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ORIGINAL ARTICLE

Middle molecule elimination in expanded haemodialysis: only convective transport?

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ABSTRACT

Background. New high-retention onset dialysers have shown improved efficacy in the elimination of uraemic toxins, and their depurative capacity has been compared with high convective volumes of online haemodiafiltration. Haemodialysis (HD) using high-flux membranes leads to convective transport by internal filtration [direct filtration (DF)/backfiltration (BF)] and allows the removal of middle molecules (MMs). The aim of this study was to assess solute transport mechanisms in expanded HD (HDx).

Methods. In 14 4-h HDx sessions with Theranova-500 dialysers under similar dialysis conditions (blood flow 400 mL/min, dialysate flow 700 mL/min, dialysate temperature 35.5°C), pressures at the inlet and outlet of both dialyser compartments (P_{bi}, P_{bo}, P_{di} and P_{do}) were collected hourly to estimate DF/BF volumes by semi-empirical methods. Uraemic toxins with various molecular weights were measured pre-dialysis, at 1 h (pre-filter and post-filter) and post-dialysis to calculate molecules' reduction over time and dialyser *in vivo* clearances.

Results. Ultrafiltration was 1.47 ± 0.9 L and Kt/V 1.74 ± 0.3. Hydrodynamic data (P_{bi}: 259 ± 39, P_{bo}: 155 ± 27, P_{di}: 271 ± 30, P_{do}: 145 ± 29 mmHg and oncotic pressure 22.0 ± 3.5 mmHg) allowed the estimation of DF/BF rates. DF flow ranged from 29.5 ± 4.2 to 31.3 ± 3.9 mL/min and BF flow ranged from 25.1 ± 2.3 to 23.4 ± 2.6 mL/min. The highest calculated DF volume was 7506.8 ± 935.3 mL/session. Diffusive clearances (K_d) of all solutes were higher than their convective transport (all $P < 0.001$) except for prolactin (23 kDa) clearances, which showed no differences. Total clearances of all solutes were correlated with their K_d ($\rho = 0.899$–0.987, all $P < 0.001$) and Kt/V correlated with all reduction rates ($\rho = 0.661$–0.941, $P = 0.010$ to <0.001). DF flow was only associated with urea ($\rho = -0.793$, $P = 0.001$), creatinine ($\rho = -0.675$, $P = 0.008$) and myoglobin clearance ($\rho = 0.653$, $P = 0.011$).

Conclusion. Results suggest that diffusive transport is a main mechanism of MM elimination in HDx. HDx offers an efficient depuration of MM without the need for high convective volumes.

Keywords: backfiltration, expanded haemodialysis (HDx), high-retention onset (HRO), medium cut-off (MCO), middle molecules

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INTRODUCTION

The elevated mortality rates of patients on haemodialysis (HD) have been related to the retention of a wide variety of uraemic toxins [1, 2]. Although small-sized (<500 Da) water-soluble solutes are easily removed by diffusive transport mechanisms, larger-sized molecules [i.e. middle molecules (MMs) sized 500 Da to 60 kDa] and protein-bound solutes are difficult to eliminate with conventional dialysis techniques [3]. However, the use of high-permeability membranes (high-flux HDx) allows MM elimination by internal filtration [direct filtration (DF)/back-filtration (BF)] [4].

The association of MMs with higher morbidity and mortality has boosted the development of convective therapies over recent years. Online haemodiafiltration (OL-HDF) with high convective volumes obtains better efficacy in MM elimination compared with high-flux HD, and it has been associated with improved outcomes [5, 6]. However, high-permeability dialysers used in high-flux HD and OL-HDF have cut-off values around 20 kDa [7], which limits the elimination of larger molecules through the membrane pores.

The development of medium cut-off or high-retention onset (HRO) dialysers has defined a new concept: expanded HD (HDx) [8]. Compared with high-flux membranes, the design of HRO membranes results in higher cut-off values, close to but lower than that of albumin, enabling the passing of larger solutes. Their tight pore size distribution results in a steep sieving coefficient (S_c) curve with molecular weight retention onset (MWRO) (where $S_c \approx 0.9$) and molecular weight cut-off (MWCO) (where $S_c \approx 0.1$) very close to each other, resulting in improved solute removal from a wide range of sizes by minimizing albumin leakage [9]. Other papers studying HDx have observed similar results of high convective volumes of OL-HDF in smaller MM removal, which are even higher for larger MMs [10, 11].

The aim of this study was to assess the transport mechanisms for uraemic toxin elimination in HDx with HRO membranes.

MATERIALS AND METHODS

Study design

A prospective observational analysis of HDx features was performed in 14 patients on maintenance OL-HDF. In a midweek dialysis session, patients underwent an HDx session with Theranova-500 (Baxter International Inc., Deerfield, IL, USA) under similar dialysis conditions: blood flow (Q_b) 400 mL/min, dialysate flow (Q_d) 700 mL/min, dialysate temperature 35.5°C and 240-min long. All sessions were carried out using a DBB-EXA dialysis monitor (Nikkiso Inc., Tokyo). Dialysate fluid composition and conductivity, system anticoagulation, and ultrafiltration pattern and volume were individualized according their usual dialysis prescription.

Inclusion criteria

Inclusion criteria were age >18 years, habitual $Q_b \geq 400$ mL/min and post-dilution OL-HDF as maintenance therapy. Exclusion criteria were vascular access issues, events or hospitalizations in the last 3 months and history of hypersensitivity reactions to synthetic HD membranes. Of the 34 patients from our dialysis unit who met all criteria, 14 patients were randomly selected for the study. The patients included signed informed consent forms and the study complies with the Declaration of Helsinki.

