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Convective and adsorptive removal of β2-microglobulin during predilutional and postdilutional hemofiltration

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Background. $Beta_2$-microglobulin (β2-m) removal in patients with end-stage renal disease (ESRD) is maximal with convective techniques, such as hemofiltration (HF) or hemodiafiltration (HDF). Although the infusion mode of the replacement solution (predilution or postdilution) is expected to influence the efficiency of HF, experimental data in this respect are scanty. We therefore investigated the impact of the fluid reinfusion mode on the efficiency of HF in 11 ESRD patients who underwent both treatments.

Methods. The dialyzer (AK 200 ULTRA) was equipped with a 3-layer polyamide membrane (Poliflux 21 S, surface 2.1 m^2) and blood flow was kept between 300 and 400 mL/min. β2-m concentrations were measured in plasma water and ultrafiltrate at appropriate times during a 240-minute treatment. The following dialytic parameters were calculated: total amount of β2-m removed (A_{tot}), β2-m removed by convection (A_{con}) and by adsorption (A_{ads}), percent reduction in β2-m plasma water concentration (% Cpw_{in}), total plasma water clearance ($CLpw_{tot}$), convective plasma water clearance ($CLpw_{con}$), adsorptive plasma water clearance ($CLpw_{ads}$), and sieving coefficient (SC).

Results. $CLpw_{tot}$, $CLpw_{ads}$, and% Cpw_{in} were similar in pre- and postdilutional conditions, whereas $CLpw_{con}$ and SC were higher and $CLpw_{ads}$ was lower in postdilution than in predilution HF. Since a significant inverse correlation was found between A_{ads} and SC, predilution probably determines greater protein fouling than postdilution.

Conclusion. The 2 techniques appear to be equivalent in terms of total β2-m removal, although this final result is obtained by different contributions of convective and adsorptive elimination.

$Beta_2$-microglobulin (β2-m) is a low-molecular-weight protein that is almost exclusively eliminated by glomerular filtration and accumulates in the plasma of patients with end-stage renal disease (ESRD) [1]. The persistently high β2-m plasma levels during long-term dialytic therapy are thought to play a pathogenetic role in amyloid deposition in the osteo-articular system and related clinical signs (carpal tunnel syndrome, peripheral osteoarthropathy) [2, 3]. The ability to remove β2-m varies considerably among the available dialysis techniques, being negligible with low-flux hemodialysis (HD), intermediate with high-flux HD, and greatest with techniques employing convective transport, such as hemodiafiltration (HDF) [4]. Although no renal replacement treatment proved to normalize β2-m plasma levels [4], it is noteworthy that ESRD patients undergoing convective procedures have a 42% reduction of risk for carpal tunnel syndrome surgery, compared with those submitted to diffusive procedures (HD) [5]. Accordingly, it seems logical to keep β2-m plasma concentrations as low as possible by means of the most suitable renal replacement therapy. By using convective transport techniques, the efficiency of solute removal chiefly depends on the membrane characteristics and the infusion site (before or after the filter). Membrane composition, structure, and thickness determine the relative amount of β2-m removed by convection or adsorption [6–8]; the 2 alternative infusion modes offer different potential advantages and drawbacks [9–12]. Investigations comparing β2-m removal in pre- and postdilution modes have mainly been carried out using the convective-diffusive HDF technique [13–15]. Little information exists on this issue concerning the pure convective HF technique [16]. The main aim of our study was to evaluate the β2-m kinetics in postdilution as well as in predilution hemofiltration. As a secondary aim, we analyzed the single separate effects of both convection and adsorption by the dialysis membrane on the β2-m overall removal. The study was planned as an in vivo study in order to take into account the effect of the plasma proteins and, consequently, the protein-cake on both the components, convective and adsorptive, of β2-m removal.

Key words: beta2-microglobulin, hemofiltration, predilution, postdilution.

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Table 1. Patient demographic and clinical characteristics ($N = 11$)

Sex	4 F; 7 M
Age *years*	66.8 ± 11.9 (46–86) [a]
Weight *kg*	64.1 ± 9.4 (45–82) [a]
Time on dialysis *months*	80.9 ± 66.9 (14–242) [a]
Renal disease	Glomerulonephritis ($N = 3$)
	Polycystic disease ($N = 1$)
	Nephroangiosclerosis ($N = 4$)
	Interstitial nephritis ($N = 2$)
	End-stage renal disease ($N = 1$)

[a] Range.

