Original Paper

Inflammatory and Angiogenic Factors Linked to Longitudinal Microvascular Changes in Hemodialysis Patients Irrespective of Treatment Dose Intensity

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Key Words

Hemodialysis • Endothelium • IL-8 • TNF- α

Abstract

Background: Cardiovascular disease is a major contributor to the poor outcomes observed in hemodialysis. We investigated the relationship between hemodialysis intensity and vascular parameters in high-dose (HDHD; >12hrs/week) and Conventional (CHD; $\leq$ 12hrs/week) hemodialysis intensity over a 6-month period. **Methods:** We present the 6-month longitudinal analysis of a 2-year multicenter study investigating the effects of HDHD on cardiovascular parameters. We used pulse wave velocity, 24hr ambulatory blood pressure and sublingual dark field capillaroscopy measurements to assess macro- and microcirculation on 6-monthly basis. Pro-inflammatory and endothelial biomarkers were also measured at 6-monthly intervals. **Results:** 47 participants (21 HDHD, 26 CHD) were studied. CHD were older (63.5 ± 14.2 vs 53.7 ± 12.6 yr; $p=0.018$), with shorter dialysis vintage (median 23 vs 61 months; $p=0.001$). There was considerable variability in the degree and direction of change of circulatory measurements over a 6-month period. Hemodialysis intensity (hrs/week) did not correlate to these changes, when adjusted for age, dialysis vintage and comorbidity. Higher levels of Interleukin (IL)-8

measured at baseline independently predicted an increase in the Perfused Boundary Region (5-25µm) of the endothelial glycocalyx ($p=0.010$) whilst higher levels of soluble Flt-1 had a significant inverse effect ($p=0.002$) in an adjusted linear model. **Conclusion:** Hemodialysis intensity did not predict changes in either macro- or microvascular parameters. Inflammation mediated through the IL-8 pathway predicted microvascular injury while Flt-1, a potential marker of angiogenesis and endothelial repair, might have a significant protective role. Further understanding of these pathways will be necessary to improve dialysis outcomes.

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Introduction

The advent of hemodialysis (HD) has provided a lifeline for more than 2 million survivors of end-stage renal disease worldwide [1]. However, despite increased uptake and technological advances, long-term survival remains worse than that of some major cancers [2]. Although equipment, membrane efficiency and HD techniques have evolved with time [3], the prescribed frequency and duration have remained the same since the 1970s [4–6]. Cardiovascular (CV) disease has been one of the main contributors to mortality and morbidity in advanced chronic kidney disease (CKD) [7]. Although a large component of the observed increased CV risk in CKD can be attributed to co-existing comorbidity such as the high prevalence of diabetes in this group, there is an emerging body of evidence that uremia itself is a pro-atherogenic state [8, 9]. The link between accelerated atherosclerosis and micro-inflammation has driven research in this area [10]. Uremia as a state of oxidative stress and impaired angiogenesis has been considered a promoter of micro-inflammation and endothelial dysfunction [11–13]. Micro- and macrocirculation injuries are believed to be distinct in their pathophysiology [14] however, the impact of inflammation on these processes in HD patients has not been studied in detail. The fact that a number of mediators implicated in inflammation and vascular dysfunction are also uremic retention products [15] could suggest their elimination from the circulation might improve outcomes. Studies of outcomes in high-dose HD (HDHD; >12hrs HD/week) have linked these type of prescriptions to a CV benefit, compared to conventional 4hr, 3 times per week HD regimes (CHD) [16–19]. However, whether this benefit is due to improved uremic toxin removal or purely due to better fluid management is yet to be established. Our prospective analysis of longitudinal data from the first 6-months of follow-up of participants in the study of Uremic Toxins, Cardiovascular Effects and Physical Activity in Intensive Hemodialysis (INTHEMO) aims to investigate the effect of HD intensity on micro- and macrocirculation over this period. Additionally, the relation between vascular and inflammatory biomarkers and changes in micro- and macrocirculatory parameters was assessed.

