

Original Article

## Reverse mid-dilution: new way to remove small and middle molecules as well as phosphate with high intrafilter convective clearance

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### Abstract

**Background.** The removal of small and middle molecules has a relevant impact on haemodialysis (HD) patient survival. Mid-dilution (MD) is a technique combining *ease of use* with high diffusive-convective clearances. However, MD may increase the intrafilter blood pressure due to the high filtration fraction. We devised a new filter configuration, reverse MD, with an *inverted* blood inlet and outlet. We compared biochemical and technical performances of reverse MD vs standard MD.

**Methods.** Eight HD patients underwent one standard MD treatment and one reverse MD. Samples for instantaneous clearance and total mass removed from dialysate spilling (urea, phosphate,  $\beta_2$ -microglobulin, angiogenin) were obtained. Dialysate and blood pressures in the circuit were monitored every 15 min. The reinfusion rate was set at 6 l/h for both treatments.

**Results.** Absolute removals were very high and statistically comparable in both the configurations. Pressures were significantly lower with the reverse compared with the standard MD: inlet blood pressure was  $731 \pm 222$  and  $595 \pm 119$  mmHg in the standard and in the reverse MD, respectively. The transmembrane pressures were lower in the reverse compared with the standard MD ( $422 \pm 90$  and  $611 \pm 136$  mmHg for 1st stage;  $188 \pm 54$  and  $307 \pm 56$  mmHg for 2nd stage).

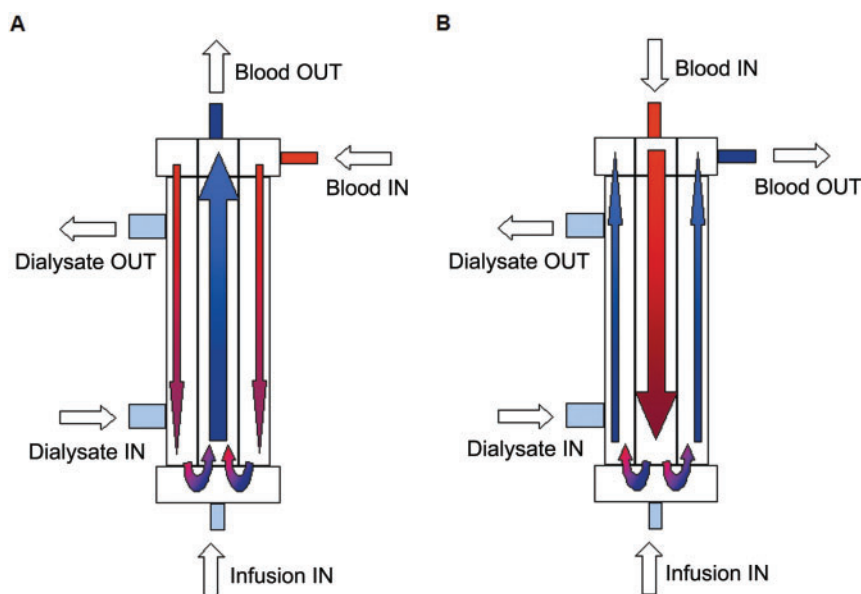
**Conclusions.** Reverse MD could be an ideal technique for high ultrafiltration routine treatments without any external fluid reinfusion. It allows a very high removal of small and middle molecules, with relatively lower intrafilter pressures.

