Ultrapure dialysis fluid slows loss of residual renal function in new dialysis patients

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Abstract

Background. Residual renal function is beneficial for adequacy of haemodialysis, quality of life and mortality in dialysis patients. Our prospective randomised investigation aimed to analyse the effects of the microbiological quality of dialysis fluid on the course of residual renal function after initiation of haemodialysis.

Methods. Thirty patients starting haemodialysis were randomly assigned to ultrapure or conventional dialysate. During the 24-month study period, creatinine clearance, CRP and IL-6 levels, hydration status, number of hypotensive episodes and blood pressure recordings were assessed every 6 months.

Results. Residual renal function declined in both groups during the study period, although there were no statistically significant differences in demographic (age, gender), renal (cause of end-stage renal disease, residual renal function, hypertension, ACE inhibitors) and treatment characteristics (K\textsubscript{t}/V urea) at recruitment. The use of mildly contaminated (up to 300 CFU/ml) dialysate resulted in higher CRP and IL-6 levels and more pronounced loss of residual renal function. Multiple regression analysis showed that the microbiological quality of the dialysate is an independent determinant of the loss of residual renal function.

Conclusions. Ultrapure dialysis fluid combined with high-flux synthetic membranes are effective components of renal replacement therapy to slow the loss of residual renal function in haemodialysis patients. These improvements of haemodialysis are desirable, but add to treatment costs.

Keywords: biocompatibility; residual renal function; ultrapure dialysate

Introduction

Residual renal function (RRF), defined as the urinary clearance of urea or creatinine, declines exponentially after initiation of dialysis therapy and vanishes in many end-stage renal disease (ESRD) patients undergoing non-transplant renal replacement therapy [1,2]. However, seemingly trivial amounts of RRF confer significant benefits to patients on maintenance haemodialysis. RRF has been shown to influence adequacy of haemodialysis, quality of life and mortality of haemodialysis patients [3].

There is substantial evidence that different modes of renal replacement therapy have an effect on RRF; for example, RRF is better preserved on continuous ambulatory peritoneal dialysis (CAPD) than on intermittent haemodialysis [4–6]. These differences between haemodialysis and CAPD are thought to be related to cardiovascular instability and to the inflammatory response induced by nephrotoxic inflammatory mediators from the use of bioincompatible components of the artificial kidney.

Some studies [7–11], but not all [5,12], have demonstrated that the potential of dialyzer membranes to activate the complement system and circulating white blood cells may affect the decline of RRF in patients commencing haemodialysis. Moreover, it has been shown that mild microbacterial contamination of the dialysate has a negative effect on anaemia, nutritional status [13,14] and AB amyloidosis [15–17].

Our prospective randomized investigation was carried out to analyse the impact of the microbiological quality of dialysis fluid on the rate of decline of residual renal function in a group of ESRD patients after dialysis initiation.

Subjects and methods

Patients with ESRD starting haemodialysis as their choice of renal replacement therapy were considered for participation in the investigations. The criteria for entering the study
were: (i) a creatinine clearance of $>5$ ml/min; (ii) no previous renal replacement therapy; (iii) no major comorbid conditions (congestive heart failure, chronic obstructive pulmonary disease, liver cirrhosis); and (iv) start of haemodialysis in the outpatient dialysis unit. Exclusion criteria were: (i) diabetes mellitus; (ii) rapidly progressive glomerulonephritis; (iii) chronic infection; (iv) inflammatory disorders; and (v) malignancy. During the study period, suitable patients were listed for renal transplantation and each patient had the chance to switch to peritoneal dialysis.

Thirty patients (16 males, 14 females, aged 26–78 years) were eligible. The diagnosis of chronic renal failure was based on history, laboratory tests, radiological signs and histology of renal biopsies. Chronic glomerulonephritis was diagnosed in 15 patients, chronic tubulo-interstitial nephritis in seven and polycystic kidney disease in eight patients. Hypertension was defined by pre-dialysis blood pressure recordings in excess of 140/90 mmHg. Repeated blood pressure recordings documented that hypertension was present in 19 patients at the start of the study. Eighteen hypertensive patients received angiotensin-converting enzyme (ACE) inhibitors at recruitment. During the study period, high blood pressure was reduced or normalized by ultrafiltration and a sodium-restricted diet in 12 patients, and the number of patients taking ACE inhibitors declined to six.