Materials and Methods

INTHEMO is a 2yr prospective multicenter international longitudinal study investigating the effects of HDHD on uremic toxin clearance, CV parameters and physical activity. HDHD was defined as >12hr of HD/week. The participating units include Maastricht University Hospital and Heerlen Dialysis Unit in the Netherlands, Central Manchester University Hospitals NHS Foundation Trust in the UK and the Hasselt Dialysis Unit in Belgium. The study received favorable ethical opinion from the Greater Manchester Research Ethics Committee (14/NW/1158) for centers in the UK, the Medical Ethics Committees of the Maastricht University Medical Centre and Jessa General Hospital (NL35039.068.10) for centers in the Netherlands and Belgium. All adult patients receiving their treatment at the above units were able to participate in the study. Patients that were planned for transplantation or for modality change within the next 12 months were excluded. Informed written consent was obtained prior to participation to the study. This analysis focuses on the baseline and 6-month follow-up measurements of the study participants.

Data Collection

The past medical history and medication lists were collected directly from participants and through their electronic medical records. These were used to calculate the Davies' Comorbidity Score [20] for all participants. The HD prescription including frequency and duration of dialysis treatment and dialysis adequacy in the form of urea clearance, expressed in standard Kt/V, were also recorded.

Study Procedures

Study measurements were performed at a single visit that either took place during the participants' attendance for their routine dialysis treatment (in-center HD patients) or during attendance at clinic (home HD patients). Participants receiving CHD (up to 4hrs, 3 times a week) had these measurements performed before their mid-week dialysis session. Follow-up measurements were performed at 6 monthly intervals for the duration of the study. All measurements were performed by 3 trained operators, 1 for the Manchester Centre and 2 for the centers in the Netherlands and Belgium.

Fluid Status Assessment

Hydration status was assessed using multifrequency bioimpedance measurements with the use of the BCM device (Fresenius, Germany). This provided measurements of Total Body Water, Extracellular (ECW), Intracellular Water and absolute overhydration index (OH). Overhydration was expressed as absolute OH or relative overhydration (OH/ECW) [21–23].

Cardiovascular Measurements

Macrocirculation was assessed by means of Pulse Wave Velocity (PWV) and blood pressure (BP) measured during the research visit and by means of 24hr Ambulatory BP Monitoring (ABPM) measurement. Microcirculation was assessed by means of dark-field sublingual capillaroscopy (DFSC). Cardiac function was assessed by echocardiography at baseline and on an annual basis for the duration of the study.

Pulse Wave Velocity

PWV was measured by means of carotid/femoral applanation tonometry using the SphygmoCor system (AtCor Medical, Australia).

24hr Ambulatory Blood Pressure Monitoring

24hr ABPM was performed using an oscillometric technique and the Mobil-O-Graph NG device (I.E.M GmbH, Germany). Measurements of BP were obtained at 15-minute intervals during the day and at 30-minute intervals during the night. Readings were analyzed using the hypertension management software of the Mobil-O-Graph NG device. Incomplete measurements (not completing the 24hr period) were included for analysis provided the data were spread across the day.

Capillaroscopy

DFSC was used to measure microvascular endothelial injury. This was performed by assessing the thickness of the Perfused Boundary Region (PBR) of the endothelial glycocalyx and red blood cell (RBC) width using Sidestream Dark Field Imaging (GlycoCheck, Netherlands). The software records an adequate sample of high quality video frames of microvasculature, identifies all available microvessels and defines small vascular segments every 10 μ m along their length. For each vascular segment 840 radial intensity profiles are obtained, which are tested for the presence of RBCs and signal quality, and then the RBC column widths (RBCW) are determined from these intensity profiles. The median RBCW and the outer edge of the RBC perfused lumen (Dperf) are determined from these measurements. The distance of the median RBCW value to the position of the outer edge of the RBC perfused lumen is defined as the PBR [$PBR = (Dperf - RBCW)/2$]. This methodology has been validated in both animal and human models [24–28] and the software has been used in a number of studies [29–32]. Both PBR and RBCW measurements have been linked with microvascular injury in CKD [24, 33].

Echocardiography

Left ventricular Ejection Fraction and Left Ventricular Mass Index were measured annually using echocardiography. This was performed by the local cardiorespiratory investigation services of the participating centers.

Blood Sampling and Residual Renal Function Assessment

All participants at the time of the research visit had their renal function, serum electrolyte concentration, urate, parathyroid hormone and β 2-microglobulin level measured by mean of venous blood sampling. Residual renal function was assessed by means of a 24-72hr urine collection. This was performed during the interdialytic period. The volume and protein concentration was measured. Residual renal function was defined as urine volume < 100ml/day. All blood measurements were repeated at 6-monthly intervals through the duration of the study.

