Long-term effects of high-efficiency on-line haemodiafiltration on uraemic toxicity. A multicentre prospective randomized study

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Abstract

Background. Haemodiafiltration (HDF) may improve survival of chronic dialysis patients. This prospective, multicentre randomized cross-over study evaluated the effects of long-term on-line HDF on the levels of solutes of different molecular weight markers or causative agents of the most common metabolic derangements in uraemia.

Methods. Sixty-nine patients from eight Italian centres were randomly assigned to two 6-month treatment sequences: A-B and B-A [A, low-flux haemodialysis (HD) and B, on-line HDF]. Comparative evaluation of basal levels of small, medium-sized and protein-bound solutes at the end of the two treatment periods and analysis of parameters dependence during the interventions were performed.

Results. On-line HDF showed greater efficiency than low-flux HD in removing small solutes (eKt/Vurea 1.60 ± 0.31 versus 1.44 ± 0.26, P < 0.0001) and in reducing basal levels of beta2-microglobulin (22.2 ± 7.8 versus 33.5 ± 11.8 mg/L, P < 0.0001), total homocysteine (15.4 ± 5.0 versus 18.7 ± 8.2 μmol/L, P = 0.003), phosphate (4.6 ± 1.3 versus 5.0 ± 1.4 mg/dL, P = 0.008) and, remarkably, of intact parathyroid hormone (202 ± 154 versus 228 ± 176 pg/mL, P = 0.03). Moreover, in on-line HDF, lower levels of C-reactive protein (5.5 ± 5.5 versus 6.7 ± 6.1 mg/L, P = 0.03) and triglycerides (148 ± 77 versus 167 ± 87 mg/dL, P = 0.008) and increased HDL cholesterol (49.2 ± 12.7 versus 44.7 ± 12.4 mg/dL, P = <0.0001) were observed. The asymmetric dimethylarginine level was not significantly affected (0.97 ± 0.4 versus 0.84 ± 0.37 μmol/L). Erythropoietin and phosphate binders’ doses could be reduced.

Conclusions. On-line high-efficiency HDF resulted in enhanced removal and lower basal levels of small, medium-sized and protein-bound solutes, which are markers or causative agents of uraemic pathologies, mainly inflammation, secondary hyperparathyroidism and dyslipidaemia. This may contribute to reducing uraemic complications and possibly to improving patient survival.

Keywords: beta2-microglobulin; haemodiafiltration; high-flux; homocysteine; parathyroid hormone

Introduction

Secondary analyses of the HEMO Study showed a reduced rate of cardiac death in patients treated with high-flux membranes, as well as longer survival in patients undergoing high-flux haemodialysis (HD) for >3.7 years [1]. Similarly, a primary analysis of the MPO study [2] showed improved survival of high-flux HD patients with albumin level ≤4 g/dL and of diabetic patients in a secondary analysis. On the other hand, large observational studies have shown a significant 35% reduction of death risk in patients on high-efficiency haemodiafiltration (HDF) compared both to low- and high-flux HD [3, 4]. While this advantage of HDF needs to be confirmed by prospective studies, a number of observations have shown that, compared to high-flux HD, convective techniques may enhance removal of compounds of different molecular weight which are
markers or causative agents of several uraemic pathologies [5-8]. Uraemic toxicity is generally attributed to the accumulation in the body of a constellation of recognized and unknown compounds [9] and a deeper knowledge of the effects of HDF therapy on their individual blood levels could help to shed light on the link between convective high-efficiency therapies and their favourable clinical results.

The aim of this prospective multicentre study was to evaluate to what extent high-efficiency on-line HDF, by enhancing convective removal of uraemic toxins to the highest levels offered by current technology, can influence the course of the most common complications of uraemia, such as anaemia, secondary hyperparathyroidism, inflammation, malnutrition and cardiovascular disease. The level of some small, middle molecular and protein-bound solutes involved in the pathogenesis or recognized as markers of such complications were compared at the end of two 6-month cross-over study periods during which on-line high-efficiency HDF and low-flux HD were evaluated in sequence. Low-flux HD was chosen as a control treatment in order to clearly differentiate the effects of a prevalent diffusive treatment (low-flux HD) from those of a convective one (HDF) performed at its highest possible efficiency. Synthetic highly biocompatible membranes and ultrapure dialysate/infusate were used in both treatment modalities to avoid confounding factors in the comparison of the effects of flux.