Laboratory

Plasmatic levels of small-sized uraemic toxins and MMs (urea: 60 Da, phosphate: 96 Da, creatinine: 113 Da, β2-microglobulin: 11.8 kDa, cystatin-C: 13 kDa, myoglobin: 17.2 kDa and prolactin: 23 kDa), and serum albumin levels (66 kDa), were assessed pre-dialysis, post-dialysis, and at 60 min at blood inlet (pre-filter) and outlet (post-filter) into the dialyser.

Dialyser excretion ratios (ER) and total dialyser clearances (K_D) of uraemic toxins and albumin were calculated from the blood compartment at 1 h. Also, solute reduction ratios (RRs) in the whole session, in the first hour and in the last 3 h were calculated to evaluate their elimination throughout the session, using the following formulas:

$$\mathrm{ER} = \frac{\left([\mathrm{Solute}]_{\mathrm{prefilter}} - [\mathrm{Solute}]_{\mathrm{postfilter}}\right)}{[\mathrm{Solute}]_{\mathrm{prefilter}}}$$

$$K_D = Qb \cdot \mathrm{ER}$$

$$\mathrm{RR} = 100 \cdot \left(\frac{[\mathrm{Solute}]_{\mathrm{pre}} - [\mathrm{Solute}]_{\mathrm{post}}}{[\mathrm{Solute}]_{\mathrm{pre}}}\right)$$

To calculate albumin RRs, albumin levels at 60 and 240 min ($[\mathrm{albumin}]_{\mathrm{post}}$) were corrected with ultrafiltration in the whole session, in the last 3 h or in the first hour according to the corresponding period [12]:

$$[\mathrm{Albumin}_{\mathrm{post}}]' = \frac{[\mathrm{Albumin}]\mathrm{post}}{1 + \left(\frac{(\mathrm{Ultrafiltration})}{0.2 \cdot (\mathrm{Predialysis\ Weight} - \mathrm{Ultrafiltration})}\right)}$$

Blood viscosity parameters [haemoglobin, haematocrit (HTC), total proteins (C_p), albumin and gammaglobulins] were evaluated at the beginning and at 60 min to calculate oncotic pressure [13] (π) and plasma and blood viscosity [14] (μ) with the formulas:

$$\pi = [2.1 \cdot C_p] + [0.16 \cdot (C_p)^2] + [0.009 \cdot (C_p)^3]$$

$$\mu = 0.6915 \cdot \left(1 + \left(\frac{(1.22 - 1) \cdot C_p}{7}\right)\right) \cdot \left(1 + 2.5 \cdot \left(\frac{\mathrm{HTC}}{100}\right)\right)$$

Monitor screen parameters

By using different pressure sensors, we collected arterial pressure (AP), blood pressure at the inlet (P_{bi}) and outlet of the dialyser (P_{bo}), dialysate pressure at the inlet (P_{di}) and outlet (P_{do}), and transmembrane pressure (TMP_m) each hour. Every hour, the average TMP (TMP_c) was calculated using inlet and outlet pressures [$TMP_c = (P_{bi} + P_{bo}) - (P_{di} + P_{do})$]. Also, the inlet TMP [$TMP_i = (P_{bi} - P_{do})$], outlet TMP [$TMP_o = (P_{bo} - P_{di})$] and pressure drop in both compartments were obtained hourly.

Other monitor screen data included Q_b, Q_d, ultrafiltration, urea clearance by ultraviolet light absorbance (K_{DDM}) (Dialysis Dose Monitor, Nikkiso), urea RR (URR_{DDM}) and Kt/V (Kt/V_{DDM}). Kt/V_{DDM} was obtained with K_{DDM} and urea distribution volume (UDV) by pre-dialysis bioimpedance, using the Daugirdas formula for spKt/V [15].

Convective volumes calculation

Convective transport was estimated using different semi-empirical models previously used to quantify internal filtration

with Theranova [16]. Models were developed using P_{bi}, P_{bo}, P_{di}, P_{do} and dialyser characteristics provided by the manufacturer (ultrafiltration coefficient, K_{UF}: 59 mL/h/mmHg; surface area: 2 m^2). The area under the TMP curve (AUC_{TMP}) on the axial axis of the dialyser represents DF/BF flow. DF flow (Q_{DF}) was obtained from the area where DF occurs (A_{DF}) and from the average TMP in that DF area, and BF flow (Q_{BF}) was obtained from the BF area (A_{BF}) and from the average TMP in that BF area:

$$Q_{DF} = K_{UF} \cdot A_{DF} \cdot TMP_{DF}$$

$$Q_{BF} = K_{UF} \cdot A_{BF} \cdot TMP_{BF}$$

The first 'linear' model (Model A1) was obtained assuming a linear pressure drop in both compartments and therefore a linear profile of TMP along the dialyser. The cut-off point (X_o) between the lines describing pressures in both compartments reflects the section of the dialyser where ultrafiltration flow changes from DF to BF, and was calculated with the formula:

$$Xo\,(A1) = \frac{P_{do} - P_{bi}}{(P_{bo} - P_{bi}) - (P_{di} - P_{do})} = \frac{TMP_i}{\Delta P_b + \Delta P_d}$$

In another version of the linear model (Model A2), blood oncotic pressure (π) was added to the previous formula:

$$Xo\,(A2) = \frac{P_{do} - (P_{bi} - \pi)}{((P_{bo} - \pi) - (P_{bi} - \pi)) - (P_{di} - P_{do})} = \frac{TMP_i - \pi}{\Delta P_b + \Delta P_d}$$