Table 2. Treatment parameters

	Predilution HF	Postdilution HF	*P*
Fluid replacement *L*	61.3 ± 15.7	25.6 ± 3.5	<0.001
Body weight decrease *kg*	2.9 ± 0.6	3.0 ± 0.6	NS
Blood flow (Qb) *mL/min*	317.3 ± 18.49	338.2 ± 26.4	<0.05
Ultrafiltration rate (Quf) *mL/min*	273.7 ± 56.4	121.4 ± 14.9	<0.001
Infusion rate (Qinf) *mL/min*	262.9 ± 56.1	109.0 ± 15.0	<0.001
Treatment duration *minutes*	240 ± 8	240 ± 3	NS

METHODS

Patients and HF set-up

Eleven uremic patients (7 males, 4 females, mean age 67 ± 12 years, weighing 64 ± 9 kg) who had been on periodical renal replacement therapy for at least 3 months entered this study after providing their informed consent. The study was approved by the local Ethics Committee and was conducted in compliance with the principles of the Declaration of Helsinki. The inclusion criteria were age >18 years and permanent blood access granting a flow rate of at least 300 mL/min. The exclusion criteria were the occurrence of severe vascular instability during treatment, active HBV or HCV hepatitis, HIV positivity, coagulation disorders, and neoplasms. Patients' demographic and clinical characteristics are listed in Table 1.

Each patient underwent 2 online hemofiltration treatments, 1 in predilution (pre-HF) and 1 in postdilution mode (post-HF). The dialyzer (AK 200 ULTRA; Gambro, Lund, Sweden) was equipped with a 3-layer polyamide membrane (Poliflux 21 S, surface 2.1 m^2) and with a system that prepared sterile substitution solution online. A reverse-bi-osmosis system was used for the treatment of the main tap water. The incoming reverse-osmosis-treated water is filtered in a polyamide ultrafilter before entering the dialysis monitor. The infusion solution is then prepared on-line by the AK200 ULTRA, as a sterile, nonpyrogenic solution, by stepwise ultrafiltration of water and bicarbonate-containing dialysis fluid (BiCart), thanks to 2 polyamide ultrafilters sterilizing water, the first inserted into the machine, the second, disposable, inserted in the infusion line.

Blood flow was kept between 300 and 400 mL/min. The exchange volume was ≈45% and ≈100% of the body weight in post-HF and pre-HF, respectively. Previous and hitherto unpublished observations of ours have shown that these exchange volumes could guarantee an equivalence in the urea removal.

The main treatment parameters are shown in Table 2.

Hematocrit (Hct), total plasma protein, plasma urea, and creatinine concentrations were measured at the start and at end of each treatment; their values are listed in Table 3.

β2-microglobulin concentration measurements

The β2-microglobulin (β2-m) plasma concentrations entering the hemofilter (Cp_{in}) have been determined immediately before starting HF (time 0) and after 30, 60, 120, 180, and 240 minutes. In pre-HF mode, blood samples were taken before dilution with replacement fluid. The sampling times for the β2-m concentrations in plasma leaving the hemofilter (Cp_{out}) and in the ultrafiltrate (C_{uf}) were the same except for the first time, which was 5 minutes instead of 0 minutes (when Cp_{out} and C_{uf} are presumed to be zero). In post-HF mode, Cp_{out} was measured before dilution with replacement fluid.

β2-m concentrations were assayed by means of a turbidimetric method using rabbit antihuman β2-m for agglutination (Cobas Mira, Roche, Rotkrenz, Switzerland).

Pharmacokinetic analysis

The time courses of Cp_{in}, Cp_{out}, and C_{uf} were empirically described by cubic spline curves. The area under the curve (AUC) of β2-m concentrations versus time (0–4 h) was calculated for CP_{in} ($AUCp_{in}$), CP_{out} ($AUCp_{out}$), and C_{uf} (AUC_{uf}) by means of the trapezoidal rule.

