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A Randomised Prospective Crossover Study on the Effects of Medium Cut-Off Membranes on FGF-23 and Inflammatory Mediators in Patients Receiving Regular Haemodialysis

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Keywords
Chronic kidney disease · Medium cut-off membrane · Haemodialysis · Fibroblast growth factor-23 · Intrasubject stability

Abstract

Introduction: In contrast to high-flux dialysis (HFD) membranes, medium cut-off membranes (MCO) can potentially remove a wide range of middle molecules. Our study aimed to compare the clearance rate (CR) of fibroblast growth factor 23 (FGF-23) and other selected inflammatory cytokines between medium MCO and HFD membranes and investigate the intrasubject stability of these biomarkers. ***Methods:*** This prospective randomised case-crossover study recruited 20 adult patients who were randomised into two groups: group A: to start with 1 week of thrice-weekly dialysis using HFD membrane followed by a 3-week washout period and then 1 week of dialysis with an MCO membrane. Group B followed the reverse sequence. Blood samples were taken before and after each dialysis session for the analysis of the assessed biomarkers (FGF-23, interleukin-6 [IL-6], interleukin-18 [IL-18], high-sensitivity C-reactive protein [hsCRP], and dephosphorylated uncarboxylated matrix Gla protein [dp-ucMGP]). Wilcoxon signed rank and paired *t* tests were used for comparison between the membranes. One-way repeated measures ANOVA or Friedman tests were used for the intrasubject stability of the biomarkers. ***Results:*** The use of both MCO and HFD membranes resulted in a significant reduction of FGF-23 levels and other selected inflammatory cytokines. However, there was no significant difference in the CR: FGF-23 (0.31 vs. 0.23], $p = 0.242$), IL-6 (0.19 vs. 0.12, $p = 0.215$), IL-18 (−0.05 vs. −0.03, $p = 0.704$), dp-ucMGP (0.33 vs. 0.33, $p = 0.903$), and hsCRP (−0.05 vs. −0.08, $p = 0.107$). There was no significant intrasubject variability for all assessed biomarkers except in pre-dialysis high hsCRP levels when using HFD membrane. ***Conclusion:*** The use of both MCO and HFD membranes resulted in a significant reduction of FGF-23 levels and other selected inflammatory cytokines. However, the MCO membrane did not demonstrate a significant advantage over the HFD in the short term. There was no significant intrasubject variability for all assessed biomarkers apart from hsCRP.

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Introduction

Haemodialysis is a critical, life-sustaining treatment for individuals with end-stage kidney disease. Despite significant technological advancements in renal replacement therapy, mortality rates among haemodialysis patients remain markedly higher than those of age- and sex-matched individuals in the general population [1]. The primary causes of death in this patient group are cardiovascular events and infections [2].

The progressive loss of kidney function leads to the accumulation of various solutes known as uraemic toxins, ultimately resulting in uraemic syndrome. These uraemic toxins can be classified into three main categories: small molecules (<0.5 kDa), middle molecules (0.5–60 kDa), and protein-bound toxins [3]. While conventional dialysis membranes are effective at removing small molecules, the removal of middle molecules presents a significant challenge. Middle molecules such as fibroblast growth factor 23 (FGF-23) and inflammatory cytokines may contribute to heightened cardiovascular morbidity and mortality in patients receiving regular haemodialysis.

FGF-23, a 32-kDa protein, plays a crucial role in the renal handling of phosphate as chronic kidney disease (CKD) progresses, maintaining normal phosphate levels until very advanced CKD. The impaired renal secretion of FGF-23 due to progressive CKD can lead to a greater than 200-fold increase in serum levels [4]. Elevated levels of FGF-23 have been associated with left ventricular hypertrophy and a significant increase in cardiovascular events in dialysis patients [5–7]. Experimental studies have shown that FGF-23 causes cardiomyocyte hypertrophy through the activation of fibroblast growth factor receptor 4 and calcineurin-nuclear factor of activated T cells signalling pathway. In endothelial cells, high levels of FGF-23 were associated with endothelial dysfunction by increasing superoxide/nitric oxide ratio through interaction with fibroblast growth factor receptor 1. The effects of FGF-23 on cardiomyocytes and endothelium are independent of FGF-23 co-receptor Klotho, which mediates its effects in the kidneys [8].

Progressive CKD is associated with a state of persistent low-grade inflammation, mediated by increased oxidative stress, increased insulin resistance, and reduced urinary secretion, meaning higher accumulation of a group of polypeptides called cytokines [9]. The latter increases inflammation by activating several pathways in the kidney such as increased expression of oxygen/nitrogen species, proliferation of resident cells, and increase in the procoagulant activity of the renal endothelium [10]. This state of systemic inflammation is a pathogenic factor in the development of atherosclerosis and ultimately cardiovascular diseases and mortality [11]. Inflammatory biomarkers with molecular weights in the middle molecule range such as interleukin-6 (IL-6), a 26-kDa protein, and interleukin-18 (IL-18), a 17-kDa protein, have been associated with mortality in dialysis patients [12].

In addition to CKD-associated systemic inflammation, vascular calcification also contributes to higher cardiovascular disease and mortality risk [13]. There is ample evidence showing functional vitamin K deficiency as an important contributor to vascular calcification. Dephosphorylated uncarboxylated matrix Gla protein (dp-ucMGP), a 10-kDa protein, is considered a biomarker of vitamin K deficiency, and its levels are significantly elevated in people receiving dialysis. Dp-ucMGP is the inactive form of matrix Gla protein, lacking both vitamin K-dependent carboxylation and phosphorylation [14]. Dp-ucMGP levels fall with vitamin K supplementation and increase with vitamin K antagonism [15]. Therefore, a higher concentration of dp-ucMGP is indicative of vitamin K deficiency and a surrogate marker for increased risk of vascular calcification.

Medium cut-off (MCO) membranes, such as Theranova®, were designed with the aim of expanding the removal of medium-sized molecules while preserving albumin due to its steep sieving curve [16]. A recent meta-analysis indicated that MCO membranes were generally more effective in removing middle-sized molecules compared to conventional high-flux dialysis (HFD) membranes [17]. In this meta-analysis, three studies (138 patients) showed no clear advantage in eliminating IL-6 using MCO membranes relative to HFD membranes. The effectiveness of MCO membranes in clearing FGF-23 remains controversial. One study involving 6 patients reported no significant difference in median FGF-23 clearance among three dialysis modalities: conventional haemodialysis using an MCO membrane, conventional dialysis with an HFD membrane, and online haemodiafiltration (OL-HDF) with an HFD membrane [18]. In contrast, another study demonstrated a significant reduction in FGF-23 levels after 3 months of dialysis with MCO membranes compared to HFD membranes [19]. To our knowledge, there are no previous studies evaluating the intradialytic profiles of IL-18 or dp-ucMGP with conventional or MCO membranes.