The second 'geometric' model (Model B1) also assumes linear pressure drops in both compartments, but with different slopes for the line that characterizes the DF and BF segments. This model assumes that, in the absence of ultrafiltration, AUC_{TMP} for DF and AUC_{TMP} for BF must be equal to ensure volumetric control; in case of ultrafiltration, AUC_{TMP} for DF must be greater than AUC_{TMP} for BF, and it is equivalent to the sum of AUC_{TMP} for BF and the TMP needed to achieve the programmed ultrafiltration (TMP_{uf}) ($Q_{DF} = Q_{BF} + Q_{UF}$). The cut-off point (X_o) between pressure lines was calculated with the formula:

$$\frac{TMP_i \cdot X_o}{2} = \frac{TMP_o \cdot (1 - X_o)}{2} + TMP_{uf}$$

So:

$$X_o\ (B1) = \frac{TMP_o + (2 \cdot TMP_{uf})}{TMP_i + TMP_o}$$

In another version of the geometric model (Model B2), π was added to the previous formula:

$$X_o\ (B2) = \frac{TMP_o + \pi + (2 \cdot TMP_{uf})}{TMP_i + TMP_o}$$

Models A2 and B2 consider the effect of oncotic pressure on convective transport, so it was subtracted from TMP_{DF} to calculate Q_{DF} and it was added to TMP_{BF} to calculate Q_{BF}. Both models understand oncotic pressure as a constant value along the dialyser and throughout the session. Pre-dialysis oncotic pressure was used to estimate overall DF/BF volumes in the whole session, and oncotic pressure at 60 min to estimate DF/BF flows in the first hour.

Efficacy measurements: transport mechanisms for molecule elimination

Convective clearances (K_c) were calculated with DF flow at 60 min and theoretical S_c ($K_c = Q_{DF} \cdot S_c$). According to membrane characteristics (MWRO ≈12 kDa and MWCO ≈50 kDa) [9], and assuming a linear reduction of S_c as the molecular weight increases, theoretical S_c values obtained for MMs were: S_c β2-microglobulin 0.90; S_c cystatin-C 0.88; S_c myoglobin 0.79; and S_c prolactin 0.67. An $S_c \approx 0.01$ was used for albumin and $S_c \approx 1$ was used for low-molecular weight solutes. Diffusive clearances (K_d) at 60 min were estimated with the difference between K_D and convective clearances at that time ($K_d = K_D - K_c$).

Overall, mass transfer (MT_{ovr}) was estimated from K_D using the formula:

$$MT_{ovr} = K \cdot \left(\frac{([Solute]pre - [Solute]post)}{Ln\ ([Solute]pre/[Solute]post)} \right) \cdot Time$$

Overall clearances (K_{ovr}) were calculated from MT_{ovr} and the AUC of plasmatic levels, with the formula [17]:

$$K_{ovr} = \frac{MT_{ovr}}{AUC}$$

$$AUC = \left(\frac{([Solute]pre + [Solute]1h)}{2} \cdot \frac{60}{240} \right) + \left(\frac{([Solute]1h + [Solute]post)}{2} \cdot \frac{180}{240} \right)$$

Overall convective clearances ($K_{ovr(c)}$) were estimated from average DF flow and theoretical S_c. Overall diffusive clearances ($K_{ovr(d)}$) were calculated with the difference between K_{ovr} and $K_{ovr(c)}$ [$K_{ovr(d)} = K_{ovr} - K_{ovr(c)}$].

Statistical analysis

Statistical analysis was performed using SPSS Statistics, version 21 (SPSS, Inc., Chicago, IL, USA). The Kolmogorov–Smirnov test was used to analyse the variables' distribution patterns. Descriptive results were expressed as mean ± standard deviation for normally distributed values, median (interquartile range) for non-normal distributed quantitative variables and percentages for qualitative variables. Given the small sample size, non-parametric tests (Spearman, Wilcoxon and Mann–Whitney) were used to analyse the association between efficacy variables, convective transport, patient features and molecule elimination. A value of $P < 0.05$ was considered statistically significant with a 95% confidence interval.

RESULTS

Patients' features

Demographic data, patient dialysis-related features, anthropometric measurements and body composition are shown in Table 1.

Dialysis features

Average effective Q_b was 401.4 ± 3.1 mL/min, with blood pump Q_b 428.3 ± 20.6 mL/min and AP −103.5 ± 42.5 mmHg. Pre-dialysis weight was 68.5 ± 18.9 kg and ultrafiltration volume was

Table 1. Patient features

Patient feature	Mean ± SD[a] Percentage; n[b]
Age (years)	64.6 ± 17.6
Gender: male (%; n)	71.4; 10
CKD aetiology (%; n)	
Diabetes	21.4; 3
Glomerular	14.3; 2
Vascular	14.3; 2
PKD	14.3; 2
Interstitial	7.1; 1
Other/unknown	28.6; 4
Previous kidney transplant	7.1; 1
Dialysis vintage (months)	49.6 ± 36.2
Residual diuresis volume > 500 mL/day (%; n)	35.7; 5
Vascular access	
AVF	78.6; 11
AVG	7.1; 1
CVC	14.3; 2
Anthropometric measurements and body composition	
Pre-dialysis weight (kg)	68.5 ± 18.9
Post-dialysis weight (kg)	67.0 ± 19.2
BMI (kg/m^2)	22.8 ± 4.93
BSA (m^2)[c]	1.77 ± 0.26
UDV (L)[d]	37.8 ± 11.7

[a]Quantitative variables are expressed as mean and SD.
[b]Qualitative variables are expressed as percentages and absolute values.
[c]BSA using DuBois and DuBois formula.
[d]Data from pre-dialysis bioimpedance spectroscopy.
AVF: arteriovenous fistulae; AVG: arteriovenous graft; BMI: body mass index; BSA: body surface area; CKD: chronic kidney disease; CVC: central venous catheter; PKD: polycystic kidney disease.

1.47 ± 0.89 L. Table 2 shows dialysis parameters obtained hourly from the monitor screen.