**Keywords:** beta-2-microglobulin; connective dialysis; hemodiafiltration; mid-dilution; middle molecules

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### Introduction

Conventional haemodialysis (HD) is associated with high mortality and morbidity and a poor quality of life [1,2]. Over the last few years, technological innovation in dialysis equipment and the quality of dialysis water has paved the way for the development of other methodologies that have maximized convection as compared with merely diffusive HD. Thus, haemofiltration and haemodiafiltration (HDF) have been developed, which in the new online versions use large amounts (>40 l) of infusion solutions in pre- or post-dilution [3]. Mid-dilution haemodiafiltration (MD-HDF) is a new haemodiafiltration technique that uses a special dialyser—MD190 (Nephros, Inc., New York, USA)—which enables both pre- and post-reinfusion [4,5]. Although the outside of the dialyser appears similar to conventional haemodialysers, the internal fibres are divided into two bundles by a special annular header that first lets the blood pass through the peripheral bundle in ‘post-dilution’, then mixes with the reinfusion fluid at the opposite end of the dialyser and finally proceeds (after ‘pre-dilution’) to the dialyser blood exit. The dialyser is able to support substantially higher reinfusion rates than traditional post-HDF (10–12 l/h). Krieter *et al.* [5] demonstrated that by means of mid-dilution (MD) it is possible to achieve a very high instantaneous clearance for urea, creatinine, phosphate and  $\beta_2$ -microglobulin. Recently, we have confirmed these results [4] and have also shown that the clearance and the mass removal of toxic molecules of larger molecular weight, such as angiogenin (14 kDa) and leptin (16 kDa), are significantly higher in MD as compared with high-flux dialysis. However, one of the drawbacks of MD may be an elevated resistance inside the filter due to the very large ultrafiltration rates. In this short note, we suggest a new version of MD that we have named *Reverse MD* because we have changed and reversed the arrangement of the access routes to the filter.



**Fig. 1.** Olpur™ 190 haemodiafilter. Panel A - Standard configuration: blood flows through the *annular* region of the fibre bundle (stage 1), mixes with substitution fluid at the opposite end, then flows in the reverse direction through the *core* region of the fibre bundle (stage 2). Panel B - Reverse configuration: first, the blood flows through the *core* region of the fibre bundle (stage 1), mixes with substitution fluid at the opposite end, then flows in the reverse direction through the *annular* region of the fibre bundle (stage 2).

The performances in terms of small-and-large-molecular-weight molecules and the pressures measured at the various exit and entry points to the filter obtained with reverse MD have been compared in an *in-vivo* study with what has been obtained with the traditional configuration of MD.

## Subjects and methods

Standard MD was performed with an Olpur MD190 dialyser (Nephros, 1.9 m<sup>2</sup>, DIAPES  $\gamma$  sterilized) and the blood path and dialysate inlets/outlets have been extensively illustrated by Krieter *et al.* [5]. The dialysers used for the present study were from the same lot. Briefly (Figure 1), in the standard configuration the blood and substitution fluid mixture flows into the hollow core fibres in a reverse direction in relation to the blood flowing through the annular hollow fibres. In the 'reverse configuration', blood flows through the core region of the fibre bundle, mixes with substitution fluid at the other end, and flows in the reverse direction through the annular region of the fibre bundle (Figure 1).

The study was approved by the Hospital's Official Scientific Board. Eight stable end-stage renal disease (ESRD) patients (4 men, 4 women mean age  $56.6 \pm 23.6$  years) on maintenance dialysis were selected and gave their informed consent to participate in the study. All the patients had well-functioning arterio-venous fistulas. The underlying renal diseases were chronic glomerulonephritis (two), hypertension (one), tubulo-interstitial nephritis (one), polycystic kidney disease (one) and other diseases (three).

The patients underwent a sequence of one mid-week treatment with traditional (standard) MD, followed by one mid-week of MD with the 'reverse configuration'.

Prescribed treatment parameters (session length, blood flow, dialysate composition and temperature) were kept unchanged as compared with the usual dialysis modalities.

The treatments, both in the standard and in the reverse configuration, were performed using the same monitor: i.e. Formula 2000 (Bellco, Italy).

Ultrafiltration volume was set according to the interdialysis body weight gain and the ideal dry weight of the patient.

Total infusion flow ( $Q_{inf}$ ) was set at 6 l/h for both techniques.

Pressures in the extra-corporeal circuit were monitored at 15-min intervals and included the following: inlet blood pressure ( $P_{bi}$ ), infusion port pressure ( $P_{inf}$ ), outlet blood pressure ( $P_{bo}$ ), inlet dialysate pressure ( $P_{di}$ ), outlet dialysate pressure ( $P_{do}$ ), stage-1 and stage-2 transmembrane pressure (TMP).

