Effective removal of protein-bound uraemic solutes by different convective strategies: a prospective trial

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Abstract

Background. Although different on-line convective removal strategies are available, there are no studies comparing the efficiency of solute removal for the three main options [post-dilution haemodiafiltration (post-HDF), pre-dilution haemodiafiltration (pre-HDF) and pre-dilution haemofiltration (pre-HF)] in parallel.

Methods. In this study, we compared post-HDF (Polyflux 170), pre-HDF (Polyflux 170) and pre-HF (Polyflux 210) in 14 patients. Parallelism of the evaluation protocols consisted in applying the same blood flow, dialysis time and effective convection (22.9 ± 1.7 versus 22.2 ± 2.0 L, P = NS) in pre-HDF versus post-HDF, and the same blood flow and dialysis time while comparing pre-HDF and pre-HF (1:1 dilution). With pre-HF, ultrafiltration was maximized and resulted in an effective convective volume of 28.5 L. We studied water-soluble compounds (urea, creatinine, uric acid), protein-bound compounds (hippuric acid, indole acetic acid, indoxylsulfate and p-cresylsulfate) and β2-microglobulin (β2M).

Results. Post-HDF was superior to pre-HDF for water-soluble compounds and β2M, whereas there was no difference for protein-bound compounds. Pre-HDF was superior to pre-HF for water-soluble compounds and protein-bound compounds. In contrast, removal of β2M for pre-HF was higher than for pre-HDF, but it did not differ from that obtained with post-HDF.

Conclusions. It is concluded that post-dilution is superior to pre-dilution HDF under conditions of similar convective volume, and that HDF is superior to HF in pre-dilution, with the exception of removal of β2M. Overall, post-HDF is the most efficient convective strategy among those tested.

Keywords: haemodiafiltration; haemofiltration; post-dilution; pre-dilution; uraemic toxins

Introduction

Secondary analyses of controlled trials show a survival advantage for high-flux dialysis membranes when used in a mainly diffusional haemodialysis (HD) mode [1–4]. Even the more substantial removal of larger molecules can be obtained by applying convective transport, pursuing additional ultrafiltration through highly permeable membranes and replacing the excess ultrafiltrate by equiluominous amounts of substitution fluid in therapies referred to as haemodiafiltration (HDF), which is a combination of diffusion and convection or haemofiltration (HF), which is a purely convective strategy. For both therapies the substitution fluid can be administered either in post-dilution at the dialyzer outlet or in pre-dilution, referring to substitution at the inlet [5]. However, post-dilution HF is no longer practiced in chronic therapy due to the limited small solute removal [6].

Although clinical benefits with convective strategies have been demonstrated in smaller controlled trials [7,8], improved survival has as yet been shown only in observational studies [9,10]. However, several large randomized studies are pending [11–13].

In view of all these data linking removal of ‘difficult to remove’ molecules to outcome, an essential task is to optimize this removal within the reach of available technical possibilities. To the best of our knowledge, however, currently available information does not allow the user to make a proper strategic choice between existing treatment options.

With the aim of optimizing the solute removal, the following questions remain largely unsolved: (1) Is there a difference between pre-dilution and post-dilution HDF, if convective volume is the same? (2) Is it sufficient to achieve maximum convection or should it be combined with diffusion? (3) What is the optimal removal strategy for protein-bound compounds? (4) Finally, which of the above strategies is the most efficient in the overall removal of uraemic retention solutes in a broad molecular weight range?

In the present study, several aspects related to adequacy of removal are evaluated in a parallel protocol, allowing comparison of three convective approaches widely...
practised today [post-dilution HDF (post-HDF), pre-dilution HDF (pre-HDF) and pre-dilution HF (pre-HF)].

Subjects and methods

Patients
Seventeen patients were selected for the study. Three patients withdrew from the study due to transplantation (n = 2) and lack of compliance (n = 1). Fourteen stable, adult age 5 kidney disease patients (7 males, 7 females, mean age 63.5 ± 17.7 years), who had been on three times weekly HD for at least 6 months and who had a permanent blood access capable of delivering a blood flow rate of at least 300 mL/min were included in the study. Mean dialysis duration was 30.2 ± 36.0 months. The primary renal diagnoses were diabetic nephropathy (n = 3), chronic interstitial kidney disease (n = 3), IgA nephropathy (n = 2), chronic glomerulonephritis (n = 2), renal vascular disease (n = 2), renal cortical necrosis (post-partum) (n = 1) and cause unknown (n = 1). Exclusion criteria were active infectious diseases, chronic inflammatory condition, pregnancy, expected interdialytic body weight gain ≥ 4 kg, expected survival < 1 year and expected kidney transplantation within 1 year.

