Exploring the Effects of Expanded Solute Removal in Haemodialysis

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List of Abbreviations

β2M	β2–Microglobulin
A1M	Alpha 1-microglobulin
aCA	Adjusted calcium
ACS	Acute Coronary Syndrome
ADMA	Asymmetric dimethylarginine
AE	Adverse event
ANOVA	Analysis of Variance
aSV	Adjusted substitution volume
ATM	Adipose tissue mass
AVF	Arterio-venous fistula
BCM	Body Composition Monitor
Bf	Backfiltration
BFR	Blood flow rate
BIS	Bioimpedance Spectroscopy
BMI	Body mass Index
BP	Blood pressure
BSA	Body Surface Area
CD	Cluster of differentiation
CE	Conformitè Europëenne
CFS	Chalder fatigue score
CHI3L1	Chitinase-3-Like Protein 1
CKD	Chronic Kidney Disease
CKD-MBD	Chronic Kidney Disease Mineral Bone Disorder
CO_2	Carbon dioxide
CONSORT	CONsolidated Standards Of Reporting Trials
CRP	C-reactive Protein
DOPPS	Dialysis Outcomes and Practice Patterns Study
DRT	Dialysis recovery time
DSI	Dialysis Symptom Index
E/I	Extracellular to Intracellular water ratio

ECW	Extraceullular water
ED	Emergency Department
eGFR	Estimated Glomerular Filtration Rate
ELISA	Enzyme-linked immunosorbent assay
EMV	Endothelial Microvesicle
EPC	Endothelial Progenitor Cell
ESRD	End Stage Renal Disease
EUDIAL	European Dialysis Working Group
EUTox	European Uremic Toxin Group
FACS	Fluorescence Activated Cell Sorting
FCS	Foetal Calf Serum
FMD	Flow-mediated dilatation
FPSA	Fractionated plasma separation and adsorption
FTI	Fat Tissue Index
FTM	Fat tissue mass
GFR	Glomerular Filtration Rate
HADS	Hospital Anxiety and Depression Scale
Hb	Haemoglobin
HCO	High cut-off
HD	Haemodialysis
HDF	Haemodiafiltration
HDx	Haemodialysis with a Medium cut-off dialysis membrane
HFHD	High Flux Haemodialysis
HHD	Home Haemodialysis
hr	Hour
HRQoL	Health Related Quality of Life
HUVEC	Human Umbilical Vein Endothelial Cells
HvHDF	High Volume Haemodiafiltration
ICAM	Intracellular Adhesion Molecule
ICW	Intracellular water
IDEAL	Initiating Dialysis Early and Late
IDH	Intradialytic Hypotension

IDPN	Intradialytic Parenteral Nutrition
IIEF	International Index of Erectile Function
IIR	Investigator Initiated Research
IL	Interleukin
IPOS	Integrated Palliative Care Outcome Scale
IS	Indoxylsulfate
kDa	Kilodalton
kg	Kilogram
kHz	Kilohertz
Kt/V	K = dialyser clearance of urea, t = time,, V = volume of Distrubution or urea
Kuf	Ultrafiltration coefficient
LDL	Low-density lipoproteins
LOCF	Last observation carried forward
LTI	Leant Tissue Index
LTM	Lean tissue mass
MID	Minimal important difference
MCO	Medium cut-off
min	Minute
ml	mililitres
mmHg	Millimetre of mercury
MoDal	A Randomised Pilot Study Investigating the Effect of Medium Cut-Off Haemodialysis On Markers of Vascular Health Compared With On-Line Haemodiafiltration
MV	Microvesicle
MW	Molecular Weight
MWCO	Molecular weight cut-off
MWRO	Molecular weight retention onset
NCDS	National Cooperative Dialysis Study
ng	Nanogram
NHS	National Health Service
nm	Nanometre

NO	Nitric Oxide
OH	Overhydration
OLHDF	On-line Haemodiafiltration
OMERACT	Outcome Measures in Rheumatology
PD	Peritoneal Dialysis
PE	Phycoerythrin
PECAM	Platelet Endothelial Cell Adhesion Molecule
PES	Polyethersulfone
PEW	Protein Energy Wasting
PFP	Platelet-free Plasma
pg	Picogram
pmp	Per million population
PO4	Phosphate
POS-S	Palliative Care Outcome Scale
PPP	Platelet poor plasma
PREM	Patient Reported Experience Measure
PROM	Patient Reported Outome Measure
PTH	Parathyroid Hormone
PTX-3	Pentraxin-3
PVP	Polyvinylpyrrolidone
PWV	Pulse Wave Velocity
Q	Quartile
RCT	Radomised Controlled Study
REC	Research Ethics Comitee
Rel OH	Relative overhydration
RKF	Residual Kidney Function
RRT	Renal Replacement Therapy
SAE	Serious advert event
SC	Sieving Coefficient
SD	Standard Deviation
SDMA	Symmetric dimethylarginine
SF-36	36-Item short form health survey

SONG	Standardised Outcomes in Nephrology
sp	Single pool
TBW	Total Body Water
TNF-a	Tumor Necrosis Factor alpha
Uf	Ultrafiltration
UK	United Kingdom
US	United States
VCAM	Vascular Cell Adhesion Molecule
VEGF	Vascular endothelial Growth Factor
VEGFR	Vascular endothelial Growth Factor Receptor
VLDL	Very-low density lipoproteins
vWF	Von Willebrand factor
WHO	World Health Organisation
*μ*g	Microgram
*μ*m	Micrometre

Abstract

Background: Patients on dialysis treatment have a significantly increased risk of cardiovascular disease. Inflammation and cardiovascular disease, characterised by endothelial dysfunction, are intimately linked in this patient group. Medium Cut-Off (MCO) Haemodialysis (HDx) provides improved clearance of larger middle molecules (up to 45kda) compared with high-flux haemodialysis. Expanded solute removal, through HDx could be biologically significant in modifying endothelial function and cardiovascular risk. This thesis focuses on research investigating the effects of HDx treatment on markers of endothelial health, inflammation and patient-reported outcome measures.

Methods: 64 patients on haemodiafiltration (HDF) treatment were recruited and randomised in a prospective open-label randomised controlled trial (RCT) with patients either continuing on HDF or switching to HDx. Outcome measures included changes in a large panel of biomarkers including EMV, inflammatory cytokines and larger middle molecules. Body composition monitoring, patient-reported outcome measures (POS-S-Renal, Chalder Fatigue Score, Dialysis Recovery Time) and pulse wave velocity were also measured. Cell culture experiments were carried out in a subset of patients.

Results: There was no difference in plasma EMV concentration between the two groups at 24 weeks. HDx was non-inferior to high volume HDF with respect to most clinical and biological markers and *in vitro* endothelial cell function. There was a signal that HDx treatment could be associated with a greater preservation of lean tissue index compared with HDF. HDx may be associated with an improvement in self-reported dialysis recovery time.

Conclusion: HDx therapy appears to be non-inferior to HDF therapy and may be associated with optimisation of nutritional status and improvements in patient-reported outcome measures. Mechanisms behind the study findings require further exploration. In an era where equipoise still exists between diffusive and convective treatment modalities, HDx, where performance is maintained at modest blood flow rates and without the need for infusion of high volumes of substitution fluid, could be an important future direction.

Declaration

No portion of the work referred to in the thesis has been submitted in support of an application for another degree or qualification of this or any other university or other institute of learning.

Copyright Statement

Acknowledgments

First and foremost, I would like to thank my supervisor Professor Sandip Mitra. I emailed Sandip completely out of the blue in 2014 whilst working in Brighton and I had no idea of the journey this email would take me on. Sandip has been a true mentor to me in every sense of the word. He has instilled into me a genuine passion for dialysis and pushed me further than I ever thought I was capable of. He is somebody that always finds time for you and listens with genuine interest, despite having the busiest diary I have ever come across. I am truly grateful for everything that Sandip has contributed to my career over the past few years and am certain we will continue to build on this great relationship in the future.

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Lastly, I would like to thank the patients who took part in this project. This study would only have been possible with their participation. Despite living their lives through dialysis, the patients that I got to know through this study were enthusiastic and obliging all the way through. I am very grateful for this.

Contributions

The contributions for the study presented in this thesis are as follows:

All of the work that I contributed to this study was under the supervision of Professor Sandip Mitra.

I conceived the study and wrote the grant application alongside my supervisor Professor Sandip Mitra. Our collaborative team at Manchester Metropolitan University (MMU) (Professor Yvonne Alexander, Dr Fiona Wilkinson, Dr Liliana Shalamanova and Ms. Annie Herring) were involved in the grant application. I designed the study and wrote the study protocol. I wrote and submitted the ethics application for the study. I implemented the study at Manchester NHS Foundation Trust (MFT) alongside Ms Victoria Jackson, renal research and staff nurse. Victoria and I performed all clinical measurements and data collection during the study and collected all blood samples. All laboratory work for the study biomarkers, endothelial microvesicle (EMV) analysis and cell culture experiments (presented in chapters 3 and 4) was performed by the MMU team. Collaboration was maintained throughout the study between the MMU and MFT teams.

Data analysis was performed by myself with input from Dr Catherine Fullwood (Statistician at MFT) and the MMU team. I wrote all chapters in this thesis, the team at MMU through their collaboration, reviewed chapters 2,3 and 4.

Dedication

To my grandparents Om Parkash Kharbanda & Satya Devi Kharbanda. Your courage, hard work and love are still with us all.

Chapter 1: Introduction Part A: Chronic Kidney Disease and the Uraemic Syndrome

1.1 Background

Chronic Kidney Disease (CKD) is common with an estimated prevalence of 8.5% in the United Kingdom (UK) for stage 3-5 disease[1]. It has a variable prognosis and staging the disease (stages 1 to 5) according to estimated glomerular filtration rate (eGFR) [2] has improved the care of patients from diagnosis through to treatment and management. The majority of patients with advanced kidney disease (Stage 5) require treatment in the form of renal replacement therapy (RRT), either as dialysis treatment or a kidney transplant.

1.2 The Rise of End Stage Renal Disease

Whilst CKD stage 5 represents the smallest proportion of those with CKD (0.1% global prevalence[3]), this group of patients require a significant proportion of health resources. In the UK, it was estimated that half of the £1.44-£1.55 billion spent on CKD in 2009-2010 was on RRT [4]. Globally, approximately 2.618 million people receive RRT [5] and modelling demonstrates a huge unmet global need. It is estimated that between 4.09 million and 9.071 million require therapy[5]. Unsurprisingly, the biggest gaps in treatment are in low-income countries.

Over a 20-year period (1990 to 2010) there has been a 1.7 fold increase in the global prevalence of end-stage renal disease (ESRD) (those requiring dialysis or a transplant) from 165 per million population (pmp) to 284 pmp[6]. Incidence of ESRD has more than doubled in the same time period(44 pmp in 1990 to

93 pmp in 2010)[6]. This trend of a rising ESRD prevalence and a global unmet need for RRT poses a huge challenge to the global Nephrology community in terms of RRT provision. Novel ways in detecting disease, preventing disease progression as well as providing RRT must be developed.

1.3 Dialysis Treatment

At present, dialysis is provided to patients in the form of peritoneal dialysis or haemodialysis. Haemofiltration is used only as a short-term treatment however, haemodiafiltration is increasingly being used as a long-term treatment. These treatments will now be briefly outlined.

