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Efficacy of medium molecular weight toxin clearance in manual mixed online hemodiafiltration vs. pre- and post-dilution online hemodiafiltration and conventional hemodialysis: A crossover observational study

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Abstract. *The effectiveness of various hemodiafiltration (HDF) modes in removing different toxins is not fully understood. The present study aimed to compare the efficacy of manual mixed online HDF with pre-dilution online HDF, post-dilution online HDF, and conventional hemodialysis in clearing medium molecular weight toxins.*

Methods. This single-center, crossover observational study included 11 patients (7 males, 4 females) aged 48-85 years (mean age 65.2±11.7) with a dialysis vintage of 24-455 months (mean duration 126±123 months), including 10-29 months (mean duration 19.5±6.4) on HDF. The study focused on the removal of two medium molecular weight molecules: beta 2-microglobulin and the larger prolactin. The effectiveness of various dialysis modes was evaluated in single sessions for each patient, including pre-dilution online HDF (Group A), post-dilution online HDF (Group B), a mixed dilution model with post-dilution during the first half of the session and pre-dilution during the second half (Group C), and conventional hemodialysis (Group D).

Results. A statistically significant difference in the reduction of serum beta 2-microglobulin levels was observed in Group B compared to Group A (70.6±3.1% vs. 64.4±2.7%, $p<0.0001$) and Group C (70.6±3.1% vs. 65.9±4.9%, $p<0.001$), with no significant difference between Groups A and C (p=NS). For serum prolactin levels, the greatest reduction was noted in Group B, with significant differences compared to Group A (52.5±8.9% vs. 36.2±9.5%, $p<0.001$) and Group C (52.5±8.9% vs. 46.9±5.2%, $p=0.03$). Additionally, Group C showed a significant difference compared to Group A (46.9±5.2% vs. 36.2±9.5%, $p<0.005$).

Conclusions. Post-dilution HDF is the most effective mode for removing beta 2-microglobulin and prolactin. Mixed dilution HDF also shows significant efficacy, surpassing pre-dilution HDF in toxin clearance. These findings highlight the advantage of post-dilution techniques in managing medium molecular weight toxins. Further research is needed to explore these results in greater detail and to confirm their clinical implications.

Keywords: *hemodiafiltration, dilution techniques, beta 2-microglobulin, prolactin, clearance.*

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Ефективність кліренсу токсинів середньої молекулярної маси під час мануальної змішаної онлайн гемодіафільтрації порівняно з онлайн пре- та пост-дилюційною гемодіафільтрацією або традиційним гемодіалізом: перехресне обсерваційне дослідження

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Резюме. *Ефективність різних моделей гемодіафільтрації (ГДФ) щодо видалення токсинів середньої молекулярної маси є невизначеною. Метою цього дослідження було порівняти ефективність їх усунення методами мануальної змішаної онлайн ГДФ порівняно з онлайн ГДФ у режимі пре- або постдилюції та гемодіалізом класичним за кліренсами бета 2-мікроглобуліну та пролактину.*

Методи. Це одноцентрове перехресне обсерваційне дослідження включало 11 пацієнтів (7 чоловіків, 4 жінки) віком 48-85 років (65,2±11,7) та тривалістю гемодіалізу 24-455 місяців (126±123 міс.), включаючи 10-29 місяців (19,5±6,4 міс.) лікування ГДФ. Дослідження було зосереджено на видаленні бета 2-мікроглобуліну та пролактину. Ефективність різних моделей діалізу оцінювалася під час окремих сеансів для кожного пацієнта, включаючи ГДФ з предилюцією (група А), ГДФ з постдилюцією (група В), мануальну змішану ГДФ (група С), а також традиційного гемодіалізу (група D).

Результати. Статистично значуща різниця в зниженні рівнів бета 2-мікроглобуліну в сироватці спостерігалася в групі В порівняно з групою А (70,6±3,1% проти 64,4±2,7%, p<0,0001) і групою С (70,6±3,1% проти 65,9±4,9%, p<0,001), без істотної різниці між групами А та С (p=NS). Концентрації пролактину в сироватці крові була статистично-значущо нижчою в групі В порівняно з групою А (52,5±8,9% проти 36,2±9,5%, p<0,001) та групою С (52,5±8,9% проти 46,9±5,2%, p=0,03). Крім того, група С подемонструвала достовірну різницю порівняно з групою А (46,9±5,2% проти 36,2±9,5%, p<0,005).

