NEFROLOGÍA. Volumen 26. Número 4. 2006

# *Depuración de grandes moléculas. Más allá de la $\beta_2$-microglobulina*

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**SUMMARY**

*The uremic toxin removal capacity mainly depends on dialyzer and hemodialysis modes. The low-flux hemodialysis only removes solutes having molecular weights less than 5.000 Da. High-flux hemodyalisis represents a form of low-volume hemodiafiltration because of the internal filtration and back-filtration that can take place within a dialyzer. Hemodiafiltration with large volumes of replacement fluid seems to be the best technique for removing all small, medium-sized and large molecules. The objective of our study was to evaluate the large molecules removal bigger than $\beta_2$-microglobuline on high flux haemodialysis and on-line hemodiafiltration with postdilutional infusion, in patients with three times a week dialysis and on short daily dialysis.*

*We studied 24 patients, 15 males y 9 females stable on haemodialysis programme, twelve on standard four to five hours three times a week dialysis and twelve on 2 to 2 1/2 hours six times a week dialysis. All patients were dialysed with Fresenius 4008 monitor, three sessions on high flux haemodialysis (HD) and three sessions on on-line hemodiafiltration (OL-HDF). Two sessions with each filter were performed (polisulfone HF80, polyethersulfone Arylane H9 and new polisulfone APS 900). Pre and postdialysis concentrations of urea, creatinine, ($\beta_2$-microglobulin ($\beta_2$-m), myoglobin, prolactin and $\alpha_1$-microglobulin ($\alpha_1$-m) were measured.*

*There was no difference in urea and creatinine small molecules removal. $\beta_2$m removal was 68% on HD and 81% on OL-HDF. Myoglobin and prolactin present a similar removal pattern, a higher removal with new filters (60% with Arylane and 59% with APS) in comparison with clasical polisulfone (22% with HF80). The mean $\alpha_1$-m reduction rate on HD was 6% and on OL-HDF 22%. OL-HDF with APS 900 filter was the most remove technique (35.4%), significatively higher than the other modes and filters.*

*We can conclude that the new filters generation reach a better uremic toxins removal, specially in large molecules higher than $\beta_2$-m and on HD modality.*

Key words: ***$a_1$-microglobulin. High-flux dialysis. On-line hemodiafiltration. Myoglobin. Prolactin.***

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## DEPURACIÓN DE GRANDES MOLÉCULAS. MÁS ALLÁ DE LA $\beta_2$-MICROGLOBULINA


### RESUMEN

*La capacidad depurativa de toxinas urémicas depende en gran medida de los dializadores y de la modalidad de tratamiento dialítico. La hemodiálisis de bajo flujo tan sólo depura solutos con un peso molecular inferior a 5.000 Da. La diálisis de alto flujo representa una forma de hemodiafiltración de bajo volumen ya que tanto el filtrado interno como el retrofiltrado se producen en el dializador. Las técnicas de hemodiafiltración con elevados volúmenes de reposición son los que consiguen mayor depuración tanto de pequeñas como de medias y grandes moléculas. El objetivo del estudio fue valorar la depuración de moléculas grandes, superiores a la $\beta_2$-microglobulina en hemodiálisis de alto flujo y hemodiafiltración on-line con infusión postdilucional, en pacientes que seguían un esquema de tres sesiones semanales y otros con hemodiálisis diaria.*

*Se estudiaron 24 pacientes, 15 hombres y 9 mujeres en programa estable de hemodiálisis, doce en régimen estándar de 4 a 5 horas 3 sesiones semanales y doce de 2 a 2 1/2 horas 6 veces por semana. Todos se dializaron con monitor 4008 (Fresenius). Cada paciente recibió tres sesiones con hemodiálisis de alto flujo (HD) y tres sesiones con hemodiafiltración on-line (HDF-OL), manteniendo el resto de parámetros habituales. Dos sesiones con cada dializador (polisulfona HF80, polietersulfona Arylane H9 y nueva polisulfona APS 900). Se determinó la concentración pre y postdiálisis de urea, creatinina, $\beta_2$-microglobulina ($\beta_2$-m), mioglobina, prolactina (PRL) y $\alpha_1$–microglobulina ($\alpha_1$–m).*