Subjects and methods

This was an open-label, prospective, multicentre, centrally randomized cross-over study designed to compare the effects of high-efficiency on-line HDF and low-flux HD on removal and basal levels of uraemic toxins in a wide molecular range. Primary end points were the levels of total homocysteine (tHCy), as a marker of uraemic protein-bound solutes and cardiovascular marker, and of beta2-microglobulin (ß2-m), as a marker of middle molecular uraemic toxins. Secondary end points were the levels of other markers/parameters of treatment adequacy (eKt/Vurea), calcium-phosphate metabolism, inflammation, anaemia, nutritional and cardiovascular status. The study was conducted in accordance with the basic principles of the Declaration of Helsinki and the rules of Good Clinical Practice and the protocol was approved by the ethic committees of all participating centres. EudraCT registry number for Protocol HD-OL-03-1: 2006-003347-23.

Patients

Eight Italian centres participated in the study. Inclusion criteria were age between 18—80 years, thrice weekly stable HD treatment for at least 3 months and native or prosthetic arteriovenous fistula with an effective blood flow (Qhmin; nominal blood flow corrected for the effect of the negative arterial pressure) > 300 mL/min. Exclusion criteria were malignancy with poor prognosis, congestive heart failure, acute myocardial infarction or stroke in the last 3 months and diabetes or lipid disorders treated pharmacologically. Established dropout causes were death, transplantation, consent withdrawal and clinical problems compromising adherence to the protocol or vascular access failure for more than six consecutive treatment sessions.

Patients who had given their written informed consent were randomized by central telephone into a 1:1 ratio to either low-flux HD (Group A) or online HDF (Group B) for a period of 6 months. Thereafter, the patients were switched to the other modality of therapy and followed for another 6 months. The total period of observation was 1 year and two sequences, A-B and B-A, were available for analysis.

Interventions

After randomization and before the start of the observation periods, patients were submitted to a 4-week run-in period, during which the first randomized treatment was performed and optimized. Qhmin was set to accomplish the protocol criteria. Dry body weight was assessed clinically and dialysate sodium concentration and temperature were arranged to prevent haemodynamic instability. Optimal acid-base and electrolyte balance of the sessions was established through individual adaptation of the dialysis bath composition. Drug prescription was managed to correct hypertension and long-term uraemic complications. Folic acid supplementation (5 mg thrice weekly) was given during the run-in period to prevent the effects of depletion on homocysteine levels. Only depleted patients were supplemented with iron, vitamin B12 and B6.

On-line HDF was the experimental intervention (Treatment B) and was compared to low-flux HD (Treatment A). The same Qhmin, dialysate surface, frequency and duration of the session (180—240 min thrice weekly) were maintained in each patient throughout both study periods. Treatments were performed with 400HS/US monitors (Fresenius Medical Care, FMC, Bad Homburg, Germany). Dialysers with low- and high-flux polysulphone membrane were used in Treatments A and B, respectively. Treatment characteristics of the experimental and control procedures are shown in Table 1. Ultrapure bicarbonate-buffered dialysis fluid was produced with water treated with a double reverse osmosis process and ultrafiltration across an endotoxin-absorbing polysulphone membrane (Diasafe; FMC). Absence of bacterial and endotoxin contamination was checked regularly and met the recommended standards [10]. Dialysate flow rate (Qd) was set at 500 mL/min in low-flux HD, while for on-line HDF, it resulted from the set value of 800 mL/min minus the amount diverted for substitution fluid production. This underwent a further ultrafiltration step before being infused at the post-dilution (post-HDF) or the pre-dilution site (pre-HDF) or simultaneously at both sites (mixed HDF). The highest possible and safe infusion/ultrafiltration rate was ensured during HDF sessions with the aid of a feedback control system described elsewhere [11, 12], which maintained the transmembrane pressure (TMP) within the optimal range of 250—300 mmHg.