Висновки. ГДФ з постдилюцією є найефективнішою моделлю ГДФ у видаленні бета 2-мікроглобуліну та пролактину. Змішана ГДФ має вищий кліренс бета 2-мікроглобуліну та пролактину, ніж ГДФ з предилюцією. Необхідні подальші дослідження для підтвердження клінічного значення отриманих нами результатів.

Ключові слова: *гемодіафільтрація, модель дилюції, бета 2-мікроглобулін, пролактин, кліренс.*

Introduction. The clearance of toxins in dialysis patients is of critical importance and is closely related to both survival and overall well-being. Existing studies have highlighted the superiority of hemodiafiltration (HDF), with post-dilution HDF showing better outcomes compared to pre-dilution HDF, as well as conventional hemodialysis and high-flux hemodialysis [1-5], although a meta-analysis of 13 studies didn't find any difference in the mortality from all causes included the cardiovascular.

Studies on the removal of medium molecular weight toxins have primarily focused on beta 2-microglobulin, with fewer examining larger molecules such as prolactin or complement. Most literature on dialysis clearance has used Kt/V or the urea reduction ratio (URR) for assessing low molecular weight toxins, without addressing the specific amounts of medium molecular weight (MW) toxins removed. From the medium MW toxins, beta 2-microglobulin is a useful biomarker of morbidity and mortality in dialyzed patients [6], but we also wanted to determine the elimination of another toxin with medium MW, such as prolactin. By determining its serum levels before and after the end of the session, the efficiency of one dialysis session for these molecules, in each dialysis method used in this study was estimated.

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It was chosen to estimate the clearance of two molecules with medium molecular weight: beta 2-microglobulin, commonly included in most studies for comparison, and prolactin, which has a significantly higher molecular weight and is less frequently studied in terms of removal during dialysis. The present study aimed to compare the effectiveness of manual mixed online hemodiafiltration (HDF) with pre-dilution online HDF, post-dilution online HDF, and conventional hemodialysis in clearing medium molecular weight toxins.

Patients and Methods. Patients. This single-center, crossover observational study included a total of 11 patients undergoing hemodialysis. All the patients were older than 48 years, stable, and uncomplicated during their dialysis sessions (none had haemodynamic instability during dialysis sessions). They maintained stable dry weight for at least four months, three times per week. None had malignancy, acute catabolic or infectious disease, and no one had evidence of gastrointestinal bleeding. None had metabolic acidosis or very high urea serum levels. Patients who failed to meet the above inclusion criteria were excluded. The study was approved by the Ethics Committee of the Scientific Council of General Hospital of Komotini (protocol number 3/2023, dated 03/01/2023) and was conducted following the guidelines for good clinical practice and ethical principles of the Declaration of Helsinki. All the patients gave written informed consent for their participation in the study.

Methods. The patients underwent one session of online HDF pre-dilution (Group A), one online HDF post-dilution (Group B), one mixed online HDF (as mentioned above manually) (Group C), and a session of conventional haemodialysis (Group D) during the midweek days (Wednesday-Thursday). The time interval between these four sessions for each patient was one week. The filter used was polyethersulfone (polynephron) with a surface area of 2.5 m^2 (high-flux) and polynephron low-flux, with a surface area of 2.1 m^2 (for conventional haemodialysis). The blood supply (pump) was 400 ml/min in all sessions and patients, the dialysate flow was 500 ml/min, and the duration of each session was ≥240 min (240 for 7 patients, 265 for 3, and 285 min for 1 patient). In pre-dilution HDF, the substitution volume was set at 50% of the blood pump flow rate (200 ml/min for all patients), while in post-dilution HDF, it was set at 24-25% (96-100 ml/min for all patients). During the dialysis session, 2,500 or 3,500 IU of low molecular weight heparin (vemiparin) was administered, depending on the patient's body weight. Nikkiso DBB EXA dialysis machines were used for the procedures.

The prescription of dialysate was personalised. Of the patients, sodium 138 mmol/L have had 4, and 7 had 140 mmol/L. Dialysate bicarbonates ranged from 30-33 mmol/L, where 1 patient had 30 mmol/L, 3 had 31 mmol/L and the remaining 7 had 33 mmol/L. Dialysate potassium was 2 mmol/L in 6 patients and 3 mmol/L in the remaining 5 and calcium was 3 mmol/L in 6 patients and 2 mmol/L in 5. In all patients, the chloride of dialysate was 110 mmol/L, magnesium 0.50 mmol/L, glucose 5.5 mmol/L, and acetate 3 mmol/L. All determinations were performed with a dialysate of fixed composition for each patient.

