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What Is the Difference between Post- and Pre-Dilution in On-Line Haemodiafiltration in the Removing of Median-Molecular-Weight Toxins?

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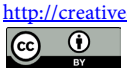

Abstract

Introduction: β_2-microglobulin and prolactin are medium-molecular-weight toxins. We believe that the rate of removal of these molecules, using filters with two different surfaces, in post- and pre-dilution haemodiafiltration, will be of interest to the literature. **Methods:** We studied, in 12 haemodialyzed patients, the removal of those, with filter with surface of 2.5 m^2 (Group A) or 2.1 m^2 (Group B) with post-dilution haemodiafiltration, and with a filter with surface 2.5 m^2 with pre-dilution (Group C). **Results:** Satisfactory removal of β_2-microglobulin (best with post-dilution) was found, and a good rate of removal of prolactin without the filter surface played a role in both cases. **Conclusion:** The elimination of β_2-microglobulin in post-dilution is not affected by the filter surface area. Pre-dilution achieves removal of β_2-microglobulin, less than that of post-dilution. Prolactin was removed satisfactorily regardless of the filter surface in post-dilution, and its removal appears to be less than that of β_2-microglobulin.

Keywords

Haemodiafiltration, Pre-Dilution, Post-Dilution, β_2-Microglobulin, Prolactin

1. Introduction

After many years, the superiority of haemodiafiltration (HDF) over high-flux haemodialysis has been confirmed [1] [2]. Of course, not all vascular accesses can provide blood, so post-dilution on-line HDF can be applied. In such cases, the duration of the dialysis session can be increased (which is usually not desired

by the patients), or on-line HDF pre-dilution can be applied. An example is the Japanese haemodialyzed patients who use this model at a rate of over 90%, because their vascular accesses do not provide a satisfactory blood supply, with the aim to improve patient survival, although until recently they have not seen any improvement [3]. Finally, the question that remains is whether the efficiency of pre-dilution on-line HDF is sufficiently satisfactory (because the researchers consider pre- and post-dilution HDF to be equal if an appropriate volume of supernatant is exchanged) [3], especially for medium-molecular-weight toxins, to consider its application as a better method of blood purification, because this will be associated with better survival. Our study conducted a comparison of post- and pre-dilution on-line HDF to determine how much is removed from two medium-molecular-weight toxins, β_2-microglobulin and the even larger molecule prolactin, with filters of different surface areas.

2. Patients - Methods

2.1. Patients

Twelve haemodialyzed patients were studied (7M, 5F). We included all patients of our unit who were in on-line HDF and did not have any exclusion criteria, as shown below. The primary renal diseases in four were glomerulonephritis, in three adult-type polycystic kidney disease, in two hypertensive nephrosclerosis, and in three the primary renal disease was of unknown aetiology. Eight of the patients had an internal arteriovenous anastomosis (fistula), three had a graft, and one had a double-lumen jugular dialysis catheter. Only four patients had residual renal function (24-hour urine output > 400 ml per day off dialysis) (**Table 1**). All patients had been on dialysis for >10 months (mean ± SD = 128 ± 150). Patients had maintained a stable dry body weight for at least four months (range 54 - 85 kg).

Those with symptomatic cardiovascular disease, haematocrit (Hct) levels > 45%, a history of on-session cardiovascular instability, or recirculation > 5%, were excluded from the study. Patients with cancer, cryoglobulinemia, gammopathies, polycythaemia, or active infection were also excluded. None of the patients were receiving drugs that increase plasma prolactin levels.

2.2. Methods

All patients underwent one post-dilution on-line HDF session with a filter of 2.5 m^2 surface area (Group A), one post-dilution on-line HDF session with a filter of 2.1 m^2 surface (Group B) and one pre-dilution on-line HDF session with a filter 2.5 m^2 surface (Group C). The substitution volume (replacement fluid) in post-dilution was 25% of the blood supply (*i.e.*, ≥24 L/session), while in pre-dilution it was 50% of the blood supply (*i.e.*, ≥48 L/session).