Haemodialysis uses a machine to pass blood through an extracorporeal circuit where it comes into contact with a sterile dialysate fluid across a semi-permeable membrane and passes back into the patient [7] [8]. This process of using a concentration gradient to separate solutes across a semi-permeable membrane is knows as diffusion or diffusive solute transport [8] [9].

Haemofiltration uses a hydrostatic pressure gradient to pass the patients' blood across a membrane with a large pore size. Solutes follow water through a process called "solvent drag"[10] and this process is known as convection. Haemodiafiltration (HDF) combines both haemodialysis and haemofiltration which allows the clearance of both smaller and larger molecules.

1.4 Outcomes in Haemodialysis

Outcomes in CKD worsen as eGFR declines[11,12] [11]. Median unadjusted survival in the UK for incident patients starting haemodialysis is just 3.5 years [12](data presented in Figure 1.1). These poor outcomes are not specific to the UK and are seen globally[13] [14] [15] [16].

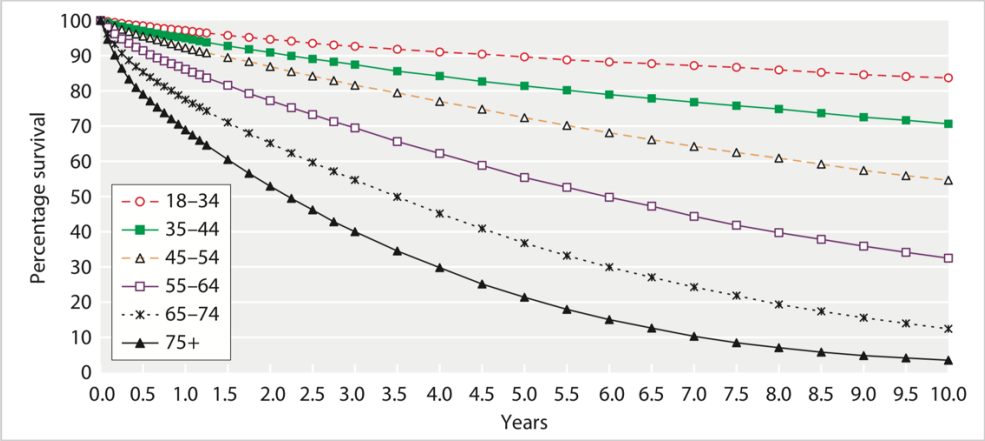

Figure 1-1: Unadjusted survival for haemodialysis patients in the UK in 1997 to 2014 cohort amongst different age groups. *Figure adapted from the 19th Annual UK Renal Registry Report, 2015 [12].*

The majority of deaths in haemodialysis patients are related to cardiovascular disease. In the UK, almost a third of deaths amongst prevalent dialysis patients are cardiovascular or cerebrovascular in nature, the next leading cause of death is infection, accounting for 20% of deaths[17].

1.5 Determinants of Haemodialysis Patient Outcomes

A number of factors have been identified which impact on the outcome of haemodialysis patients.

1.5.1 Dialysis Frequency and Treatment Duration

Bernard Charra and his group in Tassin, France showed hugely impressive survival rates of their haemodialysis patients of 87% at 5 years and 43% at 20 years which far surpassed matched patients on both European & US registries[18]. Patients in this group received extended haemodialysis as 8 hours, 3 times per week. It is likely that the survival association is related to achieving good blood pressure control (antihypertensives were seldom required in the group) through optimised ultrafiltration as well as the enhanced clearance of uraemic toxins provided by the longer treatment. Their publication played an important role in sparking interest once again in extended haemodialysis which had faded following publication of the National Cooperative Dialysis Study (NCDS) [19].

Home haemodialysis (HHD) allows the delivery of more extended and frequent haemodialysis prescriptions to be delivered. Data for HHD patients shows a significant survival benefit, even when adjustments are made for age and co-morbidity[22,23]. Figures of 90% survival at 5 years and 45% at 20 years have been quoted[20]. Figure 1-2 shows a clear survival advantage of home haemodialysis over both peritoneal dialysis and hospital-based HD. This

observational data has to be interpreted with care given the high number of confounders. Patients selected for home haemodialysis are generally younger with a low co-morbidity burden. They are usually highly motivated and take an interest in their healthcare.

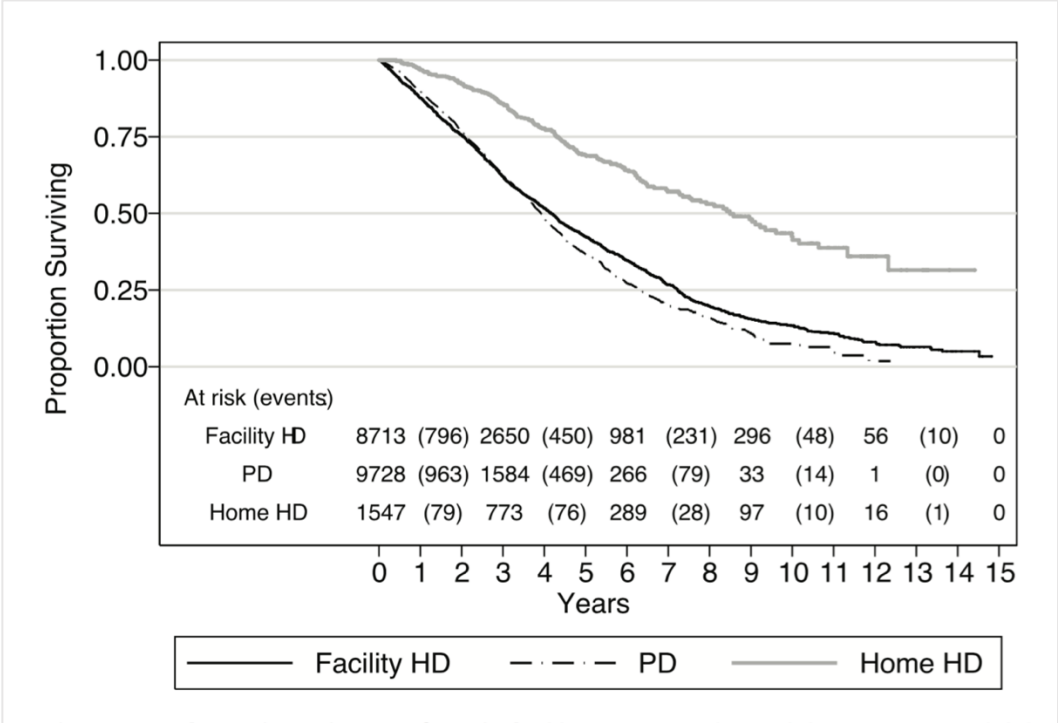

Figure 1-2: The survival of Home HD patients in New Zealand compared with Facility HD and Peritoneal Dialysis (PD). Image adapted from Marshall et al[21]

Although an RCT to investigate the benefits of more extended and frequent dialysis has been carried out[22,23], the trials faced recruitment difficulties. A benefit was only seen in patients on more frequent dialysis and a benefit was not seen in nocturnal HD patients. Additionally, access complication rates were higher and there was a significant loss in residual renal function compared with the control group in the nocturnal arm[24]. Strong data from prospective studies demonstrating a benefit from extended and frequent

dialysis persecutions is lacking, however, registry data remains strong and we are unlikely to see another large randomised study.

1.5.2 Ultrafiltration Management, Intradialytic Hypotension & Dialysate Temperature

As residual kidney function and urine output diminishes, ultrafiltration from haemodialysis treatment is increasingly relied upon to maintain euvolaemia. There is a strong association between a high ultrafiltration rate (>10ml/kg/hour) and mortality[29-31]. Ultrafiltration rate is also closely related to episodes of intradialytic hypotension (IDH) [25]. Plasma refilling rate is variable amongst patients abut has been shown to be significantly lower than ultrafiltration rates at between 2 and 6ml/kg/hour[26]. IDH is associated with myocardial stunning- a transient reduction in segmental cardiac function due to ischaemia[27]. Repeated episodes of myocardial stunning lead to a reduction in ejection fraction and a significant increase in mortality [28]. Registry data has shown improved survival associated with longer treatment times and reduced ultrafiltration rates [29]. Thermal cooling also appears to have an advantageous effect where cooled dialysate reduces IDH episodes[30], myocardial stunning[31] and the progression of HD-associated cardiomyopathy [32]. Optimizing dialysis treatment to lower ultrafiltration rates and reduce episodes of IDH are likely to improve patient outcomes, prospective studies evaluating this are however lacking.

1.5.3 Maintenance of Residual Kidney Function

The average eGFR at dialysis initiation in the UK is 8.6ml/min[33] and at this stage, the contribution of residual kidney function (RKF) is still highly significant. Amongst peritoneal dialysis patients, RKF confers a significant survival benefit with a 12% reduction in the relative risk of death for each 5 litre/week per 1.73[2] increment in glomerular filtration rate (GFR)[34]. In haemodiaysis, for each 1.0 per increase in residual function (Kt/V urea/week), the relative risk of death is 0.44 [35]. Data is conflicting as to which dialysis modality is best at preserving RKF with earlier studies suggesting PD as more beneficial[36] and more recent studies demonstrating little difference between the two [37]. What has become apparent is the importance of optimising volume status in dialysis patients and tools such as bioimpedance spectroscopy are increasingly being used to aid this[38].

1.5.4 Enhancing Solute Clearance: Diffusion vs Convection

Haemodiafiltration (HDF) has come under the spotlight recently as a promising future therapy. Newer technology offers “on-line” production of ultrapure dialysate and replacement fluid where fluid is produced by the machine using a dialysate concentrate and purified water. This allows treatment with high convective volumes (the volume of replacement fluid infused back into the patient) and makes the treatment much more feasible for both the hospital and home setting. HDF provides enhanced clearance of middle molecules

compared with haemodialysis using a high flux membrane (HFHD) [39-43]. It is associated with a reduction in pro-inflammatory cytokines such as IL-6 and TNF-alpha[44] and reduced episodes of IDH[49,50]. Data on whether HDF improves patient mortality is conflicting from 4 recent randomised controlled studies(RCT's) [51-54]. Pooled data from the trials suggests the mortality benefit associated with HDF could be related to the convective volume delivered[45]. There remains clinical equipoise as to whether HDF is truly a superior treatment modality and here are currently two large RCT's underway to explore this further.

1.6 The Uraemic Syndrome

Understanding the poor outcomes seen in CKD requires an understanding of the syndrome that emerges as the disease progresses. Patients with advanced CKD are in a so-called state of "uraemia" which is characterised by inflammation, malnutrition and significant burden of cardiovascular disease. Patients also experience a significant burden of symptoms and reduced health-related quality of life (HRQoL). This syndrome is associated with accumulating uraemic retention solutes; these are "compounds which accumulate in blood and tissues during the development of advanced kidney disease and which have an impact on biological functions" [46].

1.7 Symptom Burden

Patients with advanced CKD have a similar number of symptoms and symptom distress to those with terminal malignancy [47]. Fatigue, feeling drowsy, pain, pruritis and dry skin appear to be the most common symptoms in patients across all stages of CKD [48,49]. The mean number of symptoms in those with CKD ranges from 6 to 20 across different studies [50 51 52 53 54]. There is a significant negative correlation between symptom burden and HRQoL in patients with CKD [55].