Convective transport

Figure 1 describes blood and dialysate pressures, TMP profiles along the axial axis and estimated convective volumes obtained from each semi-empirical model. The highest DF estimated rates were 1876.7 ± 233.8 mL/h and 1717.9 ± 218.4 mL/h with geometric Models B1 and B2, respectively.

Pre-dialysis blood tests showed haemoglobin 11.1 ± 1.3 g/dL, HTC 32.7 ± 4.3% and total proteins 6.31 ± 0.67 g/dL (albumin 3.78 ± 0.43; gammaglobulins 1.14 ± 0.38). Pre-dialysis blood oncotic pressure was 22.0 ± 3.5 mmHg and blood viscosity was 1.51 ± 0.1 cP. Oncotic pressure at 60 min was 20.7 ± 3.4 mmHg.

Higher haemoglobin levels were associated with greater estimated convective transport in all models (DF volume: $\rho = 0.609$–0.825, P = 0.023 to <0.001; BF volume: $\rho = 0.561$–0.759, P = 0.037–0.002), related to higher TMP_i ($\rho = 0.829$, P < 0.001) and TMP_o ($\rho = -0.535$, P = 0.049). Total proteins were only associated with higher DF volume in Model A1 ($\rho = 0.548$, P = 0.042) and higher BF volume in Model A2 ($\rho = 0.542$, P = 0.045) and Model B1 ($\rho = 0.559$, P = 0.038). No association was observed between albumin or gammaglobulins and DF/BF volumes.

Efficacy in molecule elimination

Plasmatic levels of uraemic toxins and albumin, and their RRs throughout the session, are summarized in Table 3, whereas clearances and mass transfers of each solute are included in Table 4.

Figure 2 represents the contribution of both diffusion and convection to the K_{ovr} of each solute. For all the solutes, the K_d was greater than its transport by convection (all P < 0.001), except in the case of prolactin, where there was no difference.

Diffusive clearances of every solute were correlated with their K_D ($\rho = 0.899$–0.987, all P < 0.001). DF flow, and therefore K_c, were negatively correlated with urea and creatinine clearances in all models (urea: $\rho = -0.736$ to -0.793, P = 0.003–0.001; creatinine: $\rho = -0.565$ to -0.675, P = 0.035–0.008), being even stronger than the negative correlation with K_d (urea: $\rho = -0.855$ to -0.908, P < 0.001; and creatinine: $\rho = -0.714$ to -0.793, P = 0.004–0.001). In contrast, DF/BF flows were directly correlated with myoglobin clearance in Model A1 ($\rho = 0.653$, P = 0.011 for DF flow and $\rho = 0.604$, P = 0.022 for BF flow) and Model A2 ($\rho = 0.587$, P = 0.027 for DF flow and $\rho = 0.723$, P = 0.003 for BF flow). In Model A2, BF flow was also associated with greater myoglobin K_d ($\rho = 0.600$, P = 0.023).

K_{DDM} was correlated with urea K_D ($\rho = 0.552$, P = 0.041) and creatinine K_D ($\rho = 0.688$, P = 0.007). Kt/V_{DDM} and URR_{DDM} were correlated with all the RRs ($\rho = 0.661$–0.941, P = 0.010 to <0.001 and $\rho = 0.648$–0.943, P = 0.012 to <0.001, respectively).

Factors related to molecule elimination

Pre-dialysis albumin levels were negatively correlated with urea RR ($\rho = -0.654$, P = 0.011), creatinine RR ($\rho = -0.612$, P = 0.020), β2-microglobulin RR ($\rho = -0.592$, P = 0.026) and cystatin-C RR ($\rho = -0.601$, P = 0.023), and haemoglobin levels were negatively associated with β2-microglobulin RR ($\rho = -0.539$, P = 0.047). Blood oncotic pressure and viscosity were only associated with lesser β2-microglobulin RR ($\rho = -0.548$, P = 0.042 and $\rho = -0.530$, P = 0.05, respectively).

All the RRs were negatively correlated with weight, body surface area and UDV, with the strongest correlation with UDV measured by bioimpedance (urea RR $\rho = -0.934$, P < 0.001; creatinine RR $\rho = -0.908$, P < 0.001; phosphate RR $\rho = -0.714$, P = 0.004; β2-microglobulin RR $\rho = -0.868$, P < 0.001; cystatin-C RR $\rho = -0.846$, P < 0.001; myoglobin RR $\rho = -0.635$, P = 0.015; and prolactin RR $\rho = -0.692$, P = 0.006). In a multivariable regression analysis including UDV, pre-dialysis serum albumin and, in the case of β2-microglobulin, blood viscosity parameters, only UDV remained an independent predictor for molecule reduction (urea: $\beta = -0.835$, P < 0.001; creatinine $\beta = -0.601$, P = 0.023; β2-microglobulin $\beta = -0.679$, P = 0.016; and cystatin-C $\beta = -0.601$, P = 0.023). The final RRs of all uraemic toxins were correlated with Kt/V_{DDM} ($\rho = 0.661$–0.941, P = 0.010 to <0.001).

DISCUSSION

The present analysis of the mechanisms for uraemic toxin elimination with HRO membranes suggests that diffusion plays an essential role in the removal of a wide variety of molecules up to 23 000 Da. The use of these HRO membranes could change the widespread concept that diffusion is only useful for the elimination of small solutes [18, 19]. Therefore, high convective volumes are not necessary to achieve effective removal of MMs. In most situations where there are limitations regarding achieving efficient convective transport, patients could benefit from the prescription of HDx.