Furthermore, our understanding of the above-mentioned biomarkers is predominantly based on cross-sectional studies and there is limited knowledge regarding their intrasubject stability in haemodialysis patients. Our study aimed to compare the clearance rate (CR) of FGF-23, IL-6, IL-18, high-sensitivity C-reactive protein (hsCRP) (23 kDa) and dp-ucMGP between MCO

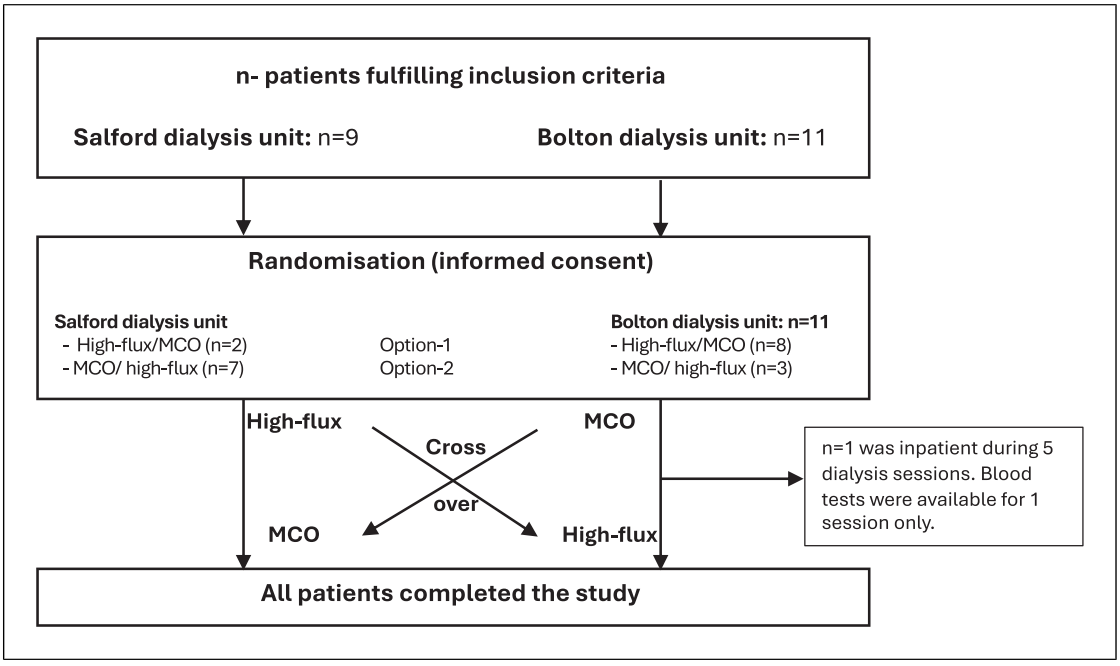

Fig. 1. The flowchart shows patient recruitment and inclusion in two participating dialysis units. All patients completed the study. MCO, medium cut-off membrane.

and conventional HFD membranes in patients receiving regular haemodialysis and investigate their intrasubject stability.

Methods

Study Design

This prospective randomised case-crossover study was conducted in two separate haemodialysis centres within the same organization – Salford and Bolton. The study was registered on the ISRCTN Registry on July 07, 2022 (ISRCTN92537009, https://www.isrctn.com/ISRCTN92537009). After obtaining informed consent, patients were randomly assigned to one of the two treatment options using sealed anonymized envelopes (Fig. 1).

Option 1: Patients began with 1 week of monitored thrice-weekly dialysis treatment using a conventional HFD membrane (Baxter). During this week, blood samples were collected before and after dialysis via the dialysis circuits. Following this, there was a 3-week washout period during which patients continued their usual dialysis treatment. Subsequently, patients underwent 1 week of monitored dialysis treatment with an MCO membrane, during which blood samples were again taken before and after dialysis for analysis. Ten patients were assigned to this option and designated as group A.

Option 2: Patients started with 1 week of monitored dialysis treatment using an MCO membrane, with blood samples collected before and after dialysis for analysis. This was followed by a 3-week washout period during which they continued their usual dialysis treatment. After the washout, patients received 1 week of monitored thrice-weekly dialysis treatment using an HFD membrane, with blood samples taken before and after dialysis for analysis. Ten patients were assigned to this option and designated as group B.

Participants

All patients aged 18 years and older receiving regular haemodialysis at the specified units were approached for informed consent. The exclusion criteria were as follows:

- Inability to provide consent for treatment.
- Significant residual urine output (>500 mL in 24 h).
- Poor dialysis adequacy (urea reduction rate <65%).
- Presence of active infection.
- Diagnosis of active malignancy.

Sample Collection and Handling

Before and after each monitored dialysis session, the following blood samples were collected: one 5 mL lithium heparin tube, one 5 mL serum tube, one 5 mL EDTA tube,

Table 1. Baseline characteristics for group A and group B

	Total population (*N* = 20)	Group A (*N* = 10)	Group B (*N* = 10)
Age, mean±SD	62.9±15.4	62.0±16.1	63.8±15.5
Gender (male), *n* (%)	15 (75)	8 (80)	7 (70)
Ethnicity (white), *n* (%)	18 (90)	8 (80)	10 (100)
Diabetes mellitus, *n* (%)	4 (20)	4 (40)	0 (0)
Heart failure, *n* (%)	3 (15)	3 (30)	0 (0)
Ischaemic heart disease *n* (%)	6 (30)	3 (30)	3 (0)
Pre-dialysis weight, mean±SD	76.1±17.3	82.0±17.8	70.2±15.5
Post-dialysis weight, mean±SD	74.4±17.3	80.3±17.5	68.5±15.7
Pre-dialysis SBP, mean±SD	148.5±22.9	151.2±26.5	145.9±19.7
Post-dialysis SBP, mean±SD	138.5±26.2	146.9±26.4	130.1±24.4
Pre-dialysis DBP, mean±SD	77.7±10.7	77.2±12.5	78.2±9.1
Post-dialysis DBP, mean±SD	74.4±11.4	76.9±12.0	71.8±10.9

SBP, systolic blood pressure; DBP, diastolic blood pressure; SD, standard deviation.

and one 5 mL 3.2% sodium citrate tube. The serum tube was allowed to stand at room temperature for 15 min to facilitate clotting before centrifugation, which separated the serum from the clot. The resulting serum was then pipetted into 1.5 mL cryovials for storage at −80°C. The lithium heparin blood tube was centrifuged to separate plasma from red blood cells. The sodium citrate tubes underwent two centrifugation steps: one at low speed and another at high speed to remove any remaining debris. The plasma was also transferred into 1.5 mL cryovials for storage at −80°C.

Analysis

Samples for FGF-23, IL-6, IL-18, dp-ucMGP, and hsCRP levels were sent to the Department of Biochemistry at the Research Institute Maastricht (CARIM), Maastricht University, for analysis. FGF-23 was measured by sandwich immunoassay from Biomedica (Vienna, Austria) for the quantitative determination of FGF-23 in human samples. The assay measures human intact and C-terminal fragments of FGF-23 with a precision in between <10% and within run of <12%. The limit of detection was 0.6 pg/mL.