Study design
Within the scope of a study evaluating the evolution of the pre-treatment concentration of several uraemic retention solutes, patients were treated with post-HDF during 9 weeks. In the fourth week of this 9-week post-HDF treatment period, blood samples were collected during a midweek session for evaluation of dialysis adequacy parameters during that single session (see below). At the occasion of the midweek session of week 5 and week 9 of the same period, post-HDF was switched once to either pre-HDF or pre-HF. The sequence of these two treatments was defined on a random basis. Similar to the procedure followed at week 4, blood samples were collected as well during these sessions at weeks 5 and 9 (see below).

The study was approved by the local Ethics Committee and conducted in accordance with the principles of the Declaration of Helsinki and rules of Good Clinical Practice. Written informed consent was obtained from all participants. The trial was registered in a public trials registry (www.clinicaltrials.gov).

Systems and treatment strategies
All treatments were performed with AK200 ULTRA S (Gambro, Lund, Sweden) dialysis machines that provide on-line ultrapure dialysis fluid and sterile, non-pyrogenic substitution fluid. In our unit, water quality is checked to ensure that it is free of endotoxins (LAL test) and that it meets the standards of microbial purity recommended by the European Best Practice Guidelines [14].

High-flux, synthetic, steam-sterilized Polyflux filters (Gambro, Lund, Sweden) were used for all treatments: Polyflux 170 (1.7 m²) for HDF treatments and Polyflux 210 (2.1 m²) for the HF treatments. All treatments were performed in each patient using the same blood flow rate (Q_b), dialysis treatment time (T_d) and dialysate/infusate composition. These parameters were set on an individual basis according to the patient’s regular treatment protocol.

For HDF treatments dialysis fluid serves both for dialysis and for substitution. The HDF treatments were performed in volume control mode with the target set on achieving an infusion flow rate (Q_inf) corresponding to ~25% of the Q_b in the post-dilution mode and 50% in the pre-dilution mode. According to our calculations this results in treatments with comparable convective volumes. When operating in volume control mode, the transmembrane pressure (TMP) is adjusted during the treatment in order to achieve the set ultrafiltration and infusion flow rates.

The HF treatments were performed in pressure control mode (TMP 200 mmHg) so that the dilution ratio between blood and fluid should be 1, i.e. Q_inf = Q_b. When operating in pressure control mode the Q_inf varies according to the ultrafiltration achieved at the selected pressure, the set TMP.

During the treatment, there was no use of predetermined profiles to modulate any parameter.

Treatment characteristics are noted in Table 1.

Sample collection and laboratory analysis
Samples were taken from the inlet dialyzer blood line at times 0 (pre-dialysis), 30, 60, 120 and 240 min and from the outlet blood line at times 30, 60, 120 and 240 min for all dialysis sessions even if the treatment duration was >240 min. Samples (20 mL) from the dialysate and/or ultrafiltrate outflow were collected at 60 min. In the pre-dilution set-up the inlet blood samples were drawn upstream from the section of the circuit where the dilution with replacement fluid took place. In the post-dilution mode, outlet blood samples were collected downstream from the section of the circuit where the substitution with replacement fluid took place; collections were sampled from an additional blood line access, which was placed directly on the dialyzer outflow tract immediately ahead of the venous fistula needle.