The total ultrafiltrate was collected from each patient in each of the four dialysis sessions in a custom-made volumetric stainless barrel. After the end of each session and after stirring the ultrafiltrate for 10 minutes with an electric stirrer, a sample was taken for urea and beta 2-microglobulin (the amount of prolactin in the dialysate was impossible to measure because of the very low concentration). Both at the beginning of each session and one hour after the end, a blood sample was taken from the arterial line for determination of serum urea, beta 2-microglobulin (mg/L) and prolactin (ng/ml) levels. The reduction ratio (RR) has been calculated by the following equation: RR = (SubstancePre - SubstancePost: SubstancePre) x 100 (where the substance was beta 2-microglobulin or prolactin).

An Abbott Alinity C analyzer was used to measure the studied parameters. Prolactin levels were measured photometrically, while beta 2-microglobulin levels were measured immunoturbidometrically.

Statistical analysis. Continuous variables were expressed as mean ± standard deviation (M ± SD) according to the data distribution. Categorical variables were expressed as absolute frequencies and percentages. Comparisons between the groups were performed using the Student's t-test. The analysis was conducted with the Statistical software MedCalc (version. 20.218). Probability values of $p<0.05$ (two-tailed) were considered statistically significant for all comparisons.

Results. The patients have been on dialysis for an average of 126 ± 123 months, with a range of 24 to 455 months (Table 1).

Table 1

The patient characteristics, dialysis history, on-line HDF duration, and hyperfiltration volume

P/ts 7M, 4F	Age (y/s)	Body weight (kg)	Body water (L)	Body surface area (m^2)	Duration on dialysis (months)	Duration on HDF (months)	Duration of dialysis session (min)	Ultrafiltrate ml/min (ml/h)			
								Group A	Group B	Group C	Group D
1	48	74.0	42.5	1.95	76	29	240	5.4 (324)	7.5 (450)	6.0 (360)	6.7 (402)
2	69	67.5	37.2	1.80	263	24	265	4.2 (252)	4.5 (270)	3.0 (180)	3.0 (180)
3	57	65.0	31.7	1.72	94	29	265	6.8 (408)	6.0 (360)	5.3 (318)	6.0 (360)
4	68	59.5	34.6	1.70	455	15	265	4.5 (270)	3.8 (228)	3.9 (234)	5.0 (300)
5	70	59.5	36.7	1.70	66	16	240	7.9 (474)	7.5 (450)	10.0 (600)	8.6 (516)
6	85	84.0	40.7	1.91	24	14	240	8.3 (498)	5.4 (324)	7.5 (450)	5.8 (348)
7	83	54.0	27.5	1.49	31	13	240	3.8 (228)	2.1 (126)	3.3 (198)	2.5 (150)

Continuation of Table 1

P/ts 7M, 4F	Age (y/s)	Body weight (kg)	Body water (L)	Body surface area (m^2)	Duration on dialysis (months)	Duration on HDF (months)	Duration of dialysis session (min)	Ultrafiltrate ml/min (ml/h)			
								Group A	Group B	Group C	Group D
8	51	70.0	33.1	1.79	56	21	240	10.4 (624)	7.1 (426)	5.0 (300)	9.2 (552)
9	53	72.0	31.7	1.67	48	26	240	5.8 (348)	2.1 (126)	7.1 (426)	5.8 (348)
10	62	78.0	41.0	1.90	124	10	240	5.8 (348)	8.7 (522)	5.8 (348)	6.3 (378)
11	71	77.5	33.8	1.95	150	17	285	9.8 (588)	10.3 (618)	7.7 (462)	11.1 (666)
Mean±SD	65.2±11.7	69.2±8.7	35.5±4.4	1.78±0.14	126±123	19.5±6.4	251±15	6.6±2.1 (396±127)	5.9±2.5 (354±150)	5.9±2.0 (352±120)	6.4±2.4 (382±145)

Abbreviations: P/ts, patients; y/s, years; kg, kilogram; L, liter; m^2, square meters; ml, milliliter.