Results in molecule elimination were similar to those found in other recent studies [10, 20] and comparable to those achieved in post-dilution OL-HDF. As seen in Figure 2, the K_D of all molecules exceeded their K_c. Discarding the convective component of total clearance and assuming the absence of molecule

Table 2. Dialysis parameters obtained from monitor screen

Dialysis parameter	5 min	60 min	120 min	180 min	240 min
Effective Q_b (mL/min)	400.9 ± 3.8	400.7 ± 4.6	401.9 ± 2.8	401.9 ± 3.1	401.4 ± 3.5
Ultrafiltration (L)	–	0.38 ± 0.23	0.74 ± 0.45	1.11 ± 0.69	1.47 ± 0.89
K_{abs} DDM (mL/min)	–	337.7 ± 61.5[a,d]	303.1 ± 51.2[a,b]	286.9 ± 51.1[b,c]	275.7 ± 51.7[c,d]
Kt/V	–	0.55 ± 0.13	0.96 ± 0.19	1.39 ± 0.26	1.74 ± 0.32
URR (%)	–	40.4 ± 7.4	58.1 ± 7.3	70 ± 7	76.9 ± 6
P_{bi} (mmHg)	255 ± 36	260 ± 43	260 ± 40	261 ± 42	260 ± 44
P_{bo} (mmHg)	155 ± 27	161 ± 32	153 ± 29	154 ± 30	153 ± 29
P_{di} (mmHg)	271 ± 34	276 ± 34	271 ± 31	269 ± 32	269 ± 32
P_{do} (mmHg)	144 ± 27	150 ± 35	144 ± 31	143 ± 32	144 ± 32
TMP_m (mmHg)	1.6 ± 2.4	1.4 ± 5.1	1.9 ± 4.5	1.6 ± 5	2.6 ± 5.6
TMP_c (mmHg)	−2.8 ± 7.8	−2.1 ± 8.7	−1 ± 7.7	1 ± 7.9	−0.4 ± 9.4
TMP_i (mmHg)	110.4 ± 17.6	110.5 ± 17.2	115.9 ± 15.4	117.5 ± 17.7	115.8 ± 18.9
TMP_o (mmHg)	−115.9 ± 12.8	-114.6 ± 4.8	−118 ± 6.1	−115.4 ± 4.9	−116.6 ± 5.1
dP_b (mmHg)	99.7 ± 19[e]	99.3 ± 19.2	106.4 ± 17.7	106.5 ± 20.3	106.9 ± 20.6[e]
dP_d (mmHg)	126.6 ± 12.1	125.8 ± 1.8	127.5 ± 2.2	126.4 ± 1.8	125.6 ± 2.9

[a]Wilcoxon test for paired samples (P=0.003).
[b]Wilcoxon test for paired samples (P=0.001).
[c]Wilcoxon test for paired samples (P=0.002).
[d]Wilcoxon test for paired samples (P=0.001).
[e]Wilcoxon test for paired samples (P=0.026).
a-e characters represent comparisons between 60 and 120 min (a), between 120 and 180 min (b), between 180 and 240 min (c), between 60 and 240 min (d), and between 5 and 240 min (e).
DDM: dialysis dose monitor; dP_b: pressure drop in the blood compartment; dP_d: pressure drop in the dialysate compartment; K_{abs}: urea clearance measured by UV absorbance; Kt/V: spKt/V by Daugirdas, from urea clearance measured by UV absorbance and urea distribution volume measured by bioimpedance; P_{bi}: pressure at the inlet of blood compartment; P_{bo}: pressure at the outlet of blood compartment; P_{di}: pressure at the inlet of dialysate compartment; P_{do}: pressure at the outlet of dialysate compartment; Q_b: blood flow; TMP_m: transmembrane pressure from monitor screen; TMPc: calculated 4-points transmembrane pressure; TMPi: transmembrane pressure at blood inlet; TMPo: transmembrane pressure at blood outlet; URR: urea reduction ratio measured by UV absorbance.

adsorption to the membrane, it can be deduced that diffusive transport mechanisms play a major role in the elimination of these solutes.

Convective transport

Numerous studies have attempted to quantify convective transport by internal filtration inside the dialyser by several different methods [21, 22]. Semi-empirical models included multiple errors in the estimation of DF/BF flows while linear models overestimated BF rates, which is not compatible with volumetric control of patients. Geometric models complied with ultrafiltration volume and were in accordance with fluid balance within the dialyser (DF = BF + UF). Here, Model B2 includes oncotic pressures aimed at increasing accuracy but probably underestimates blood pressure drops during DF (Figure 1).

Other nonlinear, more rigorous mathematical models include changes in oncotic pressure and HTC as a result of haemoconcentration along the dialyser to calculate local Q_{DF} and Q_{BF} [23, 24]. These *in vitro* models have estimated DF/BF rates of 1978 mL/h (without ultrafiltration) using Theranova-500 with Q_b 400 mL/min [16], which represents a slightly higher estimated volume than with Model B1 or B2 (5 and 13%, respectively).