*No hubo diferencias importantes en la depuración de urea y la creatinina. La depuración de $\beta_2$-m fue del 68% con HD y del 81% con HDF-OL, sin apenas diferencias entre los tres dializadores. La mioglobina y PRL presentaron un patrón de depuración similar, con una mayor depuración en HD con los dializadores de nueva generación (60% con Arylane y 59% con APS) respecto a la polisulfona clásica (22% con HF80). La reducción de $\alpha_1$–m en HD fue del 6% y en HDF-OL del 22%. Con HDF-OL y APS 900 se obtuvo una depuración del 35,4%, muy superior con el resto de modalidades y dializadores.*

*En conclusión, los cambios introducidos en los dializadores de nueva generación han facilitado una mayor depuración de toxinas urémicas de tamaño superior a la $\beta_2$-m, especialmente en la modalidad de HD. Es importante cuantificar la capacidad depurativa y en cada modalidad de tratamiento para una adecuada elección y comparación de los dializadores.*


Palabras clave: ***$a_1$-microglobulina. Hemodiafiltración en línea. Hemodiálisis alto flujo. Mioglobina. Prolactina.***

## INTRODUCTION

Renal failure produces an accumulation of substances inside the organism, known as uremic toxins. Throughout the years, we have come to know better these substances and we categorize them by their size as small (< 500 Da), intermediate (500-5000 Da), and big molecules (5000-50,000 Da); they are classified according to their inter-compartmental mass transference coefficient or Kc; we also know a group of protein-bound small toxins which clearance with conventional dialysis or with high-flow dialysis is unsatisfactory.[1-3] The European Working Group on Uremic Toxins[4] has classified a total of 90 solutes that accumulate in renal insufficiency, 45 of which are low molecular weight toxins not bound to proteins, 25 are small protein-bound molecules, and the remaining 22 are compounds with a molecular weight higher than 500 Da, 12 of which have a molecular weight higher than 12,000 Da.

The depurative capability of toxins depends on dialyzers, and on treatment modality and regimen. Low-flow hemodialysis only clears solutes with a molecular weight lower than 5000 Da. High-flow

dialysis represents a form of low-volume hemodiafiltration since both internal filtration and retrofiltration occur within the dialyzer. Hemodiafiltration techniques with high reposition volumes are the ones achieving the best depuration of small, intermediate, and big molecules.[5,6] $b_2$-microglobulin (11,800 Da) is not depurated with low-flow hemodialysis since its size is higher than the diameter of the pores of these dialyzers. There are few studies analyzing depuration of toxins with similar molecular weight.

The pharmaceutical industry has developed and improved the dialyzers to achieve a better depuration of toxins, coming closer to the clearance capability of the healthy kidney. The aim of this study was to evaluate the depuration of big molecules, higher than $b_2$-microglobulin, with different dialysis modalities, with different frequency regimens, and with the new generation of dialyzers.

## PATIENTS AND METHODS

We carried out a non-randomized prospective study comparing high-flow hemodialysis and on-line hemodiafiltration. We studied 24 patients, 15 males and 9 females, with a mean age of 71.6 ± 11 years (range 35-83), on a stable hemodialysis program, 12 on a standard regimen of 4-5 hours, 3 sessions weekly, and 12 on daily short dialysis, 2-2.5 hours, 6 times weekly.

Residual renal function was negligible in all patients. The etiologies of chronic renal failure were chronic glomerulopathy (6), nephroangiosclerosis (9), adult polycystic renal disease (3), tubulointerstitial nephropathy (3), diabetic nephropathy (1), and of unknown origin (2).

All patients were dialyzed with a 4008 Fresenius monitor, with the capability for on-line HDF. Each patient received 6 dialysis sessions while keeping the usual parameters of dialysis time 201 ± 66 min (2-5 h), blood flow (Qb) 409 ± 37 mL/min (range: 350-450 mL/min), dialysis fluid flow (QD) 800 mL/min, usual anticoagulation with low molecular weight heparin, and ultrafiltration adjusted to reach their lean weight. The only changes were dialysis modality, three sessions with high-flow hemodialysis (HD) and three sessions with on-line hemodiafiltration (OL-HDF), and/or the type of dialyzer used: two sessions with the classical high-flow polysulphone (HF80 Fresenius), two with new generation poly aril ether sulphone (Arylane H9, Hospal) and two with new generation polysulphone (APS 900, Asahi); with a surface area of 1.89, 2.01, and 1.80 $m^2$, and an ultrafiltration coefficient (UFC) of 55, 98, and 75 mL/h/mmHg, respectively. All dialyzers were sterilized by gamma radiation or water vapor.