Data collection and measurements

Blood samples were drawn at the beginning and the end of each observation period before the mid-week dialysis session, and the following parameters were measured: tHCy and asymmetric dimethylarginine (ADMA) with HPLC; ß2-m and albumin with nephelometry (Immage 800; Beckman Coulter); intact parathyroid hormone (i-PTH) with immune-chemiluminescence (Liaison PTH Assay;DiaSorin), folic acid and vitamin B12 plasma levels with chemiluminescence, CMIA method (Abbott); urea, creatinine and triglycerides with the enzymatic method (UniCel DxC Systems; Beckman Coulter); phosphate (P) with colorimetric method (UniCel DxC Systems; Beckman Coulter); total and ionized serum calcium with indirect potentiometry (GEM Systems, Instrumentation Laboratory and DxC Synchron; Beckman Coulter); haematocrit, haemoglobin and mean globular volume with a coulter counter (ADIVA 1210; Siemens); transferrin saturation and unsaturated iron binding capacity with colorimetric ferene reaction (UniCel DxC 800; Beckman Coulter); ferritin concentration with monoclonal LIA assay (Abbott); C-reactive protein (CRP) with turbidimetric method (UniCel DxC 800; Beckman Coulter); total cholesterol and its HDL and LDL fractions with kinetic colorimetry (DxC Synchron; Beckman Coulter).

Blood samples for urea were also collected at the end of dialysis with the ‘slow flow’ method, and eKt/Vurea and normalized protein catabolic rate (nPCR) were calculated with Daugirdas’s equations [13]. Pre- and post-dialysis plasma sodium, potassium and bicarbonate were measured with ion-selective electrodes (GEM Systems, Instrumentation Laboratory and DxC Synchron; Beckman Coulter). Residual renal function was calculated during the run-in period as an average of creatinine and urea clearance in patients with a daily urinary output > 500 mL.

A novel HPLC assay based on the use of naphthalene-2,3-dicarboxaldehyde as derivatizing agent [14] was used for ADMA because of its ability to better separate the stereoisomer symmetric dimethylarginine. As a consequence, this method has a lower range of normal concentration values (0.18—0.53 µmol/L) [14] than the common method using orthophthalaldehyde as derivative.

ADMA, tHCy, ß2-m, i-PTH, folic acid and vitamin B12 concentrations were determined on separated plasma samples stored at −20°C in a single central laboratory (Biochemistry Department, Bologna Hospital) certified against ISO-9001 Standards. All involved laboratories participated in the Regional Quality Assurance Programmes, which require and control strict adherence to the regulatory quality standards in a laboratory’s analytical process.

Patient characteristics, drug therapy and pre- and post-dialysis body weight and blood pressure at each session were collected and stored in a web database.
Table 1. Mean treatment and patient parameters of the two experimental proceduresa

<table>
<thead>
<tr>
<th>Dialyser membrane</th>
<th>Low-flux HD (n = 62)</th>
<th>On-line HDF (n = 62)</th>
<th>P-valueb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Material</td>
<td>Low-flux polysulphone</td>
<td>High-flux polysulphone</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>KUF, mL/h/mmHg</td>
<td>9.8 ± 1.7</td>
<td>58.3 ± 5.0</td>
<td></td>
</tr>
<tr>
<td>(range 1.6–2.4)</td>
<td>1.9 ± 0.3</td>
<td>2.0 ± 0.3</td>
<td>0.08</td>
</tr>
<tr>
<td>QBeff, mL/min</td>
<td>348 ± 37</td>
<td>346 ± 35</td>
<td>0.2</td>
</tr>
<tr>
<td>QTOT, mL/min</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Post-HDF (n = 46)</td>
<td>87 ± 17</td>
<td>164 ± 17</td>
<td></td>
</tr>
<tr>
<td>Pre-HDF (n = 3)</td>
<td>207 ± 26</td>
<td>207 ± 26</td>
<td></td>
</tr>
<tr>
<td>Mixed HDF (n = 13)</td>
<td>507 ± 45</td>
<td>616 ± 87</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Dialysate composition, mmol/L</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Na+</td>
<td>139.5 ± 3.0</td>
<td>139.9 ± 2.3</td>
<td>0.5</td>
</tr>
<tr>
<td>K+</td>
<td>2.5 ± 0.3</td>
<td>2.2 ± 0.3</td>
<td>0.002</td>
</tr>
<tr>
<td>Ca++</td>
<td>1.51 ± 0.13</td>
<td>1.50 ± 0.07</td>
<td>0.8</td>
</tr>
<tr>
<td>HCO3−</td>
<td>31.5 ± 1.6</td>
<td>31.4 ± 1.8</td>
<td>0.9</td>
</tr>
<tr>
<td>Mg++</td>
<td>0.6 ± 0.2</td>
<td>0.6 ± 0.2</td>
<td>0.8</td>
</tr>
<tr>
<td>Treatment time, min</td>
<td>227 ± 22</td>
<td>225 ± 24</td>
<td>0.6</td>
</tr>
<tr>
<td>Dry body weight, kg</td>
<td>65.3 ± 12.0</td>
<td>65.0 ± 11.9</td>
<td>0.2</td>
</tr>
<tr>
<td>Body weight loss, kg</td>
<td>2.3 ± 1.0</td>
<td>2.3 ± 1.1</td>
<td>0.9</td>
</tr>
</tbody>
</table>