IL-6 was measured by sandwich immunoassay from Biomedica (Vienna, Austria) for the quantitative determination of IL-6 in human samples. The assay measures human IL-6 with a precision in between <6% and within run of <7%. The limit of detection was 0.28 pg/mL.

IL-18 was measured by sandwich immunoassay from Sanbio (Uden, The Netherlands) for the quantitative determination of IL-18 in human samples. The assay measures human IL-18 with a precision in between <7% and within run of <8%. The sensitivity of the assay was 12.5 pg/mL.

CardioPhase® hsCRP was measured by the MUMC+ department of central diagnostics, using an automated immuno-nephelometry system (Siemens, BN ProSpec® System) = for the quantitative, IFCC-standardized determination of C-reactive protein (CRP) to screen for inflammatory processes. Polystyrene particles coated with monoclonal antibodies specific to human CRP are aggregated when mixed with samples containing CRP. These aggregates scatter a beam of light passed through the sample. The intensity of the scattered light is proportional to the concentration of the relevant protein in the sample. The result is evaluated by comparison with a standard of known concentration. The lower limit of detection of the assay is determined by the lower limit of the reference curve and therefore depends upon the concentration of the protein in the applied standards. A typical lower limit of detection for hsCRP is <0.17 mg/L for measurements performed using a sample dilution of 1:20 and is even lower if a sample is less diluted. The higher limit of detection is virtually non-existing as high CRP concentrations will be diluted and are not relevant for the CardioPhase® hsCRP assay, which is focussed on low CRP concentrations. Because the precision at low

Table 2. Comparing medium cut-off membrane with HFD membrane

	MCO	HFD	*p* value
FGF-23			
Pre-dialysis level, median pmol/L ± SD	237.4 [94.0 to 617.9]	248.3 [97.6 to 767.6]	0.398
Post-dialysis level, median pmol/L ± SD	195.3 [86.3 to 341.5]	214.7 [99.2 to 451.4]	0.136
CR, mean ± SD	0.31±0.26	0.23±0.18	0.242
Re-accumulation rate, median ± SD	0.50 [0.25 to 1.06]	0.23 [−0.12 to 0.89]	0.071
IL-6			
Pre-dialysis level pg/mL, median [IQR]	12.6 [7.6 to 24.5]	15.1 [9.2 to 25.8]	0.737
Post-dialysis level pg/mL, median [IQR]	12.4 [5.9 to 23.3]	11.1 [7.7 to 24.3]	0.911
CR, median [IQR]	0.19 [−0.10 to 0.34]	0.12 [−0.12 to 0.25]	0.215
Re-accumulation rate, median [IQR]	0.21 [−0.04 to 0.98]	0.17 [−0.28 to 0.44]	0.121
Dp-ucMGP			
Pre-dialysis level, median pmol/L [IQR]	691.1 [302.9 to 1,628.5]	859.3 [174.4 to 2022.7]	0.550
Post-dialysis level, median pmol/L [IQR]	419.7 [252.8 to 770.9]	504.2 [139.3 to 988.1	0.502
CR, mean ± SD	0.33±0.25	0.33±0.23	0.903
Re-accumulation rate, median [IQR]	0.50 [0.23 to 1.13]	0.96 [−0.06 to 1.75]	0.528
IL-18			
Pre-dialysis level pg/mL, mean ± SD	380.7±163.7	424.8±159.6	0.332
Post-dialysis level, mean ± SD	403.8±175.1	414.1±167.1	0.739
CR, mean ± SD	−0.05±0.13	−0.03±0.12	0.704
Re-accumulation rate, mean ± SD	−0.01±0.13	−0.03±0.10	0.389
hsCRP			
Pre-dialysis level mg/mL, median [IQR]	6.2 [4.1 to 14.3]	6.9 [2.9 to 23.2]	0.936
Post-dialysis level mg/mL, median [IQR]	7.2 [4.6 to 15.3]	6.1 [2.8 to 26.3]	0.879
CR, mean ± SD	−0.05±0.10	−0.08±0.10	0.107
Re-accumulation rate, median [IQR]	0.01 [−0.14 to 0.19]	−0.12 [−0.19 to −0.05]	**0.035**

MCO, medium cut-off membrane; HFD, high-flux dialysis; FGF-23, fibroblast growth factor 23, Dp-ucMGP, dephosphorylated uncarboxylated matrix Gla protein; hsCRP, high-sensitivity C-reactive protein; SD, standard deviation; IQR, interquartile range. A paired *t* test was used to determine the significance for normally distributed data and the Wilcoxon signed rank sum test was used for non-normally distributed data.

concentrations is very adequate, the lower limit of reporting is near the limit of detection and is set at <0.16 mg/L independent of the applied dilution step. The repeatability CV% within a batch analysis is <5%, while the overall reproducibility CV% is <10% including reagent lot and operator variability factors.

Plasma dp-ucMGP levels were measured in a single run by the Laboratory of Coagulation Profile (Maastricht, The Netherlands) using the commercially available IVD CE-marked chemiluminescent InaKtif MGP assay on the IDS-iSYS system (IDS, Boldon, UK). Plasma samples and internal calibrators were incubated using magnetic particles that were coated with murine monoclonal antibodies against dp-MGP, acridinium-labelled murine monoclonal antibodies against ucMGP, and an assay buffer. The magnetic particles were captured using a magnet and washed to remove any unbound analyte. Trigger reagents were added, and the resulting light emitted by the acridinium label was directly proportional to the level of dp-ucMGP in the sample. The assay measuring range was between 200 and 12,000 pmol/L and was linear up to 11,651 pmol/L. The within-run and total variations of this assay were 0.8–6.2% and 3.0–8.2%, respectively.

Statistical Analysis

The Kolmogorov-Smirnov test was used to evaluate the normality of data distribution. For continuous variables with normal distribution, data were presented as means with standard deviation and a paired *t*-test was used to compare statistical significance. When distribution was not normal, data were presented as median with interquartile range and the Wilcoxon signed rank sum test was used to compare statistical significance. Categorical values were expressed as percentages and the chi-square test was used to determine significance.