<table>
<thead>
<tr>
<th>Post-HDF</th>
<th>Pre-HDF</th>
<th>Pre-HF</th>
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<tbody>
<tr>
<td>Blood flow, Q_b (mL/min)</td>
<td>311 ± 17</td>
<td>315 ± 15</td>
</tr>
<tr>
<td>Treatment duration, T_d (min)</td>
<td>248 ± 13</td>
<td>249 ± 13</td>
</tr>
<tr>
<td>Weight loss (L)</td>
<td>2.1 ± 0.7</td>
<td>2.4 ± 1.0</td>
</tr>
<tr>
<td>Infusion volume (L)</td>
<td>20.1 ± 1.7</td>
<td>41.0 ± 3.6∗∗§§</td>
</tr>
<tr>
<td>Total UF volume (L)</td>
<td>22.2 ± 2.0</td>
<td>43.4 ± 3.8∗∗§§</td>
</tr>
<tr>
<td>Dilution factor</td>
<td>0.53 ± 0.02</td>
<td>0.38 ± 0.03§§</td>
</tr>
<tr>
<td>Effective UF volume (L)</td>
<td>22.2 ± 2.0</td>
<td>22.9 ± 1.7</td>
</tr>
<tr>
<td>Ultrafiltration flow, Q_UF (L/min)</td>
<td>89 ± 5</td>
<td>174 ± 8∗∗§§</td>
</tr>
<tr>
<td>Infusion flow, Q_inf (L/min)</td>
<td>81 ± 4</td>
<td>165 ± 8∗∗</td>
</tr>
<tr>
<td>Diallyte flow, Q_d (L/min)</td>
<td>619 ± 4</td>
<td>535 ± 8∗∗</td>
</tr>
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</table>

Pre-HDF versus post-HDF: ∗P < 0.017, ∗∗P < 0.001; pre-HF versus post-HDF: ∗P < 0.017, ∗∗P < 0.001; pre-HF versus pre-HDF: ∗P < 0.017, ∗∗P < 0.001.
After collection, blood and dialysate samples were immediately placed on ice. Blood samples were centrifuged (3000 rpm corresponds with 1250 g) and all samples were stored at −80°C until analysis.

Creatinine (MW: 113.1 Da) and urea (MW: 60.1 Da) were measured by standard laboratory methods. Analysis of uric acid (MW: 168.1 Da) was performed by RP HPLC with UV detection at 254 nm.

β2-microglobulin (β2M) (12 kDa) concentrations were quantified using a sandwich ELISA kit from Oygentec Diagnostika GmbH (Mainz, Germany) according to the manufacturer’s guidelines. Samples were analysed using the EL808 Ultra Microplate Reader from Bio-Tek Instruments (Winooski, VT, USA) by the KC4 V3.0 Analysis software.

To establish the total concentration of hippuric acid [MW: 179.2 Da, protein binding (PB): ± 50%], indole-3-acetic acid (MW: 175.2 Da, PB: ± 65%), indoxylsulfate (MW: 212.1 Da, PB: ± 90%), p-cresylsulfate (MW: 187.2 Da, PB: ± 95%) and 3-carboxyl-4-methyl-5-propyl-2-furanpropionic acid (CMPF) (MW: 240 Da, PB: ± 100%), serum and dialysate samples were deproteinized by heat denaturation and were analysed by reverse-phase high-performance liquid chromatography (RP-HPLC). Indoxylsulfate and indole-3-acetic acid (λ_ex 280 nm; λ_em 340 nm) and p-cresylsulfate (λ_ex 265 nm; λ_em 290 nm) were determined by fluorescence analysis, and hippuric acid and CMPF were analysed by UV detection at 254 nm [15].

The serum total protein (TP) content was analysed according to standard methods.

Calculations

In order to have comparable data on the degree of convection, effective UF volume was calculated from total UF volume (Table 1) corrected for dilution in the pre-dilution modes. The dilution factor (DF) for pre-HDF and pre-HF was calculated by the formula of Locatelli et al. [16]: DF = Qpw/(Qpw + Qout), which is based on the dilution of plasma water (PW).

Effective UF volume = DF × total UF volume.