At the mid-week session (Wednesday or Thursday) a blood sample was taken before the start of the dialysis session from the arterial line for urea, to determine the urea reduction ratio (URR), Hct, and albumin, but also for β_2-microglobulin

and prolactin. One hour after the end of the session, a blood sample was also taken from everyone (to redistribute the parameters we would determine) [4].

Dialysate bicarbonates were 33 mEq/L in eight and 31 mEq/L in the remaining four patients, while sodium was 138 mEq/L in seven and 140 mEq/L in the remaining five patients. The potassium of the dialysate in five patients was 2 mEq/L, and in seven 3 mEq/L, while the glucose of the dialysate was 100 mg/dl for all the patients (all dialysis sessions were done with a standard dialysate for all patients). The duration of the session was 240 min for nine patients, 255 min for two patients, and 285 min for one patient (Table 1). Polyethersulfone-polynephron filters (Elisio™ Nipro, high-flux) were used, with a sieving coefficient for β_2-microglobulin equal to 0.803. All patients were dialyzed with Nikkiso DBB EXA machines.

Low-molecular-weight heparin (vemiparin) was used as an anticoagulant in all the patients, in doses of 2500 - 3500 IU/session, depending on the patients' body weight. The blood supply (blood pump) was 400 ml/min for all patients, with a negative pressure < 200 mmHg (*i.e.*, at least 0.8 - 1 m^2/200 ml/min blood flow, meaning that at a pump supply of 400 ml/min, the filter was at least up to 2 m^2), and the dialysate flow was 500 ml/min for all patients.

Table 1. It contains the age, the dry body weight, the body water of the patients, the duration of each dialysis session/patient, the residual renal function, the months on dialysis of the patients and the months on on-line HDF of each patient.

Patient	Age (Years)	Dry BW (kg)	Body water (L)	Duration dialysis session (min)	Residual urination/24h (ml)	Months in dialysis	Months in on-line HDF
1	48	74	42.5	240	500	71	24
2	69	67	37.2	255	0	258	19
3	56	73	34.8	240	100	54	10
4	57	65	31.6	255	250	89	24
5	68	59	34.6	240	0	450	10
6	70	70	36.7	240	<150	61	10
7	85	84	40.3	240	100	19	9
8	77	85	41.7	240	750	10	4
9	72	69	31.7	240	500	29	8
10	53	79	40.3	285	0	417	6
11	83	54	27.47	240	800	26	6
12	51	70	33.12	240	200	51	15
Mean ± SD	65.75 ± 12	70.75 ± 8.8	36 ± 4.4	246.3 ± 12.9		128 ± 150	12.1 ± 6.6

All the ultrafiltrate was collected in a specially made volumetric tank, where its volume was measured. After the end of the session and after thorough stirring of the ultrafiltrate with an electric stirrer for 10 min, a sample was taken to determine the urea and β_2-microglobulin. The assessment of the provided clearance was done with the URR.

The change in concentration of a toxin before and after a dialysis session (reduction rate) is a good index of its removal from a single space, such as the vascular, but because β_2-microglobulin and prolactin are distributed in the extracellular space (*i.e.*, vascular and interstitial, which are two spaces) [5], the one-compartment model in this case overestimates their actual removal [6]. However, by determining β_2-microglobulin and prolactin in a blood sample taken one hour after the end of the session, as we did in our study, their intercompartmental movement is almost restored, and thereafter their content in the extracellular space is the same everywhere [interstitial and vascular) [7].

The Abbott Alinity C analyzer was used to determine all parameters studied. Urea was determined by an enzymatic method, prolactin photometrically (which determines the total molecule of prolactin and not only the monomer) and β_2-microglobulin immunoturbidometrically. Prolactin could not be determined in the total ultrafiltrate because of its very low concentration therein.