Quality of life, a concept discussed in the medical field for over 60 years was defined by the Who Health Organisation (WHO) in 1947 as the "state of complete physical, mental and social well-being, and not merely the absence of disease and infirmity" [56]. More recently, "Health-Related Quality of Life" [57] has been more widely adopted in medical literature. The importance of the impact of disease has on the mental and social aspects that contribute to quality of life are now better recognised and measures of HRQoL are frequently incorporated into clinical studies with the use of these data in health economic appraisals and the development of clinical services.

In an era with a growing focus on patient-centred care the voice of patients is increasingly being heard by clinicians and researchers. For example, we are hearing that dialysis patients are willing to trade off 23 months of their life expectancy in order to reduce their travel restrictions [58]. In an effort to bridge this gap between clinician directed research and the needs of patients, the

Standardised Outcomes in Nephrology (SONG) initiative was started (outlined in figure 1-3). This initiative started in 2014 with the aim of developing core outcome sets for research in haemodialysis (SONG-HD), transplantation, peritoneal dialysis, children and adolescents, polycystic kidney disease and glomerular disease. It has involved clinicians, patients, caregivers and key stakeholders from over 80 countries. The methodology used is the same validated process that has been used for a similar initiative in Rheumatology (Outcome Measures in Rheumatology- OMERACT) [59]. This method involves systematic reviews of trials, focus groups, interviews, Delphi surveys and consensus workshops [60]. SONG-HD has highlighted and ranked key outcomes and highlighted both similarities and differences between different subgroups in terms of their priorities. The initiative has identified the most important future research outcomes as cardiovascular disease, vascular access issues, dialysis adequacy and fatigue [61]. It was noted in the study that patients and caregivers understood dialysis adequacy as a quality of life outcome, ie "dialysis that is adequate for enabling patients to feel well [61]" rather than a marker of urea clearance. Outcomes favoured most by patients and their carers compared with clinicians and other stakeholders were ability to travel, dialysis adequacy and "washed out feeling after dialysis'. These outcomes clearly highlight the importance patients place on quality of life and it is important that their voice is heard and that these outcomes are considered in future research.

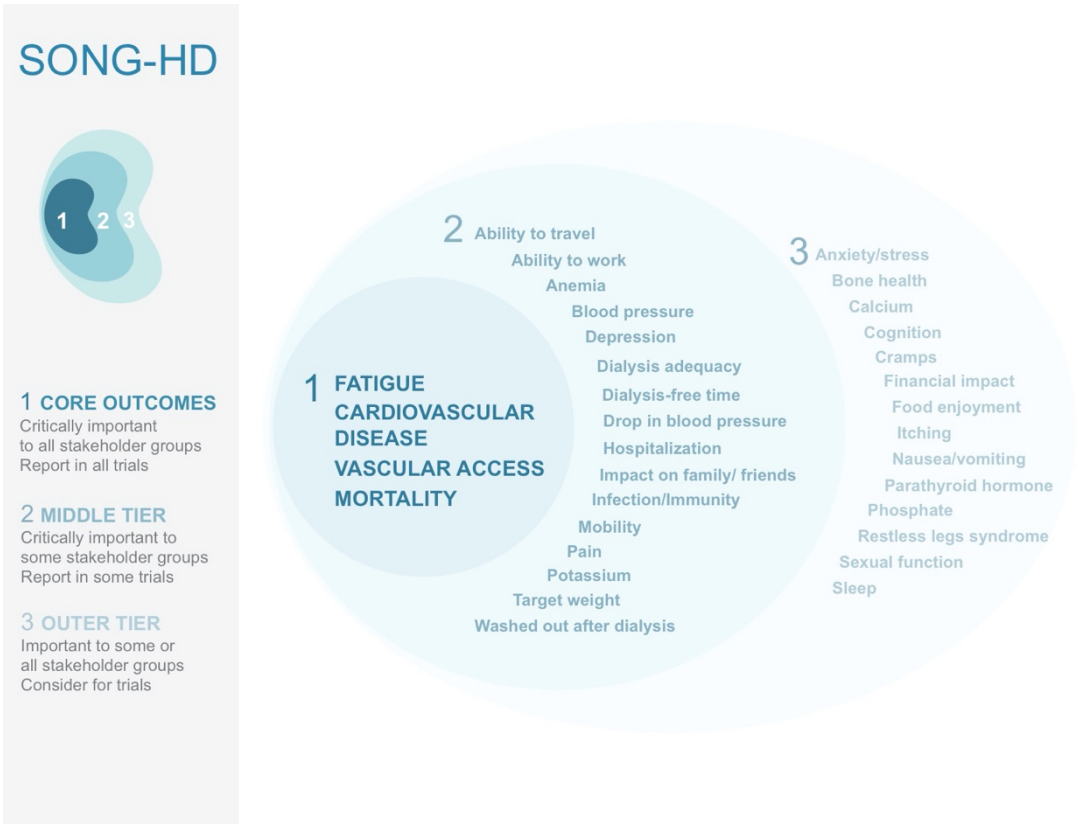

Figure 1-3: Outline of the key findings of the SONG-HD (Standardised Outcomes in Nephrology-Haemodialysis) project. *Symptoms and issues identified in the project were divided into core outcomes and a middle and outer tier. Image available on https://songinitiative.org/projects/song-hd/*

At present, there appears to be a sea-change in medical research where the voice of the patient is being increasingly heard. Research tools have been developed, such as patient-reported outcomes measures (PROM's) and patient-reported experience measures (PREM's), to capture how the patients feels and what their experience is. In learning what matters to our patients and in listening to how they feel as well as capturing what their HRQoL is, our research can be significantly improved to develop interventions that matter to our patients

1.8 Uraemic Retention Solutes

Our understanding of uraemic retention solutes has advanced considerably. The European Uremic Toxin (EUTox) group was formed in 1999 and published their first review in 2001[62]. Their 2003 publication[63] identified 90 uraemic solutes classified as either low molecular weight (<500 Daltons) without known protein binding, those that are protein bound and "middle" molecules (>500 Daltons in size) with 22 in total. This work has since been expanded by the group in several publications[64-66]. The most relevant solutes within each of these groups are listed in figure 1-4. There is more to this story however than simply eGFR. We know that eGFR is not a perfect measure of true GFR and additionally, uraemic toxin concentrations correlate poorly with eGFR[67]. This finding may in part explain the variability in symptom burden between patients who have the same eGFR. In The Initiating Dialysis Early and Late (IDEAL) study[68], 75.9% in the late start arm of the trial (eGFR 5-7ml/min vs 10-15ml/min) needed to start dialysis early before they reached their target. Current dialysis therapies are able to easily remove low molecular weight water soluble solutes such as urea however, protein-bound and larger middle molecules present more of a challenge.

1.8.1 Small Molecules

Urea, at 60 Daltons, is both water soluble and easily removed by dialysis. Whilst urea levels rise as glomerular filtration rate falls, there is debate about the direct toxicity of urea at the levels seen in CKD[69-71]. The same is true for

creatinine (113 D). Other commonly unmeasured uraemic toxins within this group have been associated with greater toxicity. Guanidino compounds such as asymmetric dimethylarginine (ADMA) and symmetric dimethylarginine (SDMA) are the most significant within this group and at their concentrations seen in CKD, they have been shown to be neurotoxic[72,73] and pro-inflammatory[74] and are associated with endothelial dysfunction[75].

Guanidino Compounds (<500 D)

α-Keto-δ-guanidinovaleric acid
α-*N*-acetylarginine
Asymmetric dimethylarginine (ADMA)
Argininic acid
β-Guanidinopropionic acid
Creatine
Creatinine
γ-Guanidinobutyric acid
Guanidine
Guanidino acetic acid
Guanidinosuccinic acid
Methylguanidine
Symmetric dimethylarginine (SDMA)
Taurocyamine

Protein-bound molecules

Advanced glycation end products (AGEs)
Carboxy methyl propyl furanpropionic acid (CMPF)
Cytokines
Interleukins
Tumor necrosis factor-α (TNFα)
Dimethylguanidines
Hippuric acid
Homocysteine
Indole-3-acetic acid
Indoxylglucuronide
Indoxylsulfate
Kynurenic acid
Kynurenine
Leptin
Phenolic compounds
P-cresylsulfate
P-cresylglucuronide
Phenolsulfate
Phenolglucuronide
Phenylacetic acid
Quinolinic acid
Retinol-binding protein

Middle Molecules (>500D)

Adiponectin	Cystatin C	Leptin
Adrenomedullin	DIP-I	Methionine-enkephalin
AGEs	Delta-sleep-inducing peptide	Motilin
AOPPs	Desacylghrelin	Myoglobin
α1-Acid glycoprotein	Dinucleoside polyphosphates	Neuropeptide Y
α1-Microglobulin	Endothelin	Orexin A
Angiogenin	FGF-23	Orexin B
Angiotensin A	Ghrelin	Osteocalcin
Atrial natriuretic peptide (ANP)	Glutathione	Parathyroid hormone (PTH)
Basic fibroblast growth factor	Guanylin	Prolactin
β-Endorphin	Hyaluronic acid	Resistin
β-Trace protein	Insulin growth factor-1 (IGF-1)	Retinol-binding protein (RBP)
β2-Microglobulin	Interleukin-1β (IL-1β)	SIAM-1
Calcitonin	Interleukin-6 (IL-6)	Substance P
Calcitonin gene-related peptide	Interleukin-10 (IL-10)	Tumor necrosis factor α (TNF-α)
Cholecystokinin	Interleukin-18 (IL-18)	Uroguanylin
Clara cell protein (CC16)	κ-Ig light chain	VEGF
Complement factor D	λ-Ig light chain	Vasoactive intestinal peptide

1

Figure 1-4: Main uraemic solutes categorised as either guanidino compound (these are small molecules <500 D, urea is also within this spectrum), protein-bound and middle molecules (>500 D). Daltons (D). *Adapted from Neirynck et [493].*

Of the protein-bound uraemic toxins, P-cresol and Indoxylsulfate (IS) are the most widely studied toxins. P-cresol sulfate is formed by conjugation in the liver of p-cresol which itself is formed by gut bacteria from the amino acids

tyrosine and phenylalanine[77]. In vivo, 95% of p-cresol circulates as p-cresol sulfate[78]. P-cresol sulfate has been shown to cause toxicity in the form of endothelial microparticle release (a marker of endothelial dysfunction and discussed in later sections) in haemodialysis patients[79], induction of free radical production[80] and vascular remodelling[81].

Indole is produced from tryptophan by gut bacteria which is then metabolised in the liver to IS[82]. Serum IS levels increase as eGFR falls and IS levels predict aortic calcification, vascular stiffness and cardiovascular mortality in CKD patients[487](although this study did not adjust for eGFR) as well as endothelial dysfunction[83] and in vitro release of endothelial microparticles[84].