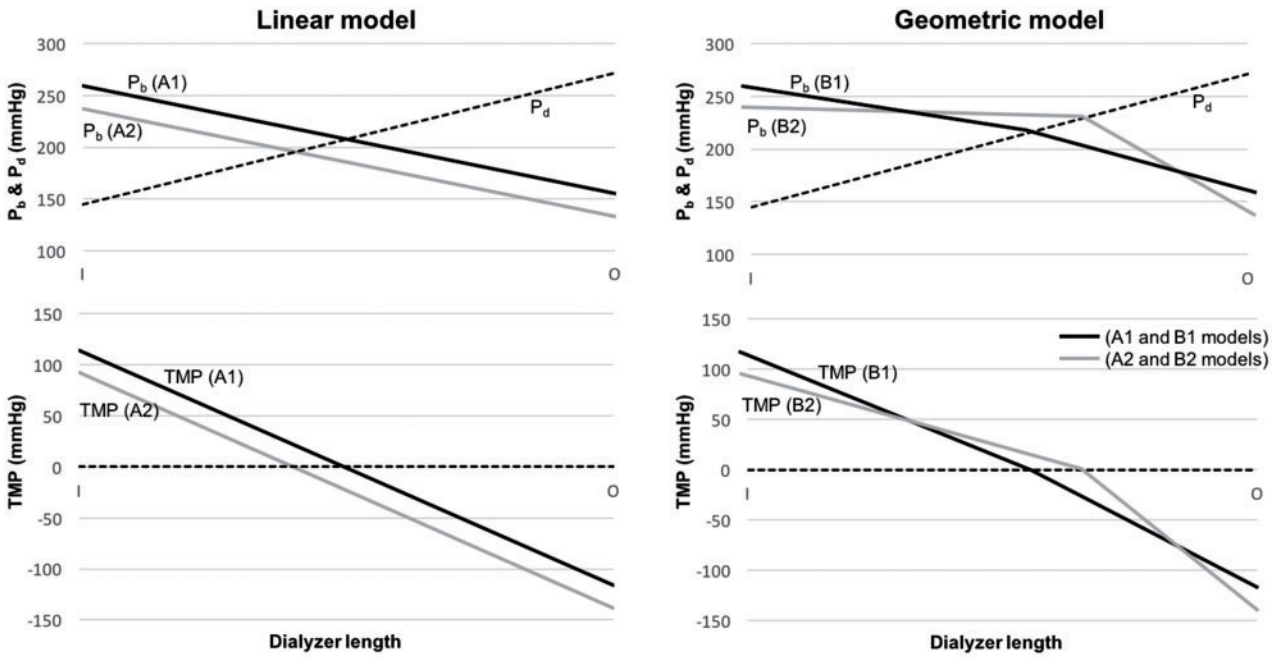
The highest estimated DF flows would suppose a maximum K_c (for molecules with $S_c \approx 1$) of 31 mL/min, but much higher DF/BF volumes would be necessary to achieve these total clearances by convection only.

Another issue to be considered is the true value of S_c during clinical performance. The value of S_c for a particular solute and membrane is empirically determined by the absence of a gradient for diffusion during isolated ultrafiltration experiments. However, effective sieving properties are different from the ideal value due to the interference of cells and proteins in the blood compartment and other phenomena, such as polarization at the blood–membrane interface [25]. Therefore, *in vitro* S_c values used for calculations probably overestimate convective clearances and, as a consequence, the role of diffusive transport may be even more important.

Diffusive transport

The development of these 'high pore size' membranes has probably led to a wider range of molecules that can be efficiently eliminated by diffusion. As shown in Tables 3 and 4, solute removal decreases as their size increases, both their elimination in the dialyser (ER and K_D) and globally throughout the session (RR and MT_{ovr}). It should be noted that this 'size-dependent' decrease in solute clearance is proportionally much greater than the decrease in S_c and, therefore, cannot be explained solely by the reduction in K_c. However, the reduction in K_d with the increase in molecular size is proportionally more consistent with and parallel to the reduction in K_D (Figure 2). The reduction in diffusive transport is probably conditioned by a decreasing mobility of larger solutes as their size increase.

For molecules over 20 kDa (such as prolactin), the contribution of K_d is reduced to be comparable with K_c, which determines an equitable contribution of both transport mechanisms in their elimination. Nevertheless, this progressive reduction in K_d with the increase in size conditions the expected diffusion of molecules >25–30 kDa to be minimal or negligible, while convective transport becomes more important. The lack of information for molecules greater in size than prolactin is a major limitation of the study, since they have been shown to be effectively eliminated with HDx. Further studies are required to



	Direct Filtration	Backfiltration
Linear model (A1)		
· Dialyzer area (%)	49.3 ± 3.0	50.7 ± 3.0
· Dialyzer area (m²)	0.987 ± 0.06	1.013 ± 0.06
· Flow / Rate (ml/min)*	27.9 ± 5.7	28.9 ± 1.7
· Volume (mL/session)*	6689.8 ± 1378.5	6937.6 ± 413.5
Linear model, including oncotic pressure (A2)		
· Dialyzer area (%)	39.7 ± 3.2	60.3 ± 3.2
· Dialyzer area (m²)	0.795 ± 0.06	1.205 ± 0.06
· Flow / Rate (ml/min)*	18.2 ± 4.5	27.8 ± 1.8
· Volume (mL/session)*	4364.7 ± 1071.1	6684.2 ± 436.1

	Direct Filtration	Backfiltration
Geometric model (B1)		
· Dialyzer area (%)	55.9 ± 3.0	44.1 ± 3.0
· Dialyzer area (m²)	1.12 ± 0,06	0.88 ± 0,06
· Flow / Rate (ml/min)*	31.3 ± 3.9	25.1 ± 2.3
· Volume (mL/session)*	7506.8 ± 935.3	6036.1 ± 562.0
Geometric model, including oncotic pressure (B2)		
· Dialyzer area (%)	65.6 ± 2.8	34.4 ± 2.8
· Dialyzer area (m²)	1.31 ± 0.06	0.69 ± 0.06
· Flow / Rate (ml/min)*	29.5 ± 4.2	23.4 ± 2.6
· Volume (mL/session)*	7093.2 ± 1005	5622.5 ± 635.4

FIGURE 1: Blood and dialysate pressures along the device (upper graphs), transmembrane pressure profile along the axial axis (middle graphs) and estimated convective volumes obtained from each semi-empirical model (lower tables).