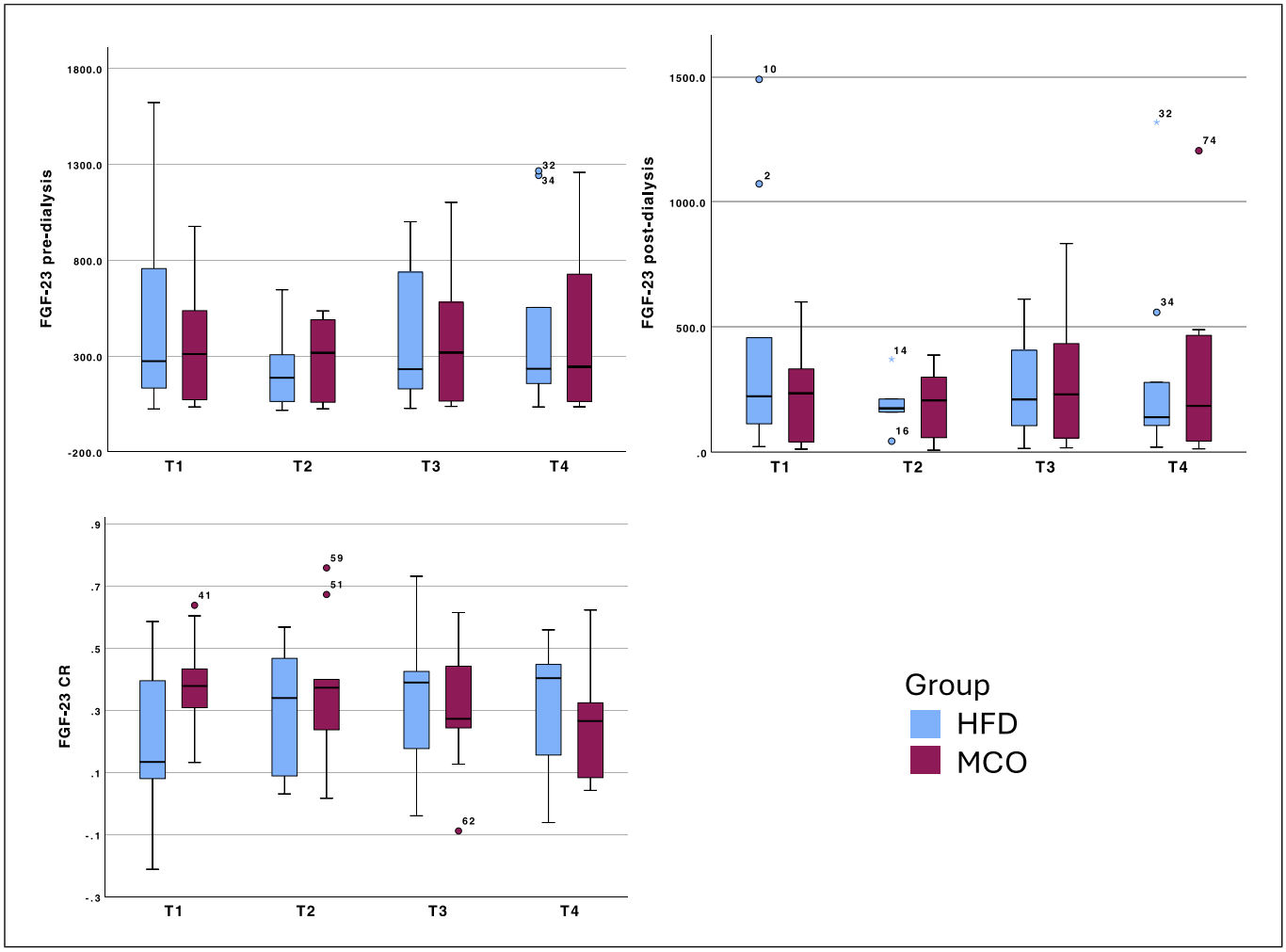

Fig. 2. Reports mean and 95% CIs of FGF-23 pre-dialysis and post-dialysis levels and CR stratified by treatment (HFD and MCO) at 4 time-points (T1 = baseline, T2 = after 1 week of treatment with either MCO or high flux, T3 = end of washout period, T4 = end of 1 week of treatment with either MCO or HFD).

CR of any of the analysed molecules (X) was calculated as (X pre – X post)/X pre for each dialysis session. The re-accumulation rate (RA) of (X) was measured as (X pre – X post previous dialysis session)/X post previous dialysis session. Average values of CR and RA for each treatment arm were used in the comparative analysis.

Within-subject analysis was constructed using analysis of variance (one-way repeated measures ANOVA for normally distributed data and the Friedman repeated measures analysis of variance test for non-normally distributed data) to determine intersubject stability of pre-dialysis and post-dialysis and for each treatment arm (MCO and HFD membranes) for the 3 consecutive dialysis sessions in each treatment arm. All statistical analyses were performed using IBM SPSS (Version 28.0.1.1), provided by the University of Manchester (2021).

Sample Size

Sample size calculation was determined for FGF-23 clearance based on the Damasiewicz et al. [20] study. With 20 patients, the study had an 80% chance of detecting a log difference of 7.47 or greater for FGF-23, at a two-sided 0.05 significance level. A p value of less than 0.05 was considered statistically significant.

Results

Baseline Characteristics

The study was conducted from October 2022 to April 2023, involving a total of 20 patients randomised into two groups (10 in group A and 10 in group B). The overall