2.3. Ethical Considerations

All procedures were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2013.

2.4. Statistical Analysis

The student *t*-test was used for the statistical analysis. Significant differences were considered with a significance level of $p < 0.05$.

3. Results

The study involved 12 patients (7M, 5F) with a median age of 68.5 years (mean ± SD = 65.75 ± 12), who had been on dialysis for 128 ± 150 months and on post-dilution on-line HDF for 6 - 24 months (mean ± SD = 12.1 ± 6.6 months). Their dry body weights, body water, and session durations are presented in **Table 1**. The weight gain between sessions, which were removed in the patients' three sessions during the study, was always < 2000 ml/session.

The mean Hct of the patients before each session of the study is shown in **Table 2**. It was found to be significantly lower before the session with the 2.1 m^2 filter (36.9% ± 1.66%) (Group B), compared to its value before the session with the pre-dilution on-line HDF (39% ± 2.96%) (Group C) [p(B − C) < 0.03] (**Table 2**).

The URR in Group A was 78.1 ± 6.7, in Group B 76.4 ± 2.8 and in Group C 75.0 ± 3.0, without finding a statistically significant difference between the

Table 2. It contains the Hct, the serum albumin levels, the changes in β_2-microglobulin, the serum levels of β_2-microglobulin before and at the end of dialysis session, the amount of β_2-microglobulin removed in each dialysis session [Changes in serum levels: p(A − C) < 0.02, p(B − C) < 0.005, amount of β_2-microglobulin removed: p(B − C) < 0.005, p(A − C) < 0.02, and Hct levels: p(B − C) < 0.03. All the other correlations were p = NS].

	25H (post) Group A						21H (post) Group B						25H (pre) Group C					
Patients	Hct (%)	Albumin (mg/dl)	Change (%)	At beginning HFD (mg/L)	At the end of HDF (mg/L)	Amount removed (mg)	Hct (%)	Albumin (mg/dl)	Change (%)	At beginning HFD (mg/L)	At the end of HDF (mg/L)	Amount removed (mg)	Hct (%)	Albumin (mg/dl)	Change (%)	At beginning HFD (mg/L)	At the end of HDF (mg/L)	Amount removed (mg)
1	38.1	4.3	69.7	26.99	8.17	245.95	38.3	4.4	71.8	27.65	7.81	260.6	37.9	4.3	66.2	25.25	16.63	259
2	38.5	4.2	71	33.92	9.83	303	38.6	4.1	71.1	33.86	9.97	306.03	40.4	3.9	66.7	29.65	9.86	233.6
3	36.4	4.3	58.4	34.55	14.36	268.64	35.5	4.2	70.7	23.34	7.56	219	44.8	4.6	66	21.3	9.14	159.8
4	38.4	4.1	71	24.21	7.05	227.9	38.6	4.2	72.9	25.6	6.94	237.1	39.3	3.9	63.4	74.58	44.91	214.8
5	42.1	4.4	69.3	22.8	7.0	232	38.0	4.0	69.4	22.2	6.76	200.1	42.4	4.0	64.7	19.33	6.83	157.9
6	37.7	4.1	66.3	19.22	6.48	187	36.3	3.9	66.4	19.33	6.5	200.6	41.6	4.1	59.5	19.52	7.91	118.7
7	39.7	4.6	74.6	32.26	8.21	286.4	36.9	4.8	70	31.09	9.32	277.4	33.0	4.2	62	33.17	16.62	271.6
8	34.7	3.8	49.9	14.25	7.14	198.8	33.3	4.1	60.6	21.41	8.44	216.08	39.3	4.0	62.1	20.36	7.71	177
9	39.7	3.4	68.8	19.63	6.13	159.1	39.3	4.0	70.5	20.88	6.16	161	38.1	3.7	71.6	19.81	5.62	133.7
10	34	3.7	71.8	28.91	8.34	290.7	35.2	3.7	70.6	28.75	8.46	121.4	36.4	3.8	67.6	27.72	8.99	140.7
11	37.6	4.0	68.8	27.57	8.6	135.8	36.7	3.9	67	25.02	8.26	173.74	36.7	4.0	67	26.13	8.62	167.58
12	37.5	4.3	76.5	25.96	6.1	226.3	36.5	4.0	72.1	24.23	6.78	211.7	38.0	4.0	65.7	25.51	8.76	176.4
7M, 5F	37.9 ± 2	4.1 ± 0.32	68 ± 6.9	25.9 ± 5.9	8.1 ± 2.2	213 ± 76.6	36.9 ± 1.66	4.1 ± 0.27	69.4 ± 3.2	25.3 ± 4.2	7.8 ± 1.1	215.4 ± 48.8	39 ± 2.96	4.04 ± 0.23	65.2 ± 3.0	28.5 ± 14.5	12.6 ± 10.3	184.2 ± 47.5