The mean TMP within the dialyser was calculated using the following equation:

$$TMP = \frac{P_{bi} + P_{bo}}{2} - \frac{P_{di} + P_{do}}{2} - \pi_0 \quad (1)$$

where  $\pi_0$  is the mean oncotic pressure exerted by the plasma protein, set by default to a constant value of 25 mmHg.

Anti-coagulation was performed according to the previous routine patient heparinization. Unfractionated sodium heparin was administered directly to the patients before the beginning of the dialysis session (50 IU/kg directly into the venous needle) followed by continuous heparin infusion ( $635 \pm 154$  IU/h).

Recirculation of the vascular access was tested in the previous routine dialysis and was <10% in all the patients.

Blood samples were taken at the beginning of dialysis ( $T_0$ ), and at the end of the treatment ( $T_{fin}$ ), in order to evaluate the removal rate (%) of  $\beta_2$ -microglobulin, angiotensin, leptin, urea, creatinine and phosphate. Then,  $\beta_2$ -microglobulin was measured with the nephelometric method (Beckman). Angiotensin was measured with an enzyme immunoassay kit (Quantikine R&D Systems). Leptin was measured with an enzyme immunoassay kit (Cayman Chemical).

**Table 1.** Treatment pressures: Standard vs reverse configuration

	Standard (mmHg)	Reverse (mmHg)
Inlet blood pressure ( $P_{bi}$ )	731 ± 222	595 ± 119
Infusion port pressure ( $P_{inf}$ )	562 ± 94	392 ± 53**
Outlet blood pressure ( $P_{bo}$ )	116 ± 16	127 ± 21
Inlet dialysate pressure ( $P_{di}$ )	68 ± 52	121 ± 34*
Outlet dialysate pressure ( $P_{do}$ )	-11 ± 67	21 ± 48
$P_{bi} - P_{bo}$	615 ± 208	397 ± 288
$P_{inf} - P_{bo}$	446 ± 79	266 ± 55***
TMP first filtration stage	611 ± 136	422 ± 90**
TMP second filtration stage	307 ± 56	188 ± 54***
TMP total filter	382 ± 101	290 ± 66*

Data expressed as mean value ± SD. \* $P \leq 0.05$ ; \*\* $P \leq 0.01$ ; \*\*\* $P \leq 0.001$ .

TMP first filtration stage =  $(P_{bi} + P_{inf})/2 - (P_{di} + P_{do})/2$ ; TMP second filtration stage =  $(P_{inf} + P_{bo})/2 - (P_{di} + P_{do})/2$ ; TMP total filter =  $(P_{bi} + P_{bo})/2 - (P_{di} + P_{do})/2$ .

Mass transfer was evaluated by measuring the concentration of  $\beta_2$ -microglobulin, angiogenin, leptin, urea, creatinine, phosphate, albumin in the sample of dialysate spilled with a pump placed at the exit of the dialyser running at the constant speed of 1 ml/min for the total duration of the treatment. Total mass removal was obtained from a sample of dialysis fluid, continuously removed from the effluent dialysate using a peristaltic pump that did not influence the pressure profile in the dialysis circuit. This pump was calibrated at the beginning and at the end of the dialysis session. The total removed dialysate was collected in a graduated cylinder and the total volume was verified during and after the treatment.

The total removed mass (Trm) for each element was calculated with the following equation:

$$\text{Trm} = C_{\text{spill}}(V_d + V_{\text{inf}} + V_{\text{uf}}) \quad (2)$$

where  $C_{\text{spill}}$  is the concentration of a given solute in the solution withdrawn by a spilling-pump;  $V_d$  is the total dialysate volume passed through the dialyser;  $V_{\text{inf}}$  is the total infusion volume;  $V_{\text{uf}}$  is the total ultrafiltration volume.

Treatment efficacy was determined by measuring the instantaneous clearance at 45 min ( $T_{45}$ ), for  $\beta_2$ -microglobulin, angiogenin, leptin, urea, creatinine and phosphate.