aKUF, ultrafiltration coefficient of the dialyser. Other abbreviations and definition of the parameters are in the text (Methods section). Low-flux dialysers (treatment A): F7, F8 and F10 (FMC, Germany). High-flux dialysers (Treatment B): HF70S, HF80S, FX80, FX100 and HDF100S (FMC, Germany). Data are means ± SDs.
bStudent’s t-test for paired data (low-flux HD versus on-line HDF).

Statistical analysis

The expected reduction of tHcy concentration in the HDF group regarded as clinically significant was 10.0 μmol/L. The sample size required to detect the mentioned effect with a power of 0.9 was determined as suggested by Fleiss [15] for cross-over trials. The mean concentration evolution of the patients allocated to the on-line HDF-low-flux HD sequence (assumed to be 10.0 μmol/L) was compared with the reverse one (−10.0 μmol/L) by an independent sample ’t’ test. The standard deviation (SD) of tHcy concentration was assumed to be of 3.0 μmol/L, equal to the average value reported in previous similar studies [16–18]. The intrapatient correlation between tHcy concentrations in the two phases of the trial was prudentially assumed to be 0.1. This results in an intrapatient SD difference of 4.05 μmol/L. Based on the above assumptions, a total sample size of 56 patients was computed with the statistical package R (’power.t.test’ function) [19]. With a predicted dropout of 15%, equal to the yearly mortality rate of the population, the final required recruitment was 66 subjects.

The descriptive analysis was based on the mean and SD values for normally distributed continuous variables. A Mixed Effect Linear Model (MLM) [20] was fitted to each primary outcome measurements obtained at both study phases. The linear predictor always contained the patient as a random intercept and treatment type, study phase and treatment sequence as fixed effects. This last explanatory variable was tested to exclude carry-over effects. The following putative prognostic factors had been identified as fixed effects in each model. When statistically significant (p < 0.05), they were kept in the models. Their P-values along with the treatment P are reported in Table 3. The treatment comparisons’ P-values shown outside Table 3 originated from paired r-tests. Analysis was carried out using SPSS version 14.0 and R versions 2.3.1 and 2.10.1.

Results

Eighty patients were assessed for eligibility. Eleven of them did not meet all the inclusion criteria. Sixty-nine patients (48 men and 21 women) with a mean age of 59.6 ± 12.9 years (range 25–80 years) and mean dialytic vintage of 76 ± 73 months (range 6–375 months) entered the study. Ten patients had a mean residual renal clearance of 2.34 ± 1.21 mL/min. Sixty-two patients completed the study. Drop-outs (four transplantations and three vascular access failures) were not replaced. The total substitution volume during on-line HDF sessions was 19.7 ± 4.2 L in post-HDF, 37.7 ± 4.8 L in mixed HDF and 46.3 ± 7.6 L in pre-HDF.

Treatment adequacy

High-efficiency on-line HDF showed greater efficiency than low-flux HD in removing β2-m, as shown by the significant 33.7% lower basal levels at the end of the HDF period of observation (Table 2). A significant 18% reduction in the basal level of tHcy was also obtained with on-line HDF, irrespective of the effect of the pharmacological therapy, which ensured adequate plasma levels of folate and vitamin B12 from the beginning of the study in both experimental arms. Similarly, the efficiency in removing small solutes (eKt/Vurea) was significantly greater during the convective treatment. The MLM showed that the treatment modality exerted the most significant effect on the efficiency index and the concentration level of the studied solutes (Table 3). Blood flow, session time and vintage on dialysis exerted additional significant effects on eKt/Vurea, β2-m and tHcy levels, respectively.

Calcium–phosphate metabolism

Total serum calcium concentration remained unchanged throughout the two periods of observation, while pre-dialysis phosphate level, calcium × phosphate product
and intact PTH were significantly lower at the end of the HDF period as compared to HD (Table 2). The MLM showed that HDF treatment, session time and blood flow were the main factors influencing significant favourable changes of the above parameters (Table 3). The daily dose of the chelating agent sevelamer was significantly lower and that of vitamin D was not significantly different at the end of the HDF period as compared to HD (Table 4).