At baseline a panel of vascular biology and pro-inflammatory biomarkers (Table 1) was also measured. Biomarker analysis was performed using an electro-chemiluminescence detection system in single and multi-arrays (MesoScale Discovery SECTOR Imager 2400, Gaithersburg, Maryland, USA). All measurements were performed in duplicate. The inter- and intra-assay coefficients of variation (CVs) for all markers were <9%, with the exception of the inter-assay CVs for bFGF (10.6%) and IL-6 (16%). The biomarker levels for the CHD and HDHD groups were also compared to those collected from 20 healthy control subjects in Maastricht (Research Ethics Reference: NL 33129.068.10).

Statistical Analysis

The cohort characteristics and measurements were explored using descriptive epidemiology. Categorical variables were reported as percentages and ratios. Continuous variables were reported as mean and standard deviation where distribution was normal and as median with values of minimum and maximum when distribution was skewed. Normality of distribution was assessed using the Shapiro-Wilk method. Comparison of variables between subgroups was performed using Pearson's χ^2 test for categorical variables, independent sample t-test and one-way ANOVA for continuous variables with normal distribution and Mann-Whitney and Kruskal-Wallis tests for continuous variables with skewed distribution. Post-Hoc analysis was performed using Tukey's Test for parametric analysis and Pairwise Comparison with Bonferroni's correction for non-parametric analysis. Longitudinal analysis for CV measurement outcomes at 6 months in relation to dialysis regime at baseline were assessed using intention-to-treat methodology and linear regression models adjusted for age, comorbidity and dialysis vintage at baseline, the CV measurement itself at baseline and the change in OH/ECW between the 2 time points. The association between CV measurement outcomes at 6 months and vascular and pro-inflammatory biomarker levels at baseline were assessed using linear regression models adjusted for age, comorbidity, dialysis vintage and the weekly number of HD treatment hours of at baseline, the CV measurement itself at baseline and the change in OH/ECW between the 2 time points. Outcome variables with skewed distribution underwent logarithmic transformation to normalize distribution and log-variables were used in the models. Biomarkers that showed a significant predictive value in univariate adjusted linear models were added to a multivariate adjusted model in a stepwise manner. Model fit was assessed using R^2 values. Statistical analysis was performed using SPSS 23 (IBM, USA).

Results

Seventy-two participants enrolled in the study. Twenty participants had to prematurely exit the study before the 6-month follow-up, while 5 missed the 6-month visit (Fig. 1). The commonest cause for early exit from the study was transplantation (57.1%) while illness at the time of follow-up was the commonest cause for missing a visit (80%). Two participants (one from each cohort) died during the 6-month period (9.5%). Of the transplanted patients 9 (75%) were receiving HDHD and 3 (37.5%) CHD (Supplementary Material- Table 1). For all supplemental material see www.karger.com/doi/10.1159/000485048.

Of the 47 participants studied at 6 months, 21 were performing HDHD and 26 CHD (Demographic and HD characteristics in Table 2). CHD participants were older (63.5 ± 14.2 vs 53.7 ± 12.6 yr; $p=0.018$), with fewer months on dialysis (median 23, max-min 6-106 vs 61, max-min 10-432; $p=0.001$) and better-preserved residual renal function (61.5% vs 9.5% ; $p<0.001$). Biomarker levels between HDHD and CHD participants were comparable. However, when compared to those from 20 healthy control subjects the biomarker profiles of both HD groups were significantly deranged (Table 3).

Baseline and 6-months CV and hydration measurements are presented in Table 4. PWV increased by an average of $10.6 \pm 31.4\%$ over the follow-up period but the increase was comparable for both HDHD and CHD cohorts.

Fig. 1. Flow-sheet of patient enrolment and 6-month follow-up in the INTHEMO study.