None of the 11 patients had residual diuresis. The underlying causes of kidney failure were glomerulonephritis in 3 patients, hypertensive nephrosclerosis in 3, polycystic kidney disease in 2, chronic pyelonephritis in 2, and unknown etiology in 1 patient. Regarding vascular access, 9 patients had an internal arteriovenous anastomosis (native arteriovenous fistula), while 2 had a vascular graft (prosthetic arteriovenous fistula). The baseline laboratory values of the patients, including urea, creatinine, electrolytes, albumin, and hematocrit, before a dialysis session are presented in Table 2.

Table 2

Laboratory values before pre-dilution online HDF

P/ts	Creatinine (mmol/L	Urea (mmol/L)	Potassium (mmol/L)	Calcium (mmol/L)	Proteins gr/L	Albumin (gr/L)	Bicarbonates mmol/L)	Hematocrit (%)
1	114.1	53.0	5.3	2.275	69	43	21.5	37.9
2	119.3	22.7	5.0	2.375	66	39	22.5	40.4
3	121.1	54.7	5.6	2.125	64	39	21.0	39.3
4	98.2	47.2	5.5	2.325	72	40	22.3	42.4
5	112.3	43.9	5.7	2.175	66	41	23.0	41.6
6	101.8	41.5	5.6	2.150	64	42	23.0	33.0
7	74.1	42.3	5.6	2.250	69	40	23.0	36.7
8	120.5	54.3	6.2	2.125	76	42	22.0	38.0
9	75.0	70.2	5.9	2.350	65	39	23.0	32.9
10	89.8	25.1	5.3	2.300	77	43	21.0	36.1
11	77.9	24.9	4.4	2.275	65	38	21.8	33.3
Mean±SD	100.4±17.8	43.6±14.1	5.46±0.45	2.247±0.08	68.5±4.5	40.5±1.7	22.2±0.75	37.4±3.2

In the first half of the post-dilution HDF session, the substitution volume was 49.2 ± 1.8 L (range 48–53.4 L) for Group A, 25.0 ± 1.36 L (range 23–27.1 L) for Group B, and 13.3 ± 0.56 L (range 12–13.4 L) for Group C. During the second half of the session, using pre-dilution online HDF, the substitution volume was 24.7 ± 1.03 L (range 24–26.7 L) (Table 3).

As presented in Table 3, URR was significantly higher only in Group B compared to Group D ($p<0.05$). Similarly, Kt/V was also higher in Group B than in Group D ($p<0.05$). In all other comparisons of URR and Kt/V, the differences were not statistically significant.

Serum levels of beta 2-microglobulin decreased by 64.4 ± 2.7% in Group A, 70.6 ± 3.1% in Group B, and 65.9 ± 4.9% in Group C (p values: A-B < 0.0001, A-C = NS, B-C < 0.001). The amount of beta 2-microglobulin removed per session was 223 ± 66 mg in Group A, 267 ± 92 mg in Group B, and 242 ± 61 mg in Group C (p values: A-B = NS, A-C = NS, B-C = NS) (Table 4). The serum prolactin levels were decreased per session by 36.2±9.5% in Group A, 52.5±8.9% in Group B, and 46.9±5.2% in Group C (p, A-B<0.001, A-C<0.005, B-C<0.03) (Table 5).

Table 3

Comparison of ultrafiltrate, Kt/V, URR, and substitution volumes across dialysis models