groups (perhaps for the numerically lower URR value in Group C, the higher Hct of this group played a role) (Table 2)

Regarding β_2-microglobulin, no difference was found in its percentage change between Groups A and B, while a significant difference was found between Groups A and C (68 ± 6.9 vs 65.2 ± 3.0, $p < 0.02$) and Groups B and C (69.4 ± 3.2 vs 65.2 ± 3.0, $p < 0.005$). The amount of β_2-microglobulin removed did not differ between Groups A and B (213 ± 76.6 vs 215.4 ± 48.8, p = NS), while it differed between Groups A and C (213 ± 76.6 vs 184.2 ± 47.5, $p < 0.02$) and Groups B and C (215.4 ± 48.8 vs 184.2 ± 47.5, $p < 0.005$) (Table 2).

For prolactin, no difference in percentage change was found between Groups A and B (54.6 ± 8.89 vs 58.05 ± 9.1, p = NS), while a difference was found between Groups A and C (54.6 ± 8.89 vs 42.3 ± 8.2, $p < 0.002$) and Groups B and C (58.05 ± 9.1 vs 42.3 ± 8.2, $p < 0.0002$) (Table 3), a fact indicating that less prolactin is removed in pre-dilution on-line HDF (about 12% - 16%).

Table 3. It contains the changes in serum levels of prolactin (%) and the serum levels of prolactin at the beginning and at the end of dialysis session [change in serum prolactin levels between the groups: p(A − C) < 0.002, (B − C) < 0.0002, all the other correlations had p = NS].

	25H (post) Group A			21H (post) (Groum B)			25H (pre) (Group C)		
Patients	Change serum prolactin (%)	At beginning HFD (mg/L)	At the end of HDF (mg/L)	Change serum prolactin (%)	At beginning HFD (mg/L)	At the end of HDF (mg/L)	Change serum prolactin (%)	At beginning HFD (mg/L)	At the end of HDF (mg/L)
1	50.9	26.15	12.85	58.5	29.87	12.4	43.1	29.25	16.63
2	73.5	9.82	2.6	75.9	9.94	2.4	58.6	12.79	5.3
3	57	21.53	9.25	63.5	23.34	8.53	41.4	21.3	12.49
4	55.3	68.14	30.47	61.6	72.97	28.04	39.8	74.58	44.91
5	45.8	13.74	7.45	48.6	12.95	6.66	37.7	17.76	11.07
6	39	10.86	6.62	42.4	13.45	7.75	34.8	10.78	7.03
7	58.4	32.81	13.64	56.9	36.58	15.78	28.9	31.26	22.24
8	42.5	17.36	10.0	48.3	16.98	8.78	42.6	17.21	9.87
9	60.1	25.11	9.81	64.9	25.2	8.84	52.4	20.46	9.74
10	61.7	17.98	6.88	64.9	17.73	6.23	52.2	21.43	10.25
11	53.5	63.05	29.34	53	50.02	23.53	41.5	64	37.41
12	57.6	173.87	73.81	60.6	181.16	71.33	34	197.06	130.13
7M, 5F	54.6 ± 8.89	40.0 ± 44.3	17.73 ± 18.83	58.05 ± 9.1	40.85 ± 45.72	16.9 ± 17.95	42.3 ± 8.2	43.2 ± 50.2	26.4 ± 33.4