Instantaneous dialyzer clearances were calculated by the formula of Ouseph et al. [17]: K = QBE × (CPi − Cpw)/Cpi + QUF × Cpw/Cpi = (Qp + QUF) × Cpw/Cpi, where QBE is the effective blood flow rate, QUF is the instantaneous ultrafiltration rate, CPi and Cpw are the concentrations at the inlet and outlet of the dialyzer, respectively, and Cpw is the outlet dialysate concentration. QBE was assumed to be the blood water flow rate (Qbw) for urea and the PW flow rate (Qpw) for β2M. The Qbw and Qpw were calculated from Qbw = 0.93 × [1 − Hct + 0.86 × Hct] × Qp and Qpw = 0.93 × [1 − Hct] × Qbw, where Qp is the blood flow rate. Hct is the fractional haematocrit and the constants 0.93 and 0.86 correct for the protein content of PW and the fractional water content of the red blood cells, respectively. Clearances of the protein-bound solutes, creatinine and uric acid were calculated using the dialysate concentration and flow rate to avoid the uncertainty associated with any plasma water–red blood cell disequilibrium that developed as blood transited the dialyzer. Clearances were calculated at 60 min because this allowed studying serum and dialysate in equilibrated status with concentrations in dialysate that were still sufficiently high for reliable measurements.

Reduction rates (RR) were calculated according to the formula RR (%) = (C0 − C240c)/C0 × 100. For all the protein-bound compounds and β2M, concentration at 240 min (C240c) was corrected for haemoconcentration by a correction factor (CF) based on TP concentration at start versus that at 240 min: CF = TP0/TP240 [18].

Statistics

Statistical evaluation was performed with the SPSS statistical package 12.0 (SPSS Inc., Chicago, IL, USA). Data are shown as mean ± standard deviation. Differences between post-HDF, pre-HDF and pre-HF were analysed with the Friedman test followed when necessary (two-sided P-value of < 0.05 was considered as statistically significant) by the Wilcoxon test including Bonferroni correction (which means an alfa level of 0.05/3 < 0.017 to accept significance) for multiple comparisons.

Results

Comparison of pre-HDF versus post-HDF

As listed in Table 1, there were no significant differences in blood flow rate (Qp), dialysis treatment time (T_d), weight loss and effective UF volume. The latter results in a similar convective volume.

In Figure 1, the evolution of the concentration of the small water-soluble compounds at the inlet and outlet of the dialyzer is illustrated. The start concentration (0 min) was the same for all compounds. Only for uric acid was the inlet concentration at 120 min and 240 min lower with post-HDF. The outlet concentration over the entire time span (30–240 min) was lower with post-HDF for all compounds.

In Figure 2, the evolution of the middle molecule, β2M, is presented. Although the start concentration was lower for pre-HDF than for post-HDF, the inlet concentration at 240 min and the outlet concentrations (30–240 min) were lower with post-HDF.

Figure 3 illustrates the evolution of the protein-bound compounds. The inlet and outlet concentrations at different time points were similar for all compounds except for CMPF, where at 30 min (outlet) and 60 min (inlet) the concentrations with pre-HDF were significantly lower than with post-HDF. Only for CMPF did the start concentration differ such that pre-HDF.

Table 2 includes the instantaneous clearances at 60 min. Clearance values were higher for post-HDF for all water-soluble compounds and β2M. For the protein-bound solutes only the clearance of hippuric acid was higher with post-HDF. For CMPF, however, we could not calculate clearances because only the free fraction can pass through the dialyzer into the dialysate. Since CMPF has a protein binding of almost 100%, the CMPF concentration in dialysate was below the detection limit.

In Table 3, the RR (%) at 240-min treatment, which is the percentage reduction of concentration at 240 min versus pre-dialysis concentration, are noted. For the water-soluble compounds, only the RR of urea was higher with post-HDF. The RR of β2M was higher as well with post-HDF.
Effective removal of protein-bound uraemic solutes by different convective strategies

Fig. 1. Evolution of the inlet and outlet concentration of the small water-soluble compounds at different time points (0, 30, 60, 120 and 240 min). Post-dilution haemodiafiltration data are illustrated by white bars, pre-dilution haemodiafiltration data by grey bars and pre-dilution haemofiltration by black bars. *Pre-dilution haemodiafiltration versus post-dilution haemodiafiltration, † pre-dilution haemofiltration versus post-dilution haemodiafiltration, ‡ pre-dilution haemofiltration versus pre-dilution haemodiafiltration; 1 symbol $P < 0.017$, 2 symbols $P < 0.001$.

