The Medium Cut-Off Membrane Does Not Lower Protein-Bound Uremic Toxins

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BACKGROUND

The concentration of gut-derived protein-bound uremic toxins (PBUTs) is increased in the circulation of patients with chronic kidney disease (CKD) due to alterations caused by uremic conditions. Some PBUTs such as indoxyl sulfate (IS) and p-cresyl sulfate (pCS) are associated with increased cardiovascular disease in CKD.

Even though PBUTs are small (<500 Da), they tend to bind to larger-sized proteins, particularly albumin, and this bound form is difficult to remove by diffusive dialysis. IS and pCS are classified as PBUTs that are strongly bound to albumin (>90%), with a very small fraction as free form.

The kidney primarily removes PBUTs by tubular secretion via organic anion or cation transporters. Conventional hemodialysis (HD) partially replaces glomerular filtration but cannot reproduce tubular function, therefore PBUTs build up in the plasma of patients on HD despite HD therapy. Free forms of PBUTs are easily removed by HD, however they represent a small fraction. Various HD strategies have been investigated to remove PBUTs, for example, prolonged HD duration using nocturnal dialysis eliminated PBUTs to a greater extent. Adding a convective method with hemodiafiltration (HDF) enhanced the removal of middle molecular uremic toxins, but did not consistently remove PBUTs. A recently developed medium cut-off (**MCO** dialyzer) selectively removes larger-sized uremic toxins, but the efficacy of PBUT removal remains unclear.

OBJECTIVE

This study was performed to test the effectiveness of high-flux HD (HF-HD), high-volume post-dilution online HDF (post-OL-HDF), and **MCO** membrane-HD dialysis for removing PBUTs.

METHODS

Study Design

This was a prospective, randomized, cross-over study conducted at three tertiary dialysis centers in Korea.

Participants

The study included adult patients who were anuric, had permanent vascular access, and received maintenance HDF 3x/week for >3 months. Patients were excluded if they had residual urine of more than 100 cc/day, were pregnant, received dialysis with a catheter, had malignancy, congestive heart failure, or hemodynamic instability.

Treatments

Patients received all dialysis treatments: HF-HD, post-OLHDF, and **MCO** membrane-HD 3x/week for three consecutive weeks each. The order in which patients received the treatments was randomly assigned. Plasma and dialysate samples were collected during the mid-week treatment in the third week. The dialysis duration was 4 h, with a dialysate flow of 500 mL/min and a blood flow of 250–320 mL/min. HDF was performed in post-dilution mode with a target convective volume of >23 L.

Characteristics of the dialyzers tested are shown in Table 1. Please note the difference in surface area of the membranes used with the **MCO** membrane having the smallest surface area.

Outcomes

The following plasma levels were measured before and after dialysis:

- Blood urea nitrogen (BUN, 60 Da)
- λ-free light chain (λ -FLC, 45,000 Da)
- β2-microglobulin (β2MG, 11,800 Da)
- Albumin and protein levels

Pre-dialysis serum concentration, post-dialysis concentration, reduction rate (RR), dialysate mass removal and clearance were measured for IS and pCS.

Sample Collection and Analysis

Blood samples were taken before the dialysis onset and immediately after the dialysis session in a mid-week treatment in the 3rd week. Dialysate mixtures were collected from the

	HF-HD	Post-OL-HDF	MCO-HD
Dialyzer	Fx CorDiax 80	Fx CorDiax 800	**Theranova** 400 dialyzer
Inner diameter (μm)	185	210	180
Wall thickness (μm)	35	35	35
Membrane polymer	polysulphone-PVP blend	polysulphone-PVP blend	polyarylethersulphone-PVP blend
Effective surface area (m2)	1.8	2.0	1.7
Sieving coefficients of albumin	<0.001	<0.001	0.008
Clearance of inulin	135	178	183
UF coefficient (mL/h/mmHg)	64	62	48

PVP, polyvinylpyrrolidone; UF, ultrafiltration.

TABLE 1. The characteristics of the dialyzers

inverse pump at 60, 120, 180, and 240 min of the dialysis session. A total of 10 mL of the dialysate sample from the mixture was obtained and stored at -80° C until further use.