**Study design**

Eligible patients who had given their informed consent were randomly assigned either to a group treated with commercial (potentially microbiologically contaminated) dialysate or to a group treated with online (dialysis machine MTS 4008 H; Fresenius, Bad Homburg, Germany)—produced ultrapure dialysate by an additional step of ultrafiltration using high-flux polysulfone filters (Diasafe; Fresenius, Bad Homburg, Germany). After randomization, patients were dialysed with the same microbiological quality of the dialysate during all outpatient treatment sessions throughout the study period of 24 months. There were no other differences in haemodialysis treatment among the two patient groups. Regular haemodialysis was performed with volumetrically controlled ultrafiltration. Bicarbonate was used as buffer. Each treatment session lasted 3–5 h, with blood flow rates between 200 and 300 ml/min, and dialysate flow rates were set at 500 ml/min. All patients received single-use biocompatible synthetic high-flux membranes (APS 650, polysulfone; Asahi, Tokyo, Japan). Dialysis was prescribed and monitored using a single-pool kinetic model to ensure a dialysis treatment among the two patient groups. Regular haemodialysis was performed with volumetrically controlled ultrafiltration. Bicarbonate was used as buffer. Each treatment session lasted 3–5 h, with blood flow rates between 200 and 300 ml/min, and dialysate flow rates were set at 500 ml/min. All patients received single-use biocompatible synthetic high-flux membranes (APS 650, polysulfone; Asahi, Tokyo, Japan). Dialysis was prescribed and monitored using a single-pool kinetic model to ensure a dialysis dose (Kt/V dialysis) of at least 1.2 per dialysis for thrice weekly dialysis.

**Study parameters**

Microbiological tests of the dialysis fluid. Samples of standard dialysis fluid were analysed by the pour-plate method using thioglycolate and trypticase soy agars at 20 and 37°C for at least 5 days. Ultrafiltered dialysis fluid was filtered through a bacteria filter (0.22 mm) and the entire filter was placed on the agar plate. Bacterial growth in the dialysate was tested every 6 months in all probes. Determinations of the endotoxin concentrations were performed with the LAL assay (Coatest Endotoxin Chromogenix, Mölndal, Sweden) at the start, after 12 months and at the end of the investigations in six selected probes of each group. The detection limit of the test system was 0.03 EU/ml [14]. The standards for ultrapure dialysis fluid were 0.1 CFU/ml and <0.03 EU/ml.

**Renal function.** Residual creatinine clearance was calculated according to the standard formula. Urine collections were performed over a 67–69 h period following the preceding dialysis session. Blood samples for determination of serum creatinine were drawn at the end of the dialysis session and at start of the following session. Three clearances (1 week intervals) were measured every 6 months. The mean value of repeated measurements was used.

**Laboratory parameters**

Serum IL-6 concentrations were measured using a commercially obtained immunoassay (IL-6, Quantikine; R&D Systems, Abingdon, UK). Using this assay, the upper limit of normal for human serum IL-6 concentrations was 12.5 pg/ml. Serum C-reactive protein (CRP) levels were determined by particle-enhanced immunoturbidimetry (COBAS Integra 700; Roche, Mannheim, Germany). The expected values of serum CRP are <0.5 mg/dl for healthy adults. These markers of inflammation were measured at the start of the study, and after 6, 12 and 24 months.

**Clinical parameters**

During the 24-month study period, the attending nephrologists determined the post-dialytic dry weight by clinical acumen (pre- and post-dialysis blood pressure recordings, or other signs of hyper- and hypovolaemia). The diameter of the vena cava inferior was determined every 6 months. Over- and underhydration were defined by a post-dialysis vena cava diameter of $>12$ mm and $<8$ mm, respectively. The number of hypotensive episodes occurring during dialysis and necessitating therapeutic intervention was counted.

**Statistics**

The mean±standard deviation was calculated for each group. The significance of effects was tested using the analysis of variance (ANOVA). $P$ values $<0.05$ were considered significant. Multiple regression analyses were used to investigate the relationship between RRF and different independent variables, such as RRF at baseline, age and gender of the patients, aetiology of ESRD, blood pressure, use of ACE inhibitors and microbiological quality of the dialysate. Statistical analyses were performed with SAS software (SAS Institute, Cary, NC, USA).

**Results**

All 30 haemodialysis patients completed the 24-month study period. Four patients (two in each group) needed hospitalization for the treatment of pneumonia (two patients), thrombosis of the Cimino fistula or upper gastrointestinal bleeding (one patient each). Three patients underwent angiography and three patients had cardiac catheterization. Three patients received oral antibiotics for acute infections of the upper respiratory tract. However, neither the number of
patients treated with antibiotics nor that receiving radio contrast media differed markedly among the two groups. None of the patients developed sudden anuria or had significant restoration of RRF.

There were no statistically significant differences in age, gender, cause of renal disease, residual renal function (6 months prior to and at initiation of haemodialysis), hypertension, number of patients receiving ACE inhibitors, CRP levels and IL-6 levels among the two patient groups at the beginning of the study (Tables 1 and 2). Conventional dialysis fluid showed a wide range of bacterial growth (0–230 CFU/ml). Bacterial growth was 0 CFU/ml in 50% of the probes from conventional dialysate, 1–10 CFU/ml in 35%, 10–50 in 10% and >100 CFU/ml in 5% (n = 225).