Mass transfer and clearance calculations

The total amount of β2-m removed from plasma during the 4-hour HF period (A_{tot}) was calculated by the formula:

$$A_{tot} = (AUCb_{in} - AUCb_{out}) \times Qb \qquad \text{(Equation 1)}$$

where $AUCb_{in}$ is the AUC of β2-m concentrations in the blood entering the hemofilter, $AUCb_{out}$ is the AUC of β2-m concentrations in the blood leaving it, and Qb is blood flow. Since β2-m is distributed outside red cells, β2-m blood concentrations (Cb) can be calculated by using the β2-m plasma concentration (Cp) and the hematocrit value expressed as a decimal fraction (Hct):

$$Cb = Cp \times (1 - Hct) \qquad \text{(Equation 2)}$$

The Hct of the blood entering the hemofilter (Hct_{in}) was directly measured, and that leaving it (Hct_{out}) were

Table 3. Hematochemical parameters at start and end of HF

	Start				End			
	Hct %	Proteins g/dL	Urea mg/dL	Creat mg/dL	Hct %	Proteins g/dL	Urea mg/dL	Creat mg/dL
Pre-HF	32.7 ± 4.3	6.40 ± 0.66	145.1 ± 30.0	8.91 ± 1.78	36.6[b] ± 5.0	7.43[a] ± 0.84	59.4[b] ± 19.6	3.88[b] ± 1.15
Post- HF	32.7 ± 3.5	6.57 ± 0.56	135.6 ± 49.0	8.30 ± 1.74	35.9[a] ± 4.7	7.28[a] ± 0.87	61.3[b] ± 21.3	3.45[b] ± 1.03

[a] $P < 001$; [b] $P < 0.0001$.

calculated from Hct_{in}, Qb, and the input/output fluid balance, according to the following formulas:

$$\text{for predilutional HF} : Hct_{out} = (Qb \times Hct_{in})/(Qb - Quf + Qinf) \quad \text{(Equation 3)}$$

$$\text{for postdilutional HF} : Hct_{out} = (Qb \times Hct_{in})/(Qb - Quf) \quad \text{(Equation 4)}$$

where Quf is the ultrafiltration flow and Qinf is the infusion rate. (See **Appendix** for calculations yielding Equations 3 and 4).

The amount of β2-m removed by convection [i.e., recovered in the ultrafiltrate during the 4-hour HF period (A_{con})], was calculated by the formula:

$$A_{con} = AUC_{uf} \times Quf \quad \text{(Equation 5)}$$

The amount of β2-m removed by membrane adsorption (A_{ads}) was calculated as the difference between A_{tot} and A_{con}.

The mean dialytic β2-m plasma water clearances for total, convective, and adsorptive removal were calculated using A_{tot}, A_{con}, and A_{ads}, respectively:

$$CLpw_{tot} = A_{tot}/AUCpw_{in} \quad \text{(Equation 6)}$$

$$CLpw_{con} = A_{con}/AUCpw_{in} \quad \text{(Equation 7)}$$

$$CLpw_{con} = A_{ads}/AUCpw_{in} \quad \text{(Equation 8)}$$

where $AUCpw_{in}$ is the AUC of β2-m plasma water concentrations entering the hemofilter (Cpw_{in}). Cpw_{in} was obtained from Cp_{in} and the total plasma protein concentration (Pt) using Colton's formula [17]:

$$Cpw_{in} = Cp_{in}/(1 - 0.0107 \times Pt) \quad \text{(Equation 9)}$$

where the Pt unit is g/dL.

To investigate possible time-dependent changes in β2-m clearances, $CLpw_{tot}$, $CLpw_{to}$, $CLpw_{tot}$ were also determined at each sampling interval (0–0.5, 0.5–1, 1–2, 2–3, 3–4 hours).

As an additional index of β2-m removal, the percentage difference between Cpw_{in} measured at the end and start of HF (% Cpw_{in}) was calculated.