Simultaneous quantitative analyses of the total IS (212 Da, protein-bound ~90–95%) and pCS (187 Da, protein-bound ~95%) in the plasma and dialysate were performed using liquid chromatography-tandem mass spectrometry (LC-MS/MS). Briefly, 25 µL of human plasma or 125 µL of dialysate was precipitated with acetonitrile, including internal standards (pCS-d7), and vortexed for 30 s, followed by centrifugation for 10 min at 19,500 g at 4° C. Next, the supernatant (200 µL plasma sample and 300 µL dialysate sample) from each tube was transferred to a new microcentrifuge tube and evaporated using a Speed-Vac (HyperVac, VC2200, Gyrozen Co., LTD, Deajeon, Korea). Subsequently, 50 µL and 25 µL of solvent A (5 mmol/L ammonium acetate solution) were used for the dried plasma and dialysate samples, respectively. For quantification of target uremic toxins, LC-MS analysis was performed using a triple quadrupole mass spectrometer (6490 series, Agilent Technologies, Wilmington, DE, USA) coupled to a 1200 series high-performance liquid chromatography system (Agilent Technologies, Wilmington, DE, USA) with a Hypersil GOLD column (2.1 100 mm ID; 1.9 µm, Thermo Fisher Scientific, Waltham, MA, USA). The injection volume was 2 µL for serum and 3 µL for dialysate analyses. The total run time was 14.5 min for each analysis and the LC gradient system was performed as follows: 0–1 min, 20% solvent B (100% methanol); 1–2.5 min, 20–60% solvent B; 2.5–3 min, 60–95% solvent B; 3–5 min, 95% solvent B; 5–5.5 min, 95-20% solvent B; and 5.5–14.5 min, 20% solvent B with a fixed LC flow rate of 0.15 mL/min. IS and pCS were eluted at 2.8 min and 4.3 min, respectively. The electrospray (ESI) MS method was used to analyze IS and pCS, and all acquisition method parameters were set as follows: capillary voltage: 3000 V in negative mode, drying gas flow: 12 L/min at 290° C, sheath gas flow: 12 L/min at 400° C, and nebulizer gas flow at 30 psi. Multiple reaction monitoring (MRM) conditions, including MS/MS collision energy and computed transitions, were optimized for each molecule to analyze the target uremic toxins in individual samples. The MRM transition was selected at the following transitions: *m/z* 212.04→80.14, 132.05 for IS, *m/z* 186.98→80.02, 107.03 for pCS, and *m/z* 194.04→80.02, 114.04 for pCS-d7. The charcoal-stripped human plasma and dialysate before dialysis served as blank matrices for constructing the calibration curves. All experiments were performed in triplicate.

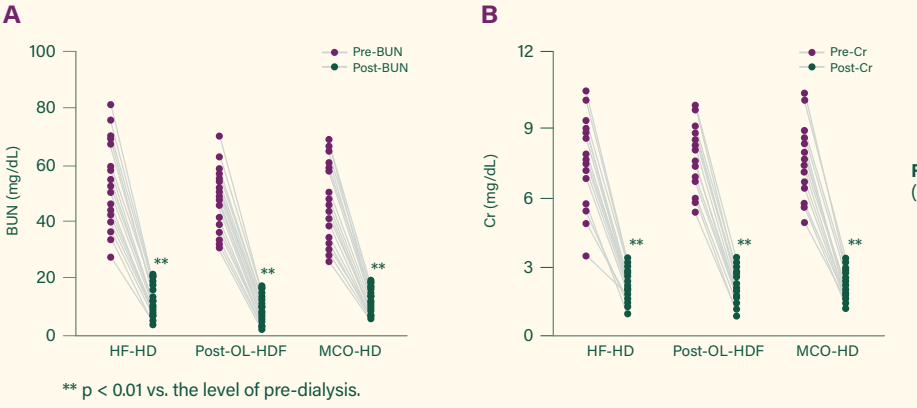
The reduction rate (RR) of the solutes was defined as ($RR = [1 - (C_{post}/C_{pre})] \times 100$, C_{post} = post-dialysis plasma concentration, C_{pre} = pre-dialysis plasma concentration). C_{post} was calculated using a reference formula reflecting hemoconcentration.

For the middle-molecular-weight toxins, C_{post} was replaced by $C_{post\text{-}corr}$ ($C_{post\text{-}corr} = C_{post} / [1 + (BW_{pre} - BW_{post})/(0.2 \times BW_{post})]$, BW_{pre} = body weight before dialysis, BW_{post} = body weight after dialysis).