Endotoxin concentrations in selected probes (n = 18) were not measurable in 15 patients, and ranged from 0.05 to 1.2 EU/ml in three patients. Only one patient had an endotoxin concentration of >0.25 EU/ml. None of the probes from ultrapure dialysate (n = 225) showed bacterial growth or had measurable endotoxin concentrations (n = 18).

Use of ultrapure dialysis fluid was associated with significantly lower CRP levels throughout the study period. CRP levels above the normal range were detected in only 7% of the patients treated with ultrapure dialysis fluid compared with 40% of the patients using conventional dialysis fluid. There was a statistically significant difference between the CRP levels of patients treated with online-produced ultrapure and conventional dialysate at any time during the study period. Moreover, patients with mildly contaminated dialysate had significantly elevated IL-6 levels (Table 2). The two groups of patients did not differ in the quality of blood pressure control or in their hydration status. There were no statistically significant differences in the percentage of the number of hypertensive episodes among groups (9% in the ultrapure group vs 10% in the conventional group).

RRF, as determined by remaining creatinine clearance and daily urine output, declined in both groups, but the decline after initiation of dialysis was faster in the group treated with standard dialysate (Table 3). When post-initiation residual creatinine clearance values were plotted on a semi-log scale, the half-life of RRF was 33 months for the ultrapure group and 16.5 months for the standard dialysate group.

Multiple regression analysis showed that the microbiological quality of dialysis fluid was an independent determinant of the loss of RRF in haemodialysis patients (Table 4).

### Discussion

Preserving residual renal function after initiation of haemodialysis is desirable for improving quality of life and dialysis efficiency. However, prospective evaluation and monitoring of potentially modifiable factors that protect RRF in patients who have just started haemodialysis have not received the same level of attention as preservation of renal function in patients with progressive mild or moderate chronic renal disease. Our prospective randomized comparison of contaminated and ultrapure dialysate demonstrated that even mild contamination of the dialysate resulted in a faster decline of RRF compared with ultrapure dialysate. The negative impact of the haemodialysis procedure on RRF could be lessened by improving the quality of this exchangeable component of extracorporeal renal replacement therapy.

The accelerated rate of decline of RRF in patients receiving conventional dialysate could not be explained by differences in demographic characteristics. The two groups of patients were well matched according to age, gender, aetiology of renal disease, severity of renal failure, quality of blood pressure control or number of patients receiving ACE inhibitors. Moreover, there were no statistically significant differences in other factors known to affect RRF, such as prescription of diuretics (number of patients and dose) or hydration status. In fact, multiple regression analysis showed that the quality of dialysate was an independent determinant for the preservation of RRF.

Multiple mechanisms, either patient-specific characteristics, uraemia-related factors or treatment-associated factors, induce chronic low-grade inflammation in haemodialysis patients [18]. The intensity of microinflammation, confirmed by elevated CRP or IL-6 levels, is correlated with the use of bioincompatible cellulosic membranes [19], as well as endotoxins derived from contaminated dialysate [20,21]. There is evidence, albeit not undisputed, that one major contributing factor is the biocompatibility of dialysis membranes, as the loss of RRF is accelerated by inflammatory nephrotoxic mediators generated by cellulosic bioincompatible membranes. Our notion that the decline of RRF is associated with inflammation is corroborated by independent findings in peritoneal dialysis patients. Chung and co-workers...
have shown that declining RRF was linked with systemic inflammation [22]. Moreover, in haemodialysis/haemodiafiltration patients, the use of ultrapure dialysate or substitute has been shown to preserve RRF as well as in CAPD patients [23]. The role of elevated cytokine levels in low-grade inflammation is supported by the finding that cytokines may cause glomerular sclerosis and tubular fibrosis, and their circulating levels or urinary concentrations may reflect the rate of decline in different progressive renal diseases [24–27].

It is obvious that we are unable to replace the benefits of RRF with enhanced haemodialysis, regardless of all our technological advancements of extracorporeal renal replacement therapy. Therefore, dialysis techniques should aim to reduce the systemic