Whilst the majority of protein-bound uraemic toxins are small in size (<500 D), their protein binding limits their removal due to the pore size of haemodialysis membranes. There are reports of enhanced removal through convection[85,86], dialysis membrane surface area and dialysate flow[87], however these parameters make only modest improvements. Fractionated plasma separation and adsorption (FPSA) provides much greater removal of protein-bound solutes compared with high-flux haemodialysis(double) [88] however achieving adequate anticoagulation for the circuit is challenging. Protein-leaking membranes offer loss of some of these toxins at the expense of albumin loss- we are yet to determine how much albumin loss is acceptable in dialysis patients.

1.8.2 Middle Molecules

In the most recent review by the EUTox group[76], over 50 solutes have been identified within this group. These toxins tend to have adverse cardiovascular effects through mediating inflammation, endothelial dysfunction, activation of coagulation cascades and accelerating CKD mineral bone disorder. Clearance of larger middle molecules by current dialysis techniques are poor-whilst convective therapies provide enhanced clearance of smaller middle molecules compared with HFHD, clearance of middle molecules greater than 25 kDa is limited for HDF and >15kDa for HFHD[40,89,90]. Figure 1-5 summarises a recent review of middle molecules by size, highlighting those that are not removed by current treatment modalities.

Removed by High Flux (<15 kD)	Molecular Mass, kD	Removed by HDF (15–24.9 kD)	Molecular Mass, kD	Not Currently Removed (>25 kD)	Molecular Mass, kD
Methionine-enkephalin	0.5	Clara cell protein	15.8	Hyaluronic acid	25
Glutathione	0.6	Leptin	16	β-Trace protein	26
Angiotensin A	0.8	Myoglobin	17	Soluble TNF receptor-1	27
δ–Sleep-inducing peptide	0.8	TNF-α	17	Adiponectin	30
Dinucleoside polyphosphates	1	Soluble TNF receptor-2	17	FGF-23	32
Substance P	1.3	IL-1β	17.5	α1-Microglobulin	33
Motilin	2.7	FGF-2	18	VEGF	34.2
Orexin B	2.9	IL-10	18	YKL-40	40
Atrial natriuretic peptide	3	Retinol binding protein	21.2	Pentraxin-3	40.2
Desacylgherlin	3.2	Prolactin	22	α1-Acid glycoprotein	43
Vasoactive interstinal peptide	3.3	κ-Ig light chain	22.5	AGEs	45
Calcitonin	3.4	Complement factor D	23.75	λ-Ig light chain	45
Gherlin	3.4	IL-18	24	Visfatin	55
β-Endorphin	3.4	IL-6	24.5	AOPPs	>60
Orexin A	3.5				
Calcitonin gene–related peptide	3.7				
Cholecystokinin	3.8				
Endothelin	4.2				
Neuropeptide Y	4.2				
SIAM-1	4.2				
Adrenomedullin	5.7				
Osteocalcin	5.8				
IGF-1	7.6				
IL-8	8				
Parathyroid hormone	9.5				
Guanylin	10.3				
β2-Microglobulin	11.8				
Uroguanylin	12				
Resistin	12.5				
Cystatin C	13.3				
Degranulation inhibiting protein[a]	14.1				

Thirty-one molecules had molecular mass under 15 kD, and therefore, they can be removed by high-flux dialysis. Fourteen molecules had molecular mass between 15 and 25 kD, and therefore, they can be removed by HDF. Fourteen molecules had molecular mass >25 kD. HDF, hemodiafiltration; FGF, fibroblast growth factor; VEGF, vascular endothelial growth factor; AGE, advanced glycosylation end product; AOPP, advanced oxidative protein products.
[a]Degranulation inhibiting protein corresponds to angiogenin.

Figure 1-5: Middle molecules (15-60kDa) identified by EUTox database *(adapted from review by Wolley et al [91]). Solutes divided by size based on removal by current dialysis therapies.*

There will now be a focus on some of these middle molecules in more detail particularly those where clearance in current treatment modalities remains poor.

1.8.2.1 β2-Microglobulin (11.8 kDa)

Since the link between β2-Microglobulin (β2M) and dialysis-related amyloidosis was made[92], this molecule has been of interest as a target for enhanced solute removal during dialysis and this stimulated a shift away from

focusing purely on urea clearance. β2M is synthesized by all nucleated cells and has a role in antigen presentation[93]. Serum levels are increased in high turnover states such as autoimmune disease (Crohn's, Sjogrens, systemic lupus erythematosus, rheumatoid arthritis) [94,95], solid organ malignancy [96,97] and lymphoproliferative disorders[98,99]). It is freely filtered in the renal glomerulus and serum levels are therefore influenced by both production and renal elimination. In the context of chronic kidney disease, residual renal function is the most significant determinant of β2M levels compared with dialysis clearance in HD or HDF[100 101]. In fact, dialytic removal of β2M only impacts serum levels when residual renal clearance is less than 2ml/min[102]. In this context, there is also a strong positive correlation between β2M, cystatin C and creatinine[103]. Levels also predict cardiovascular events and mortality across different stages of CKD[104,105]. Removal of β2M is significantly enhanced by high-flux membranes compared with low-flux [106,107] and HDF provides even greater removal [108,109]. Despite these advancements in technology that have enabled improved clearance, the translation into definitive improvements in clinical outcomes are still lacking.

1.8.2.2 Leptin (16 kDa)

Leptin has been recognised for playing a major role in energy balance. This small 16kDa peptide hormone suppresses food intake and increases energy expenditure through receptor binding primarily in the hypothalamus[110]. Leptin is freely filtered by the glomerulus and the kidney is the primary site of leptin

clearance [111]. Concentrations of leptin in advanced kidney disease are significantly raised[112], including those on both peritoneal dialysis (PD) and HD [113,114] and although this is most likely due to renal clearance, leptin is also intimately linked with inflammatory cytokines and potentiates the secretion of tumor necrosis factor (TNF), interleukin(IL)-1 , IL-2 & IL-6[115], therefore increased production may also play a part. Additionally, patients on haemodialysis with cachexia have been found to have high CRP and leptin levels with a reduced serum albumin[115] suggesting a close link with the protein energy wasting syndrome. Despite this, data linking leptin concentrations with nutritional parameters is conflicting[116] [117] [118] and no studies have demonstrated that reducing leptin concentrations results in improved nutrition. High-flux HD and HDF both enhance leptin clearance[119,120] however translation into clinical nutritional parameters has again not been definitively demonstrated.

1.8.2.3 α1-microglobulin (33 kDa)

Alpha 1-microglobulin (A1M) is a protein around 30kDa that is predominantly synthesised in the liver. It has a protective role in preventing damage caused by oxidative stress[121] and urinary concentration has been utilised as a biomarker of renal tubular function[122]. It is freely filtered by the glomerulus and reabsorbed and catabolized by the proximal tubule and serum concentrations correlate with creatinine clearance[123]. Given these properties, it is a useful biomarker for assessing middle molecule clearance.

1.8.2.4 YKL-40 (Chitinase-3-Like Protein 1) (40 kDa)

YKL-40, also known as chitinase-3-like protein 1 (CHI3L1), is a glycoprotein expressed by several cell types however macrophage is a major source and macrophage expression of YKL-40 has been implicated in atherosclerosis. Levels of YKL-40 have been closely linked with the angiographic severity of coronary artery disease[124] and have been found to be elevated alongside IL-6[125,126] suggesting a link with inflammation. Higher YKL-40 levels have been found in patients suffering with myocardial infarction[127-129], atrial fibrillation[130] and diabetes[131-133]. In CKD, elevated levels have been found in both PD[134] and HD patients[134,135] and there is a negative correlation between YKL-40 levels and flow-mediated dilatation in HD patients[135]. YKL-40 predicts mortality in HD patients[136] and clearance by haemodialysis is poor[136] Given the findings in non-CKD patients, elevated YKL-40 levels are likely to be a result of both increased production and reduced clearance. Whether increasing YKL-40 removal can improve outcomes is yet to be determined.

1.8.2.5 Pentraxin-3 (40.2 kDa)

Pentraxin-3 (PTX-3) is an acute-phase protein which is a predictor of cardiovascular mortality in patients with advanced CKD, independent of CRP[137,138]. Production is stimulated by inflammatory mediators such as TNF-α[139] and there is a strong suggesting it is actively involved in the process of

atherogenesis[140,141]. Higher PTX-3 levels are seen in patients with protein-energy wasting and cardiovascular disease[489] and there is also an association with endothelial dysfunction in patients with CKD[138,142]. Current extracorporeal dialysis modalities (HFHD & HDF) do not appear to alter concentrations of PTX-3 [143] and the long-term clinical effects of enhancing removal are unknown.

1.9 Inflammation in CKD

As previously mentioned, it is clear that patients with CKD have significant cardiovascular disease, this risk increases as eGFR declines and cardiovascular disease is the leading cause of death in dialysis patients. Whilst patients with CKD are subject to the "traditional" cardiovascular risk factors, there are also many "non-traditional" risk-factors that are specific to CKD and which contribute to the huge excess in mortality seen in this patient group. These factors include anaemia, CKD mineral bone disorder (CKD-MBD), increased oxidative stress and chronic inflammation.

There is a strong relationship between eGFR and inflammation such that levels of pro-inflammatory cytokines rise considerably as eGFR declines[144]. IL-6 concentration, for example, is over five times higher in CKD Stage 5 (pre-dialysis) compared with CKD stage 2[145](figure 1-6) and of the inflammatory cytokines, it appears to be the strongest predictor of outcome in CKD[146 147 148].

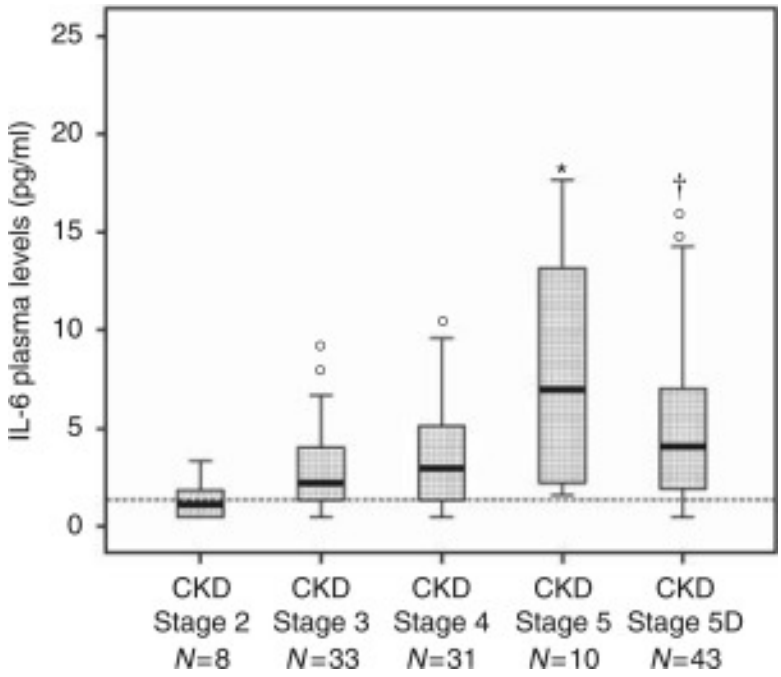

Figure 1-6: Concentrations of IL-6 at different stages of CKD(Adapted from Barreto et al[145]). *There is a steady increase in IL-6 concentration as CKD progresses followed by a reduction in dialysis patients.*

The aetiology of this intimate relationship between CKD and inflammation is complex, clearly reduced cytokine clearance associated with a falling eGFR plays a part- as previously discussed, many pro-inflammatory cytokines fall into range of uraemic toxins which have limited removal by current dialysis therapies. There is much more to this story though beyond impaired cytokine clearance. Several stimulators of inflammation have been identified in CKD. Firstly, in dialysis patients, these factors include membrane compatibility[149,150], CVC infections and water purity[151,152] all of which have been shown to influence inflammation. Endotoxinaemia stimulated through dialysis [153] as well as through changes in gastrointestinal permeability from volume overload[154], have also been implicated as inflammatory stimulators. The high burden of

oxidative stress is another factor hypothesised as another stimulator of inflammation in CKD[155], as well as periodontal inflammation[156], uraemic retention solutes (previously discussed) and co-morbidities which co-exist alongside CKD.