Fig. 2. Evolution of the inlet and outlet concentration of middle molecule at different time points (0, 30, 60, 120 and 240 min). Post-dilution haemodiafiltration data are illustrated by white bars, pre-dilution haemodiafiltration data by grey bars and pre-dilution haemofiltration by black bars. *Pre-dilution haemodiafiltration versus post-dilution haemodiafiltration, † pre-dilution haemofiltration versus post-dilution haemodiafiltration, ‡ pre-dilution haemofiltration versus pre-dilution haemodiafiltration; 1 symbol $P < 0.017$, 2 symbols $P < 0.001$. 
Fig. 3. Evolution of the inlet and outlet concentration of protein-bound compounds at different time points (0, 30, 60, 120 and 240 min). Post-dilution haemodiafiltration data are illustrated by white bars, pre-dilution haemodiafiltration data by grey bars and pre-dilution haemofiltration by black bars. *Pre-dilution haemodiafiltration versus post-dilution haemodiafiltration, †pre-dilution haemofiltration versus post-dilution haemofiltration, ‡pre-dilution haemofiltration versus pre-dilution haemodiafiltration; 1 symbol $P < 0.017$, 2 symbols $P < 0.001$. 
The protein-bound compounds showed no differences in RR.
In summary, post-HDF does not differ from pre-HDF for protein-bound molecules whereas it is superior for water-soluble compounds and $\beta_2$M.

**Comparison of pre-HF versus pre-HDF**

$Q_b$, $T_d$ and weight loss (Table 1) were the same for both treatments. Effective UF volume, i.e. convective volume, was ~25% higher in pre-HF treatment compared with pre-HDF.

The start value of the water-soluble compounds (Figure 1) was similar. From the 30th min on, the inlet and outlet concentrations were all lower with pre-HF except for uric acid, for which the concentration at the inlet was only lower from 60 min on.

There was no difference in $\beta_2$M start concentration between pre-HF and pre-HDF (Figure 2). In contrast with the water-soluble compounds the inlet (120 and 240 min) and outlet (30–240 min) concentrations were lower with pre-HDF than with pre-HDF.

The start concentration of the protein-bound compounds (Figure 3) was the same. The inlet concentrations were not different except for hippuric acid, where the 120-min and 240-min concentrations were lower with pre-HDF. The outlet concentrations from 30 min to 240 min of hippuric acid were also lower with pre-HDF. For the other compounds only the outlet concentrations at 120 min and 240 min of $p$-cresylsulfate and indoxylsulfate were lower with pre-HDF.

The clearance values (Table 2) were significantly higher with pre-HDF; for $\beta_2$M, however, clearance was higher with pre-HF.

In agreement with the clearance data, the RR data (Table 3) were also higher with pre-HDF with the exception of CMPF; again, for $\beta_2$M, clearance was higher with pre-HF.

In summary, pre-HDF provides significantly more removal of small solutes as well as of several protein-bound solutes than pre-HF in spite of the lower convective removal. The exception is $\beta_2$M, which is removed better with pre-HF.

**Table 2. Instantaneous clearance (mL/min) at 60 min**

<table>
<thead>
<tr>
<th></th>
<th>Post-HDF</th>
<th>Pre-HDF</th>
<th>Pre-HF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urea</td>
<td>243.0 ± 18.7</td>
<td>230.1 ± 10.5*</td>
<td>150.7 ± 15.0*§§</td>
</tr>
<tr>
<td>Creatinine</td>
<td>179.4 ± 48.3</td>
<td>148.9 ± 22.3*</td>
<td>103.7 ± 19.9*§§</td>
</tr>
<tr>
<td>Uric acid</td>
<td>166.4 ± 14.1</td>
<td>153.4 ± 9.8*</td>
<td>104.8 ± 8.9*§§</td>
</tr>
<tr>
<td>$\beta_2$M</td>
<td>82.8 ± 16.1</td>
<td>67.2 ± 18.5*</td>
<td>87.5 ± 9.6*§§</td>
</tr>
<tr>
<td>Hippuric acid</td>
<td>131.2 ± 15.6</td>
<td>121.4 ± 13.1*</td>
<td>68.7 ± 23.9*§§</td>
</tr>
<tr>
<td>Indole acetic acid</td>
<td>66.6 ± 8.6</td>
<td>67.5 ± 9.3</td>
<td>38.8 ± 5.4*§§</td>
</tr>
<tr>
<td>Indoxylsulfate</td>
<td>33.4 ± 7.4</td>
<td>34.7 ± 9.9</td>
<td>18.7 ± 6.6*§§</td>
</tr>
<tr>
<td>$p$-Cresylsulfate</td>
<td>23.5 ± 4.6</td>
<td>24.6 ± 6.4</td>
<td>12.9 ± 2.5*§§</td>
</tr>
</tbody>
</table>