The sieving coefficient was calculated for convective β2-m removal as the ratio between measured $CLpw_{con}$ and maximal theoretical plasma water clearance ($CLpw_{max}$, i.e., the CLpw expected if S = 1), which are:

$$\text{for postdilutional HF} \quad CLpw_{max} = Quf; \quad \text{(Equation 10)}$$

$$\text{for predilutional HF} \quad CLpw_{max} = Qpw \times Quf/(Qpw + Qinf) \quad \text{(Equation 11)}$$

where Qpw is mean the plasma water flow during the 4-hour HF period. Qpw was calculated from the mean plasma flow [$= Qb \times (1 - Hct_{mean})$] corrected by the mean plasma protein concentration:

$$Qpw = Qb \times (1 - Hct_{mean}) \times (1 - 0.0107 \times Pt_{mean}) \quad \text{(Equation 12)}$$

Statistical analysis

Data are reported as mean ± SD. Comparisons between HF parameters measured in pre-HF and post-HF conditions were carried out by means of Student *t* test for paired data or the Wilcoxon paired rank test (for not normally distributed data). Time-related differences in fractional clearances and sieving coefficients were tested by means of a one-way analysis of variance (ANOVA) for repeated measures, followed by the Newman-Keuls post-hoc test. Correlations between parameters were assessed by linear regression analysis and the determination coefficient (r^2). The level of significance was considered as $P < 0.05$.

RESULTS

The urea and creatinine reduction ratios were 59 ± 11% and 56 ± 10%, respectively, in pre-HF, and 54 ± 7% and 58 ± 10% in post-HF, with the differences between the 2 modes not being significant.

The time courses of the mean β2-m concentrations in the plasma (Cp_{in}, Cp_{out}) and the ultrafiltrate (C_{uf}), after

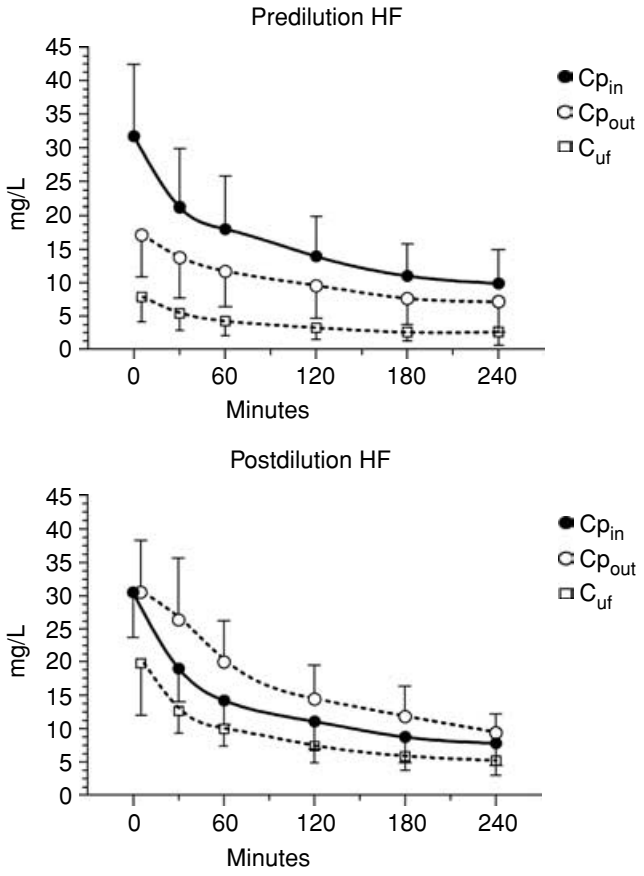

Fig. 1. Time courses of mean β2-m concentrations in plasma (Cp_{in}, Cp_{out}) and ultrafiltrate (C_{uf}).

Table 4. Model-independent pharmacokinetic parameters of β2-m based on Cp_{in}, Cp_{out}, and C_{uf}

	Predilution ($N = 11$)	Postdilution ($N = 11$)	P
Cp_0 *mg/L*	31.7 ± 10.7	30.4 ± 6.8	NS
Cp_{4h} *mg/L*	9.8 ± 5.1	7.7 ± 3.3	NS
$AUCp_{in}$ *mg/L · min*	3705 ± 1556	3074 ± 945	NS
$AUCp_{out}$ *mg/L · min*	2463 ± 1160	4191 ± 1174	<0.0005
AUC_{uf} *mg/L · min*	907 ± 471	2090 ± 656	<0.0001

both pre- and postdilution modes are shown in Figure 1. No significant differences between pre- and postdilution were found for pharmacokinetic parameters Cp_0, Cp_{4h}, and $AUCp_{in}$, but $AUCp_{out}$ and AUC_{uf} were higher in post-HF than in pre-HF (Table 4). As expected, Hct and Pt increased, and urea and creatinine plasma concentrations decreased from the start to the end of each procedure (Table 3).