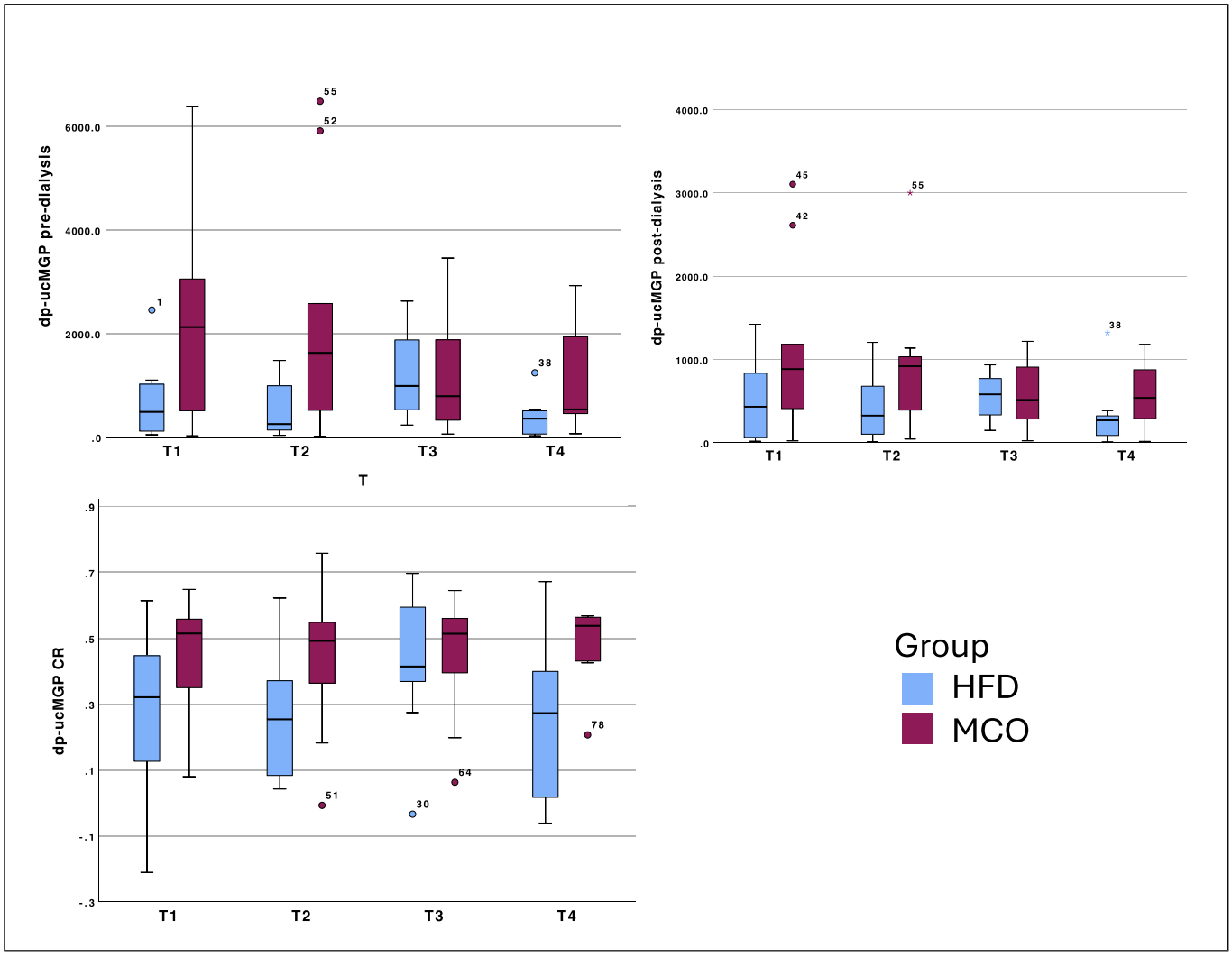

Fig. 3. Reports mean and 95% CIs of dp-ucMGP pre-dialysis and post-dialysis levels and CR stratified by treatment (HFD and MCO) at 4 time-points (T1 = baseline, T2 = after 1 week of treatment with either MCO or HFD, T3 = end of washout period, T4 = end of 1 week of treatment with either MCO or HFD).

median age of participants was 64.9 years [interquartile range 59.3–70.8], with a predominance of male gender (75%) and white ethnicity (90%). Baseline characteristics of the study population are presented in Table 1.

Medium-Cut-Off Membrane versus High-Flux Dialysis Membrane

The use of both membranes resulted in a significant reduction in FGF-23 serum levels (MCO: pre-dialysis, 237.4 [94.0–617.9] vs. post-dialysis 195.3 [86.3–341.5], $p < 0.001$; HFD: pre-dialysis, 248.3 [97.6–767.6] pmol/L vs. post-dialysis, 214.7 [99.2–451.4] pmol/L, $p < 0.001$). There was no significant difference in the CR of FGF-23 when using the MCO membrane compared to the HFD membrane (0.31 ± 0.26 vs. 0.23 ± 0.18, respectively, $p = 0.242$) (Table 2). The RA of FGF-23 prior to dialysis sessions was not statistically different when using MCO or HFD membrane (0.50 [0.25–1.06] vs. 0.23 [−0.12–0.89], respectively, $p = 0.071$) (Fig. 2).

A similar trend was observed with dp-ucMGP (MCO: pre-dialysis, 691.1 [302.9–1,628.5] vs. post-dialysis 419.7 [252.8–770.9], $p < 0.001$; HFD: pre-dialysis, 859.3 [174.4–2022.7] pmol/L vs. post-dialysis, 504.2 [139.3–988.1 pmol/L, $p < 0.001$). The CR was comparable between the two membranes (MCO: 0.33 ± 0.25 vs. HFD: 0.33 ± 0.23], $p = 0.903$) (Fig. 3).

HFD membrane rather than MCO membrane was significantly effective in reducing post dialysis levels of

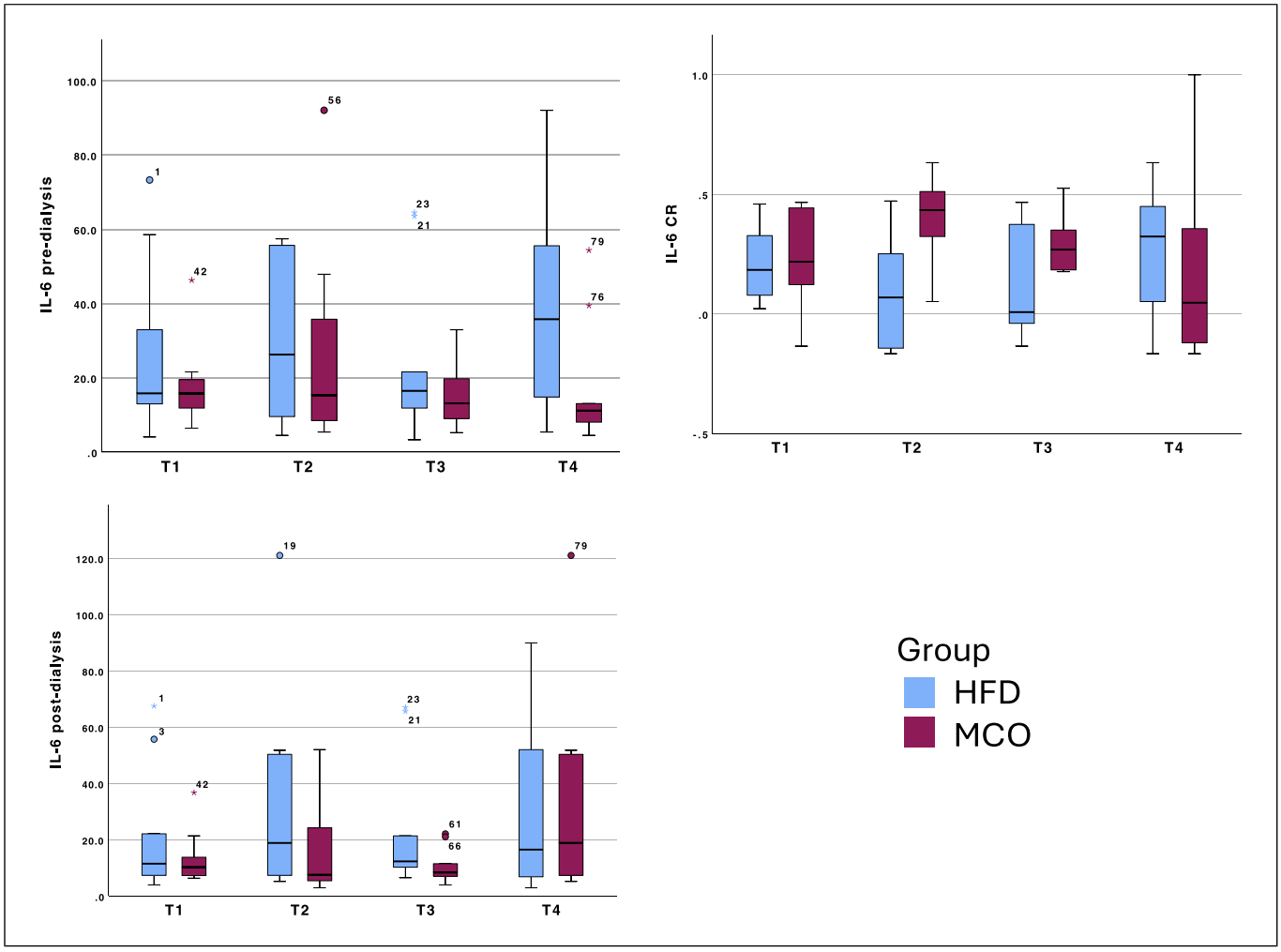

Fig. 4. Reports mean and 95% CIs of IL-6 pre-dialysis and post-dialysis levels and CR stratified by treatment (HFD and MCO) at 4 time-points (T1 = baseline, T2 = after 1 week of treatment with either MCO or HFD, T3 = end of washout period, T4 = end of 1 week of treatment with either MCO or HFD).