Table 1. Vascular and Pro-inflammatory Biomarkers Tested in the study. kDa= kilo-Dalton, ml= milliliter, ng= nanogram, pg= picogram, µg= microgram

Biomarker	Molecular Weight
Intracellular Adhesion Molecule-1 (ICAM-1) (ng/ml)	75-115 kDa
Vascular Cell Adhesion Molecule-1 (VCAM-1) (ng/ml)	100-110 kDa
E-selectin (ng/ml)	107-115 kDa
P-selectin (ng/ml)	86 kDa
Intracellular Adhesion Molecule-3 (ICAM-3) (ng/ml)	95 kDa
Thrombomodulin (ng/ml)	74 kDa
Matrix Metalloproteinase (MMP)-1 (ng/ml)	52 kDa
MMP-3 (ng/ml)	54 kDa
MMP-9 (ng/ml)	82-92kDa
C- Reactive Protein (CRP) (µg/ml)	110-144 kDa
Soluble Amyloid A (SAA) (µg/ml)	12 kDa
IL-6 (pg/ml)	21-28 kDa
IL-8 (pg/ml)	11 kDa
Tumor Necrosis Factor-alpha (TNF-α) (pg/ml)	17 kDa
Basic Fibroblast Growth Factor (bFGF) (pg/ml)	22-24kDa
Placenta Growth Factor (PIGF) (pg/ml)	30-45 kDa
Soluble fms-like tyrosine kinase-1 (Flt-1) (pg/ml)	100 kDa
Vascular Endothelial Growth Factor (VEGF) (pg/ml)	38 kDa

Fig. 2. Distribution of increase and decrease in circulatory measurements over 6-months follow-up period.

Changes to DFSC measurements were also similar between the 2 cohorts (Supplementary Materials- Table 2). Although the average percentage change for these measurements was <5%, there was a considerable variability in the degree and direction of change between individuals (Fig. 2).

The association between HD intensity, expressed as hours of HD per week, and the change in CV measurements was assessed through linear regression. HD intensity status at baseline did not predict any of the CV measurements at 6 months, adjusted for age, comorbidity, dialysis vintage and the CV measurement at baseline. This persisted when change in OH/ECW was added to the models (Table 5).

The associations between baseline levels of individual vascular endothelial and pro-inflammatory biomarkers with CV measurements at 6 months, adjusted for baseline value of the assessed CV measurement, were evaluated using linear regression. The model was also adjusted for age, dialysis vintage, comorbidity and the number of hours of prescribed HD/week at baseline and the change in OH/ECW over 6 months. Univariate analysis is shown in Supplementary Tables 2-6. There was no correlation between biomarker levels and change in PWV measurements over 6 months.

A number of biomarkers correlated to changes in PBR measurements. TNF- α showed a significant association with increased PBR (5-25mcg) thickness (β = 0.336, 95% CI 0.044 to 0.915, p =0.032) and sFlt-1 showed an inverse negative association with the same measurement (β = -0.366, 95% CI -0.465 to -0.061, p = 0.012). The association of sFlt-1 with PBR did not change when adjusted for PIGF and VEGF levels (β = -0.368, 95% CI -0.474 to -0.056, p = 0.015). Other biomarkers showed weaker associations that were only significant with increases in PBR thickness in specific capillary size ranges (CRP with PBR 5-9mcg: β = 0.329, 95% CI 0.020 to 0.779, p = 0.040; IL8 with PBR 10-19mcg: β = 0.346, 95% CI 0.159 to 1.786, p = 0.020). Thrombomodulin was associated with increase in the RBCW (β = 0.452, 95% CI 0.235 to 1.438, p = 0.008).

Baseline CRP, IL-8, TNF- α , Thrombomodulin and sFlt-1 levels were added to a stepwise multivariate linear regression model for prediction of PBR (5-25mcg) at 6 months adjusted

Table 2. Cohort Demographic and Dialysis Profiles Stratified by Dialysis Dose. Categorical variables are presented as frequencies and percentages. Continuous variables with normal distribution are presented as mean and standard deviation (SD) and variables with skewed distribution as median and minimum-maximum values. Sig= Statistical Significance (p-value). * Highlights Result with statistical significance at the level of $p < 0.05$. AV= Arteriovenous, Kg= Kilogram, L= Litre, mg= milligram, pmol= picomol