The parameters characterizing the ultrafiltration process with the 2 techniques are listed in Table 5. CL_{tot} was slightly but not significantly ($P = 0.064$) higher during post-HF. By contrast, CL_{con} and CL_{ads} significantly differed between the 2 techniques [i.e., CL_{con} was significantly higher (79.6 mL/min vs. 60.8 mL/min) and CL_{ads} was significantly lower (3.2 mL/min vs. 12.6 mL/min) in post-HF than in pre-HF]. The same trend was observed for the amounts of β2-m removed (A_{tot}, A_{con}, A_{ads}), although only A_{ads} was significantly lower in post-HF than in pre-HF (52.3 mg vs. 12.7 mg). Using pooled pre- and post-HF data, a significant inverse correlation ($r^2 = 0.43$; $P = 0.0009$) was found between the amount of β2-m adsorbed by the membrane (A_{ads}) and the sieving coefficient.

Table 5. Mean values ± SD of the main parameters of HF efficiency

	Predilution ($N = 11$)	Postdilution ($N = 11$)	P
Plasma water clearance *mL/min*			
CL_{max}	114.5 ± 14.1	121.3 ± 14.9	NS
CL_{tot}	73.4 ± 12.4	82.8 ± 12.5	NS
CL_{con}	60.8 ± 13.1	79.6* ± 11.8	<0.005
CL_{ads}	12.6 ± 9.3	3.2* ± 8.3	<0.005
Sieving coefficient	0.53 ± 0.10	0.65* ± 0.07	<0.005
% Cpw_{in}	−70.5 ± 7.4	−74.7 ± 6.6	NS
β2-microglobulin removal *mg*			
A_{tot}	288.9 ± 113.8	273.2 ± 77.6	NS
A_{con}	236.6 ± 93.9	260.5 ± 65.1	NS
A_{ads}	52.3 ± 40.0	12.7* ± 28.6	<0.005

The percentage decrease in Cpw_{in} from the start to the end of the treatment was similar (−70.5% in pre-HF vs. −74.6% in post-HF). The sieving coefficient was significantly higher in post- than in predilution (0.65 vs. 0.55).

The changes in $CLpw_{tot}$, $CLpw_{con}$, $CLpw_{ads}$, and sieving coefficient throughout the 4-hour HF period are shown in Figures 2 and 3. The one-way ANOVA revealed that $CLpw_{tot}$, $CLpw_{con}$ underwent a significant time-dependent decrease in both HF modes, whereas $CLpw_{ads}$ decreased only with post-HF and the sieving coefficient decreased only with pre-HF (Table 6).

DISCUSSION

Different strategies have been adopted in the course of time in order to prevent the accumulation of β2-m. The routine use of polyacrylonitrile or, more in general, the switch from conventional to high-flux dialysis membranes have proven to be useful to reduce the risk of carpal tunnel syndrome [18]. The bacteriologic control of all the dialysis fluids, avoiding the endotoxin contamination, may also play a preventative role. In actual fact, some studies have demonstrated that the use of high-quality dialysis fluids alone may reduce the incidence of carpal tunnel syndrome [19, 20]. The use of convective dialysis therapies, in addition to the control of the biocompatibility aspects of dialysis, may add the strength of an enhanced removal, to the reduced production of β2-m, thus resulting to be the best strategy to prevent β2-m–related pathologies.