P/ts	Total ultrafiltrate (L)				Kt/V				URR (%)				Substitution volume (L)			
	Group A	Group B	Group C	Group D	Group A	Group B	Group C	Group D	Group A	Group B	Group C	Group D	Group A	Group B	Group C	
1	168	146	167	122	1.30	1.41	1.40	1.24	69.2	70.4	70.4	66.1	48.0	24.0	12.0	25.5
2	177	152	165	126	1.58	1.67	1.65	1.58	74.7	76.5	76.5	75.2	49.8	26.5	13.2	26.4
3	179	154	165	128	1.73	2.30	1.71	1.54	77.1	85.5	77.1	73.2	51.0	26.5	12.8	25.5
4	168	145	159	123	1.77	1.72	1.80	1.66	78.2	77.6	79.3	76.5	51.0	26.5	12.0	24.0
5	174	146	167	124	1.52	1.56	1.71	1.48	72.7	74.0	76.4	71.4	48.0	24.0	12.0	24.0
6	173	149	159	122	1.66	1.65	1.41	1.58	76.2	75.6	70.8	75.2	48.0	24.0	12.0	24.0
7	171	146	157	125	1.72	1.73	1.38	1.76	78.0	78.6	70.6	78.9	48.0	24.0	11.5	24.0
8	168	146	164	124	1.61	1.79	1.69	1.67	74.4	78.7	77.3	75.9	48.0	24.0	12.0	24.0
9	170	145	159	123	1.75	2.02	1.87	1.85	78.3	83.2	80.1	80.0	48.0	23.0	12.0	24.0
10	170	152	168	129	1.42	1.54	1.50	1.36	71.3	72.9	73.1	69.6	48.0	27.1	12.0	24.0
11	192	162	186	137	1.49	1.70	1.61	1.51	71.1	75.5	74.4	71.6	53.4	25.5	13.4	26.7
Mean±SD	174±6.8	149±5.0	165±7.6	126±4.2	1.59±0.14	1.74±0.23	1.61±0.16	1.56±0.16	74.7±3.06	77.1±4.16	75.1±3.31	74.0±3.90	49.2±1.8	25.0±1.36	13.3±0.56	24.7±1.03
p-value	A-B=0.00001 A-C=0.0065 A-D=0.00001 B-C=0.00001 B-D=0.00001 C-D=0.00001				A-B=NS A-C=NS A-D=NS B-C=NS B-D<0.05 C-D=NS				A-B=NS A-C=NS A-D=NS B-C=NS B-D<0.05 C-D=NS							

Abbreviations: P/ts, patients; URR, urea reduction ratio.

Table 4

Serum beta 2-microglobulin levels, percentage change, and amount removed per dialysis session with intergroup statistical analysis

P/ts	Beta 2-microglobulin											
	Group A				Group B				Group C			
	Pre-dilution	Post-dilution	Change (%)	Removed amount (mg)	Pre-dilution	Post-dilution	Change (%)	Removed amount	Pre-dilution	Post-dilution	Change %)	Removed amount
1	25.5	8.5	66.7	259.0	27.0	8.2	69.6	246.0	24.1	7.5	68.9	230.5
2	29.7	9.9	66.7	233.6	33.9	9.8	71.1	303.0	33.5	10.6	68.4	298.7
3	25.5	9.3	63.5	214.8	24.2	7.1	70.7	227.9	26.7	7.8	70.3	222.8
4	19.3	6.8	64.8	157.9	22.8	7.0	69.3	232.0	21.1	7.1	66.4	201.9
5	19.5	7.9	59.5	118.7	19.2	6.5	66.1	187.0	21.3	6.4	70.0	188.7
6	33.2	12.6	62.0	271.6	32.3	8.2	74.6	286.4	28.1	10.2	63.7	240.1
7	26.1	8.6	67.0	167.6	27.6	8.6	68.8	135.8	17.8	8.5	52.2	186.8
8	25.5	8.8	65.8	176.4	26.0	6.1	76.5	226.3	24.6	7.7	68.7	209.9
9	29.0	9.1	68.6	192.1	31.2	8.1	74.0	216.0	28.8	9.3	67.7	197.2
10	34.7	13.1	62.2	309.4	45.4	14.7	67.8	474.2	29.2	10.6	63.7	287.3
11	33.6	13.0	61.3	348.7	35.9	11.6	67.7	397.3	36.5	12.7	65.2	399.9
Mean±SD	27.4±5.0	9.8±2.1	64.4±2.7	223±66	29.6±6.9	8.7±2.4	70.6±3.1	267±92	26.5±6.3	8.9±1.8	65.9±4.9	242±61
p-value			A-B<0.0001 A-C=NS B-C<0.001	A-B=NS A-C=NS B-C=NS								

Table 5

Serum prolactin levels before and after online HDF, and percentage change with intergroup statistical analysis