Pre-HDF versus post-HDF: *P < 0.017, **P < 0.001; pre-HF versus post-HDF: *P < 0.017, **P < 0.001; pre-HF versus pre-HDF: †P < 0.017, ‡P < 0.001.

Pre-HDF versus post-HDF: *P < 0.017, **P < 0.001; pre-HF versus post-HDF: *P < 0.017, †P < 0.001; pre-HF versus pre-HDF: †P < 0.017, ‡P < 0.001.

¢240-min treatment concentrations were corrected for haemoconcentration.

**Table 3. Reduction ratio (%) at 240 min dialysis**

<table>
<thead>
<tr>
<th></th>
<th>Post-HDF</th>
<th>Pre-HDF</th>
<th>Pre-HF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urea</td>
<td>80.3 ± 3.9</td>
<td>77.3 ± 3.9*</td>
<td>64.2 ± 4.3*§§</td>
</tr>
<tr>
<td>Creatinine</td>
<td>73.0 ± 4.9</td>
<td>70.2 ± 5.2</td>
<td>60.2 ± 4.4*§§</td>
</tr>
<tr>
<td>Uric acid</td>
<td>80.6 ± 3.5</td>
<td>78.9 ± 2.6</td>
<td>70.2 ± 3.6*§§</td>
</tr>
<tr>
<td>$\beta_2$M</td>
<td>77.8 ± 4.4</td>
<td>67.2 ± 8.8**</td>
<td>76.2 ± 6.8*§§</td>
</tr>
<tr>
<td>Hippuric acid¢</td>
<td>73.6 ± 9.5</td>
<td>74.3 ± 10.5</td>
<td>61.1 ± 11.7*§§</td>
</tr>
<tr>
<td>Indole acetic acid¢</td>
<td>48.0 ± 9.6</td>
<td>50.4 ± 11.6</td>
<td>41.6 ± 8.1*§§</td>
</tr>
<tr>
<td>Indoxylsulfate¢</td>
<td>44.8 ± 12.1</td>
<td>48.5 ± 10.0</td>
<td>33.8 ± 9.9*§§</td>
</tr>
<tr>
<td>$p$-Cresylsulfate¢</td>
<td>40.0 ± 8.8</td>
<td>41.9 ± 6.3</td>
<td>30.6 ± 7.3*§§</td>
</tr>
<tr>
<td>CMPFa</td>
<td>7.1 ± 5.7</td>
<td>5.9 ± 6.7</td>
<td>4.0 ± 7.8</td>
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</table>

Pre-HDF versus post-HDF: *P < 0.017, **P < 0.001; pre-HF versus post-HDF: *P < 0.017, †P < 0.001; pre-HF versus pre-HDF: †P < 0.017, ‡P < 0.001.

Discussion

This study evaluates the removal of uraemic solutes by different convective strategies under parallel conditions. On one hand, pre-HDF is compared to post-HDF with identical $Q_b$, $T_d$ and convective volume. On the other hand, pre-HF is compared to pre-HDF, with the same $Q_b$ and $T_d$, but for HF, convective volume is maximized. Essentially, the following
questions are raised: (1) Is there a difference between pre-dilution and post-dilution if the amount of convection is the same? (2) Does convection alone suffice or should diffusion be added?

We evaluated the removal of uraemic toxins based on three parameters: the evolution of the concentration at the inlet and outlet of the dialyzer, the reduction ratio after 240-min treatment and the instantaneous clearances at 60 min.