$b_2$-microglobulin was determined by immunoturbidimetry (Quantex $b_2$-microglobulin immunoturbidimetry) with a normal range of 1.1-2.4 mg/L. Myoglobin levels were determined by "sandwich" immunoenzimatic assay (Access", Beckman), with a normal range of 0-70 mg/mL. Prolactin levels were determined by "sandwich" immunoenzimatic assay (Immulite 2000", Beckman), with a normal range of 2-30 ng/mL. $a_1$-microglobulin levels were determined by immunoturbidimetry (Turbitex $a_1$-microglobulin", Biocon), with a normal range of 5-25 mg/L.

In order to correct hemoconcentration during dialysis, percentages of plasma level reduction pre-/post-therapy of $b_2$-microglobulin, myoglobin, prolactin and $a_1$-microglobulin were calculated by the Bergström and Wehle formula.[7]

The results are expressed as arithmetic mean ± standard deviation. For statistically significant analysis of quantitative parameters the Student's t test (paired and non-paired data), and the analysis of variance (ANOVA) for repeated variables have been used. A p value $< 0.05$ has been considered as statistically significant.

## RESULTS

Tolerability of dialysis sessions during the study was satisfactory. There were no relevant complications during the plug-in, session, and plug-out that would rendered difficult the performance of the planned regimens and laboratory workouts.

**Table I.** Percentage of reduction of different molecules and with the different conditions studied

| | Urea | Cr | $\beta_2$-m | Mio | PRL | $\alpha_1$-m |
|---|---|---|---|---|---|---|
| HF 80 (HD) | 74.0 ± 10 | 64.8 ± 10 | 62.0 ± 9 | 21.5 ± 7 | 23.3 ± 9 | -1.9 ± 13 |
| HF 80 (HDF-OL) | 74.3 ± 11 | 66.3 ± 10 | 78.8 ± 9 | 56.5 ± 7 | 54.6 ± 9 | 12.9 ± 8 |
| Arylane H9 (HD) | 74.0 ± 9 | 65.3 ± 9 | 71.4 ± 9 | 60.6 ± 10 | 57.7 ± 11 | 6.9 ± 12 |
| Arylane H9 (OL-HDF) | 75.7 ± 9 | 66.9 ± 8 | 83.1 ± 7 | 74.2 ± 6 | 71.6 ± 8 | 17.6 ± 11 |
| APS 900 (HD) | 73.1 ± 10 | 65.3 ± 9 | 71.9 ± 7 | 59.3 ± 6 | 56.6 ± 8 | 13.1 ± 8 |
| APS 900 (O-HDLL) | 74.8 ± 11 | 66.7 ± 9 | 81.2 ± 8 | 75.7 ± 6 | 74.6 ± 7 | 35.4 ± 16 |