Table 3. Uraemic toxins plasmatic levels and reduction throughout the session

Solute	Pre-dialysis levels	Levels at 60 min	Post-dialysis levels	RR (0–60 min) (%)	RR (60–240 min) (%)	RR (0–240 min) (%)
Urea (mg/dL)	109.9 ± 23.5	52.4 ± 12.6	17.0 ± 8.4	52.1 ± 8.1	69.2 ± 10.7	84.7 ± 7.4
Phosphate (mg/dL)	4.0 ± 0.74	1.99 ± 0.44	1.69 ± 0.69	49.1 ± 11.3	17.3 ± 24.2	58.2 ± 15.6
Creatinine (mg/dL)	7.18 ± 2.14	3.65 ± 1.25	1.64 ± 0.82	49.5 ± 6.2	57.0 ± 11.9	77.9 ± 7.9
β2-microglobulin (mg/L)	20.2 ± 6.4	8.9 ± 2.9	4.1 ± 1.5	55.7 ± 6.7	53.1 ± 9.9	79.1 ± 5.8
Cystatin C (mg/L)	6.01 ± 0.89	2.82 ± 0.67	1.59 ± 0.4	53.3 ± 7.6	43.3 ± 9.4	73.5 ± 5.9
Myoglobin (ng/mL)	209.1 ± 105.4	115.9 ± 67.7	72.9 ± 40.4	46.1 ± 6.7	35.6 ± 9.0	65.4 ± 5.7
Prolactin (mg/L)	16.4 ± 10.8	9.4 ± 6.2	5.7 ± 3.4	40.5 ± 9.8	37.1 ± 11.3	62.4 ± 10.9
Albumin (g/dL)	3.78 ± 0.43	3.64 ± 0.5	3.75 ± 0.41	6.5 ± 8.2	5.1 ± 6.2	10.9 ± 5.9

Albumin levels after each period (0–60, 60–240 and 0–240 min) were adjusted with the corresponding ultrafiltration for its RR calculation.

evaluate solute transport mechanisms for those large-sized MMs with HDx.

Similar to the interaction mechanisms between diffusion and convection described with other techniques [26], a negative correlation was found between the diffusion of small solutes (urea and creatinine) and convective transport. In contrast, the association of myoglobin clearance, both with its K_d and with DF flow, suggests that both mechanisms are complementary for the elimination of MMs rather than competitive.

While blood viscosity data influenced convective transport volumes, they had no association with molecules elimination, which is probably explained by the predominant role of diffusion in their removal. On the other hand, the association of Kt/V with small-sized solutes and MM (at least up to 23 kDa)

Table 4. Uraemic toxin ERs, clearances at 60 min, mass transfer and overall clearances

Solute	ER (%)	K_D (mL/min)	K_c (mL/min)	K_d (mL/min)	MT_{ovr} (mg)	K_{ovr} (mL/min)	$K_{ovr(c)}$ (mL/min)	$K_{ovr(d)}$ (mL/min)
Urea	84.9 ± 1.9	341.8 ± 7.2	30.6 ± 3.7	311.2 ± 9.6	40.2 ± 11.1 ($\cdot 10^3$)	357.8 ± 34.3	31.3 ± 3.9	326.5 ± 34.9
Phosphate	75.4 ± 6.8	304.1 ± 27.5	30.6 ± 3.7	273.5 ± 27.2	1918 ± 463	375.9 ± 37.9	31.3 ± 3.9	344.6 ± 38.4
Creatinine	68.8 ± 3.8	278.3 ± 15.8	30.6 ± 3.7	247.7 ± 18.2	2488 ± 965	307.2 ± 27.0	31.3 ± 3.9	275.9 ± 28.9
β2-microglobulin	35.9 ± 3.6	147.9 ± 13.9	27.7 ± 3.3	120.2 ± 13.2	359 ± 126	175.5 ± 21.0	28.3 ± 3.5	147.2 ± 20.4
Cystatin C	31.1 ± 2.1	129.4 ± 8.5	26.9 ± 3.3	102.5 ± 7.8	103 ± 20	156.3 ± 11.4	27.5 ± 3.4	128.9 ± 11.3
Myoglobin	18.2 ± 2.8	78.1 ± 11.3	24.2 ± 2.9	53.9 ± 10.1	2399 ± 1228 ($\cdot 10^{-3}$)	92.1 ± 14.9	24.7 ± 3.1	67.4 ± 13.8
Prolactin	10.1 ± 8.0	46.1 ± 30.8	20.4 ± 2.5	25.7 ± 31.0	113 ± 93 ($\cdot 10^{-3}$)	52.3 ± 34.9	20.9 ± 2.6	31.4 ± 35.2
Albumin	−0.77 ± 4.05	−9.4 ± 17.1	–	–	–	−9.5 ± 17.9	–	–

ER by dialyser at 60 min; K_D at 1 h measured from blood compartment; K_c and $K_{ovr(c)}$ were calculated with the highest DF flow observed (Model B1) using theoretical sieving coefficients ($S_c = 0.90$ for β2-microglobulin; $S_c = 0.88$ for cystatin C; $S_c = 0.79$ for myoglobin; $S_c = 0.67$ for prolactin; $S_c = 0.01$ for albumin; and $S_c = 1$ for urea, creatinine and phosphate).