<table>
<thead>
<tr>
<th>Months</th>
<th>0</th>
<th>6</th>
<th>12</th>
<th>24</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRP (mg/dl)</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Ultrapure</td>
<td>0.6 (0.3)</td>
<td>0.4 (0.2)</td>
<td>0.4 (0.2)</td>
<td>0.3 (0.2)</td>
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<td>Conventional</td>
<td>0.5 (0.3)</td>
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<td>0.8 (0.3)*</td>
<td>0.8 (0.4)*</td>
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<tr>
<td>IL 6 (pg/ml)</td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>Ultrapure</td>
<td>14 (4)</td>
<td>12 (4)</td>
<td>10 (3)</td>
<td>11 (4)</td>
</tr>
<tr>
<td>Conventional</td>
<td>16 (6)</td>
<td>36 (6)*</td>
<td>32 (12)*</td>
<td>38 (14)*</td>
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<tr>
<td>Blood pressure (mmHg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ultrapure</td>
<td>135/85 (15/8)</td>
<td>140/88 (9/6)</td>
<td>138/82 (11/9)</td>
<td>136/88 (8/4)</td>
</tr>
<tr>
<td>Conventional</td>
<td>130/82 (22/11)</td>
<td>132/82 (8/8)</td>
<td>142/87 (6/7)</td>
<td>143/85 (12/9)</td>
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<tr>
<td>Diameter of vena cava inferior (mm)</td>
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<td></td>
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<tr>
<td>Ultrapure</td>
<td>9.9 (1.2)</td>
<td>10.0 (0.9)</td>
<td>9.5 (1.4)</td>
<td>10.2 (0.9)</td>
</tr>
<tr>
<td>Conventional</td>
<td>10.0 (0.5)</td>
<td>9.4 (1.2)</td>
<td>10.0 (1.4)</td>
<td>9.8 (1.1)</td>
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<td>Kt/V</td>
<td></td>
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<tr>
<td>Ultrapure</td>
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<td>1.0 (0.2)</td>
<td>1.2 (0.1)</td>
<td>1.1 (0.1)</td>
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<tr>
<td>Conventional</td>
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<td>1.1 (0.1)</td>
<td>1.2 (0.1)</td>
<td>1.1 (0.1)</td>
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\*P < 0.05 vs corresponding value in the ultrapure group.

<table>
<thead>
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<th>6</th>
<th>12</th>
<th>24</th>
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<tbody>
<tr>
<td>Residual creatinine clearance (ml/min)</td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>Ultrapure</td>
<td>7.9 (2.0)</td>
<td>7.1 (1.6)</td>
<td>6.0 (1.6)</td>
<td>4.3 (1.8)</td>
</tr>
<tr>
<td>Conventional</td>
<td>7.9 (1.8)</td>
<td>5.4 (1.6)*</td>
<td>4.3 (1.5)*</td>
<td>2.5 (1.8)*</td>
</tr>
<tr>
<td>Daily urine volume</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ultrapure</td>
<td>2085 (375)</td>
<td>1890 (300)</td>
<td>1470 (300)</td>
<td>970 (330)</td>
</tr>
<tr>
<td>Conventional</td>
<td>2000 (280)</td>
<td>1540 (250)</td>
<td>1130 (260)</td>
<td>490 (180)</td>
</tr>
</tbody>
</table>

\*P < 0.05 vs corresponding value in patients treated with ultrapure dialysis fluid.

Table 4. Multivariate linear regression analysis of variables affecting RRF

<table>
<thead>
<tr>
<th></th>
<th>Not standardized coefficient B</th>
<th>Standardized coefficient beta</th>
<th>95% CI for B (lower limit)</th>
<th>95% CI for B (upper limit)</th>
<th>P value</th>
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<td>Constant</td>
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<td>-</td>
<td>2.451</td>
<td>8.567</td>
<td>0.001</td>
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<td>Dialysis fluid</td>
<td>-1.33</td>
<td>-0.450</td>
<td>-2.415</td>
<td>-0.251</td>
<td>0.018</td>
</tr>
<tr>
<td>Age</td>
<td>0.02</td>
<td>0.136</td>
<td>-0.025</td>
<td>0.056</td>
<td>0.443</td>
</tr>
<tr>
<td>Gender</td>
<td>-0.06</td>
<td>-0.211</td>
<td>-0.167</td>
<td>0.046</td>
<td>0.252</td>
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<tr>
<td>Cause of renal failure</td>
<td>-0.613</td>
<td>-0.337</td>
<td>-1.306</td>
<td>0.081</td>
<td>0.080</td>
</tr>
<tr>
<td>Blood pressure</td>
<td>0.06</td>
<td>0.021</td>
<td>-1.055</td>
<td>1.180</td>
<td>0.909</td>
</tr>
<tr>
<td>Use of ACE inhibitors</td>
<td>-0.04</td>
<td>-0.168</td>
<td>-0.152</td>
<td>0.072</td>
<td>0.465</td>
</tr>
<tr>
<td>Creatinine clearance at initiation of dialysis</td>
<td>-0.01</td>
<td>-0.035</td>
<td>-0.100</td>
<td>0.084</td>
<td>0.863</td>
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</table>
inflammatory response in patients with ESRD maintained on regular haemodialysis by using synthetic membranes and ultrapure dialysis fluid despite the additional treatment costs.

References


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