Total solute removal (TSR) was calculated by multiplying the solute concentrations in the 10 mL dialysate by the effluent volume (dialysate, ultrafiltration, and substitution volume). Dialytic clearance was attained by dividing the TSR by dialysis duration. Single-pool Kt/V(spKt/V) was assessed using the reference method.

RESULTS

The mean patient age was 62.18 ± 11.42 and the most common causes of ESRD were diabetes (45.5%) and hypertension (40.9%). Blood pressure and body weight were similar, and there were no differences in baseline dialysis parameters across the three methods. Average blood flow was 302.73 ± 6.92 mL/min. Mean blood and dialysate flow rates were approximately 300 and 530 mL/min, respectively. Mean convection volume was 21.50 ± 1.90 L in post-OL-HDF. The mean ultrafiltration volume was 1.8–2.0 L/session.



** p < 0.01 vs. the level of pre-dialysis.

FIGURE 1. Plasma concentrations of blood urea nitrogen (BUN) and creatinine (Cr) before and after dialysis.

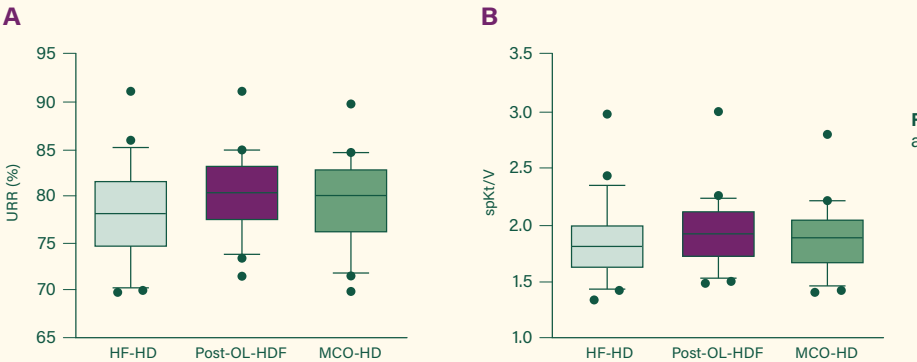


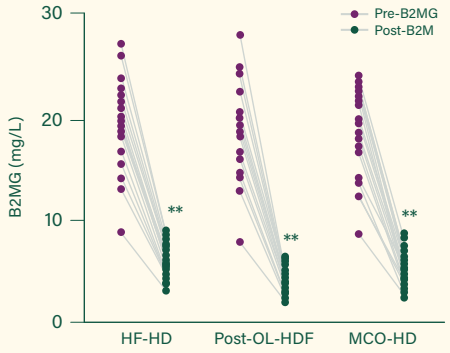
FIGURE 2. The reduction rates of urea reduction rate (URR) and single pool Kt/v (spKt/v).

Clearance of Small and Middle Molecular Weight Toxins

Blood urea nitrogen and creatinine were significantly reduced after dialysis, but there was no difference in post-dialysis levels across the different dialysis methods (Figures 1A and 1B).

URR and spKt/V were similar across dialysis methods (Figures 2A and 2B).

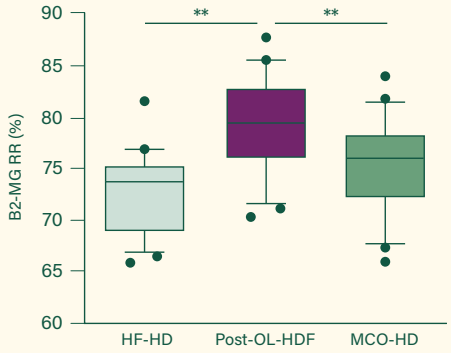
Post-dialysis β2MG level was lowest in post-OL-HDF (4.32 ± 1.21 mg/L, $p < 0.001$), followed by **MCO**-HD (5.27 ± 1.45 mg/L) and HF-HD (6.27 ± 1.70 mg/L) (Figure 3).



** p < 0.01 vs. the level of pre-dialysis.

FIGURE 3. Plasma concentrations of β2-microglobulin (β2MG) before and after dialysis.

RR of B2MG was higher in post-OL-HDF (79.54 ± 4.72%; $p < 0.001$) than in HF-HD (72.87 ± 3.98%) and **MCO**-HD (75.32 ± 4.72%). There was no statistical difference between HF-HD and **MCO**-HD groups (Figure 4).