P/ts	Prolactin								
	Group A			Group B			Group C		
	Pre-dilution	Post-dilution	Change (%)	Pre-dilution	Post-dilution	Change (%)	Pre-dilution	Post-dilution	Change %)
1	29.6	16.6	43.9	26.2	12.9	50.8	25.6	13.5	47.3
2	12.8	5.3	58.6	9.8	2.6	73.5	5.6	2.6	53.6
3	74.6	44.9	39.8	68.1	30.5	55.2	81.7	41.7	49.0
4	17.8	11.1	37.6	13.7	7.5	45.3	19.5	11.9	39.0
5	10.8	7.0	35.2	10.9	6.6	39.4	10.1	6.1	39.6
6	31.3	22.2	29.1	32.8	13.6	58.5	28.7	13.3	50.5
7	64.0	37.4	41.6	63.1	29.3	53.6	72.2	39.4	45.4
8	197.1	130.1	34.0	173.9	73.8	57.6	195.7	98.8	49.5
9	16.8	11.6	31.0	19.0	10.5	44.7	18.4	8.3	54.9
10	29.3	22.3	23.9	27.1	15.2	43.9	32.6	19.5	40.2
11	18.7	14.3	23.5	14.4	6.5	54.9	17.9	9.5	46.9
	45.7±51.8	29.3±33.9	36.2±9.5	41.7±45.9	19.0±19.3	52.5±8.9	46.2±52.6	24.1±26.6	46.9±5.2
			A-B<0.001 A-C<0.005 B-C<0.03						

Discussion. It has been established to estimate the attributable clearance by determining the Kt/V or the URR, which are traditional indexes for low MW (<500 D) toxins. However, there are toxins that are much more important, related to the quality of life and survival [1-5,7], which have higher MW and their clearance is not perceived by Kt/V or URR. These can be determined either by calculating their clearance, after a dialysis session by collecting the total ultrafiltrate, but also by the change of their serum levels (%). In this study, the removal of two medium MW toxins, beta 2-microglobulin (MW=11,800 D) and prolactin (~23,000 D) was assessed.

Post-dilution is the most effective method of HDF for toxins removal, but needs to achieve a satisfactory convection volume, which is not always possible due to hemoconcentration and the coagulation problems of the filter it causes [8]. Today, current recommendations for an adequate dialysis dose in this model is a substitution volume of ≥23 L/session in a 3-times-weekly, 4-hour/session dialysis schedule [7, 9, 10]. If the blood pump supplies 400 ml/min, a substitution volume of 25% of the pump can achieve this aim (infusion volume 100 ml/min). If the pump cannot reach this blood flow, can reduce the pump, and increase the duration of the session, to achieve the same substitution volume [11]. Of course, the recommended volume of ≥23 L/session pertains to patients with a body surface area (BSA) of 1.73 m^2 or an estimated total body water of 35 L [12]. In our opinion, it makes sense that if the body surface area is larger than 1.73 m^2 or if the body weight is above the normal range, the convection volume should probably also increase, as others have argued [12, 13]. In our study, more than 23 L were exchanged in post-dilution HDF (25.0±1.36 L).

The pre-dilution online HDF model avoids filter thrombosis and can achieve ultrafiltration volume equal to 100% of the blood pump supply. Thus, excellent elimination of medium MW toxins is achieved, and good removal of low MW toxins. But which will be the right substitution volume in any patient on this model? Tattersall et al. suggest that the substitution volume should be at least twice that of post-dilution HDF (approximately 46 L/session) [14], while others have found 50.5 L/session to be optimal [15]. From a clinical perspective, it is crucial to determine the total volume of ultrafiltrate that must be exchanged to achieve optimal survival in patients undergoing HDF. Ideally, this volume should be at least equivalent to the patient's total body water. Given these studies and recommendations, let's consider two different patients based on their body weight. The first is a 55-year-old woman with a body weight of 50 kg and a height of 160 cm. According to the Watson equation, she has 27.34 L of body water [16]. It is evident that, following any of the above guidelines, during pre-dilution online HDF, the water exchange per session would significantly exceed her total body water (46 or 50.5 L/session). If she were undergoing online HDF with a blood pump rate of 400 mL/min and an ultrafiltration rate at 53% of the pump for a 4-hour session, she would exchange about 51 L/session. This volume is nearly twice her total body water.

On the other hand, consider a 60-year-old man with a height of 170 cm and a body weight of 100 kg, who is also on a three-times-weekly dialysis program (pre-dilution online HDF), with 4-hour sessions. His total body water, according to the Watson equation, would be 48.6 L. Under the same dialysis conditions (blood pump rate of 400 mL/min, 4-hour session, and ultrafiltration rate at 53% of the pump), the convection volume would be approximately equal to his total body water.