IL-6 and hsCRP. However, this has not resulted in difference in CR between the two membranes (Table 2): IL-6 (MCO: pre-dialysis, 12.6 [7.6–24.5] pg/mL vs. post-dialysis 12.4 [5.9–23.3] pg/mL, p = 0.064; HFD: pre-dialysis, 15.1 [9.2–25.8] pg/mL vs. post-dialysis, 11.1 [7.7–24.3] pg/mL, p = 0.037) and hsCRP (MCO: pre-dialysis, 6.2 [4.1–14.3] mg/mL vs. post-dialysis 7.2 [4.6–15.3] mg/mL, p = 0.159; HFD: pre-dialysis, 6.9 [2.9–23.2] mg/mL vs. post-dialysis, 6.1 [2.8–26.3] mg/mL, p = 0.010) (Fig.4, 5).

Neither membrane was effective in reducing levels of IL-18 post-dialysis (Table 2) (MCO: pre-dialysis, 380.7 ± 163.7 pg/mL vs. post-dialysis 403.8 ± 175.1 pg/mL, p = 0.042; HFD: pre-dialysis, 424.8 ± 159.6 pg/mL vs. post-dialysis, 414.1 ± 167.1 pg/mL, p = 0.723) (Fig. 6). Representation of means values of all the assessed biomarkers at 4 time-points (T1 = baseline, T2 = after 1 week of treatment with either MCO or HFD, T3 = end of washout period, T4 = end of 1 week of treatment with either MCO or HFD) is presented in Figures 2–6.

Intrasubject Variability

There was no significant intrasubject variability between average pre- and post-levels for each membrane for all assessed biomarkers except in pre-dialysis hsCRP levels when using HFD membrane (Table 3). To determine significance, one-way repeated measures ANOVA used for FGF-23, IL-6, hsCRP, and dp-ucMGP and the Friedman repeated measures test was used for IL-18.

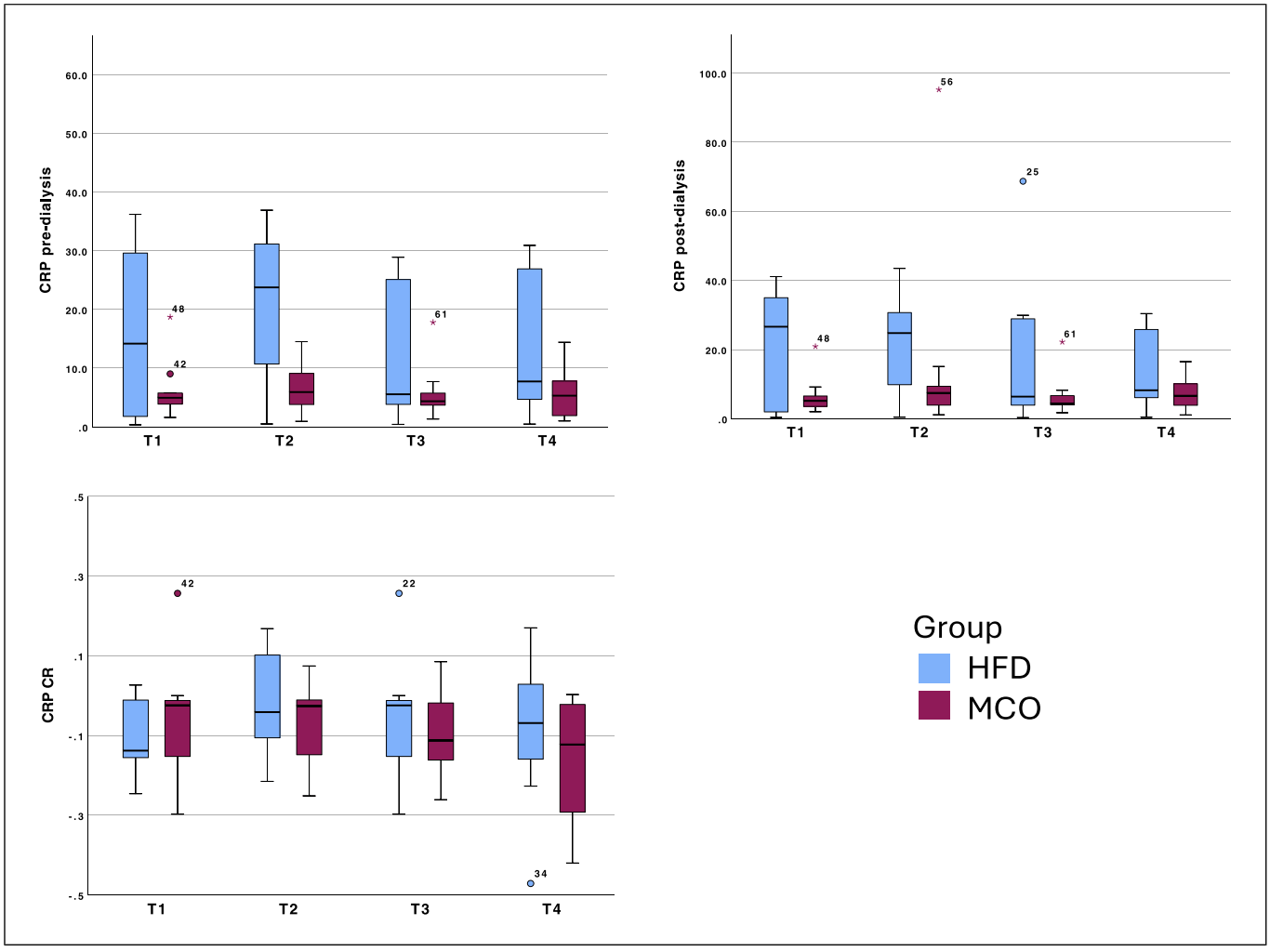

Fig. 5. Reports mean and 95% CIs of hsCRP pre-dialysis and post-dialysis levels and CR stratified by treatment (HFD and MCO) at 4 time-points (T1 = baseline, T2 = after 1 week of treatment with either MCO or HFD, T3 = end of washout period, T4 = end of 1 week of treatment with either MCO or HFD).