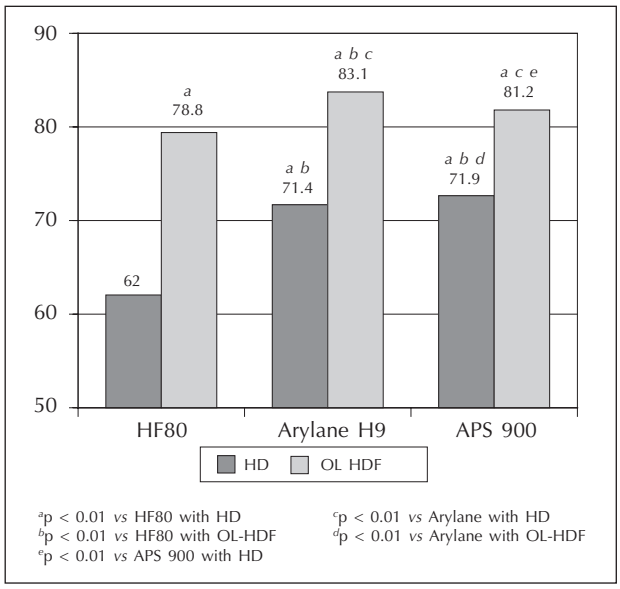


*Fig. 1.—Percentage $\beta_2$-m (11,800 Da) reduction.*

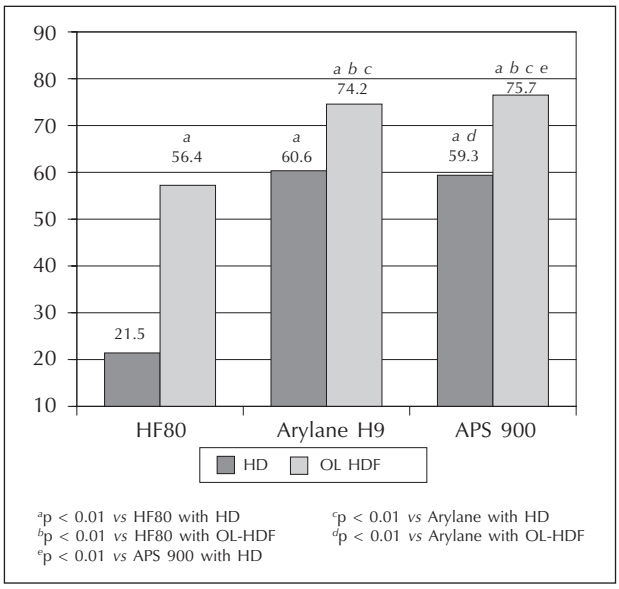


*Fig. 2.—Percentage of myoglobin (17,200 Da) reduction.*

There were no important differences for the depuration of small molecules, such as urea and creatinine, between the different modalities and dialyzers used (Table 1). The percentage of urea reduction was always higher with OL-HDF than with HD, independently of the dialyzer used, although it did not reach an statistically significant difference. The only difference observed was that OL-HDF with the Arylane H9 dialyzer achieved a slightly higher percentage of urea reduction ($p < 0.01$) than that achieved with the APS 900 dialyzer.

Mean pre-dialysis $_2$m value was 25.8 ± 8 mg/L (range: 13-41 mg/L). When we assessed $_2$m depuration, we did find clear differences between HD and OL-HDF, this difference being 13-30% higher for OL-HDF (Figure 1). We did not find important differences between the three dialyzers studied, with the only exception that HF80 in the HD modality had a lower percentage of reduction, 62 ± 9%, as compared to the other two new generation dialyzers, 71 ± 8% (Figure 1).

About higher molecular weight molecules, myoglobin and prolactin had a similar depuration pattern. Pre-dialysis myoglobin levels were 3-4 fold higher than the normal upper limit (220 ± 156 ng/mL), whereas prolactin levels were kept within the normal range (23.9 ± 25 ng/mL). We observed that HF80 dialyzer with HD had a greatly reduced depuration of myoglobin and prolactin, 21.5% and 23.3%, respectively, as compared to the new generation dialyzers assessed, which reached 55-60% depuration (Figures 2 and 3). We also observed differences, although not significant, between OL-HDF modality with HF80 dialyzer (55%), that went up to 70-75% with other dialyzers studied (Figures 2 and 3). Surprisingly depuration of these two molecules with HF80 and OL-HDF was similar to that achieved with new generation dialyzers in HD (Figures 2 and 3).