What was also found is that the change in serum prolactin levels was at least 11% - 23% less than that of β_2-microglobulin, apparently due to its greater molecular weight. If it is considered that 20% of its quantity is macromolecules (60,000 - 150,000 Da), it is obvious that the removal mentioned is very important.

4. Discussion

This study initially indicated that with both filters, but also with both methods (post- and pre-dilution on-line HDF), the clearance provided was very satisfactory for low-molecular-weight toxins. This is attributed to the high blood supply to the filter (400 ml/min), the duration of the sessions (>4 hours), and the large surface area of the filters (2.1 m^2 and 2.5 m^2).

Successful on-line HDF of large volumes with post-dilution depends on adequate vascular access blood supply (≥350 ml/min), that is, an excellent arteriovenous anastomosis with blood supply ≥ 600 ml/min, with the ability to achieve adequate anticoagulation throughout the session and in the absence of any clinical disorder that increases blood viscosity (cryoglobulinemia, gammopathy, or

polycythaemia). Basically, in post-dilution on-line HDF, if the blood supply is 400 ml/min, a replacement volume of 25% of this can be achieved, that is, 24 L/session.

Regarding patients with dialysis catheters, in a multicentre study, only one third of patients achieved the minimum substitution volume target of 21 L [8]. However, it is also possible for these patients, when they have a blood supply of 250 - 300 ml/min, to apply on-line HDF with post-dilution if the duration of the session is increased [9]. Consequently, patients with vascular access problems who refuse to extend the duration of their sessions are not suitable for on-line HDF of large substitution volumes.

β_2-microglobulin has a molecular weight of 11,800 Da and circulates as a free monomer (unbound molecule) in the extracellular space. It is a protein found on the surface of all nucleated cells (monocytes, but mainly lymphocytes) and is a Class I human leukocyte antigen (HLA). Its production increases as cells are multiplying or destroyed.

During metabolism, β_2-microglobulun is released into the blood and then found in plasma, urine, and other bodily fluids. Its production rate is 2 - 4 mg/kgBW/24 hours [10], or 0.159 mg/kgBW/hour, which is about 200 - 300 mg/day [11]. It is a well-known and well-studied index of uremic toxins of medium molecular weight and is also known for its role in haemodialysis amyloidosis [12].

β_2-microglobulun is freely filtered in the glomeruli (by 95%). Of this amount, approximately 99.9% is reabsorbed by pinocytosis and metabolized by the lysosomes of the proximal tubular cells (only 1% of β_2-microglobulin is removed extra-renally), without being able to return to the blood. As a result, its serum levels are inversely related to the rate of glomerular filtration [13]. When the glomerular filtration rate is also affected, β_2-microglobulin increases in the blood, and in patients with end-stage renal disease, its levels increase up to 60-fold [14]. At this point it is deposited as amyloid fibrils in the tissues. Its levels in the plasma of these patients can range from 30 mg/L to 50 mg/L, that is, much higher than normal values, which for men and women up to 50 years of age is 1.2 - 2.5 mg/L and for people over 50 years of age it is 1.4 - 3.2 mg/L (it has a half-life time of 1 - 3 hours) [15]. In fact, about 40 years ago it was found that patients on conventional (classic) haemodialysis had plasma β_2-microglobulin levels ranging from 12.5 mg/L to 92 mg/L, with the highest values noted in anurics [16]. Residual renal function is probably the most important determinant of its levels in blood, and obviously it is good to preserve it as much as possible, but in our patients, we found no correlation between serum β_2-microglobulin levels and the presence or absence of residual renal function.