Table 2. Laboratory values and dialysis adequacy parameters at the end of the two observation periods

<table>
<thead>
<tr>
<th></th>
<th>Low-flux HD (n = 62)</th>
<th>On-line HDF (n = 62)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>eKt/Vurea</td>
<td>1.44 ± 0.26</td>
<td>1.60 ± 0.31</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>Urea, basal, mg/dL</td>
<td>143 ± 25</td>
<td>133 ± 23</td>
<td>0.004</td>
</tr>
<tr>
<td>End session, mg/dL</td>
<td>36 ± 12</td>
<td>29 ± 10</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Creatinine, mg/dL</td>
<td>9.8 ± 2.2</td>
<td>9.1 ± 2.2</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>β2-m, mg/L</td>
<td>33.5 ± 11.8</td>
<td>22.2 ± 7.8</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>tHcy, μmol/L</td>
<td>18.7 ± 8.2</td>
<td>15.4 ± 5.0</td>
<td>0.003*</td>
</tr>
<tr>
<td>ADMA, μmol/L</td>
<td>0.97 ± 0.40</td>
<td>0.84 ± 0.37</td>
<td>0.2</td>
</tr>
<tr>
<td>Calcium, mg/dL</td>
<td>9.6 ± 0.8</td>
<td>9.7 ± 0.7</td>
<td>0.3</td>
</tr>
<tr>
<td>Phosphate, mg/dL</td>
<td>5.0 ± 1.4</td>
<td>4.6 ± 1.3</td>
<td>0.008</td>
</tr>
<tr>
<td>Ca × P product</td>
<td>47.6 ± 13.1</td>
<td>44.4 ± 13.0</td>
<td>0.03*</td>
</tr>
<tr>
<td>iPTH, pg/mL</td>
<td>228 ± 177</td>
<td>203 ± 154</td>
<td>0.03*</td>
</tr>
<tr>
<td>Triglycerides, mg/dL</td>
<td>167 ± 87</td>
<td>148 ± 77</td>
<td>0.008*</td>
</tr>
<tr>
<td>Total cholesterol, mg/dL</td>
<td>175 ± 45</td>
<td>176 ± 45</td>
<td>0.9</td>
</tr>
<tr>
<td>HDL, mg/dL</td>
<td>47.4 ± 12.4</td>
<td>49.2 ± 12.7</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>LDL, mg/dL</td>
<td>100 ± 36</td>
<td>98 ± 36</td>
<td>0.5</td>
</tr>
<tr>
<td>Albumin, g/dL</td>
<td>4041 ± 391</td>
<td>3919 ± 393</td>
<td>0.004*</td>
</tr>
<tr>
<td>CRP, mg/L</td>
<td>6.65 ± 6.07</td>
<td>5.49 ± 5.46</td>
<td>0.03*</td>
</tr>
<tr>
<td>Sodium, mmol/L</td>
<td>138.3 ± 3.3</td>
<td>138.5 ± 3.6</td>
<td>0.4</td>
</tr>
<tr>
<td>Potassium, mmol/L</td>
<td>5.2 ± 0.6</td>
<td>5.2 ± 0.7</td>
<td>0.8</td>
</tr>
<tr>
<td>Bicarbonate, mmol/L</td>
<td>21.8 ± 2.1</td>
<td>21.7 ± 1.9</td>
<td>0.6</td>
</tr>
</tbody>
</table>

aData are means ± SDs. *P value from the MLM or from the Student’s t-test for paired data (low-flux HD versus on-line HDF). Significance at P < 0.05.

Table 3. Results of the mixed linear model

<table>
<thead>
<tr>
<th>Dependent variable</th>
<th>Explanatory variables</th>
<th>Estimate 95% CI</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>eKt/Vurea</td>
<td>Treatment (HD versus HDF)</td>
<td>−0.16 −0.03 −0.01</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>β2-m (mg/L)</td>
<td>Treatment (HD versus HDF)</td>
<td>11.2 8.4 14.0</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>tHcy (μmol/L)</td>
<td>Treatment (HD versus HDF)</td>
<td>3.29 1.17 5.41</td>
<td>0.003</td>
</tr>
<tr>
<td>Phosphate (mg/dL)</td>
<td>Treatment (HD versus HDF)</td>
<td>0.40 0.10 0.70</td>
<td>0.008</td>
</tr>
<tr>
<td>Ca × P</td>
<td>Treatment (HD versus HDF)</td>
<td>3.34 0.37 6.30</td>
<td>0.03</td>
</tr>
<tr>
<td>iPTH (pg/mL)</td>
<td>Treatment (HD versus HDF)</td>
<td>35.0 4.1 65.9</td>
<td>0.03</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td>Treatment (HD versus HDF)</td>
<td>19.4 5.3 33.6</td>
<td>0.008</td>
</tr>
<tr>
<td>HDL cholesterol (mg/dL)</td>
<td>Treatment (HD versus HDF)</td>
<td>−3.34 −5.87 −0.81</td>
<td>0.01</td>
</tr>
<tr>
<td>Albumin (g/dL)</td>
<td>Treatment (HD versus HDF)</td>
<td>0.13 0.04 0.21</td>
<td>0.004</td>
</tr>
<tr>
<td>C-reactive protein (mg/L)</td>
<td>Treatment (HD versus HDF)</td>
<td>1.14 0.09 2.20</td>
<td>0.03</td>
</tr>
</tbody>
</table>