The most remarkable differences were noticed

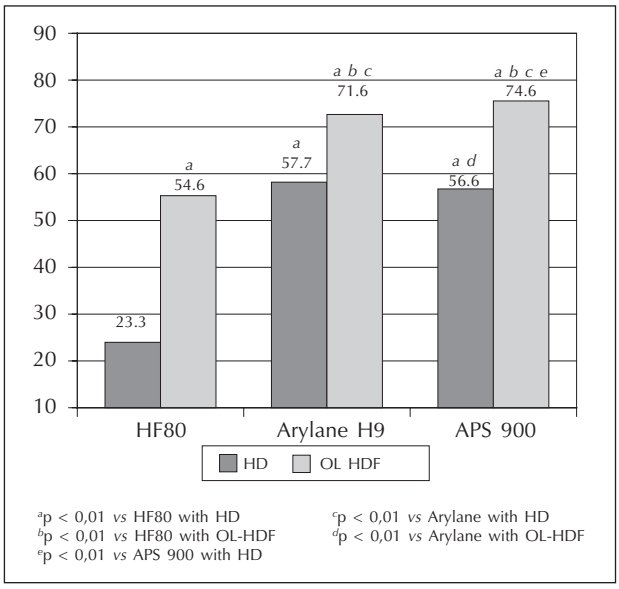


*Fig. 3.—Percentage of prolactin (23,000 Da) reduction.*

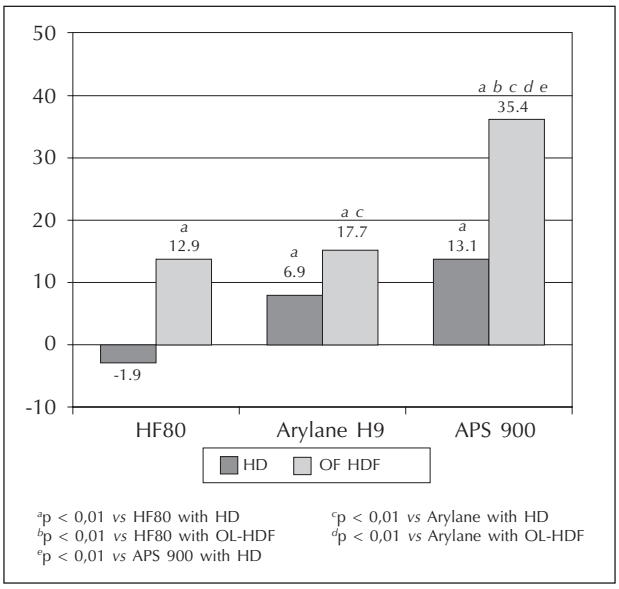


*Fig. 4.—Percentage of $\alpha_1$-m (33,000 Da) reduction.*

with $a_1$-microglobulin, which molecular weight is 3 times higher than that of $_2$m. Mean pre-dialysis levels were 213 ± 133 mg/L (range: 11-448 mg/L), about 8 fold higher the upper normal limit. The percentage of $a_1$-microglobulin reduction with HD modality was naught with HF80 dialyzer, and very discrete with Arylane H9 and APS 900 dialyzers, 6.9 ± 11% and 13.1 ± 8%, respectively (Figure 4). With the OL-HDF modality, maximal depurations were achieved with differences between the three studied dialyzers (Figure 4). The best depuration was achieved with APS 900 dialyzer, which was just 35%, although it represented twice that achieved with Arylane H9 and three fold that of HF80 dialyzers.

Finally, we compared the different frequency groups: Twelve patients with a three sessions per week regimen and mean duration of 262 ± 23 minutes and a re-infusion volume of 26.3 ± 2 L with the OL-HDF; the remaining 12 patients with six weekly sessions regimen, with a mean duration of 139 ± 13 minutes and a re-infusion volume of 14.4 ± 2 L with the on-line HDF modality. As shown in Table 2, depuration of urea, creatinine, and $_2$m was higher with conventional regimen dialysis, logically because of its longer duration. However, it is striking that depuration of myoglobin, prolactin and $a_1$-microglobulin was similar with HF80 and Arylane H9 dialyzers, in spite of the fact that dialysis duration was twice longer. Only the APS 900, with OL-HDF modality showed a greater depuration according to time.

**Table II.** Comparison of depuration of several studied molecules with the 3 sessions per week regimen or daily dialysis regimen