This example demonstrates that the woman would exchange her total body water twice per session, while the man would do so once. In our study, during pre-dilution online HDF, we exchanged each patient's water volume at least once. Specifically, we exchanged more than 40 L (mean ± SD = 49.2 ± 1.8 L), while the mean body water of our patients was 35.5 ± 4.4 L.

In mixed dilution HDF, as known from the literature, the substitution fluid is infused both pre- and post-filter simultaneously [17]. This method can increase the total substitution volume while reducing blood viscosity due to the inclusion of pre-dilution. The ratio of pre- and post-infusion can be adjusted to achieve optimal results. In this study, instead of using the classic mixed online HDF, which requires a specialized dialysis machine and specific blood lines (with an extra pump), we used a standard dialysis machine for HDF with blood lines suitable for either pre- or post-dilution HDF. We began with post-dilution online HDF for the first half of the session, then switched to pre-dilution (manually) for the remaining half in patients known to experience increased transmembrane pressure (TMP) during post-dilution online HDF, typically around the midpoint of the session. This approach allowed us to complete the HDF without thrombotic events and with excellent toxin clearance, as noted by others [14, 18] and ourselves.

Mixed HDF is found to be better than pre-dilution, in terms of beta 2-microglobulin clearance, with blood flow 272.8±39.7 in pre-dilution and 256.2±31.8 in the mixed [19] (i.e. much lower than ours) as have shown also by Schiffl in a characteristic table where he compared the clearance of low and medium MW toxins, which were similar in post- and mixed dilution, and the pre-dilution was following [9].

Beta 2-microglobulin is a well-known and pretty good, studied index of medium weight uremic toxins, and is also known for its role in hemodialysis amyloidosis, cardiovascular side effects, and morbidity [15, 20]. It is distributed in the extracellular space and there is minimal increase in its serum levels after the end of the session (rebound), which is estimated at about 4% [21].

Studies have shown that online HDF is associated with better clearance, about 20-40% of beta 2-microglobulin [1] compared to conventional hemodialysis despite the same Kt/V [1,2], which is greater in longer duration of dialysis sessions. Also, when in pre-dilution online HDF the substitution volume is >46-60 L/session [**1, 8, 9, 15**], and in post-dilution >23 L [**22**], is established better survival. So, studies showed that survival increases with increasing substitution volume in post-dilution online HDF [**1-4**], and others found an increase in survival with post-dilution online HDF at 55-75 L of substitution volume/week while arguing that >75 L/week adds no survival benefit [**13**].

In our study, the serum beta 2-microglobulin levels were decreased from 64.4±2.7% to 70.6±3.1%, with the best removal noted in post-dilution and the worst in pre-dilution. The amount of beta 2-microglobulin removed/session was higher in post-dilution and worst in pre-dilution, without any difference between pre- and mixed dilution, as noted and by others who determined the changes in the serum levels [**19**]. Investigators noted better removal of medium MW toxins with mixed dilution HDF in comparison with the pre-dilution [**23**], and especially for beta 2-microglobulin others found the removal of 72.1% in mixed dilution, and 69.2% in pre-dilution online HDF [**17**]. These findings show that the mixed dilution is comparable to the pre-dilution in the removal of medium MW toxins, without problems in the TMP and filter thrombosis [**8**] but is less effective than post-dilution [**23**].

In our study with membranes polynephron (with SC for the beta 2-microglobulin 0.803) and surface area of 2.5 m^2 we noted very good removal of beta 2-microglobulin (from 64.4±2.7% to 70.6±3.1%) in the three groups of HDF, with the best one in post-dilution and the worst in pre-dilution, as found and others [**19**], where removed from 223±66 to 267±92 mg of beta 2-microglobulin/session, with the greatest removal with the post-dilution HDF and the worse with pre-dilution.

Monomeric prolactin (MW=23,000) is the most common form of circulating molecule in healthy individuals, but there are also higher molecular mass forms such as the large prolactin which is its dimer (MW=60,000) and macroprolactin (MW=150,000). The latter two constitute the 20% of serum prolactin [**24**]. With the method used in our study, the total molecule of prolactin and not only the monomer was determined. As a hormone involved in reproductive behavior, it could play a role in the sexual dysfunction of uremia. Thus, it causes loss of libido, erectile problems, and infertility. In men, it can cause gynecomastia and galactorrhea and in women menstrual disturbances and galactorrhea. Hyperprolactinemia is likely a contributing factor to atherosclerosis in chronic kidney disease (CKD) patients. Its role in these patients has been positively associated with mortality and cardiovascular death.