The monomer of prolactin has a molecular weight of 23,000 Da; it is the most common form that circulates in healthy individuals and in most patients with true hyperprolactinemia [17]. However, there are also forms with a higher molecular weight, such as large prolactin, which is its dimer and has a molecular weight of 60,000 Da, and macroprolactin with a molecular weight of 150,000 Da

[17]. The latter two constitute 20% of serum prolactin [18]. Its serum levels in normal men range from 8 ng/ml to 18 ng/ml and in normal (non-pregnant) women from 2 ng/ml to 29 ng/ml.

The actions of prolactin are varied. At the endothelial level it stimulates monocyte adhesion in response to inflammatory cytokines [19]. Its main fraction is a stimulator of angiogenesis [20]. Its increased concentrations are associated with increased peripheral insulin resistance [21]. Among other things, as a hormone involved in reproductive behaviour, it is also responsible for the sexual dysfunction found in uraemia, expressed as loss of libido, erectile problems, and infertility. In men, it may be associated with gynecomastia and galactorrhea, and in women with menstrual disorders (amenorrhea and oligomenorrhea) and galactorrhea [22].

The kidneys play an important role in endocrine regulation, not only producing hormones (erythropoietin, renin), but also metabolizing others, such as insulin, cortisol, and prolactin. Hyperprolactinemia in patients with chronic kidney disease is very common, a fact that has been known for many years [23]. In uraemia, it accumulates due to a decrease in renal clearance [24], but mainly due to its increased production and secretion [25].

β_2-microglobulin is the first medium-molecular-weight toxin to be identified. The polyacrylonitrile membrane with large pores was found to have a higher removal capacity compared to the classical cellulose membrane [26]. It was thought that patients dialyzed exclusively with this had a lower incidence of haemodialysis amyloidosis [27].

Although HDF achieves better clearance of β_2-microglobulin (30% - 40% higher compared to low-flux dialysis), its production rate is higher than the removal capacity of each dialysis method [5] [10], so with this method, its levels before each session are corresponding to those of the previous session. **Table 2** shows that the amount lost with each method ranges from about 120 mg/session to 300 mg/session, meaning that this can easily be reproduced between sessions.

The ways to control the removal of β_2-microglobulin from the patient with on-line HDF are to determine its clearance (by collection of the ultrafiltrate) or determining the percentage of reduction of this in serum [10] [28], which we selected and used in this study.

Ward *et al.* studied 44 dialyzed patients (HDF or high-flux haemodialysis). The patients were dialyzed with polyamide filters, surface 1.7 m^2, with a blood supply of 250 - 300 ml/min (mean ± SD = 281 ± 4 ml/min) and a substitution volume of 25% of the blood supply, with an average session duration of 247 ± 3 min. They found better β_2-microglobulin removal with post-dilution on-line HDF compared to high-flux haemodialysis (73% ± 1% vs 58% ± 1%, $p < 0.01$) [1], where the post-dilution blood sample was taken from the arterial line after 20 sec of reducing the blood supply rate to 80 ml/min. Lornoy *et al.* (with a filter with a surface area of 1.8 m^2 and a blood pump of 300 ml/min) also found in the post-dilution on-line HDF with substitution volume of 24 L/session, a reduction of β_2-microglobulin by 72.7% (the blood sample at the end of dialysis was taken

from the arterial line after reducing the blood pump to 50 ml/min for 2 min) [10]. Compared to the above studies, we found on average with a filter of 2.5 m^2 and 2.1 m^2 surface area (*i.e.*, larger), with a higher blood flow (400 ml/min), a reduction of 68% ± 6.9% and 69.4% ± 3.2%, respectively. The differences found in the percentage of β_2-microglobulin removal by other investigators [1] [10] [29] compared to ours, in our opinion, was due to the different way of taken the blood sample at the end of the session. We took it one hour after the session had ended, by which time the redistribution of β_2-microglobulin is almost complete, while the others took it by simply slowing down the pump for a few seconds at the end of the session [1] [10] [29]. This means that more β_2-microglobulin exits from the cells in our case compared to the other researchers, and so its value in the blood at the end of the session in our case will be higher, so its reduction rate would be less, as it was.