Whilst there is a strong association between levels of inflammatory markers and cardiovascular outcomes, the pathogenic mechanisms by which chronic inflammation accelerates cardiovascular disease are still poorly understood as is our understanding of how much is cause or effect. There is a close relationship between the immune system and atherosclerosis (discussed later) and therefore chronic inflammation may well have an impact on this process. Additionally, inflammation impacts on vascular endothelial function and activation, this again is discussed in more detail in a later section.

Strategies at improving the inflammatory burden in CKD must be targeted at both improving clearance of cytokines and reducing cytokine production. Whether reducing the inflammatory burden in CKD will improve cardiovascular outcomes is yet to be fully determined. Given the number of factors involved, it is likely that only multitargeted interventions will make a meaningful impact on patient outcomes.

1.10 Malnutrition in CKD

Protein Energy Wasting (PEW) is a syndrome of nutritional and metabolic derangements in CKD which ultimately leads to loss of muscle and fat mass as well as cachexia[157]. A significant proportion of patients with advanced CKD see a longitudinal reduction in their anthropometric parameters (ie. muscle and fat mass) [158] [159] and during this time, a rise is seen in inflammatory cytokines such as IL-6 and CRP [160] [161]. Inflammatory markers such as IL-6 correlate with muscle mass in HD patients[162] and there is an "obesity paradox" such that patients with a higher BMI (>25) have an increased survival compared with a low BMI[163-166]. There are several factors contributing to malnutrition to CKD including poor appetite associated with chronic inflammation[167,168], amino acid loss during dialysis[169] and increased resting energy expenditure[170-172].

Recognising malnutrition and targeting interventions aimed at preventing malnutrition is an important part of patient care. There are a number of ways of assessing and monitoring malnutrition in dialysis patients- no single tool in isolation is perfect as the ideal measure should be easy to perform, reproducible and not affected by factors such as inflammation and systemic disease. The main tools used for assessing malnutrition in CKD are outlined in table 1-1. Many of these tools have been validated in dialysis patients and are used alongside assessments of energy requirements and dietary intake. Serum albumin remains one of the strongest predictors of outcomes in dialysis patients [173] [174] [175,176] and correlates with several nutritional measures [177]. Low serum albumin levels are driven by both poor nutritional intake and chronic

inflammation. Changes in weight and body mass index are often late markers of malnutrition in dialysis patients due to changes in body composition as overhydration and loss of muscle mass is common.

ANTHROPOMETRIC	BIOCHEMICAL	COMPOSITE	BODY COMPOSITION
Body Mass Index (BMI)	Serum albumin	Subjective global assessment (SGA)	Dual energy X–ray absorptiometry (DEXA)
Mid Upper Arm Circumference (MUAC)	Calcium & phosphate	Malnutrition inflammation score (MIS)	Bioelectrical impedance
Skin fold thickness	Urea		Near Infrared Interactance (NIR)
Handgrip strength	CRP		

Table 1-1: Common nutritional assessment tools used in Chronic Kidney Disease (CKD).

Interventions aimed at improving outcomes for patients with PEW have limited efficacy. Logically, measures which may provide benefit would include either those aimed at enhancing nutritional intake or reducing the burden of inflammation. Whilst it would seem sensible to provide oral supplements in haemodialysis patients, particularly in those with poor oral intake and high energy requirements, the evidence supporting their benefit is limited. A recent meta-analysis of 15 studies showed very low quality evidence supporting a benefit from energy and protein supplements for short term outcomes such as improving serum albumin and BMI[178]. The impact on longer term outcomes such as mortality is unknown. Parenteral nutrition in the form of intradialytic parenteral nutrition (IDPN) does not seem to provide benefit compared with oral nutrition[179]. Recombinant growth hormone, anabolic steroids and appetite

stimulants such as megesterol acetate have also all been used but with limited effect.

Chapter 1: Introduction Part B: The Role of the Vascular Endothelium in Inflammation and Cardiovascular Disease in CKD

1.11 The Role of the Endothelium: Vascular Endothelium, Endothelial Dysfunction & Endothelial Activation

The key role that the endothelium plays in vascular health and cardiovascular disease has been a breakthrough over the past few decades. The notion that the vascular endothelium is purely an inert lining of cells of the vascular tree has now well and truly gone. The endothelium is now known to be a large functional organ weighing 1kg with a surface area up to 7000m2 [180] (the size of a football pitch). The endothelium maintains vascular tone, permeability, platelet and leucocyte adhesion and thrombosis [181]. Mediators such as nitric oxide (NO), prostacyclin and endothelin are central to this homeostasis[182]. The terms endothelial dysfunction and endothelial activation are frequently used, sometimes interchangeably and there still lacks a consensus on the definitions of either term. Endothelial dysfunction is known to be a key feature in the early stages of atherosclerosis [183] [184] and is characterised by reduced release and/or activity of endothelium-derived NO [185]. Endothelial activation on the other hand, is characterised by endothelial expression of cell-surface adhesion molecules such as Intracellular Adhesion Molecule-1 (ICAM-1), Vascular Cell Adhesion Molecule (VCAM)-1 and E-Selectin [186]. Despite these two processes having distinct differences, the two terms are often (incorrectly) used interchangeably owing to their involvement in atherosclerosis.

Low-density lipoproteins (LDL) enter the subendothelial space in susceptible regions of the vascular endothelium [187]. These lipoproteins are modified and contribute to endothelial activation. Activated endothelial cells promote the

recruitment of other immune cells via adhesion molecules (such as selectins, ICAM and VCAM) [188] and this leads to the attachment and transmigration of monocytes into the intimal space [189] [190]. Monocytes differentiate into macrophage and become foam cells[191] by internalizing modified low-density lipoproteins (LDL), very-low density lipoproteins (VLDL) and apolipoprotein E (apoE) remnants [192]. Foam cells are the hallmark of atherosclerosis and this process progresses whereby recruited smooth muscle cells proliferate and stable fibrous plaques are formed. Endothelial activation is thought to be induced by inflammatory cytokines such as IL-6 and TNF-α[193,194] [195] as well as "traditional" risk factors such as smoking [196] [197], reduced physical activity[198] and hypercholesterolaemia [199] [200] and in environments where significant oxidative stress is observed [201]. Heparan sulfate and thrombomodulin are lost from the endothelial cell surface during endothelial activation thus promoting thrombosis [202]. Cytokines, such as IL-6 are produced by activated endothelial cells, which can then trigger an acute phase response [203]. Class II HLA antigens are expressed which are then able to act as antigen presenting cells [204]. The resting state of the endothelium has now become one that is pro-inflammatory, pro-thrombotic with impaired vasodilation. Measures of endothelial function and activation may present an opportunity to offer earlier targeted interventions as well as predict disease outcomes.

1.12 Invasive & Non-Invasive Measures of Endothelial Function

The measurement of endothelial-dependent vasomotion has dominated the assessment of endothelial function. In general, healthy arteries dilate in

response to hyperaemia or pharmacological stimuli (such as bradykinin and acetylcholine). This response is typically dependent on NO release [205] [206] [207] and is reduced or absent in disease states [207] [208]. One of the most widely used invasive measured of endothelial function (mainly due to its non-invasive nature) is flow-mediated dilatation (FMD) where the brachial artery is occluded for 5 minutes with a blood pressure cuff and ultrasound and pulse-wave doppler images of the brachial artery are acquired [183]. FMD independently predicts long-term cardiovascular events [209] [210] including those with advanced CKD [211] [212] (although in ESRD results are conflicting [213] [214]).

More invasive measures of endothelial function include coronary epicardial vasoreactivity, venous occlusion plethysmography and coronary microvascular function [215]. EndoPAT (Peripheral Arterial Tone) offers an alternative non-invasive measure to FMD [215].

1.13 Circulating Biomarkers of Endothelial Function

A wide range of potential biomarkers of endothelial dysfunction and activation have been identified. These include asymmetrical dimethylarginine (ADMA), an endogenous inhibitor of NO production [216] [217] [218] [219], adhesion molecules such as ICAM, VCAM and E-Selectin which, under stimulation by inflammation, have a key role in leucocyte recruitment to the endothelium [220] and microvesicles (MV's).

1.14 Microvesicles

There has been a growing interest in cell microvesicles (MVs) after their existence was discovered in 1967 and were described as “platelet dust” after observing “minute particulate material” in plasma following platelet activation [221]. There has been a considerable growth of research within this field since this time and the structure, function and potential significance of these vesicles as biomarkers is unravelling. It is now recognised that small particles are released from almost all eukaryotic cells during normal functioning, activation or stress (consider reference) [222-224]. As such, they provide a snapshot of the state of the cell. There are a wide range of particles released by cells and these extracellular vesicles are now subdivided into exosomes, microvesicles and apoptotic bodies based on their size (figure 1-7).