Baseline Characteristics		Entire Cohort (n=47)	HDHD (n=21)	CHD (n=26)	Sig
Age (yr)		59.1 (SD 14.2)	53.7 (SD 12.6)	63.5 (SD 14.2)	0.018*
Gender: Male		36 (76.6%)	15 (71.4%)	21 (70.8%)	0.452
Ethnicity	White	40 (85.1%)	18 (85.7%)	22 (84.6%)	0.236
	Black	3 (6.4%)	0	3 (11.5%)	
	Asian	3 (6.4%)	2 (9.5%)	1 (3.8%)	
	Other	1 (2.1%)	1 (4.8%)	0	
Weight (kg)		80.5 (SD 15.3)	81.0 (SD 16.5)	80.1 (SD 14.6)	0.853
BMI (Kg/m ²)		27.1 (17.4-44.6)	27.1 (20.2-44.6)	27.5 (17.4-37.8)	0.974
Dialysis Vintage (months)		36 (6-432)	61 (10-432)	23 (6-106)	0.001*
Previous Transplant		19 (40.4%)	11 (52.4%)	8 (30.8%)	0.133
Residual renal function		18 (38.3%)	2 (9.5%)	16 (61.5%)	<0.001*
Diabetes		15 (31.9%)	7 (33.3%)	8 (30.8%)	0.851
Cardiovascular Disease		17 (36.2%)	5 (23.8%)	12 (46.2%)	0.113
Smoking		6 (12.8%)	1 (4.8%)	5 (19.2%)	0.139
Davies Comorbidity Score		1 (0-4)	1 (0-4)	1 (0-4)	0.144
Number of medication		11.5 (SD 4.30)	10.2 (SD 4.34)	12.5 (SD 4.04)	0.062
Echocardiography	LV EF (%) (n=15:25)	57.7 (SD 15.0)	54.93 (SD 17.8)	59.40 (SD 13.1)	0.368
	LVMi (Kg/m ²) (n=14:22)	102.5 (62-248)	120.0 (88-248)	89.5 (62-182)	0.005*
	Hrs HD/week	12.0 (10.5-32)	17.5 (13.5-32.0)	12.0 (10.5-12.0)	<0.001*
HD prescription	Frequency of HD/week	3 (3-4)	3.5 (3-4)	3 (3.0-3.5)	<0.001*
	Duration of HD session	4 (3-9)	5 (4-9)	4 (3-4)	<0.001*
	Standard Kt/V	2.38 (SD 0.40)	2.63 (SD 0.41)	2.15 (SD 0.20)	<0.001*
Vascular Access	AV Fistula/Graft	44 (93.6%)	20 (95.2%)	24 (92.3%)	0.683
	Venous Catheter	3 (6.4%)	1 (4.8%)	2 (7.7%)	
B2-microglobulin (mg/L)		24.1 (SD 7.8)	26.4 (SD 6.3)	22.4 (SD 8.5)	0.092
Parathyroid Hormone (pmol/L)		26.6 (0.6-93.8)	19.9 (0.6-93.8)	28.8 (2.4-69.7)	0.160

for age, dialysis vintage and comorbidity at baseline, the number of hours of prescribed HD/week and changes in OH/ECW. IL-8 showed a significant positive association ($\beta = 0.367$, 95% CI 0.273 to 1.821; $p = 0.010$) and sFlt-1 a negative association ($\beta = -0.422$, 95% CI -0.491 to -0.115; $p = 0.002$) with change in PBR (5-25mcg), in the best-fit model ($R^2 = 0.474$). CRP, Thrombomodulin and TNF- α were excluded from the final model.

Discussion

Our study did not show a significant association between HD intensity and changes in the macro- and microcirculation over the 6-month period. A number of pro-inflammatory and endothelial biomarkers predicted microcirculatory but not macrocirculatory changes in HD patients over a 6-months period. Thrombomodulin showed an association with increased RBCW, while high IL-8 was a significant predictor of increase in PBR (5-25mcg) thickness. On the other hand, sFlt-1 showed a potentially protective effect on the microcirculation and was linked with a reduction in PBR (5-25mcg) thickness. Thus, irrespective of HD intensity micro-inflammation and angiogenesis are likely to be important predictors of vascular and, in particular, microvascular injury in HD.

The PBR of the endothelial glycocalyx visualized by DFSC has been identified as a marker of microvascular injury in CKD [24, 25] and increase to its thickness and RBCW is

Table 3. Vascular Endothelium and Pro-inflammatory Biomarker Profiles of High-dose and Conventional dialysis Participants in comparison to Healthy Controls. Continuous variables with normal distribution are presented as mean and standard deviation (SD) and variables with skewed distribution as median and minimum-maximum values. Group comparison was performed using One-way ANOVA for normally distributed variables and Kruskal-Wallis test for non-normally distributed ones. Post-Hoc analysis for parametric tests was performed using Tukey's and for non-parametric pairwise comparison with Bonferroni's correction. Sig= Statistical Significance (p-value). * Highlights Result with statistical significance at the level of p<0.05. ^a Indicates difference from the HDHD group, ^b indicates difference from the CHD group and ^c indicates difference from the health control group at post-hoc analysis