Instantaneous clearances were calculated by the equation:

$$K = \frac{(Q_b C_{\text{inlet}}) - [(Q_b - Q_{\text{uf}}) C_{\text{outlet}}]}{C_{\text{inlet}}} \quad (3)$$

where  $Q_b$  and  $Q_{\text{uf}}$  are the blood flow and the ultrafiltration rate (ml/min), respectively.  $C_{\text{inlet}}$  and  $C_{\text{outlet}}$  are the concentration of a given solute in plasma obtained from pre-haemodiafilter and post-haemodiafilter port, respectively.

Reduction ratios were determined by the equation:

$$\frac{[(C_{\text{pre}} - C_{\text{post}})]}{C_{\text{pre}}} \times 100 \quad (4)$$

where  $C_{\text{pre}}$  and  $C_{\text{post}}$  were the concentration of a given solute at the beginning and at the end of the dialysis session.

### Statistical analysis

The analysis of continuous normally distributed variables was based on the mean ± SD. The effects of the two

procedures (with the different configuration of MD) on parameters of treatment efficiency and pressures in blood circuit and pressures in the dialysate circuit were compared with the Student's *t*-test for paired data. A probability value <0.05 was considered significant.

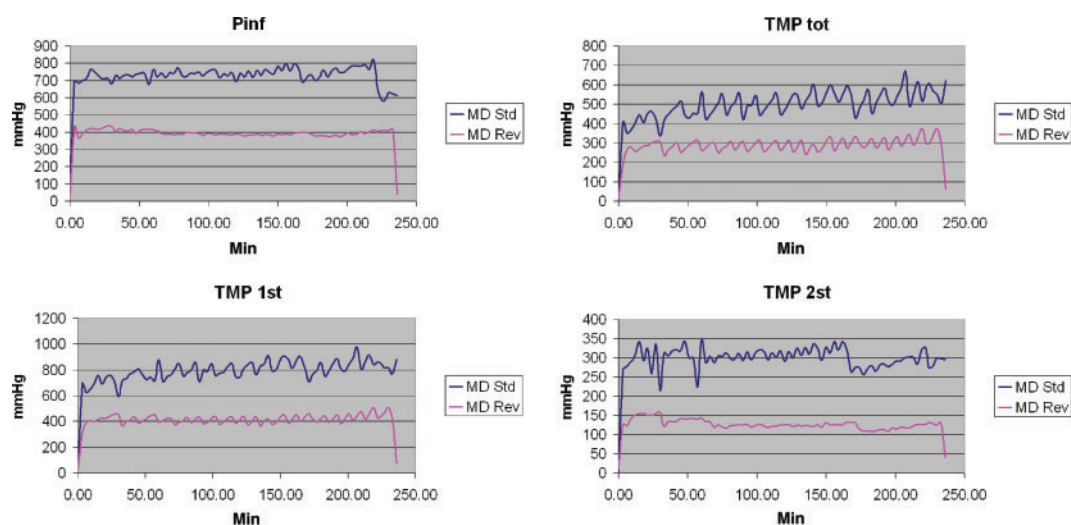
### Results

All the patients completed the study, without experiencing any adverse events. The mean dialysis session time in the eight standard MD sessions was 231 ± 11.2 min, and 231 ± 10.0 min in the eight sessions of reverse MD ( $P = \text{NS}$ ).

Blood flow was 305 ± 9.6 ml/min for the standard configuration and 308 ± 11.7 ml/min for the reverse one ( $P = \text{NS}$ ). Patient water loss rate was 0.77 ± 0.18 l/h and 0.79 ± 0.25 l/h for the standard and reverse configurations, respectively ( $P = \text{NS}$ ).