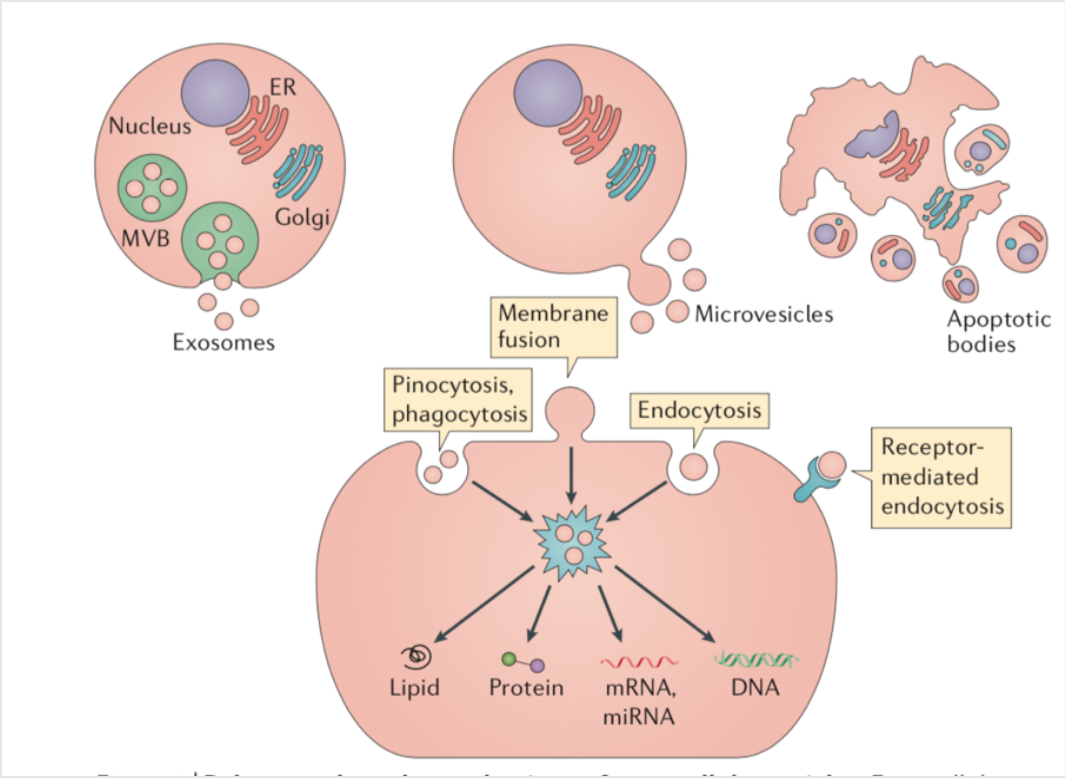

Figure 1-7: Release and uptake mechanisms of extracellular vesicles, adapted from Karpman et al, 2017[225]. *Extracellular vesicles can be taken up by other cells through endocytosis, phagocytosis, pinocytosis or membrane fusion. Ligands on the membranes of extracellular vesicles are able to bind to receptors on recipient cells.*

Briefly, exosomes are the smallest of these MVs and are formed by inward blebbing of the plasma membranes forming intracellular vesicles. These vesicles then fuse with multivesicular bodies [226] [227] (organelles in the endocytic pathway of cells [228]) after which they are either degraded within the cell or released from the cell as extracellular vesicles [229]. Apoptotic bodies are formed during the later stages of apoptosis [227,230] and are the largest of the three extracellular vesicles ranging in size from 1 to 5μm. They have a high content of nuclear material and organelles [231]. Microvesicles (MVs) are generated from the outward budding of the plasma membrane [232] into the extracellular space and they range in size from 100nm to 1μm[233]. All 3 types

of vesicles are released from the lipid rich cell membrane [234]. In addition to allowing cells to remove unwanted substances, microvesicles are able to act as important messengers and they play an important role in thrombosis[235], inflammation[236] and vascular injury[237].

In earlier literature, the term microparticle was used predominantly to describe what is now referred to as microvesicle. For the purposes of this review, only the term microvesicle will be used and this will remain the focus of this study.

1.15 Structure, Function and Release of Microvesicles

Microvesicles are composed of a lipid bilayer and can contain a wide range of material from their parent cell including receptors, genetic material (mRNA, microRNA), cytokines, chemokines, lipids and proteins [238]. They are able to interact with target cells through several mechanisms; firstly they can fuse with the membrane of a target cell and transfer their cargo[239]. Secondly, they can interact with cells via surface receptor signalling pathways[240]. Thirdly, they can be internalised by cells through endocytosis and their contents can either be released into the target cell or degraded[241]. These processes are outlined in figure 1-7.

Under usual conditions, the distribution of plasma membrane lipids is asymmetric. Phosphatidylserine (PS) is located almost exclusively on the inner surface of the membrane and phosphatidylcholine and sphingomyelin are located on the external surface [242]. This distribution is maintained by 3

enzymes (flippase, floppase and scrambalase [243]). Calcium influx into the cell (as seen in cellular activation [244]) can stimulate both floppase and scrambalase and inhibit flippase which then allows PS to be exposed on the cell surface [243] (illustrated in figure 1-8). This process allows membrane budding and thus the release of MVs into the extracellular space [243].

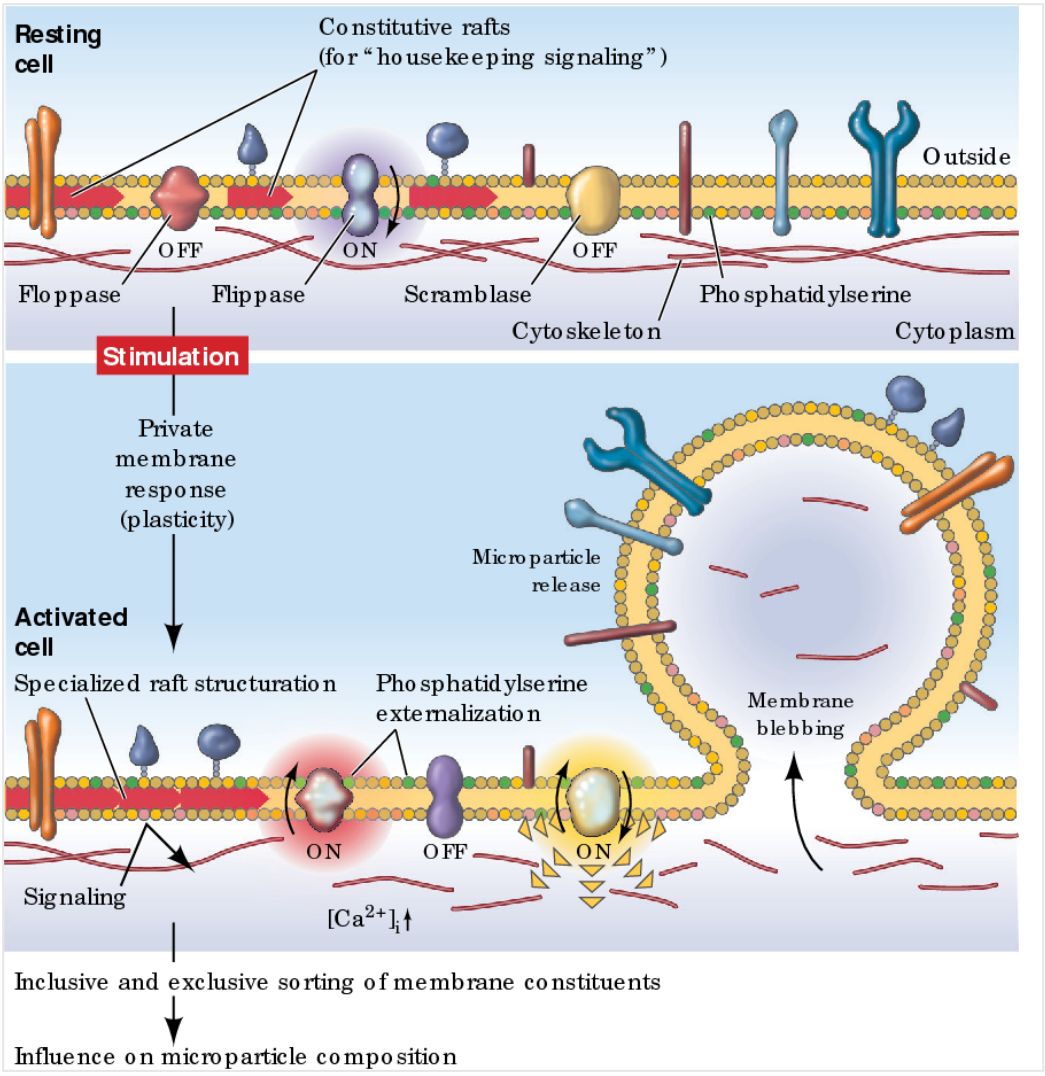

Figure 1-8: The plasma membrane response to stimulation, adapted from Hugel et al, 2005[224]. *The plasma membrane is highly structured and is made of 2 lipid bilayers. Following stimulation, redistribution of lipids takes place which leads to externalization of phosphatidylserine and microvesicle release.*

MVs have many roles including involvement with the processes of inflammation[245-248], coagulation[239,249-251], cancer [252-256] and angiogenesis[257-259]. Activated MVs have pro-coagulant properties which can be up to 100-fold higher than that of activated platelets [260] [261]. The half-life of MVs in the circulation is anything between minutes and hours [241] and further characterisation and determinants of half-life is still required. Whilst typically isolated from peripheral blood, MVs have also been isolated been from other fluids including urine [262], cerebrospinal fluid [263] and saliva [264].

1.16 Detection of MVs

There has been variation in the preparation of samples for MV analysis in the pre-analytical phase however, there is growing standardisation of techniques and groups such as the International Society of Thrombosis work to reach consensus amongst the global scientific community[265]. Differences in blood handling, freezing and centrifugation can all impact on the measurement of MVs [266] [267]. In general, whole blood samples are collected in citrated blood tubes and processed in the laboratory within 2 hours. Plasma samples are prepared using 2-step centrifugation whereby the blood sample is centrifuged, plasma is removed (leaving 100 μl above the buffy layer) followed by an additional higher speed centrifugation. This platelet-free plasma (PFP) is then snap-frozen using liquid nitrogen and stored at -80°c until analysis [265].

Once PFP samples have been prepared, there are a number of techniques that can be used to detect MVs including nanoparticle tracking analysis [268],

dynamic light scattering [269], ELISA [270] [271] and transmission electron microscopy [272]. The most widely used technique by far is flow cytometry [272].

Flow cytometry uses a flow cell which is able to pass cells suspended in liquid in single file through an optical system (figure 1-9). Based on the scatter of light, the cell size and the number of cells can be detected [273]. Further identification can take place through the use of monoclonal antibodies tagged with fluorescent dyes (typically phycoerythrin- PE) that bind with specific antigens on the cell surface membrane. Annexin-V has also been commonly used to identify MVs since it binds to PS and therefore would appear a good differentiator of MVs. However, half of all extracellular vesicles are Annexin V negative [274] and thus, the use of this agent may miss significant numbers of MVs. Annexin V is therefore not always used by groups to identify MVs and this gives further rise to variation in reporting by laboratories.

Cellular events are calculated using counting beads at a known concentration[275] and this allows the quantification of MVs. The analysis is optimised by using gating to set boundaries and include only the events of interest in the analysis [275]. Some instruments are able to physically sort cells into different populations using Fluorescence Activated Cell Sorting (FACS). At present, flow cytometry is unable to detect MVs smaller than 300nm in diameter [276] [277].