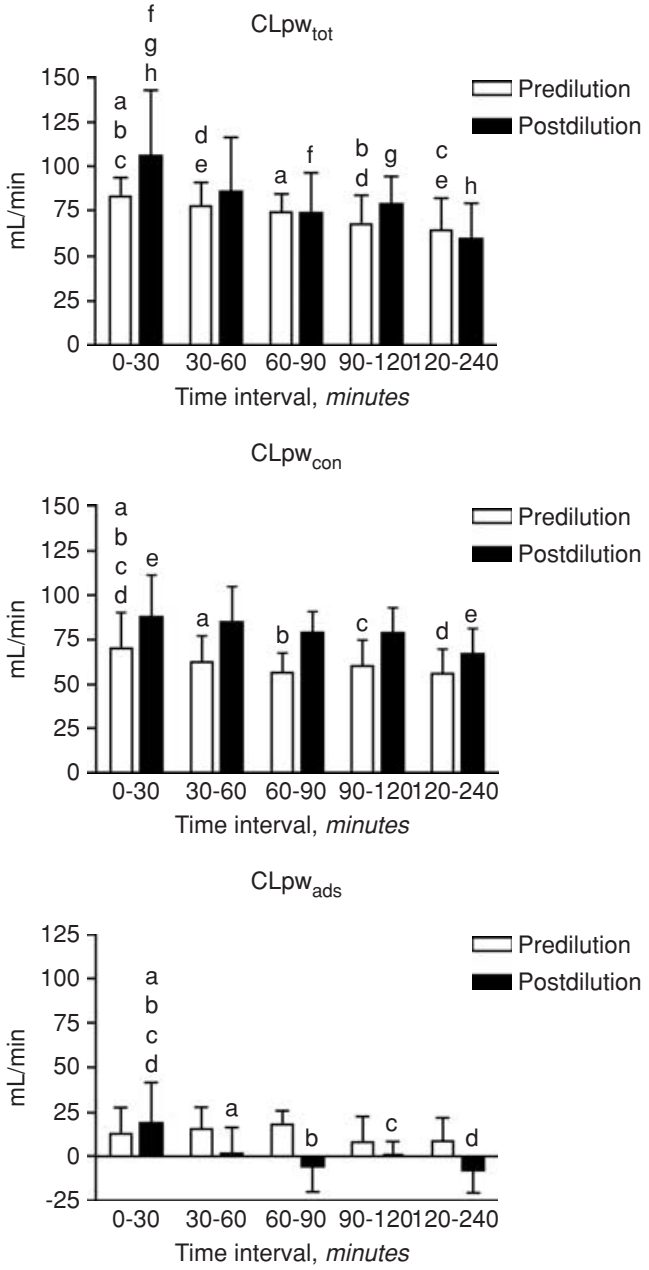

Fig. 2. Changes in fractional CLpw$_{tot}$, CLpw$_{con}$ and CLpw$_{abs}$ during HF procedure. Columns labeled with same letter differ significantly (post-hoc test).

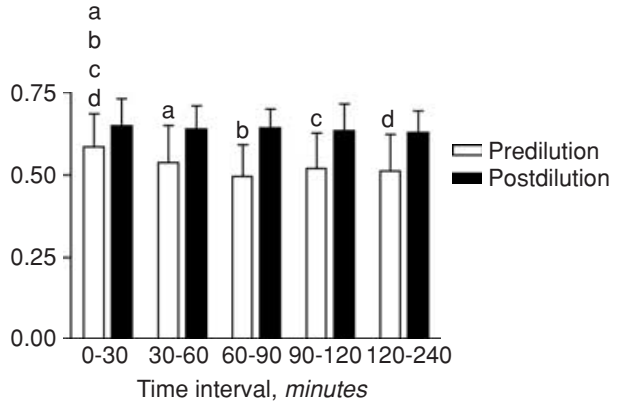

Fig. 3. Changes in sieving coefficient during HF procedure. Columns labeled with same letter differ significantly (post-hoc test).

Table 6. Influence of time on HF parameters

	Predilution HF	Postdilution HF
CLpw$_{tot}$	$P < 0.0001$	$P < 0.005$
CLpw$_{con}$	$P < 0.005$	$P < 0.05$
CLpw$_{ads}$	NS	$P < 0.005$
Sieving coefficient	$P < 0.005$	NS

One-way ANOVA.

The main result of our study is that the overall HF efficiency in removing β2-m from plasma, evaluated as CLpw$_{tot}$, A$_{tot}$, and% Cpw$_{in}$, is similar in both pre- and postdilutional conditions. These findings are in contrast with what is generally stated in the literature (i.e., that the removal of middle molecules is higher in predilution modes) [21–24]. However, another recent study [15] reported no substantial differences between pre- and postdilution HDF in regards to the total amount of β2-m removed from plasma.