The pressure values (mmHg, mean ± SD) recorded during the treatments are shown in Table 1. Pressures proved significantly lower with the reverse configuration as compared with the standard MD configuration. Specifically,  $P_{bi}$  values were 731 ± 222 mmHg and 595 ± 119 mmHg in the standard and in the reverse configurations, respectively (-18.6%). Similarly,  $P_{inf}$  values decreased from 562 ± 94 mmHg in the standard configuration to 392 ± 53 mmHg in the reverse one (-30.2%). The TMP, evaluated during the first and the second filtration stages, were largely lower in the reverse compared with the standard configuration (422 ± 90 and 611 ± 136 for the first stage; 188 ± 54 and 307 ± 56 for the second stage). Figure 2 shows the behaviour of the  $P_{inf}$  and the TMP at the first and second stages of the filtration as well as the total TMP during a session of standard and during a session of reverse MD. Throughout the reverse MD procedure, all of the aforementioned pressures were at significantly lower values than the ones obtained during the standard configuration procedure.

The instantaneous whole blood clearance and the percentage removal ratio for small and middle size solutes are summarized in Table 2. No significant differences were observed between the two MD configurations. Both procedures resulted in better clearances and removal rates for small and middle-sized solutes compared with traditional HD and HDF. The only exception was found for instantaneous phosphate clearance, that was lower in the reverse MD compared with standard MD. Also, the efficiency in removing small and middle-size solutes measured in the spent dialysate was not statistically different in the two configurations. During a single session of both procedures, at least 45 g of urea, 2.8 g of creatinine, 1 g of phosphate, 180 mg of  $\beta_2$ -microglobulin and 11 mg of angiogenin were found in the fractionated spent dialysate. The mean amount of albumin detected in the collected dialysate was 5.02 ± 1.63 g in the standard configuration and 3.09 ± 1.99 g in reverse MD. While it is true that there was a minor protein loss, this small loss would not be expected to be clinically relevant



**Fig. 2.** Behaviour of infusion pressure (Pinf), total transmembrane (TMP) pressure and TMP in the first and second stages of filtration obtained during a standard MD session and a reverse MD session.

**Table 2.** Instantaneous clearance and removal ratio of small- and middle-molecular weight toxins and phosphate: standard vs reverse configuration

	Instantaneous clearance (ml/min)		Removal ratio (%)	
	Standard	Reverse	Standard	Reverse
Urea	275 ± 17	265 ± 13	74 ± 5	71 ± 6
Creatinine	254 ± 16	250 ± 18	68 ± 4	70 ± 10
Phosphate	264 ± 17	233 ± 23*	60 ± 11	55 ± 7
β <sub>2</sub> -microglobulin	221 ± 25	221 ± 27	80 ± 4	83 ± 2
Angiogenin	184 ± 24	182 ± 21	41 ± 7	44 ± 11
Leptin	188 ± 39	186 ± 20	47 ± 17	40 ± 7

Data expressed as mean value ± SD. \* $P \leq 0.01$ .

**Table 3.** Total mass removed: standard vs reverse configuration

	Standard (mg)	Reverse (mg)
Urea	50779 ± 15781	44955 ± 13059
Creatinine	2938 ± 891	2795 ± 832
Phosphate	1011 ± 728	1058 ± 998
β <sub>2</sub> -microglobulin	184 ± 83	180 ± 62
Angiogenin	11 ± 2.5	11 ± 1.9
Albumin	5020 ± 1630	3090 ± 1990

Data expressed as mean value ± SD.  $P \leq 0.05$ .

towards affecting the patients' nutritional status [6,7]. However, the difference failed to reach statistical significance probably owing to the small number of cases and the wide SD.

## Discussion

The results of the present study show that the behaviour of blood and dialysate pressure inside the

filter and the behaviour of TMP is by far better with the reverse MD configuration as compared with conventional MD [5]. In spite of a substantial reduction in internal pressure, treatment efficiency remains the same, with a high removal rate of small and middle-size solutes. In convective therapies, two elements favour the loss of large molecules: i.e. the use of highly permeable membranes and elevated convection fluxes. In MD, the DIAPES membrane is highly permeable [8] and convection fluxes are elevated in pre- as well as in post-dilution. Under some treatment conditions, however, and in some patients, elevated work pressure may be reached. Reverse MD allows us to lower these pressures, thus allowing a more favourable pressure profile. In fact, in MD190 dialysers the central and peripheral regions are different in terms of the amount of fibres and, consequently, in terms of surface (central region: 0.8–0.9 m<sup>2</sup>, peripheral region: 1.0–1.1 m<sup>2</sup>). The incoming blood flow through the central part shows a higher-pressure fall because that blood flow must pass through a narrower cross-section. This represents the most important advantage of the reverse



