the future. Firstly, TAM used for plate count culture was not the poor nutrient recommended by guidelines (R2A, TGA). Secondly, due to the low number of endotoxin determination \( n = 22 \) in water samples, no attempt was performed to correlate bacteriometry (solid phase cytometry or plate count number) and endotoxin content. In two samples originated from the same dialysis facility, very high concentrations of endotoxin (>4 EU/mL) were found to be associated with very high levels of bacteria both by HPC and TVC by solid phase cytometry. This observation suggests that endotoxin content was positively correlated to the degree of contaminated dialysis water of viable cells.

In summary, solid phase cytometry revealed by epifluorescence microscopy offers a new and promising microbiological alternative for rapid and precise assessment (<3 h) of water and dialysis fluid microbial contamination. Although preliminary, specificity and sensitivity of results achieved in this study, provide a high degree of confidence to this new method, which deserves to be assessed more extensively in a multicentre trial including both water and dialysis fluid. At this stage of the discussion, the solid phase cytometry method offers a quite reliable, fast and very sensitive tool for assessing water purity in dialysis facilities being unfortunately restricted to some rare expert centres.

References


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