Discussion

This randomised crossover study demonstrated no significant difference in the CRs when using MCO membranes and HFD membranes for the assessed biomarkers: FGF-23, IL-6, IL-18, dp-ucMGP, and hsCRP. In addition, there was not a significant intra-subject variability for the mentioned biomarkers for pre- and post-dialysis levels except for pre-dialysis hsCRP levels.

The use of MCO membranes during dialysis has emerged as a potentially beneficial strategy for patients receiving regular haemodialysis. The initial demonstration of FGF-23 removal using MCO membranes was conducted by Patrier and colleagues in 2013 [21] comparing its efficacy to OL-HDF. It showed a higher reduction rate of FGF-23 using MCO membranes after a single session (55.7% vs. 36.2%, p = 0.001). However, a more recent study reported no significant difference in FGF-23 reduction rates between MCO membranes, conventional HFD membranes, and OL-HDF (55.5%, 34.6%, and 35.8%, respectively; p = 0.13). Our study findings are in line with this study and showed no significant difference in the CR of FGF-23 when comparing MCO to HFD membranes (31% vs. 23%, respectively, p = 0.242). A crossover study involving 40 patients found a greater reduction in FGF-23 levels with the MCO membrane compared to HFD the membrane (41% vs. 20%, p = 0.002) [19]. However, this study involved a dialysis duration of 3 months for each membrane type,

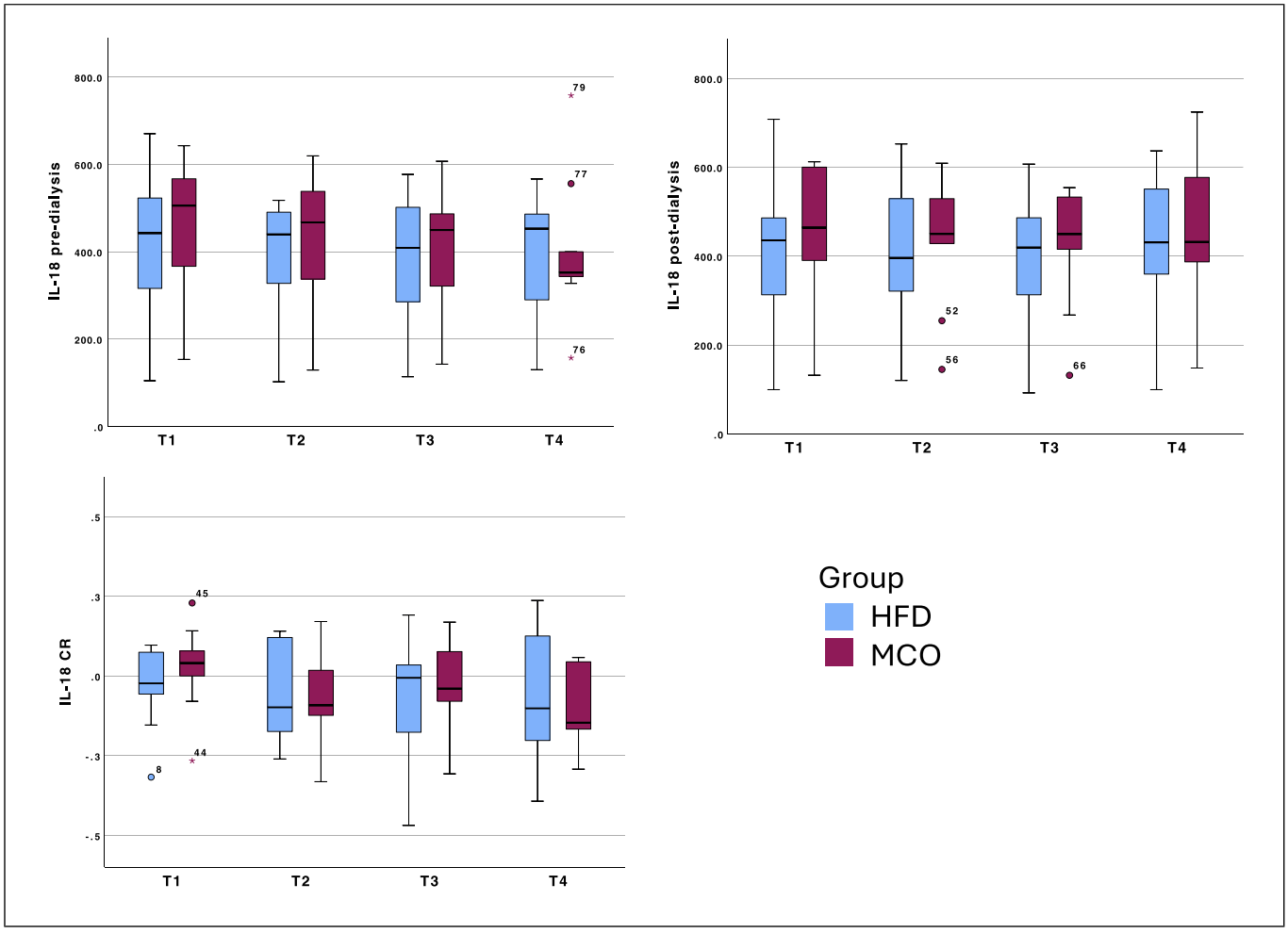

Fig. 6. Reports mean, and 95%-CIs of IL-18 pre-dialysis and post-dialysis levels and CR stratified by treatment (HFD and MCO) at 4 time-points (T1 = baseline, T2 = after 1 week of treatment with either MCO or HFD, T3 = end of washout period, T4 = end of 1 week of treatment with either MCO or HFD).

whereas our study investigated the effect of 1 week (three dialysis sessions) for each membrane.

Several studies have shown minimal or no difference in clearance of IL-6 and hsCRP when comparing different haemodialysis modalities: HFD, MCO, and OL-HDF [22–26]. Our study similarly showed no difference in IL-6 removal when using MCO membranes compared to HFD membrane (0.19 vs. 0.12, respectively, $p = 0.215$).

Our study found intrasubject stability of dp-ucMGP for the period of the study and no statistically significant difference between pre- and post-dialysis levels with both HFD and MCO membranes providing variability information for its use as a biomarker for vitamin K deficiency in dialysis patients [26]. To our knowledge, there is limited literature regarding the impact of dialysis and the intrasubject stability of this biomarker in dialysis patients. In our study, we report the use of both membranes resulted in a significant reduction in post-dialysis dp-ucMGP serum levels but with no difference in CR between the two membranes.

tOur study confirmed intrasubject stability over 3 sessions in pre- and post-dialysis values by analysis of variance for all the assessed biomarkers except for hsCRP. Our results are in keeping with a previous study examining the intrasubject stability of FGF-23 by one-way repeated measures ANOVA [20]. To our knowledge, there is no previous literature investigating the intrasubject stability of other biomarkers in dialysis settings.