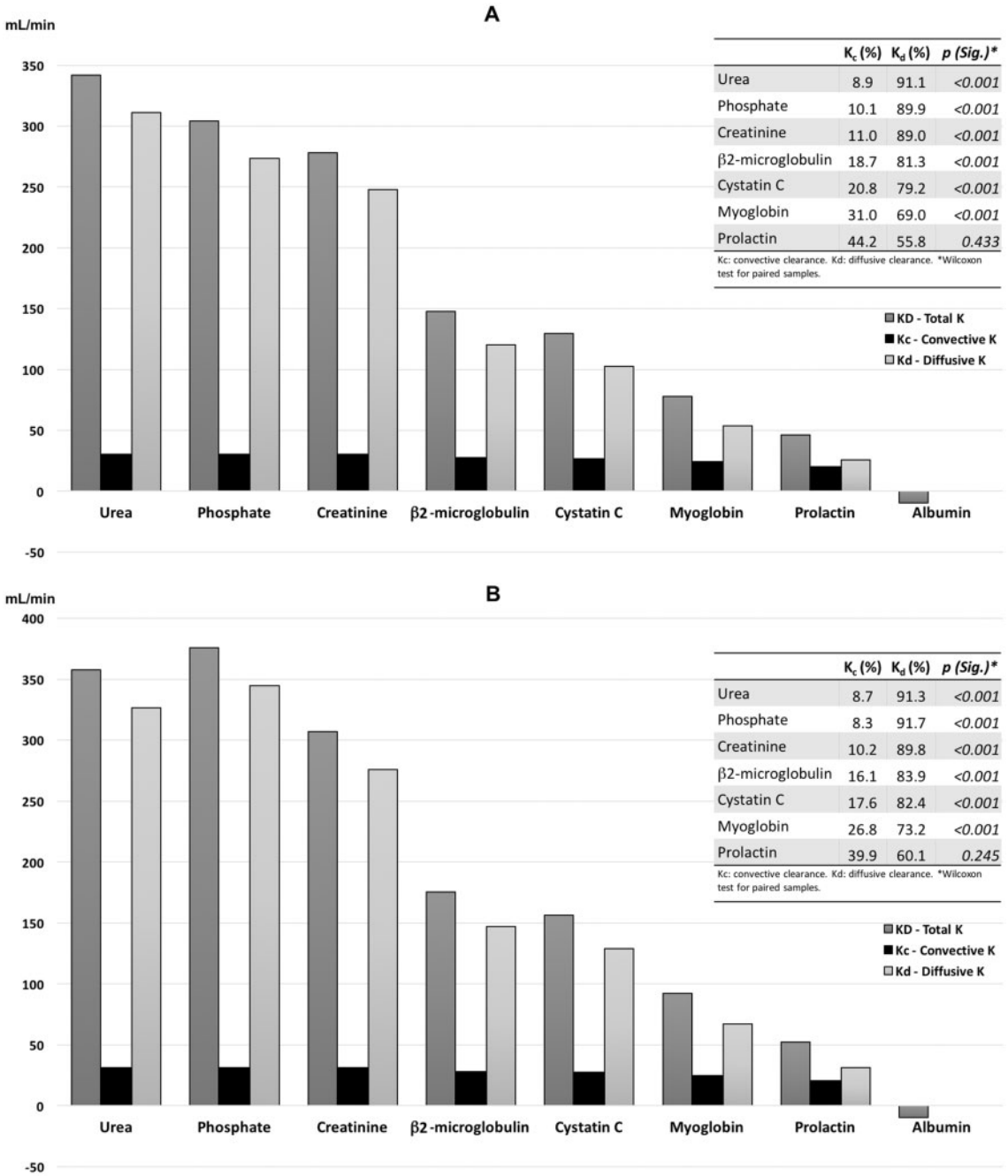


FIGURE 2: Total, convective and diffusive clearances. (**A**) Measurements at 60 min; (**B**) overall clearances.

elimination could also reflect the importance of diffusive transport. As Kt/V continues to be the reference for dialysis dose standardization [27, 28], this adds the possibility of using Kt/V as a non-invasive, adjusted to patient, on-line/real-time method for monitoring the efficacy of HDx in the removal not only of small solutes, but also of MM.

Adsorption

Results were obtained assuming the absence of absorption to the membrane. However, the exposure of blood to the membrane surface results in significant protein adsorption, which can have a significant impact on solute removal [29]. Given the progressive reduction of both diffusive and convective mechanisms as molecule sizes increase (due to slower motion of the solutes and a reduction in membrane S_c, respectively), adsorption may have greater relative importance regarding larger molecules. Although hard to measure in a clinical setting, other studies comparing clearances calculated from the blood compartment with clearances obtained from dialysates could estimate roughly adsorptive mechanisms with these membranes.

Applicability

The main advantage of HRO membranes is the possibility of performing HDx with classic conventional HD systems, providing similar or even superior depurative capacity to high convective volumes obtained with high-flux membranes, without the need for replacement systems or solutions. Most situations in which efficient convective transport cannot be achieved (limitations in Q_b, haemoconcentration, etc.) could probably benefit from the prescription of HDx with HRO membranes to obtain appropriate uraemic toxin depuration, but more studies are necessary to evaluate the clinical benefits of HDx.

A relevant issue to be clarified about estimated convective transport, which seems to be similar to that found in other techniques such as high-flux HD or low-efficiency convective therapies [30], is whether HRO membranes can be used when dialysis water conditions needed for high-flux HD are met but not for OL-HDF. Nevertheless, given the results, it should be remembered that endotoxin (lipopolysaccharides, 5227 Da) or other dialysate solutes could be transferred to the patient, not only by convective transport with BF, but also by diffusion through the membrane.

CONCLUSION

The results presented suggest that diffusive transport is a main mechanism of MM elimination in HDx. HDx offers efficient depuration of MMs without the need for high convective volumes, so it could benefit patients in which the ability to attain an effective convective dose is limited.

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AUTHORS' CONTRIBUTIONS

N.M. participated in the conception and the design of the study, in the collection, analysis and interpretation of data, and have written the original manuscript. A.S., E.T. and A.M.G.P. contributed in the collection of the data. A.V., S.A., I.A., A.M.G.P., A.S. and J.L. contributed in the analysis and interpretation of data, and provided intellectual content of critical importance. A.V., S.A. and J.L. revised the article and approved the final version to be published.

CONFLICT OF INTEREST STATEMENT

None declared.

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