The main findings are: (1) for the protein-bound molecules, post-HDF and pre-HDF are equal when applied with the same convective volume; compared to pre-HF, both HDF modes are superior, except for CMPF; (2) for the small water-soluble compounds, post-HDF is superior to both pre-HDF and pre-HF and pre-HDF is superior to pre-HF, both pre-HDF and pre-HF are again superior to pre-HDF; be it markedly more pronounced than for the small water-soluble compounds; compared to pre-HF, pre-HDF is less efficient, but comparing pre-HF to post-HDF, there is no difference, in spite of the larger convective removal in pre-HF; and (4) overall, our data suggest that under comparable conditions, post-HDF is the most efficient removal approach.

When comparing different convective therapies, it is important to consider the amount of convective volume. The recent analysis of DOPPS data for patients treated by HDF showed that only treatments in which UF volume exceeded 15 L were associated with improved survival [9]. Hence, in a comparison of convective strategies, the effective ultrafiltration volume, i.e. total ultrafiltration corrected for dilution effects, is the parameter that is most representative to quantify convective transport.

Although up to now a number of studies compared solute removal in convective strategies (Table 4), only one evaluated a protein-bound solute. Bammens et al. compared high-flux HD with post-HDF and pre-HDF, the latter at low and at high substitution volumes. The study, in contrast to ours, did not include an evaluation of pre-HF. The convective removal of only one protein-bound solute, p-cresol, was evaluated [19]. While the intra-dialytic behaviour of p-cresol has been the subject of several studies [15,18–23], more recent data show that under the uraemic condition, not p-cresol, but its conjugates p-cresylsulfate and p-cresylglucuronide are present [24,25]. For that matter, in this present study we concentrate on p-cresylsulfate, which recently was shown to have a pro-inflammatory effect [26]. In addition, our study evaluates several other patho-physiologically relevant protein-bound solutes [27–29]. Hence, our study extends the scanty knowledge on the convective removal of protein-bound molecules by comparing several different strategic options and by evaluating several protein-bound solutes with different degrees of protein binding, and shows post-HDF and pre-HDF to be equally superior to pre-HF (Tables 2 and 3, Figure 3). Thus, it appears that diffusive transport should be combined with convection to obtain an essential tool to improve the removal of protein-bound uraemic solutes. CMPF shows a moderately different behaviour compared to the other protein-bound substances, showing a very low RR during the treatment. The reason for the low removal of CMPF compared to the other protein-bound solutes is probably its protein binding of ±100%.

The mechanism for removal of protein-bound solutes is not well known. The low molecular weight of the free fraction of these compounds indicates that diffusion could play a major role. In this case pre-dilution of the blood could have two opposite effects. Because dilution lowers the concentration, diffusion would be reduced. But dilution might also increase the diffusive removal by shifting the equilibrium of the protein binding, making more of the free fraction available for removal. Our results confirm that diffusion is an important removal mechanism for protein-bound solutes, but when comparing the two modes of HDF at equal convective flow, we find no major extra impact of pre-dilution. This could be interpreted as the two effects caused by dilution of the blood compensating for each other. Our data further show that convection is also an important removal mechanism for protein-bound solutes, because HF in which no diffusion takes place also reduces the levels of some of the protein-bound solutes. However,
this does not occur to the same extent as for HDF. This again confirms the important impact of diffusive removal for protein-bound solutes because the convective volume in the HF treatments was even larger than in the HDF sessions.

The comparison of convective strategies for small watersoluble compounds and for larger ‘middle’ molecules has been performed more frequently (Table 4), especially as far as pre-HDF versus post-HDF is concerned. No study compares the three strategies together. Our data point to a slightly but significantly better removal of the small watersoluble compounds with post-HDF over both pre-HDF and pre-HF (Tables 2 and 3, Figure 1). This superiority of post-HDF can be attributed to the fact that pre-dilution reduces the concentration gradient and by that reduces diffusive transport. The \( Q_d \) of pre-HDF was lower compared to the \( Q_d \) of post-HDF. One could hypothesize that this lower \( Q_d \) results in a lower clearance. However, according to calculations using clearance formulae [30] that also consider ultrafiltration, mass transfer area coefficient (KoA) and blood flow, the difference in dialysis flow (555 versus 619 mL/min) has virtually no impact (< 0.4%) on the clearance of small solutes. For \( \beta_2 \)M a distinct superiority of post-HDF could be proven in our study, at least as compared to pre-HDF. This is in accordance with the outcome of four other studies [19,31–33].