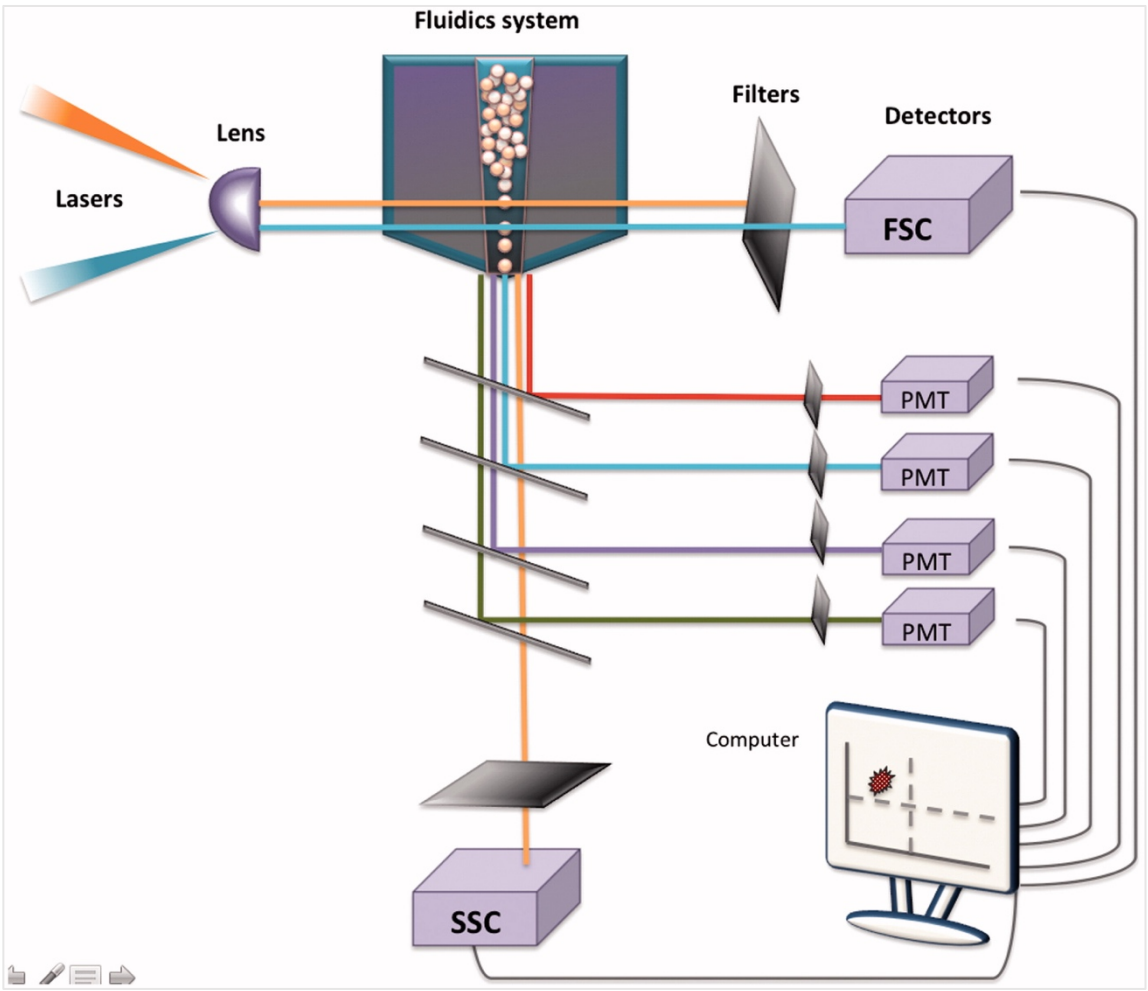

Figure 1-9: Components of a flow cytometer, signal processing & detection (Illustration adapted from Adan et al[273]). *When the laser hits the cells, 2 types of light scatter are produced- forward scatter (FSC) and side scatter (SSC). The type of scatter is dictated by the cell complexity and size. Photomultiplier tubes (PMT) convert light to a voltage which is then amplified and converted into a digital signal for processing by a computer.*

1.17 The Role of Microvesicles in Inflammation, Vascular Injury & Cardiovascular Disease

Microvesicles, particularly those derived from endothelial cells, are intimately linked to inflammation They upregulate pro-inflammatory mediators in both immune and non-immune cells[278,279] and their release (platelet, endothelial and leucocyte derived MVs) is increased in several inflammatory conditions

where vascular injury and cardiovascular disease is common such as Chronic Kidney Disease, Diabetes, Systemic Lupus Erythematosus and Acute Coronary Syndromes [223] [230] [280,281] [281]. Additionally, physical stimuli such as shear stress also appear to regulate MV release [282] [283].

Platelet and leucocyte derived MV's are involved in the release of several pro-inflammatory cytokines such as IL-1, IL-6, IL-8 and TNF-α[246]. They promote monocyte adhesion to the endothelium through promotion of cell adhesion molecule (CAM) expression [284]. Interestingly, MVs may also have an anti-inflammatory role[247,285]. The role and significance of endothelial derived microvesicles (endothelial microvesicles or EMV) will remain the focus in this section.

1.18 Endothelial Microvesicles

Endothelial microvesicles (EMV) are identified by the presence of various cell surface glycoproteins including CD144 (VE-Cadherin), CD146 (Melanoma Cell Adhesion Molecule- MCAM), CD106 (Vascular Endothelial Cadherin- VE-cadherin), CD106 (Vascular Cell Adhesion Molecule 1- VCAM-1), CD31 (Platelet Endothelial Cell Adhesion Molecule 1- PECAM-1), CD105 (endoglin) and CD62E (E-Selectin) [286]. These cell markers are not all exclusive to microvesicles of endothelial origin and so the presence of different combinations of glycoproteins can be utilised to provide specificity. Alternatively, the presence of one glycoprotein with the absence of another such as CD144+ and CD42b (a platelet marker) can also be used to

differentiate EMVs from MVs that originate from other cell types [287]. Given that EMVs are released directly from the endothelium and that quantification in peripheral blood is possible there has been growing interest in their use as a potential biomarker of endothelial activation.

1.19 Clinical Relevance of EMV

As previously mentioned, the clinical relevance of EMV has been studied in a wide range of diseases and pathological processes. For example, in Acute Coronary Syndrome (ACS), EMV levels are significantly elevated (2.5 fold higher than those with stable angina and 12 fold higher than healthy controls) [288]. MV levels have been shown to correlate with the degree of coronary artery stenosis [289] and EMV levels have been demonstrated as an independent predictor of cardiovascular events in those with stable coronary artery disease [290]

In SLE, EMV levels are elevated in active disease (compared with healthy controls) and these levels correlate with flow-mediated dilation and improve following treatment with immunosuppressive therapy [291] The relation of EMV levels to disease activity however is less clear cut [292].

In diabetes, children with type 1 diabetes have significantly elevated EMV levels compared with healthy controls [293] as well as in adults with diabetes [294]. EMV is an independent predictor of International Index of Erectile (IIEF) Dysfunction Score in diabetic men [295]. EMV levels are higher in those with

macroangiopathy (ie. coronary artery disease, cerebrovascular disease and peripheral artery disease) compared with microangiopathy (ie. retinopathy, nephropathy and neuropathy) [296].

Overall, there appears to be a strong signal that EMV levels, measured in peripheral blood samples, is clinically relevant and a marker of poor vascular health.

1.20 Clinical Relevance of EMV in CKD

In children with CKD, EMV (CD144+) levels are significantly raised (and highest in those on dialysis) compared with healthy controls. In the same study in children, EMV was shown to correlate with pulse wave velocity (PWV), age, dialysis duration, blood pressure, dialysis vintage, CRP and PTH[297], all of which have been previously identified as surrogate markers for poor cardiovascular outcomes in HD patients. Uraemic plasma, in the same study, stimulated increased release of EMV from Human Umbilical Vein Endothelial Cells (HUVEC) compared with controls. This study in children, highlights the potential relevance of EMV as a potential biomarker in a setting where more traditional cardiovascular risk factors have less of an influence, it also shines a light on uraemic toxins as a possible mediator of EMV release. Further studies supporting these findings in children are still however lacking.

In adults, EMV levels are also higher in CKD compared with controls[298,299], particularly in haemodialysis patients[84,300-304]. There appears to be a

correlation between eGFR and EMV level[305]. Given their size (a minimum of 100nm), EMVs are unable to pass through the glomerular basement membrane (as a comparator, the size of albumin is approximately 8 x 8 x 3nm in dimension[306]) and thus the higher EMV levels seen in advanced CKD are likely to reflect the increased presence of mediators of EMV production rather than relating to any change in renal clearance. MVs, when detected in the urine tend to originate from podocytes, tubular cells and epithelial cells from the urinary tract[307].

CKD patients with vascular calcification (as determined by CT angiography) appear to have higher EMV levels than those without vascular calcification[308]. When MVs from these CKD patients are incubated with Endothelial Progenitor cells (EPC), they interfere with angiogenesis and cause an increase in osteocalcin (OC) expression [308]. OC has been implicated in inducing vascular calcification [309] suggesting that MVs could contribute to vascular calcification. EMVs correlate with pulse wave velocity (PWV) in HD patients[304] as well as brachial artery flow-mediated dilation[304]. There is a loss of flow-induced dilation in rat aortic rings exposed to EMV supernatant (at a similar concentration to that in plasma) from HD patients and impaired relaxation to acetylcholine (not seen with health controls), suggesting that EMVs induce endothelial dysfunction [304]. These studies further link EMV as a marker and mediator of vascular health and function in patients with CKD.

In haemodialysis patients, EMV appears to predict both all-cause[287,300] and cardiovascular mortality[287] in HD patients such that for each log increase in EMV (events/μl) there is a 20-fold increase in cardiovascular mortality[287]. Haemodialysis itself appears to trigger EMV release although results are conflicting. In one study, the rise detected during haemodialysis appeared to be less when using a high flux membrane compared with a low flux membrane[310]. In another study, a rise in EMVs was observed in those treated with HD but not those treated with HDF [303], suggesting a potential cardiovascular benefit of convective therapy. A third study showed no rise at all from HD with cellulose-based membranes[84] and finally, a fall in EMV levels were identified in a study where patients were treated with HFHD[282]. Correlations with age[282,287] and dialysis vintage[287] are conflicting. The number of studies are however small.

Dialysis modality appears to influence EMV levels. Switching from HFHD to a convective therapy results in a reduction in EMV levels [301,303,311] within as little as 8 weeks (see figure 1-10). EMV levels appear to rise again after switching back to HFHD[301,303].

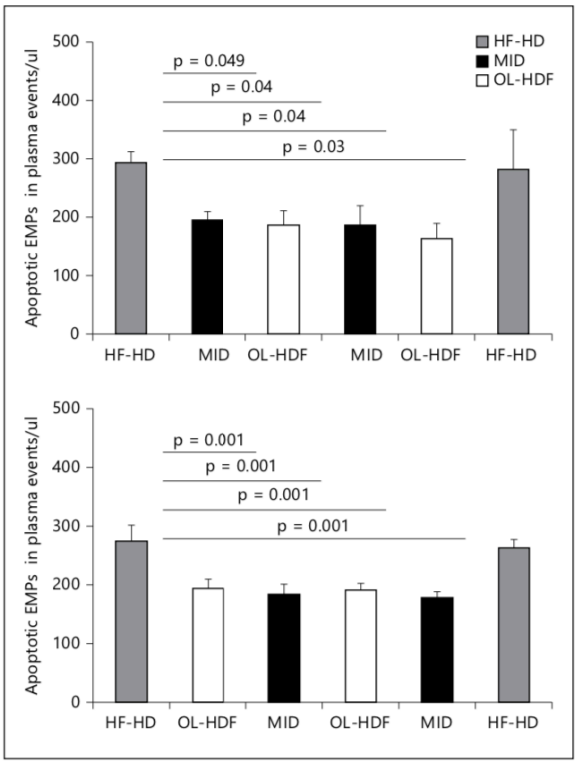

Figure 1-10: Plasma levels of EMV after switching from high-flux haemodialysis (HF-HD) to haemodiafiltration of different modes *(online haemodiafiltration post-dilution (OL-HDF) and mid-dilution (MID)) for 8-week periods. Adapted from Ariza et al 2013[301].*

Uraemic toxins are likely candidates for stimulating EMV release and potential modulators of endothelial dysfunction. In vitro, indoxyl-sulfate and p-cresol (both of which are uraemic toxins) cause an increase in EMV release from HUVEC's [84]. These uraemic toxins are poorly removed by current dialysis modalities which may also explain why the highest EMV levels are seen in dialysis patients[312-314]. The relationship between EMV level and dialysis modality (diffusive vs convective), mentioned in the previous paragraph, adds further weight to the role that larger uraemic toxins may have in modulating endothelial dysfunction. Interestingly, following renal transplantation, EMV levels fall [315].