| | 3 sessions/week (263 ± 23 min.) | Daily dialysis (139 ± 13 min.) | Significance |
|---|---|---|---|
| **HF80** | | | |
| *Urea* | | | |
| HD | 82.7 ± 5 | 65.4 ± 6 | P < 0.001 |
| OF-HDF | 83.2 ± 5 | 65.5 ± 7 | P < 0.001 |
| *Creatinine* | | | |
| HD | 72.4 ± 6 | 57.2 ± 6 | P < 0.001 |
| OL-HDF | 74.1 ± 5 | 58.5 ± 6 | P < 0.001 |
| *$\beta_2$m* | | | |
| HD | 68.5 ± 7 | 55.6 ± 6 | P < 0.001 |
| OL-HDF | 84.7 ± 6 | 72.8 ± 9 | P < 0.001 |
| *Myoglobulin* | | | |
| HD | 24.6 ± 8 | 18.4 ± 6 | P < 0.05 |
| OL-HDF | 54.6 ± 7 | 58.3 ± 8 | NS |
| *Prolactin* | | | |
| HD | 24.7 ± 7 | 21.9 ± 11 | NS |
| OL-HDF | 54.8 ± 9 | 54.4 ± 8 | NS |
| *$\alpha_1$m* | | | |
| HD | 0.2 ± 11 | -3.8 ± 15 | NS |
| OL-HDF | 11.3 ± 5 | 14.5 ± 11 | NS |
| **Arylane H9** | | | |
| *Urea* | | | |
| HD | 81.6 ± 6 | 66.6 ± 5 | P < 0.001 |
| OL-HDF | 83.3 ± 6 | 68.2 ± 5 | P < 0.001 |
| *Creatinine* | | | |
| HD | 72.5 ± 5 | 58.1 ± 5 | P < 0.001 |
| OL-HDF | 73.3 ± 6 | 60.5 ± 4 | P < 0.001 |
| *$\beta_2$m* | | | |
| HD | 76.0 ± 9 | 66.9 ± 5 | P < 0.01 |
| OL-HDF | 85.9 ± 6 | 80.2 ± 7 | P < 0.05 |
| *Myoglobulin* | | | |
| HD | 63.7 ± 7 | 57.6 ± 11 | NS |
| OL-HDF | 75.1 ± 7 | 73.5 ± 5 | NS |
| *Prolactin* | | | |
| HD | 59.0 ± 10 | 56.4 ± 12 | NS |
| OL-HDF | 72.7 ± 10 | 70.5 ± 7 | NS |
| *$\alpha_1$m* | | | |
| HD | 7.6 ± 13 | 6.2 ± 11 | NS |
| OL-HDF | 21.2 ± 12 | 14.4 ± 10 | NS |
| **APS 900** | | | |
| Urea | | | |
| HD | 81.8 ± 5 | 64.4 ± 5 | P < 0.001 |
| OL-HDF | 84.2 ± 5 | 65.5 ± 5 | P < 0.001 |
| *Creatinine* | | | |
| HD | 73.0 ± 6 | 57.7 ± 5 | P < 0.001 |
| OL-HDF | 74.4 ± 6 | 59.0 ± 4 | P < 0.001 |
| *$\beta_2$m* | | | |
| HD | 76.8 ± 6 | 67.0 ± 5 | P < 0.001 |
| OL-HDF | 87.1 ± 6 | 75.4 ± 7 | P < 0.001 |
| *Myoglobulin* | | | |
| HD | 62.2 ± 7 | 56.4 ± 4 | P < 0.05 |
| OL-HDF | 80.0 ± 4 | 71.6 ± 6 | P < 0.001 |
| *Prolactin* | | | |
| HD | 59.3 ± 7 | 53.9 ± 8 | NS |
| OL-HDF | 78.4 ± 6 | 70.8 ± 7 | P < 0.01 |
| *$\alpha_1$m* | | | |
| HD | 15.5 ± 8 | 11.0 ± 9 | NS |
| OL-HDF | 44.6 ± 12 | 27.0 ± 15 | P < 0.01 |

## DISCUSSION

Clearance of volume and solutes overload is the primary goal in dialysis. An appropriate depuration of uremic toxins may prevent or slow uremia-related complications. The choice of the dialyzer and treatment modality is essential for depuration of substances. In this study we have checked the improvement in clearance capabilities of new generation dialyzers of a wide range of different molecular weight molecules. This progression has been particularly evident with molecules bigger than $_2$m with high-flow HD modality. Besides, as it was expected, the superiority of OL-HDF over HD has been verified with all kinds of molecules and dialyzers assessed.

In a previous study,[8] we assessed the clearance capability with OL-HDF of eleven high-permeability dialyzers, comparing the relationship between their *in vitro* (ultrafiltration coefficient, clearances and screening coefficient for $b_2$m) and *in vivo* (percentage of reduction of several molecules) performance. Although we could observe differences between filters and their convective capability, the study was limited because molecules bigger than $_2$m were not evaluated.