Baseline Biomarkers Profiles	Entire HD Cohort (n=47)	HDHD (n=21)	CHD (n=26)	Healthy Controls (n=20)	Sig
ICAM 1 (ng/ml)	418.0 (259-718)	385.0 (301-718) ^c	429.0 (259-620) ^c	339.0 (244-503)	0.027*
VCAM 1 (ng/ml)	1019 (543-2040)	1101 (690-1870) ^c	985 (543-2040) ^c	488.0 (386-583)	<0.001*
MMP 1 (ng/ml)	31 (2-105)	31 (8-84)	29 (2-105)	20 (6-56)	0.144
MMP 3 (ng/ml)	49.0 (11-255)	52.0 (11-133) ^c	47.5 (13-255) ^c	17.5 (8.0-35.0)	<0.001*
MMP 9 (ng/ml)	107 (22-348)	87 (22-169)	113.5 (26-348)	122 (43-261)	0.057
E-Selectin (ng/ml)	11.0 (3.5-28.6)	11.4 (6.9-20.5) ^c	10.4 (3.5-28.6) ^c	8.8 (4.4-11.9)	0.008*
P-Selectin (ng/ml)	41.8 (13.7-101.6)	41.6 (13.7-70.4)	42.9 (21.7-101.6)	41.3 (20.5-76.7)	0.546
ICAM 3 (ng/ml)	1.0 (0.2-4.9)	1.0 (0.7-2.9) ^c	0.9 (0.2-4.9)	0.9 (0.5-1.1)	0.028*
Thrombomodulin (ng/ml)	12.7 (SD 2.8)	13.7 (SD 2.8) ^{b,c}	11.9 (SD 2.6) ^{a,c}	2.7 (SD 0.5)	<0.001*
CRP (µg/ml)	3.5 (0.5-86.9)	2.8 (0.5-86.9) ^c	3.6 (0.6-68.1) ^c	1.1 (0.2-13.1)	<0.001*
SAA (µg/ml)	8.6 (0.7-210.3)	9.2 (0.7-210.3) ^c	8.3 (0.7-165.4) ^c	2.3 (0.7-19.6)	0.001*
IL-6 (pg/ml)	1.8 (0.8-131.5)	1.2 (0.9-131.5) ^c	2.0 (0.8-7.8) ^c	0.6 (0.1-10.4)	<0.001*
IL-8 (pg/ml)	15.4 (3.5-92.3)	14.1 (3.5-62.0)	15.6 (4.5-92.3)	12.7 (5.7-34.0)	0.243
TNF-α (pg/ml)	6.0 (4.2-15.5)	6.4 (5.0-15.5) ^c	5.8 (4.2-9.3) ^c	2.1 (0.9-3.6)	<0.001*
bFGF (pg/ml)	5.0 (0-28)	6.0 (0-17)	4.5 (0-28)	5.0 (3-23)	0.233
PIGF (pg/ml)	25.0 (14-55)	28.0 (14-55) ^c	25.0 (17-45) ^c	19.0 (13-28)	0.001*
Flt1 (pg/ml)	256 (139-1434)	256 (139-319)	256 (155-1434)	246 (170-303)	0.436
VEGF (pg/ml)	759.9 (SD 352.0)	762.5 (SD 266.0) ^c	757.7 (SD 414.0) ^c	533.2 (SD 260.0)	0.043*
PIGF/Flt-1 ratio	0.11 (SD 0.04)	0.11 (SD 0.04) ^c	0.10 (SD 0.03)	0.08 (SD 0.02)	0.019*
VEGF/Flt-1 ratio	2.88 (SD 1.24)	2.95 (SD 0.84) ^c	2.82 (SD 1.50) ^c	2.11 (SD 0.89)	0.047*

a phenomenon that has been observed in states of inflammation and oxidative stress such as in critically ill intensive care patients and patients with septic shock [27, 29, 32]. The relation between serum-soluble thrombomodulin at baseline and changes to the endothelial glycocalyx over the 6-months period, assessed by DFSC, may confirm this association. Thrombomodulin, in addition to being a uremic retention product, is also an endothelial membrane glycoprotein, cleaved and released into the circulation during glycocalyx injury [34, 35].