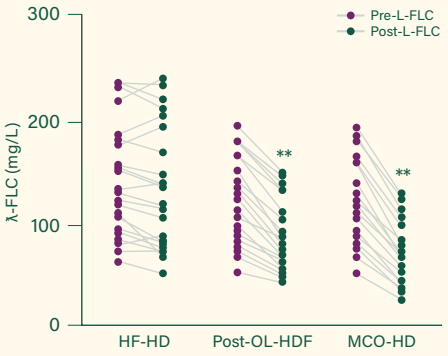


** p < 0.01 vs. other dialysis methods.

FIGURE 4. The reduction rates of β2-microglobulin (β2MG).

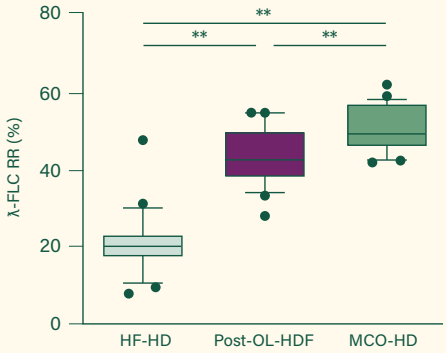
Post-dialysis λ -FLC level was lower in post-OL-HDF (89.23 ± 34.09 mg/L, $p < 0.001$), and **MCO**-HD (71.59 ± 29.61 mg/L) than in HF-HD (137.73 ± 60.71 mg/L) (Figure 5).

RR of λ -FLC was highest in **MCO**-HD (51.52 ± 6.08%; $p < 0.001$) followed by post-OL-HDF (43.48 ± 7.41%) and HF-HD (20.8% ± 8.14%) (Figure 6).



** p < 0.01 vs. the level of pre-dialysis.

FIGURE 5. Plasma concentrations of λ-free light chain (λ -FLC) before and after dialysis.



** p < 0.01 vs. other dialysis methods.

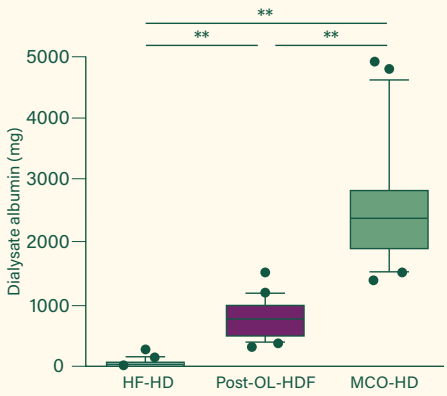
FIGURE 6. The reduction rates of λ-free light chain (λ -FLC).

Dialysate Albumin Removal

There was no significant difference across dialysis methods in:

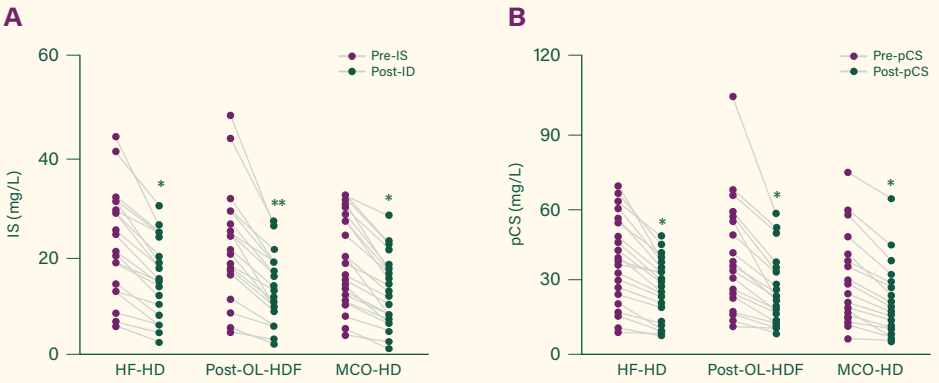
- Pre-dialysis plasma albumin (HD: 3.95 ± 0.21 g/dL, post-OL-HDF: 4.09 ± 0.29 g/dL, and **MCO**-HD: 3.91 ± 0.29 g/dL)
- Post-dialysis plasma albumin (HF-HD: 4.18 ± 0.50 g/dL, post-OL-HDF: 4.18 ± 0.50 g/dL, and **MCO**-HD: 4.09 ± 0.43 g/dL)
- RRs of albumin (HF-HD: −9.77 ± 11.09%, post-OL-HDF: −5.26 ± 7.73%, and **MCO**-HD: −5.50 ± 9.17%)

However, the dialysate albumin mass was highest in **MCO**-HD (2547.32 ± 968.31 mg/session, $p < 0.001$), followed by post-OL-HDF (778.32 ± 313.17 mg/session) and HF-HD (59.91 + 79.82 mg/session) (Figure 7).