configuration, since the infusion of the substitution fluid occurs at a point of lower pressure due to the higher loss of the aforesaid load. This is also demonstrated by the fact that the Pbi-Pinf is higher in the reverse than in the standard configuration (203 vs 169 mmHg). Transmembrane pressure is a relevant factor conditioning membrane permeability and protein loss. It induces protein polarization on the inner membrane surface, plasma protein interaction with the internal skin layer as well as with the outer layers of the membrane, leading to modifications in the transmembrane passage. In a previous study [9], we found a linear correlation between TMP and protein loss in both pre- and post-dilution HF. However, the impact of TMP is much more important in determining the protein loss in post- rather than in pre-dilution. In the pre-dilution mode, the lower protein concentration and the larger expansion of plasma water due to the dilution of the blood arriving at the dialyser minimizes the need for higher TMP and the concomitant protein loss. In post-dilution, more commonly than in the pre-dilution mode, a vicious circle can clearly be seen, being triggered with the formation of the protein cake, a reduction in membrane permeability and a subsequent increase in TMP. The increase in TMP leads to a consequent increase in the membranous and sub-membranous protein concentration that in turn induces a major protein loss and a further reduction in hydraulic permeability [10]. The continuous increase in TMP during the treatment may, particularly in the post-dilution mode, favour protein loss. Shinzato *et al.* [11] found a  $18.9 \pm 3.5$  g loss of albumin per treatment in HDF. Hillion *et al.* [12] showed an albumin loss over 10g per session in post-HDF. Our previous data obtained in pre- and post-dilution HF are more comforting with no more than 3.0 g/treatment in post-dilution haemofiltration and 2.0 in pre-dilution [9]. In the present study, we have found an albumin loss of  $5.02 \pm 1.63$  g in the standard MD configuration and only  $3.09 \pm 1.99$  g in the reverse configuration. Thus, they are broadly within the limits recently believed to be clinically acceptable by Nensel [13] with highly permeable membranes.

Another interesting fact regarding the reverse configuration is that it is possible to obtain the same clearances, and above all, high removals without varying the total infusion volume. Hence, in the treatments in which there are particularly low pressures it is possible to increase the infusion flow, and therefore maximize the convective removal. In MD, the removal, both of middle molecules as well as the phosphates is extremely high given the combination of pre- and post-dilution [4,5]. High removal of middle molecules could be useful for the implication that this could have in regard to the many aspects of the pathophysiology of uraemia [14]. Maximizing the middle molecule removal is one of the aims included among the new criteria set for defining dialysis adequacy [15]. A more recent analysis of 2165 patients

stratified into four groups: i.e. low- and high-flux HD, and low- and high- efficiency HDF, has suggested that HDF may improve patient survival [3]. High-efficiency HDF patients had a significant lower mortality risk (−35%) than those receiving low-flux HD.

In addition, the high phosphate removals (in the range of 1 g per treatment), are particularly interesting in MD. Phosphate also enters aspects of the dialysis pathology that relate not only to the bone, but can actually influence the entire cardio-vascular sphere [16]. Hence, having at our disposal a dialysis technique that removes even more than the daily dietary phosphate intake, could represent an extra weapon to be deployed together with the phosphate binders in the fight against this metabolic enemy of the bone, as well as the vessels and the heart.

In conclusion, reverse MD preserves all the advantages of standard MD with the addition of much lower intra-filter pressure regimes. On the one hand, this enhances the safety and the user-friendliness of this technique, while on the other it leaves room for the use of higher convective flows, allowing us to further increase the removal of middle molecules and phosphate.

*Conflict of interest statement.* M.V., L.S. and M.L. Wratten are full-time employees at Bellco, a company that distributes Nephros mid-dilution dialysers.

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