To the best of our knowledge, only one other study, by Altieri et al., compares pre-HF and pre-HDF [34] (Table 4). Our study conforms to these data, showing superiority of pre-HDF in removing small water-soluble compounds (Tables 2 and 3, Figure 1) as diffusion is the main transport mechanism. On the other hand, in our study, pre-HF was superior to pre-HDF for the removal of \( \beta_2 \)M. Altieri et al. found no difference between pre-HF and pre-HDF, probably due to the fact that they did not evaluate intrinsic removal per session but only the evolution of pre-dialysis \( \beta_2 \)M concentration [34]. In spite of its superiority to pre-HDF regarding \( \beta_2 \)M removal, pre-HF was not superior, however, to post-HDF in the present study. \( \beta_2 \)M is mainly cleared by convection and one could therefore expect the removal to be directly related to the convective volume provided by the therapies. Our results show, however, that the RR for \( \beta_2 \)M in pre-HF is not superior to post-HDF in spite of the larger convective volume in the former case (28.5 L versus 22.2 L). An explanation for this could be that the intercompartmental distribution of \( \beta_2 \)M limits the removal at high convective volumes [35]. Once the \( \beta_2 \)M concentration of plasma is reduced to a certain level, increasing the convection seems to have little effect.

Hence, adding diffusion to convection is at least as efficient as using convection alone, also for the difficult to remove molecules.

High-volume convective strategies as evaluated here can only be applied under technically correct conditions. Infusion of large volumes of substitution fluid implies on-line applications necessitating state of the art hardware and fluid quality [36]. The risk of infusing very large volumes in the circulation should not be underestimated and it is therefore important to apply the fluid optimally. Dilution of blood before it enters the filter requires approximately twice the fluid volume compared to when applied after the filter, when aiming for similar convective transport, and is an additional source for potential contamination if pre-dilution is not applied properly. In the same line of thought, the current evidence-based [37] trend to avoid high haemoglobin levels as guidance for erythrocyte stimulating agent (ESA) treatment might be an extra asset for the post-dilutional approach.

Since our study essentially aimed at a comparison of convective strategies under similar conditions, pre-HDF was not evaluated at extremely high convective volumes. Maximized ultrafiltration and substitution might lie more frequently at the origin of therapeutic errors and might jeopardize the fate of patients further by the unnecessarily high volumes of fluid being infused into the patient, which might become not only a health hazard but also an economic and ecologic issue in the future. Pre-HDF, as applied in the present study, is more practical and therefore commonly used; under these conditions, pre-HDF is inferior to post-HDF.

Post-HDF and pre-HDF were performed in volume control mode because we aimed at identical conditions except for the location in circuit where the substitution fluid was applied; in this context, volume control made it possible to achieve exactly the desired volume. For HF we aimed at maximum convection and therefore used both a larger membrane (2.1 m²) and pressure control mode. Our purpose was to avoid that the membrane surface area would limit UF rate; in addition this strategy prevented us from selecting an unachievable exchange volume. Instead a threshold pressure (200 mmHg) was set, which is the usual approach to achieve the maximal possible UF rate in proportion to blood flow, composition and in function of the properties of the dialyzer.

Due to the design of this study, some aspects related to convection were not evaluated. Firstly, the present publication does not contain a comparison against standard approaches such as low-flux or high-flux HD. A comparison with high-flux HD is contained in the longitudinal part of the present study that will be reported separately. Also, \( \beta_2 \)M was the only middle molecule studied, whereas previous protocols showed its behaviour is not always similar to other middle molecules [8,17].

The main conclusions are: (1) when comparing pre-dilution to post-dilution HDF with similar convective volume, pre-dilution shows a stronger removal of small watersoluble compounds and \( \beta_2 \)M, whereas there is no difference for protein-bound molecules; (2) solute removal with pre-HDF is superior to that with pre-HF with the sole exception of \( \beta_2 \)M, but the \( \beta_2 \)M removal with pre-HF is not superior to post-HDF; in spite of a higher convective volume in HF; and (3) overall, post-HDF is the most efficient convective strategy among those tested; adding diffusion to convection is more effective than using convection alone, even if maximized.

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