** p < 0.01 vs.other dialysis methods.

FIGURE 7. Dialysate albumin.



* p < 0.05 vs. the level of pre-dialysis, ** p < 0.01 vs. the level of pre-dialysis.

FIGURE 8. Plasma concentrations of indoxyl sulfate (IS) and p-cresyl sulfate (pCS) before and after dialysis.

Clearance of Protein-Bound Uremic Toxins

The post-dialysis IS and pCS were significantly reduced compared to pre-dialysis in the three different dialysis methods (Figure 8).

The plasma concentrations of IS and pCS were similar across the three methods both pre-dialysis and post-dialysis (Table 2). There was no statistical difference in the RR, dialysate mass removal, and dialytic clearance of IS and pCS among the three different dialysis methods (Table 2 and Figure 9).

There was no correlation between the dialysate mass of IS or pCS and dialysate albumin concentration (IS R=-0.126, p=0.242; pCS R=-0.169, p=0.174).

	HF-HD	Post-OL-HDF	MCO-HD
IS (mg/dL)			
Pre-dialysis plasma concentration (mg/dL)	22.59 ±10.41	21.39 ±11.05	20.08 ± 9.46
Post-dialysis plasma concentration (mg/dL)	15.27 ± 7.60	12.90 ± 6.83	13.12 ± 7.12
RR (%)	33.50 ± 11.48	40.03 ± 10.16	36.31 ± 12.75
Dialysate mass (mg)	94.98 ± 96.01	83.63 ± 100.50	74.31 ± 66.79
Clearance (mL/min)	21.12 ± 19.91	19.50 ± 17.22	19.49 ± 15.81
PCS (mg/dL)			
Pre-dialysis plasma concentration (mg/dL)	37.79 ±18.45	38.70 ± 22.65	33.09 ± 17.47
Post-dialysis plasma concentration (mg/dL)	26.60 ± 12.32	24.80 ± 14.08	23.39 ± 13.68
RR (%)	27.06 ± 10.74	34.44 ± 10.00	29.49 ± 10.28
Dialysate mass (mg)	114.56 ± 12.39	126.19 ± 69.54	101.71 ± 57.43
Clearance (mL/min)	13.58 ± 2.76	15.33 ± 3.17	14.10 ± 3.94