After a more in-depth analysis of removal mechanisms, it appears that the equivalence of CLpw$_{tot}$ arises from balanced, opposite changes in convective and adsorptive clearances with the 2 techniques, as testified by the higher CLpw$_{con}$ and lower CLpw$_{ads}$ values found in post-HF in comparison with pre-HF. The former was almost entirely attributable to a higher sieving coefficient (0.65 vs. 0.55), as CLpw$_{max}$ was virtually the same in both infusion modes (114 mL/min vs. 121 mL/min). In line with this finding, we have recently observed [25] that, at equivalent transmembrane pressure, the loss of albumin through the dialysis membrane is greater in post-HF than in pre-HF. In this respect, it is noteworthy that the sieving coefficient was similar at the beginning of the 2 procedures and then significantly decreased only during pre-HF treatment (Fig. 3). Therefore, the sieving coefficient may change during HF depending on the technique used. Interestingly, using pooled data, a significant inverse correlation was found between the amount of β2-m adsorbed to the membrane (A$_{ads}$) and the sieving coefficient. If we accept that β2-m adsorption is a marker of the more general phenomenon of protein deposition on the filter surface, we could conclude that the reduced convective β2-m removal in predilution is due to greater protein fouling. This finding contrasts with the intuitive notion that plasma and blood concentrations occurring during post-HF should reduce HF efficiency by increasing blood viscosity and clogging the membrane pores. However, ultrafiltration is a process ruled by several factors

[10, 12], whose analysis may provide explanations. To justify the greater A_{ads} found with pre-HF than with post-HF, it may be assumed that the true adsorbing surface is different with the 2 techniques, even though the same hemofilter is used. As membrane pores are structured like water channels rather than simple holes, they probably oppose differing degrees of hydraulic resistance. If so, the higher ultrafiltration flow used in pre-HF may open a greater number of channels, thus increasing the actual membrane surface and the amount of β2-m adsorbed.

Regarding the differences in sieving efficiency, it is known that the sieving coefficient is not constant for any given solute-membrane combination, but depends on the blood and the ultrafiltration flows. The high blood flows used in predilution (true Qb + Qinf) are expected to increase the wall shear rate and decrease the solute polarization (i.e., the rise in β2-m concentration at the membrane surface that improves the sieving coefficient) [10, 21]. Solute polarization is considered to be particularly important for macromolecules, like β2-m, which are confined to plasma water and accumulate on the membrane surface more than small molecules. Indeed, experimental data indicate that, if Qb is gradually increased in post-HF while Quf is kept constant, the sieving coefficient for β2-m first increases (for Qb up to 450–500 mL/min) and then decreases [10]. Furthermore, it has been shown that, if Quf is increased in constant Qb conditions, the β2-m sieving coefficient uniformly decreases [10]. Therefore, on the whole, the high blood flow and ultrafiltrate rate typical of pre-HF tend to reduce the sieving coefficient for β2-m.

CONCLUSION

Our data show a substantial equivalence between pre-HF and post-HF in removing β2-m from plasma, although the contribution of convective and adsorptive mechanisms is different in the 2 techniques. In the long term, predilution HF could prove more useful in patients with high hematocrit levels. This is a frequent condition today in view of the routine use of erythropoetin to maintain the hemoglobin levels within an ideal range (11–12 g/dL), and may favor the clotting of the hemofilter in the post-dilution modality.

Since the efficiency of urea and creatinine removal, measured as percent decreases in baseline plasma concentrations, is also comparable, the choice between the 2 HF modes should be solely based on feasibility and economic assessment.

APPENDIX

Hct_{out} may be calculated using the formula:

$$Hct_{out} = Qe/Qb_{out} \qquad \text{(Equation a)}$$

where Qe is erythrocyte flow through the hemofilter and Qb_{out} is blood flow leaving the hemofilter. Qe is equal to

$$Qb_{in} \times Hct_{in} \qquad \text{(Equation b)}$$

Qb_{out} depends on the amount of fluid infused and/or lost and on the HF technique:

$$Qb_{out} = Qb_{in} - Quf + Qinf, \text{ for predilutional HF} \qquad \text{(Equation c)}$$

$$Qb_{out} = Qb_{in} - Quf, \text{ for postdilutional HF} \qquad \text{(Equation d)}$$

Substituting Qe and Qb_{out} in Eq. a for Eqs. b, c, and d yields Eqs. 3 and 4 (see **Methods**).

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