Also inflammation, as indicated by the association of high CRP, TNF-α and IL-8 levels at baseline, was associated with increasing PBR thickness in our study population. CRP, an acute phase inflammatory mediator [36] has been extensively studied in the context of

Table 4. Cardiovascular and Hydration Measurements for the High-dose and Conventional Hemodialysis Cohorts at Baseline and 6 months of follow-up. Continuous variables with normal distribution are presented as means and standard deviations (SD) and variables with skewed distribution as medians and minimum-maximum values. Group comparison was performed using independent sample t-test for normally distributed variables and Mann-Whitney test for non-normally distributed ones. Sig= Statistical Significance (p-value). * Highlights Result with statistical significance at the level of p<0.05

		Baseline Measurements			6-Month Measurements		
		HDHD (n=21)	CHD (n=26)	Sig	HDHD (n=21)	CHD (n=26)	Sig
Visit Blood Pressure (mmHg)	Systolic	137.1 (SD 34.94)	141.9 (SD 28.80)	0.608	143.6 (SD 29.27)	141.0 (SD 32.60)	0.779
	Diastolic	73.3 (SD 15.63)	73.4 (SD 14.58)	0.982	75.1 (SD 13.45)	75.4 (SD 15.09)	0.901
	Mean Arterial Pressure	115.8 (SD 27.74)	119.0 (SD 22.24)	0.659	98.0 (SD 17.40)	96.8 (SD 19.05)	0.823
PWV		8.7 (SD 2.74)	10.5 (SD 2.44) (n=22)	0.025*	9.5 (SD 2.79) (n=20)	11.6 (SD 3.57) (n=23)	0.041*
24hr Ambulatory Blood Pressure Monitoring (mmHg)	n	19	26		14	19	
	Systolic	136.7 (SD 29.9)	128.2 (SD 23.2)	0.287	130.9 (SD 29.99)	127 (SD 17.68)	0.687
	Diastolic	83.0 (SD 17.6)	77.7 (SD 16.2)	0.298	78.0 (SD 17.61)	73.3 (SD 10.63)	0.349
	Mean Arterial Pressure	100.9 (SD 20.8)	94.5 (SD 16.8)	0.261	95.6 (SD 21.44)	91.37 (SD 11.94)	0.473
DFSC	n	20	25				
	PBR 5-25	1.85 (SD 0.24)	1.99 (SD 0.28)	0.085	1.94 (SD 0.32)	1.92 (SD 0.35)	0.858
	PBR 5-9	0.92 (0.79-1.11)	1.03 (0.88-1.37)	0.079	0.98 (SD 0.12)	0.99 (SD 0.11)	0.644
	PBR 10-19	2.02 (SD 0.23)	2.13 (SD 0.29)	0.194	2.14 (SD 0.34)	2.07 (SD 0.37)	0.540
	PBR 20-25	2.36 (SD 0.52)	2.57 (SD 0.44)	0.151	2.42 (SD 0.57)	2.47 (SD 0.60)	0.762
	RBCW	11.04 (7.42-12.33)	10.56 (7.49-17.42)	0.749	10.60 (7.87-11.80)	10.24 (7.86-16.88)	0.315
	OH (L)	1.3 (SD 1.8)	1.4 (SD 1.8)	0.866	1.7 (-2.2-4.3)	1.1 (-0.7-5.5)	0.684
Hydration	OH/ECW (%)	7.13 (SD 9.69)	7.03 (SD 8.93)	0.972	8.30 (SD 9.39)	8.17 (SD 7.88)	0.959
	Total Body Water (L)	37.0 (SD 7.89)	37.6 (SD 6.22)	0.769	37.5 (SD 7.84)	38.1 (SD 6.32)	0.795

micro-inflammation and adverse CV [37, 38] and overall [12, 38, 39] outcomes in HD. TNF- α and IL-8 are both cytokines secreted by macrophages as part of acute phase response of inflammation [40]. Both have been linked with the micro-inflammation associated with CKD and HD [15, 41, 42]. IL-8 can also be secreted by vascular endothelial cells and is a chemokine that promotes neutrophil trans-endothelial migration [43]. Its secretion is thought to be influenced by TNF- α levels [43, 44].