*aThe following explanatory variables have been evaluated in the MLM: age, sex, vintage, session duration, blood and dialysate flow rates, infusion volume, membrane surface area, dry body weight, intradialytic weight loss and residual diuresis. Only those with significant effect on the outcome measurements have been reported. CI, confidence interval.

*P from the MLM. Significance at P < 0.05. For HDL cholesterol statistical test for carry-over effect was marginally significant (P = 0.035).
Table 4. Phosphate binders and 1-25 OH Vit D dose during the two observation periods\(^a\)

<table>
<thead>
<tr>
<th></th>
<th>Low-flux HD</th>
<th>On-line HDF</th>
<th>P-value(^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ca (acetate/carbonate), g/day</td>
<td>(n = 35)</td>
<td>(n = 34)</td>
<td></td>
</tr>
<tr>
<td>Al hydroxide, g/day</td>
<td>(2.54 \pm 1.36)</td>
<td>(2.67 \pm 1.45)</td>
<td>0.6</td>
</tr>
<tr>
<td>Sevelamer, mg/day</td>
<td>(n = 16)</td>
<td>(n = 16)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(2.98 \pm 1.99)</td>
<td>(3.10 \pm 1.97)</td>
<td>0.9</td>
</tr>
<tr>
<td>1-25 OH cholecalciferol, (\mu g/week)</td>
<td>(n = 38)</td>
<td>(n = 38)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(2.25 \pm 1.64)</td>
<td>(2.38 \pm 1.83)</td>
<td>0.8</td>
</tr>
</tbody>
</table>

\(^a\)Data are means ± SD of the periods.
\(^b\)Student’s t test for paired data (low-flux HD versus on-line HDF). Mean dialysate calcium concentration was 1.49 and 1.50 mmol/L in low-flux HD and on-line HDF, respectively.

**Anaemia**

The haemoglobin level did not differ at the end of the on-line HDF period versus low-flux HD (11.8 ± 1.1 versus 11.7 ± 1.2 g/dL, \(P = 0.5\)) nor did it show any significant change over the observation time. The same trend was observed for the mean red cell volume. Folic acid and vitamin B12 concentration remained stable largely beyond the lower recommended limits and iron transport and metabolism parameters (transferrin saturation and ferritin) ensured repletion of iron stores throughout both study periods. Notwithstanding this, the mean maintenance dose of erythropoietin was 13.2% lower at the end of the on-line HDF period (5510 ± 3671 versus, 6348 ± 3585 U/week, \(P = 0.04\)) with an erythropoietin resistance index not significantly lower than that on low-flux HD (7.69 ± 4.90 versus 8.34 ± 4.90 U/week/kg/g/dL).

**Inflammation and nutritional status**

Ultrapure dialysate was used in both experimental treatments. Plasma CRP achieved significantly lower values on HDF than on low-flux HD (Table 2), as a unique effect of treatment modality evidenced with the MLM analysis (Table 3). Plasma albumin was significantly lower after the HDF period (4.04 ± 0.4 versus 3.92 ± 0.4 g/dL, \(P = 0.004\)), as an effect of the technique elicited with the linear model in addition to that exerted by the treatment vintage. The difference seems not to be clinically relevant, also taking into account that the nPCR, taken as an estimate of nutritional status, was unaffected by the treatment modality (1.15 ± 0.19 versus 1.16 ± 0.19 on on-line HDF and low-flux HD, respectively).