TABLE 2. IS and pCS results

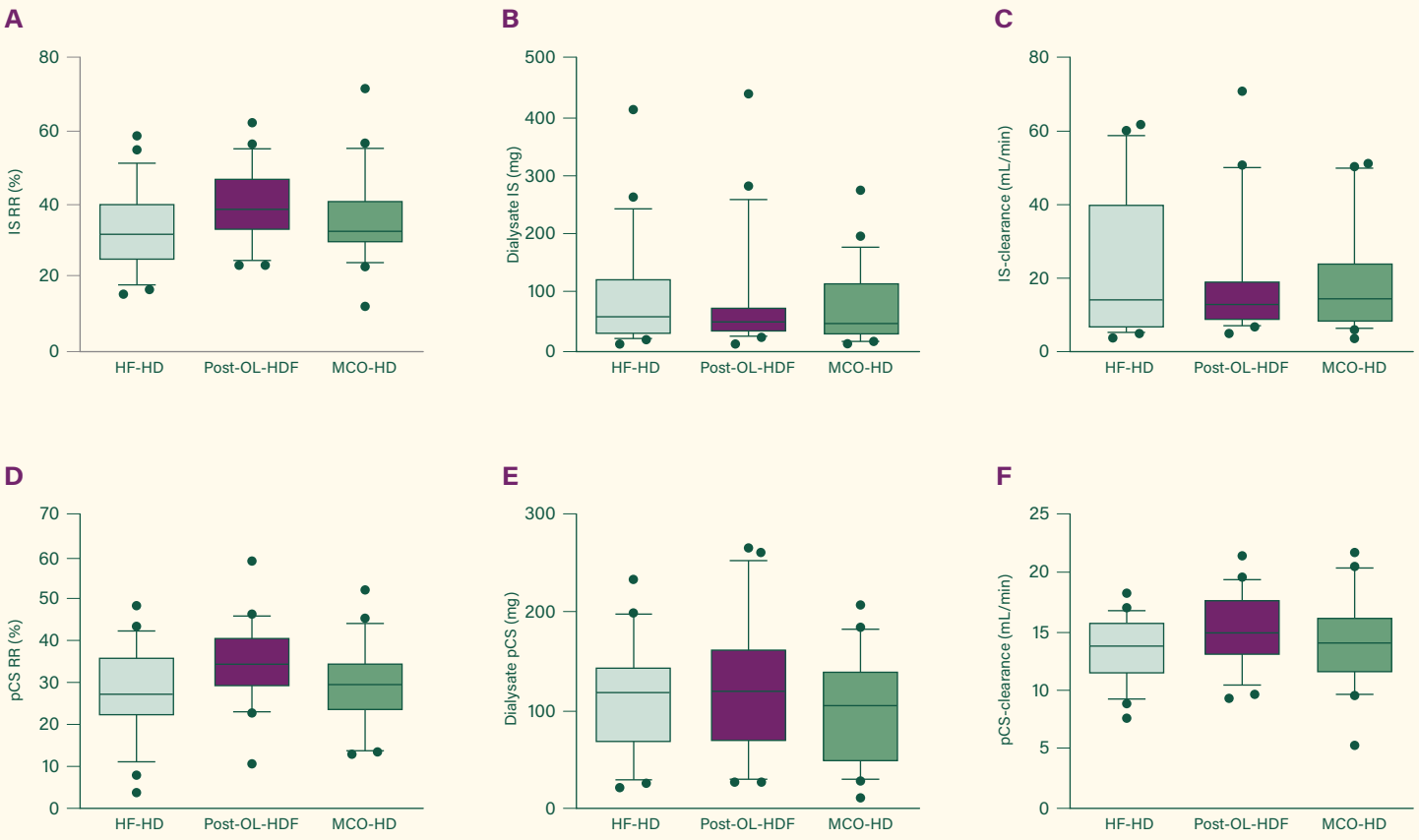


FIGURE 9. The clearing efficacy of IS and pCS. (**A,D**): Reduction rate (RR) of Indoxyl sulfate (IS) and p-cresyl sulfate (pCS); (**B,E**): Dialysate removed IS and pCS; (**C,F**): Dialytic clearance of IS and pCS mass in high-flux hemodialysis (HF-HD), post-dilution online hemodiafiltration(post-OL-HDF), and medium cut-off hemodialysis (**MCO**-HD).

DISCUSSION AND CONCLUSION

High-efficiency HD, such as **MCO** membrane-HD and high-volume HDF, was expected to have better performance in clearing protein-bound toxins by greater removal of albumin during a dialysis session. Despite higher albumin removal, **MCO** membrane-HD and post-OL-HDF did not show better performance in eliminating IS and pCS compared to that of HF-HD.

IS and pCS are known to be highly bound to albumin, with their free forms accounting for only approximately 2% in the circulation of normal individuals. Because HD cannot replace renal tubular secretion, the plasma levels of IS and pCS are 50–100 times higher in patients on dialysis than in those with normal kidney function.

The native kidney removes PBUTs mainly as free forms, and the clearance of total forms of IS and pCS are only 2.0 and 1.7%, respectively. Dialytic clearance of total forms was slightly lower or similar to kidney clearance, but dialytic clearance of the free forms was only 20–30% of that of the native kidney. The current study confirmed no correlation between dialysate albumin and IS or pCS, supporting that removal of albumin in HDF may not help eliminate PBUTs.

Limitations

The study did not measure the free forms of IS and pCS. Since the solute kinetics of bound and free PBUTs are different, more precise dialytic clearance might be obtained if the free forms are measured. However, dialytic clearance was mainly accomplished with free PBUTs and their blood level was low, thus the effect of their changes compared with total ones might be subtle. In addition, the clearance of the free forms of IS and pCS might be similar since their removal pattern was similar to those of total forms even after dialysis methods (HD vs. HDF) or time (4 vs. 8 h) were changed. Second, dialysate was collected four times every hour. Albumin elimination is higher in the first 90 min of the HD session. Considering albumin kinetics, the actual albumin loss via dialysis might be larger than was measured in the study.

Clearance of the protein-bound uremic toxins (PBUTs) indoxyl sulfate (IS) and p-cresyl sulfate (pCS) was not different across the three different dialysis methods (HF-HD, post-OL-HDF, MCO-HD).