We acknowledge a number of limitations. First, the study was powered for FGF-23 and not for the inflammatory cytokines or dp-ucMGP. In addition, the study

Table 3. Intrasubject variability for each dialysis membrane

Biomarker	Session 1	Session 2	Session 3	*p* value
	median [IQR]	median [IQR]	median [IQR]	
FGF-23				
Pre-dialysis MCO	246.6 [110.2–737.8]	273.7 [80.3–562.2]	233.8 [65.2–508.5]	0.623
Post-dialysis MCO	211.2 [40.9–407.2]	181.3 [46.0–338.3]	139.6 [86.5–299.8]	0.504
Pre-dialysis HFD	273.1 [64.9–394.4]	225.7 [84.8–352.2]	215.6 [62.8–398.5]	0.282
Post-dialysis HFD	161.9 [51.8–381.0]	194.5 [64.1–266.8]	163.3 [44.5–210.5]	0.338
IL-6				
Pre-dialysis MCO	15.8 [8.6–26.7]	09.9 [7.6–19.9]	14.8 [8.6–33.6]	0.291
Post-dialysis MCO	12.4 [7.0–31.2]	09.2 [6.7–19.9]	13.7 [6.2–20.4]	0.291
Pre-dialysis HFD	19.8 [9.0–32.9]	13.1 [8.5–28.8]	11.2 [8.1–24.5]	0.057
Post-dialysis HFD	21.1 [7.9–47.5]	09.4 [7.5–29.9]	12.4 [7.1–31.9]	0.125
Dp-ucMGP				
Pre-dialysis MCO	976.0 [327.6–1,880.0]	739.1 [332.1–1,852.0]	489.2 [175.9–1,242.4]	0.229
Post-dialysis MCO	560.8 [284.3–845.0]	535.0 [271.5–812.0]	299.8 [097.2–791.0]	0.114
Pre-dialysis HFD	580.6 [093.1–2455.0]	637.0 [160.1–2449.0]	518.0 [101.9–2145.0]	0.766
Post-dialysis HFD	431.5 [070.9–980.8]	424.8 [129.9–945.3]	391.1 [073.4–912.0]	0.338
hsCRP				
Pre-dialysis MCO	5.4 [3.8–09.0]	7.5 [3.7–14.6]	7.3 [3.8–14.5]	0.841
Post-dialysis MCO	5.5 [3.6–09.2]	8.3 [4.1–16.4]	7.9 [4.4–15.1]	0.949
Pre-dialysis HFD	4.4 [21–23.1]	6.9 [1.9–27.1]	7.8 [1.3–22.9]	**0.031**
Post-dialysis HFD	6.7 [2.2–26.8]	8.2 [3.5–30.2]	7.8 [2.4–20.9]	0.168
Biomarker	Session 1	Session 2	Session 3	*p* value
	mean±SD	mean±SD	mean±SD	
IL-18				
Pre-dialysis MCO	416.3±154.4	395.2±141.6	409.2±147.3	0.448
Post-dialysis MCO	418.7±153.7	421.7±148.3	434.6±156.7	0.507
Pre-dialysis HFD	406.4±133.0	401.1±136.0	381.4±124.1	0.803
Post-dialysis HFD	423.2±147.5	392.4±128.9	404.0±144.9	0.828

MCO, medium cut-off membrane; HFD, high-flux dialysis membrane; FGF-23, fibroblast growth factor 23, Dp-ucMGP, Dephosphorylated uncarboxylated matrix Gla protein; hsCRP, high sensitivity c-reactive protein; SD, standard deviation; IQR, interquartile range. Data for IL-18 were normally distributed. Hence, one-way repeated measures ANOVA was used to determine significance. Data for FGF-23, Dp-MCUGP, and hsCRP were non-normally distributed. Hence, the Friedman test was used to determine significance. Bold values denote statistical significance at the $p < 0.05$ level.

has a small sample size ($n = 20$) with relatively short observation period (1 week per intervention). Therefore, the relevant results should be considered with caution due to the exploratory nature of the research design. Second, the study did not include the analysis of the concentration of the biomarkers in the dialysate. Third, the study could not account for other factors not related to membrane clearance, which may contribute to intradialytic changes of inflammatory cytokines such as intradialytic endogenous production or redistribution phenomenon during dialysis sessions. Studies with larger sample sizes and longer follow-up periods are needed to validate our findings and to further explore the potential long-term advantages of one membrane over the other.

Conclusion

Our study indicated that the use of both MCO and HFD membranes led to a significant reduction in serum levels of FGF-23 and other inflammatory markers. However, over a short observation period, the MCO

membrane did not confer a significant advantage over the HFD membrane in reducing FGF-23 levels and other selected inflammatory cytokines. There was no significant intrasubject variability for all assessed biomarkers apart from hsCRP.

Statement of Ethics

Written informed consent was obtained for participation in this study. The study was reviewed and approved by the Health Research Authority (HRA) (REC reference: 19/NW/0638). This research was conducted in accordance with the World Medical Association Declaration of Helsinki.

Conflict of Interest Statement

Professor Sinha reports grants from AstraZeneca, Johnson & Johnson, and Amgen; consulting fees from Amicus, Stada, Santhera, Novartis, AstraZeneca, GSK, Boehringer Ingelheim, Bayer, Sanofi-Genzyme, Inozyme Pharma, and CSL Vifor; honoraria from Chiesi, Bayer, Menarini, AstraZeneca, GSK, Novartis, Sanofi-Genzyme, CSL Vifor, Boehringer Ingelheim, and Medscape; and support for attending meetings from AstraZeneca, Novartis, and CSL Vifor. Professor Sinha is the National Clinical Director for Renal Services, NHS England. Professor Kalra reports research grant support from CSL Vifor and Astellas; consulting fees from AstraZeneca, CSL Vifor, Unicyte, and UCB; honoraria from CSL Vifor, AstraZeneca, Bayer, Pharmacosmos, Medice, GSK, and Pfizer; and support to attend meetings from Pharmacosmos and Vifor. Professor Darren Green reports speaker fees from AZ, BI, Novartis, GSK, and Lilly; advisory fees from AstraZeneca and Bayer, CWA with AstraZeneca, Boehringer Ingelheim, and Novartis. Professor Schurgers reports grants from Lesaffre and Bayer; own shares in Coagulation Profile BV. No other authors have anything else to declare.

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Author Contributions

S.A.-C.: data curation, data analysis, and manuscript writing. S.S.: conceptualization, study design, and refinement of protocol. P.A.K.: conceptualization, study design, and refinement of protocol. D.E.: patient recruitment and sample organisation. D.G.: conceptualization and study design. L.S.: analysis of samples. D.P.: conceptualization, study design, refinement of protocol, supervision, and consent. All authors have reviewed and approved the final manuscript.

Data Availability Statement

The data that support the findings of this study are not publicly available due to privacy reasons but are available from the corresponding author upon reasonable request.

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