**Cardiovascular parameters**

As shown in Table 2, the ADMA level was not significantly different at the end of the on-line HDF period versus low-flux HD. The triglycerides level was significantly lower and the HDL fraction of cholesterol was significantly higher as a result of on-line HDF as compared to low-flux HD. The treatment modality (on-line HDF versus low-flux HD) was the main explanatory variable elicited by MLM, with additional independent effects of blood flow rate, session duration and vintage (Table 3).

At the end of the HDF period, the mean pre-dialysis systolic and diastolic blood pressure was significantly lower than values of the low-flux HD period (systolic 140 ± 22 versus 147 ± 22 mmHg, \(P = 0.014\) and diastolic 75 ± 13 versus 80 ± 13 mmHg, \(P = 0.005\)), while end-session values did not differ significantly (systolic 138 ± 25 versus 138 ± 21 and diastolic 77 ± 14 versus 76 ± 13 mmHg). Two patients experienced symptomatic intradialytic instability (>1 hypotensive episode per week) during the HDF period versus five patients during the HD period. Dialysate composition in Na and Ca was similar between the two observation periods, as was serum Na concentration, taken as a surrogate of body sodium pool in the absence of significant body volumes changes throughout the study.

**Discussion**

In this study, a cross-over comparison between high-efficiency on-line HDF and low-flux HD was performed under matched operating conditions and with dialyser membranes of different permeability but with similar composition, surface and high biocompatibility. Low-flux HD was chosen as a control treatment in order to clearly differentiate the effects of a prevalently diffusive treatment (low-flux HD) from those of a convective one (HDF) performed at its highest possible efficiency. Purity of dialysis fluids was ensured systematically during the study. These conditions prevented the results of the comparison being influenced by factors other than membrane flux.

On-line high-efficiency HDF proved to be more efficient than low-flux HD in establishing lower basal levels of tHcy, independently of the effect of pharmacological therapy. When planning this study, tHcy was chosen for its dual role of marker of protein-bound solutes and cardiovascular risk predictor, even though the latter has subsequently been challenged in a recent meta-analysis [21]. The role of other protein-bound solutes, such as uraemic toxins, has been described over the last few years. Their accumulation in uraemic blood may exacerbate chronic inflammation, oxidative and carbonyl stress [22] and has been associated with increased death risk [23, 24]. Their different sites and degree (percentage) of binding may induce different kinetics and removal during extracorporeal treatment [6] but on-line HDF has been shown to remove some of these uraemic toxins [6]. The results of our prospective study revealed that, when compared to low-flux HD, on-line HDF was more able to establish significantly lower long-term basal levels of tHcy, a solute that is almost totally bound to albumin. While taking into account the limits outlined above, this result might be extended to some toxic solutes of similar binding properties and similar intradialytic kinetics. tHcy has been shown to be efficiently removed with HD when employing super-flux membranes [25, 26], but the application of these membranes is still unsuitable for routine dialysis treatment, due to their significant protein leakage.

In this study, on-line HDF was also shown to be more efficient in removing small solutes than low-flux HD and establishing lower levels of β2-m in the long term. β2-m is
not to lower the level of serum
dialysis patient outcomes and an excellent surrogate for
middle molecules [27]. In fact, its level was shown to pre-
dict mortality in dialysis patients of the HEMO Study [28] and the European Best Practice Guidelines [10] indicate
β2-m as a marker for middle molecules of similar weight. Our findings, in line with the results of a previous study in
which convective treatments were prospectively compared with low-flux HD [29], confirm the important role of on-
line HDF in removing this middle molecular solute, while high-flux HD, compared with low-flux HD in a large pro-
spective study [2], was only able to maintain constant but not to lower the level of serum β2-m.

Improved control of phosphate level was also achieved at the end of the HDF observation period. High phosphate
levels have been associated with increased death risk in HD patients [30, 31]. While similar results have been reported
in other studies [7, 31], to our knowledge this is the first time that a significant reduction of intact parathyroid hor-
mone levels has been reported in a prospective long-term randomized study, as a likely consequence of the efficiency
of HDF in keeping phosphate level under control and generally reducing uraemic toxicity. It is also of note that
this result was obtained with a lower dose of phosphate binders and that vitamin D prescription could be increased,
even if not significantly, with respect to the low-flux HD period thanks to the improved control of phosphate (and calcium) levels in several patients. As shown with the mixed linear analysis, higher blood flow rates and longer session times contributed significantly, along with the treat-
ment modality, to achieving the favourable effects on the efficiency index and the concentration level of the studied solutes.