Depuration of small molecules is more dependent on the dialysis fluid flow rather than Qb, whereas depuration of big molecules only improves when infusion flow is increased.[5,6,8] High-flow HD is a low-volume HDF modality in which the estimated internal infusion volume is 30 mL/min or 4-6 L during four hours.[9]

Several studies have quantified _2m depuration. Kerr *et al.*[10] reported within 3 hours of dialysis session a 54.8% reduction with HD, and 62.7% with OL-HDF. Within four hours of dialysis session, Lornoy *et al.*[11] observed a 49.7% reduction with HD and 72.7% with OL-HDF. After 245 minutes of dialysis session, Maduell *et al.*[12] observed -0.2%, 60%, and 75% reductions with low-flow HD, high-flow HD, and OL-HDF, respectively. In the present study, we have observed a similar depuration with classical polysulphone (62% with HD and 78% with OL-HDF) and slightly higher with new generation dialyzers (71% with HD and 82% with OL-HDF).

There are few studies assessing depuration of molecules with higher molecular weight. In the present study we have evaluated myoglobin and prolactin, observing a similar depuration pattern between molecules with molecular weight of 15,000-25,000 Da. Lepenies *et al.*[13] presented a 7% depuration of leptin (16,000 Da) with HD, 31% with HDF (10 L), and 56% with hemofiltration (18L) at 240 minutes. Maduell *et al.*[12] observed a depuration of myoglobin at 4 hours of session with HD and OL-HDF of 24.7% and 62.2%, respectively. Kim *et al.,*[14] in their study performed with 1.6 $m^2$ polysulphone membrane, showed a 22% reduction of prolactin with HD and 50% with OL-HDF. However, with the new generation dialyzers 60% reduction of myoglobin and 57% reduction of prolactin are achieved with HD, showing that improvements in dialyzers are especially reflected in clearance of molecules bigger than $_2$m with HD modality.

Depuration beyond 30,000 Da is really difficult and there are very few articles on that. Kim *et al.*[14] observed null depuration of $a_1$-microglobulin with HD and 20% with OL-HDF with post-dilution infusion of 20 L. $a_1$-microglobulin clearance was associated with an improvement of pruritus and joint pain. In our study, $a_1$-microglobulin reduction with HD has been null with classical polysulphone, and 13% and 17% with new generation dialyzers, showing the advances brought. With OL-HDF a 13% reduction was observed with classical polysulphone that was increased to 35% with the APS 900 dialyzer.

During the last years, the pharmaceutical industry has put in the market new generation dialyzers with higher surface area, better geometric disposition of the fibers, lower wall width, and/or bigger sizes of the pores in order to achieve better toxins depuration. Van Tellingen *et al.*[15] observed better clearance of protein-bound solutes with "super flux" membranes. Samtleben *et al.*[16] observed improved albumin depuration with poly ether sulphone membranes as compared with classical polysulphone membranes, suggesting the better depuration capability of high molecular weight molecules.

The difficulty for depuration of big molecules may be explained by the low distribution volume due to diffusion resistance between organs or tissues; sometimes, depuration is limited to the intravascular compartment. This resistance may be quantified by the inter-compartmental mass transference coefficient, or Kc, and it depends on the molecular size, dialysis time, and dialysis frequency.[17,18] In the present study, we have observed that depuration of small molecules, even with a molecular weight up to that of $b_2$m, was higher with the three sessions weekly regimen because of longer duration of the sessions. However, with higher size molecules (myoglobin, prolactin, and $a_1$-microglobulin) depuration with conventional dialysis and with daily dialysis was similar, although dialysis time was double with the latter. Only the APS 900 dialyzer with the OL-HDF modality achieved better depuration with increasing time. Therefore, the increase in dialysis frequency would improve depuration of big molecules due to

this low Kc, since similar clearance percentages would be achieved more times during the week.

To conclude, the changes introduced in new generation dialyzers have facilitated a better depuration of uremic toxins with a molecular weight higher than that of $b_2m$, especially with the HD modality. In spite of this improvement, there still exist some difficulty for depurating molecules higher than 30,000 Da. It is important to quantify the depurative capability *in vivo* and with each treatment modality to adequately select and compare among the different dialyzers.

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