Other studies have also reported a significant decrease in serum β_2-microglobulin levels after switching from haemodialysis to HDF [30] [31] [32]. In the CONTRAST study, serum β_2-microglobulin was significantly reduced with HDF, especially in cases where residual renal function was low [33]. Maduell *et al.* studied 16 dialyzed patients who were on a post-dilution on-line HDF program (substitution volume > 30 L) and filters of two different capillary inner diameters (surface area 1.4 - 2.0 m^2, with sieving coefficient for β_2-microglobulin = 0.9) and found a reduction rate for β_2-microglobulin of just over 82% [34].

A meta-analysis by Roumelioti *et al.* found an average β_2-microglobulin removal of 151.66 mg/session (from 126.98 mg/session to 176.34 mg/session) [2], while we found in post-dilution with a filter with surface area of 2.5 m^2, 213 ± 76.6 mg/session, with filter 2.1 m^2, 215.4 ± 48.8 mg/session, and in pre-dilution with filter 2.5 m^2, 170.2 ± 67.9 mg/session, that is, considerably higher with each method, but clearly higher in post-dilution.

Of course, another parameter that must be considered is the adsorbed amount of β_2-microglobulin on the filter membrane, which is not the same for all membranes [7], ranging from 13.5% to 43.8% [35]. Thus, a decrease in serum β_2-microglobulin was also found by Zehnder *et al.* during on-line HDF; they demonstrated its adsorption by the polysulfone membrane [36].

Greater blood supply to the filter was significantly associated with higher β_2-microglobulin clearances (maximal benefit was seen with a replacement supply of 100 ml/min or 24 L/4-hour session) [2], as found by others [37]. Other researchers have also shown that increasing the blood supply to the filter is perhaps the best method to achieve a higher conventional volume. In fact, for every 50 ml/min increase in blood supply, the conventional volume increases by more than half a litre per hour, so the capacity to remove toxins (of small and medium molecular weight) also increases [38]. Today high-volume post-dilution on-line HDF is that in which substitution volumes > 23 L/session are used, in subjects who are dialyzed three times per week [28]. In all our patients we had a blood flow rate of 400 ml/min, which agrees with the above data (substitution volume ≥ 24 L).

In post-dilution on-line HDF and large substitution volumes, the other key determinants of conventional volume besides blood supply are session duration, filtration fraction, and Hct levels [39]. But Morena *et al.*, who studied 32 patients under post-dilution on-line HDF compared to conventional haemodialysis, found a removal of β_2-microglobulin of 84.7% ± 0.8%, and that the larger the filter surface area, the thinner the capillary wall and the consequent very high ultrafiltration coefficient, should be considered advantages in performance [29]. We did not find this, at least with respect to the filter surface area. It is noted that the blood samples in this study were taken immediately after the end of the session from the arterial line.

Still other researchers have found that increasing the filter surface area causes a small increase in conventional volume, without demonstrating an improvement in toxin-removal capacity, especially if the ultrafiltration coefficient is higher than 45 ml/hr/mmHg [40]. In fact, on-line HDF with a larger filter is numerically associated with a greater clearance of β_2-microglobulin, and this correlation is marginally statistically significant ($p = 0.06$) [2]. However, in the present study no correlation of the amount of β_2-microglobulin removal was noted with the filter surface area, since an average of 213 ± 76.6 mg was removed per session with a filter of 2.5 m^2, compared to 215.4 ± 48.8 mg with a filter of 2.1 m^2.