On HDF, the actual dialysate flow rate as measured with the machine flow meter proved to be lower than that ex-
pected (800 mL/min minus the planned infusion), but it was certainly higher in HDF than in HD sessions. Although higher dialysate flow rates in HD were shown to increase removal of free and protein-bound small solutes [32, 33], statistical analysis with the mixed model did not show any significant effect of the dialysate flow tested as explana-
tory variable of each primary outcome measurement in our study. A possible explanation is that reduction of the
diffusion gradient due to the solute content of the ultrafil-
trate partly counterbalanced the effect of the increased dial-
ysate flow. On the other hand, convective solute transfer of
larger solutes mainly depends on the pressure gradient and membrane permeability and, as expected theoretically, it
was not significantly influenced by the dialysate flow rate.

Anaemia was satisfactorily controlled throughout both observation periods at similar vitamin intake and iron stores
replenishment, but with significantly lower erythropoietin dose during HDF, as also reported in another study [7]. A
minority of patients (15), equally distributed between the two arms of the study, took angiotensin convertin enzyme
inhibitors during the observation periods. According to Bonomini et al. [34], enhanced elimination by convection of
uraemic bone marrow inhibitors may explain this result. In addition, patients with the highest CRP levels were
shown to require significantly higher erythropoietin doses to achieve comparable haemoglobin levels [35]. Indeed,
significantly lower CRP levels were established in our study by on-line HDF at similar biocompatible conditions
with low-flux HD. This observation is in line with other studies which have shown that on-line HDF is able to re-
store some host mechanisms against different inflammatory stimuli and to promote a lower degree of chronic inflam-
mation than low-flux HD [36–38]. The lower albumin level found in our patients on HDF might not be in agreement
with the above observation, even if the difference between the techniques can hardly be retained as clinically relevant. Albumin is an inflammatory and nutritional marker. Its leakage during HDF has been shown to be trivial if very high TMP do not excessively stress the high-flux mem-
brane [12], but it may lead to depletion in the long term, if adequate protein intake is not ensured. However, nutri-
tional status was not affected in the HDF group, as shown by the protein catabolic rate values monitored throughout
the study periods.

The role of homocysteine as a cardiovascular risk factor has been reviewed elsewhere [21], and it could not be evalu-
ated in this study, during which no major cardiovas-
cular events occurred. Asymmetric dimethylarginine, a
powerful predictor of cardiovascular events and death in
End-stage renal disease and RRT patients [39], may be
removed during sessions of different dialysis modality
[40], but no treatment was shown to be effective in lower-
ing its blood level in the long term [29]. In this study, the
difference in ADMA levels between low-flux HD and on-
line HDF did not achieve statistical significance. On the
contrary, a significant reduction in triglyceride levels
and an increase in the HDL fraction of cholesterol were
observed in our HDF patients. To our knowledge, this is the
first time that additional benefits have been reported on
some lipid abnormalities as a result of HDF treatment.
High-flux HD with polysulphone membranes was shown to
have a favourable impact on uraemic dyslipidaemia [41,
42], as a possible result of their high biocompatibility in-
ducing a lower degree of inflammation and oxidative stress
[43] and/or removal by convection of circulating inhibitors
of lipoprotein lipase [44]. High-efficiency HDF may help
substantially in improving lipid profile by enhancing the
latter mechanism.

The effect of on-line HDF in reducing basal blood pres-
sure is difficult to explain in the absence of apparently
different hydroelectrolytic balances between the two treat-
ments. The temperature of the infusion solution might have
exerted some benefit in some of the few patients suffering
from haemodynamic instability [45].

In conclusion, medium–long-term application of on-line
high-efficiency HDF compared to low-flux HD in this
study resulted in enhanced removal and lower basal levels
of small, medium and protein-bound uraemic solutes, some
of which are retained as markers or causative agents of
several uraemic derangements, mainly inflammation, sec-
ondary hyperparathyroidism, dyslipidaemia and cardiovas-
cular disease. Probably, many of the benefits attributed to
HDF may also be obtained with high-flux HD, but the
findings of this study about the effects of on-line HDF on
the levels of different uraemic makers and compounds,
including PTH and lipids, add knowledge to the growing
information now available about the greater efficiency of
high-efficiency convective treatments in solute removal, which potentially results in a general reduction of the uraemic toxicity. This might be the link with the clinical benefits reported in patients undergoing chronic HDF and eventually contribute to improving their survival, as suggested by published observational studies [3] to be prospectively confirmed.

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