We also found that sFlt-1 levels at baseline predicted an improvement in PBR (5-25 μ m) at 6 months. Flt-1 is a receptor for VEGF and PlGF and the significance of circulating levels of sFlt-1 is controversial [45]. Generally considered an inhibitor of angiogenesis and credited for maintaining tissues such as the cornea avascular [46], sFlt-1 is also considered a major player in the development of pre-eclampsia [47]. Its levels are increased significantly by

Table 5. Assessing the effect of Dialysis Intensity, Expressed as Hours of Hemodialysis per Week on Cardiovascular Measurements over a 6-months period using Linear Regression Modeling. Sig= Statistical Significance (p-value), Δ= Change (6-month measurement-baseline measurement)

Outcome Variables (month 6)		Model Adjusted for baseline measurement, age, dialysis vintage and comorbidity		+ ΔOH/ECW	
		B (95% CI)	Sig	B (95% CI)	Sig
PWV		0.042 (-0.20-0.28)	0.728	0.036 (-0.18-0.36)	0.740
24hr Ambulatory Blood Pressure Monitoring	Systolic	0.146 (-0.38-0.67)	0.572	0.073 (-0.38-0.57)	0.680
	Diastolic	0.128 (-0.36-0.72)	0.501	0.090 (-0.36-0.61)	0.598
	Mean Arterial Pressure	0.125 (-0.38-0.82)	0.524	0.086 (-0.37-0.61)	0.623
DFSC	PBR 5-25	-0.080 (-0.55-0.36)	0.669	-0.037 (-0.47-0.39)	0.835
	PBR 5-9	0.044 (-0.36-0.47)	0.808	0.052 (-0.36-0.48)	0.778
	PBR 10-19	0.048 (-0.38-0.50)	0.792	0.077 (-0.32-0.50)	0.652
	PBR 20-25	-0.222 (-0.71-0.18)	0.238	-0.164 (-0.63-0.24)	0.368
	logRBCW	-0.175 (-0.61-0.21)	0.329	-0.147 (-0.35-0.30)	0.391

heparin. This is thought to be due to displacement of the sFlt-1 heparin-binding site from heparan sulfate proteoglycans [48, 49]. However, this increase in sFlt-1 does not seem to be pathogenic [50]. In CKD sFlt-1 levels tend to be increased [51, 52] but show a blunted response to heparin compared to that of healthy controls [52]. While some animal models of CKD suggest that high levels of sFlt-1 are associated with increased CV disease [53], other show the opposite with low levels being linked with oxidative stress and endothelial dysfunction [52, 54]. The controversy regarding the role of Flt-1 in CV disease extends beyond CKD with studies showing increased levels in heart failure [55] while others demonstrating a protective role to the vasculature [56]. Our findings suggest a protective role on the microcirculation in HD patients. This is despite levels of sFlt-1 not being dissimilar to those of healthy controls. Also the effects of Flt-1 on the endothelial glycocalyx were independent from PIGF and VEGF levels. Understanding the exact implication of Flt-1 levels on the development of CV disease and its regulatory function on different CV tissue remains a work in progress.

The relation between PWV and inflammation in HD patients also remains inconclusive. Although some studies have linked these 2 processes [57, 58], others failed to confirm this [59, 60] with some suggesting that blood pressure [61] and fluid overload [62] are more important determinants of arterial stiffness. We did not find any association between baseline levels biomarker of inflammation and vascular health and changes in PWV over a 6-month period. This could be because changes to the arterial stiffness develop over a longer period and are influenced to a greater degree by factors other than inflammation [63, 64].

Our study is observational and, as such, carries some limitations. To preserve power, the performed analysis used an intention-to-treat approach and change to dialysis prescription was not censored. Although this had no effect at the 6-month follow-up point, it might influence the results of future follow-up. Although longitudinal analysis would minimize the inherent clinical selection bias that exists between HDHD and CHD, and has limited most studies comparing outcomes between these 2 groups, it is likely that early follow-up cessation for different reasons in these groups will introduce an attrition bias.

Conclusion

The study failed to show any impact of varying HD treatment dose and intensity on changes in the macro- and microvascular systems over a 6-month period. On the other hand, several inflammatory and angiogenic factors, most notably IL-8 and Flt-1 were predictive of microvascular but not macrovascular changes. The exact role of these mediators in microvascular disease in HD is yet unknown. The impact of their selective removal through enhanced blood purification warrants further investigation.

Disclosure Statement

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