Membrane composition, structure, and thickness determine the relative amount of β_2-microglobulin removed by convention or adsorption [41], although its removal in pre- and post-dilution in most studies refers to its diffusion rather than its adsorption in the capillaries of the filter [42]. The latter study, in fact, in nine haemodialyzed patients with filters of 2.1 m^2 and a blood supply of 300 - 400 ml/min, found that there is no difference in the clearance of β_2-microglobulin between pre- and post-dilution, with a substitution volume equal to 45% of body water in the post- and 100% in the pre-dilution on-line HDF [42], which we do not agree with, since we found with a filter of 2.5 m^2 and post-dilution (Group A) a mean removal of 213 ± 76.6 mg/session, while in pre-dilution with the same filter (Group C) 184.2 ± 47.5 mg/session were removed [$p(A - C) < 0.02$]. The newer membranes, however, did not appear to be superior at all, although they did show a greater reduction in β_2-microglobulin (percentage reduction), but not in clearance [2] [40].

Blood prolactin levels are elevated in 50% - 80% of haemodialyzed patients and are not affected by the application of conventional haemodialysis, regardless of the period of time applied and for how many hours/week [43]. Fathalla *et al.*, who studied 50 haemodialyzed patients, found prolactin levels average to 45.0 ± 9.7 ng/ml (33.00 - 68.00 ng/ml), versus 15.1 ± 5.1 ng/ml in normal controls [22].

Haemodiafiltration improves the clearance of toxins with a molecular weight of up to 25 kDa, sometimes up to 50 kDa, resulting in the reduction of prolactinemia as well. However, it has been shown that a few hours after the HDF session, prolactin levels return to pre-dialysis levels [44], both due to its redistribu-

tion [5] and due to its continued production during the dialysis session (20 - 40 mg in 4 hours of the session).

Maduell *et al.* studied eight patients in post-dilution on-line HDF (blood pump 445 ± 59 ml/min, duration session 274 ± 25 min, with substitution volume 30.7 ± 6 L) and found prolactin removal in 65.6% ± 4% (the blood samples were taken from the arterial line 60 sec after the blood pump was reduced to 50 ml/min, at the end of the session) [45]. The same researchers studied 16 patients who were in a post-dilution on-line HDF program (453 ± 34 ml blood pump and filters with capillaries of two different internal diameters, with surface area 1.4 - 2.0 m^2), and found that during a session of 240 - 300 min duration and substitution volume of about 32 L/session, with blood samples drawn immediately after the end of the session (prolactin redistributed one hour after the end of the session), the reduction rate for prolactin was about 68% [34]. We may agree with them, since in a 4-hour session, with a blood pump of 400 ml/min and a substitution volume of 24 L/session for all patients, we found a reduction of about 50% (taking the blood sample one hour after the end of the session).

Sakurai *et al.* studied eight Japanese patients (body weight, mean ± SD = 54.5 ± 7.8), pre- and post-dilution (blood pump of 250 ml/min) with various filters (polysulfone and asymmetric cellulose triacetate), with surfaces area of 2.1 m^2 and 2.2 m^2, with 12 L of substitution volume/session at post-dilution, and found prolactin removal of 77.9% ± 7.3% to 80.2% ± 3.2% [46].

From our study, it appears that the removal of β_2-microglobulin by post-dilution on-line HDF is not affected by the filter surface area. Pre-dilution achieves a significantly great removal of β_2-microglobulin, but less than that of post-dilution. The removal of prolactin is not affected by the surface area of the filters in post-dilution, while less is removed in pre-dilution (about 12% - 16%) than with post-dilution, and its reduction appears to be less than that of β_2-microglobulin, apparently due to its higher molecular weight.

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Conflicts of Interest

The authors have no conflicts of interest relevant to this article to disclose.

Data Availability Statement

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

Patient Consent Statement

Written informed consent was obtained from the patient before the study.

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