Hyperprolactinemia is very common in hemodialyzed patients [**25**]. HDF with high-flux membranes improves the clearance of molecules, with MW up to 25,000 Da, and sometimes up to 50,000 Da, resulting in the reduction of hyperprolactinemia as well. However, it has been shown that a few hours after the HDF session, prolactin levels return to pre-dialysis [**26**], due to its continued production.

We determined the removal of prolactin, a molecule with MW bigger than twice beta 2-microglobulin. The fact that it is removed better with mixed dilution than with pre-dilution (it is noted that prolactin is removed better with post-dilution) is of particular importance since even with toxins with larger MW, mixed dilution proves to be very good. Santos et al. in a study of 19 stable patients on post-dilution online HDF (filter surface area 2.1 m^2, blood pump 400 ml/min), and with convective volume 27.4±3.4 L found loss of prolactin 73.7% (67.3-77.5%) [**27**], as found and others [**28**]. This difference with our results may be due to higher substitution volume in these studies and to the time the blood samples were taken at the end of the session (immediately after the end, and not after the equilibration of prolactin between body compartments, as we did).

Also, Maduell et al. who studied 21 dialyzed patients evaluated prolactin removal with a Helixone filter in a pre-dilution online HDF session. Their blood flow during the study was 350-500 ml/min (from 434±36 ml/min), dialysate flow 400 ml/min, session duration was on average 291±17 min (from 240 - 300 min) and they found prolactin removal by 74.6±9% [**29**]. This percentage was clearly higher than ours, but it should be considered that the blood supply to the filter in their study was greater than ours, the duration of the session was also longer, and the blood samples for prolactin determination were taken immediately after the end of the session.

In the current study also a significant % reduction of serum prolactin levels was noted (removal amount ranged from 36.2±9.5% to 52.5±8.9% per session), more with post-dilution and mixed model and less with pre-dilution. That means that mixed dilution online HDF is the second-best choice in the removal of medium MW toxins, while in the removal of low MW toxins, the worst of all is conventional hemodialysis and the best is pre-dilution online HDF (again the mixed online HDF was the second-best method). So, in cases where post-dilution cannot be completed (if it cannot achieve the substitution volume of ≥23 L), it is preferable to use the manual mixed model. The most probable cause that manual mixed HDF is very effective (better than pre-dilution HDF) is because both prolactin and beta 2-microglobulin are in both the extracellular and intracellular spaces, so within the first two hours of post-dilution HDF the amount of the extracellular space is removed, and then a significantly smaller amount is removed with both (pre- and post-dilution), due to the slow movement of these toxins from the intracellular space to extracellular.

Our study has several limitations that should be acknowledged. First, the study involved a small sample size from a single center, which could introduce collection bias and limit the generalizability of the findings. Second, while the study results suggest that the substitution volume used was effective, we cannot be certain that it was the optimal volume. Further research is needed to confirm this. Finally, we were unable to measure prolactin levels in the hyperfiltrate to determine the exact amount removed during the sessions.

Conclusions. In summary, our study demonstrates that post-dilution and mixed dilution HDF methods were more effective at removing medium molecular weight toxins compared to other approaches. There was no significant difference in the removal of beta 2-microglobulin across the different HDF techniques. For prolactin removal, post-dilution HDF was superior to pre-dilution, and manual mixed online HDF also proved to be more effective than predilution. These findings are clinically significant as they demonstrate that effective clearance of medium molecular weight toxins can be achieved without the need for specialized dialysis machines, and without encountering issues that might prevent the completion of the HDF session. Further research is needed to explore these results in greater detail and to confirm their clinical implications.

Conflicts of interest statement. The authors have no conflict of interest to declare. They had full access to all the data in the study and took responsibility for the integrity of the data and the accuracy of the data analysis. The results in this paper have not been published previously in whole or in part.

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The authors' contributions.

Konstantinos Mavromatidis: Contributed to the study design, analyzed and interpreted the data, drafted and revised the manuscript, and approved the final version;

Irini Kalogiannidou: Contributed to the study design, collected and analyzed the data, revised the manuscript, and provided critical input to the study;

Ploumis Passadakis: Assisted with data collection and literature searches;

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