EMV appears to be a robust biomarker of endothelial function and activation several disease groups including CKD. Addressing the removal of larger and protein-bound toxins, which are currently poorly removed by current dialysis treatments, could perhaps lead to improved endothelial function and clinical outcomes.

Chapter 1: Introduction Part C Innovation in Haemodialysis: Dialysis Membranes

1.21 Introduction

Whilst the principles of dialysis treatment have been remained the same for decades; solute removal through diffusion across a membrane, there has been ongoing advancement in the technology used to enable treatment. Since their development in the late 1960's[316], hollow-fibre membranes provided an alternative to the original drum kidney[317] and coil dialyser[318]. Hollow-fibre membranes remain in widespread use worldwide to deliver haemodialysis treatment however in place of cellulose-based membranes, synthetic membranes now dominate.

1.22 Structure and Manufacturing Process

Modern synthetic membranes are composed of numerous hollow fibres. Each fibre is approximately 18-24cm in length with an internal diameter of 180-200μm[319]. Fibres are manufactured in a clean environment to minimise exposure to environmental toxins. The process involves passing a polymer solution containing polyethersulfone (PES) and polyvinylpyrrolidone (PVP), through the outer ring of a specialised nozzle known as a spinneret (figure 1-11). Another liquid is, known as "bore liquid", a coagulant solution, is passed through the inside of the tube. The solutions that are extruded from the spinneret make contact with a coagulation bath which results in controlled precipitation and forms a two-phase structure [320]. The membrane permeability properties are determined by the type of heat treatment used during this process and the PVP content of the casting solution. The process is as much

of an art as it is a science. Following creation of the fibres, 10,000-17,000 fibres are wound to form a bundle and placed in housing. A “potting” compound, typically composed of polyurethane, is applied to each end of the bundles to provide a seal and the bundle is then sliced to provide patent fibres. The case is finally sealed closed and sterilised, most commonly using steam or irradiation.

Typically, each fibre has three layers: the first layer has a thickness <1 μm containing pores, the second layer is around 2-5 μm in thickness, it has no sieving function but provides membrane stability, the third layer is a “finger-type” layer 40-45μm thick and provides further mechanical stability[490]. The internal diameter of the fibres is typically around 200 μm. Hollow fibre structure demonstrated in figure 1-12.

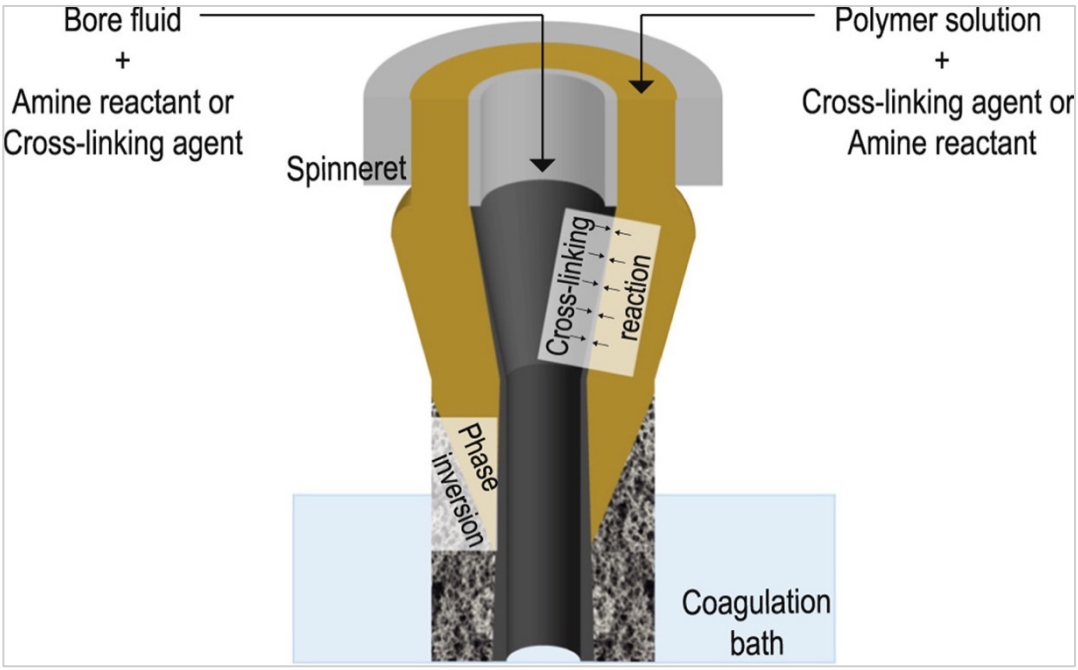

Figure 1-11:Manufacturing process of hollow-fibre demonstrating extrusion of polymer solution and bore fluid from spinneret into coagulation bath. *As the liquids enter the coagulation bath, there is immediate fibre precipitation and solidification with the formation of a two-phase structure. Image adapted from Roth el al 2018*[321] .

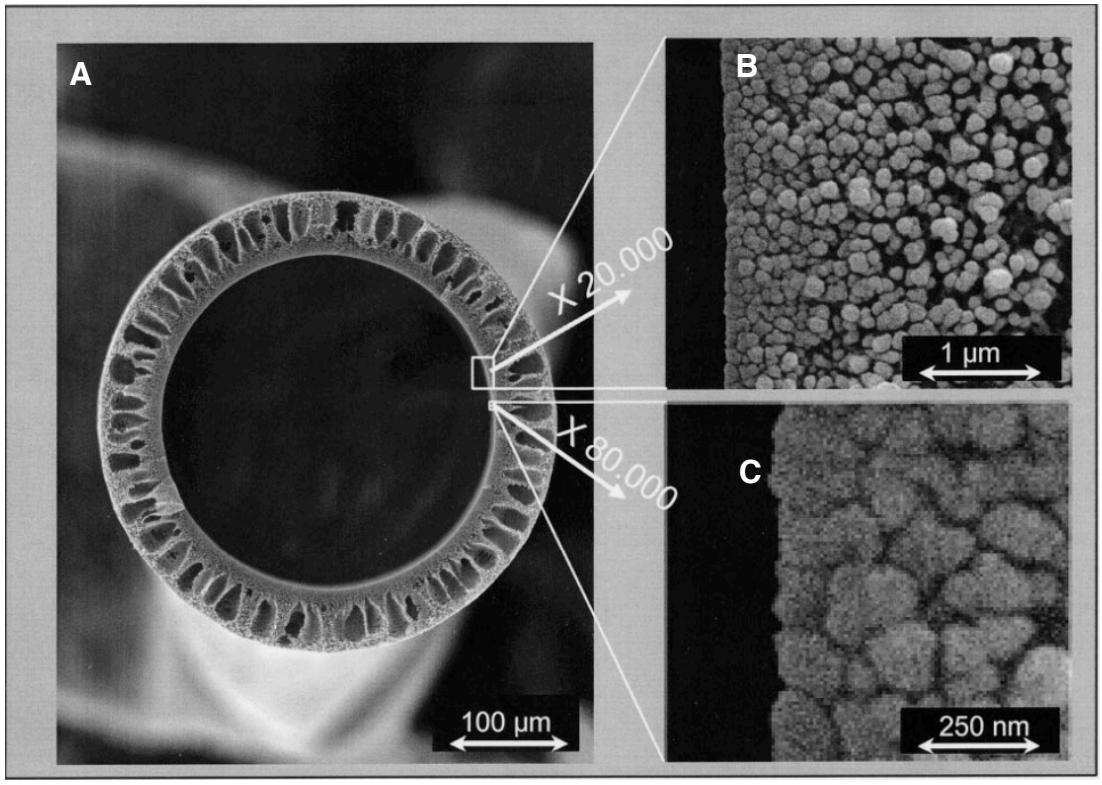

Figure 1-12: 3-layer structure of hollow fibre. *This image shows a cross-section from a high-flux membrane. 'A'- an overview of the structure, the diameter of the inner fibre is typically around 200 µm. The fibre has a thin first layer shown in 'C' which is typically <1 µm, it contains pores and this layer controls the diffusion properties of the membrane. The second layer shown in more detail in 'B' is 2-5 µm in thickness and provides support. The third sponge-like layer provides further support and is 40-45µm in thickness. Image adapted from Ronco et al 2003[490].*

1.23 Defining Membrane Characteristics

Membrane permeability is largely determined by pore size and pore size distribution. Several other features such as membrane wall thickness and hydrophilicity [322] also impact on diffusion characteristics. The hydraulic permeability of a given membrane is expressed at the ultrafiltration coefficient (Kuf). It is a measure of the ultrafiltration volume of a membrane in an in-vitro setting using animal blood with a controlled protein content and haematocrit

[323]. The measure is ml/hour/mmHg and is used to differentiate a high flux (>20ml/h/mmHg) from a low-flux membrane[324]. Solute clearance in a membrane is not directly related to the Kuf and will depend on the size and properties of the solute, particularly with larger molecules. The sieving coefficient (SC) quantifies the permeability of the membrane to a solute of a given molecular weight (MW) as a value between 0 and 1 where 1 implies full permeability and 0 implies no permeability. SC is most commonly measured using dextran solutions with compounds in a range of molecular weights [325]. SC is also used to classify dialysis membranes, in the case of high-flux membranes, the EUDIAL definition suggests an SC for β2-Microglobulin of >0.6[326]. Whilst the properties of membranes can be defined, their properties change significantly after contact with blood owing to adsorption of proteins onto the membrane and formation of a protein layer (known as membrane fouling) [327] [328]. This process is promoted by higher ultrafiltration rates which increase delivery of proteins to the membrane surface.

Two further terms to aid classification have also been proposed- these are molecular weight cut-off (MWCO) and molecular weight retention onset (MWRO). MWCO is defined as the highest MW solute where there SC is at least 0.1 and MWRO is at least 0.9. Figure 1-13 demonstrates the MWCO and MWRO of different classes of membrane.

Whilst haemodialysis membranes primarily provide clearance through diffusion, their relatively high water permeability also provides convective

clearance. There is a significant pressure differential in the blood compartment. At the proximal end where the pressure is higher ultrafiltration occurs alongside solutes through solvent drag which provides convective clearance in addition to diffusion. At the distal end, the pressure is significantly lower and drops below the hydrostatic pressure of the dialysate compartment such that dialysate flows into blood compartment [329] [330] [331]. This process of filtration and backfiltration (also known as internal filtration) results in no net fluid loss, the concentration of toxins flowing into the blood compartment is negligible and up to 3.5 litres of filtration can take place per hour [332]. The process is depicted in figure 1-14. Given that this process takes place, it is vital that the dialysate fluid is free form endotoxins and contaminants. The filtration volume can be increased by using a fibre with a smaller internal diameter thus creating a larger pressure differential along the length.

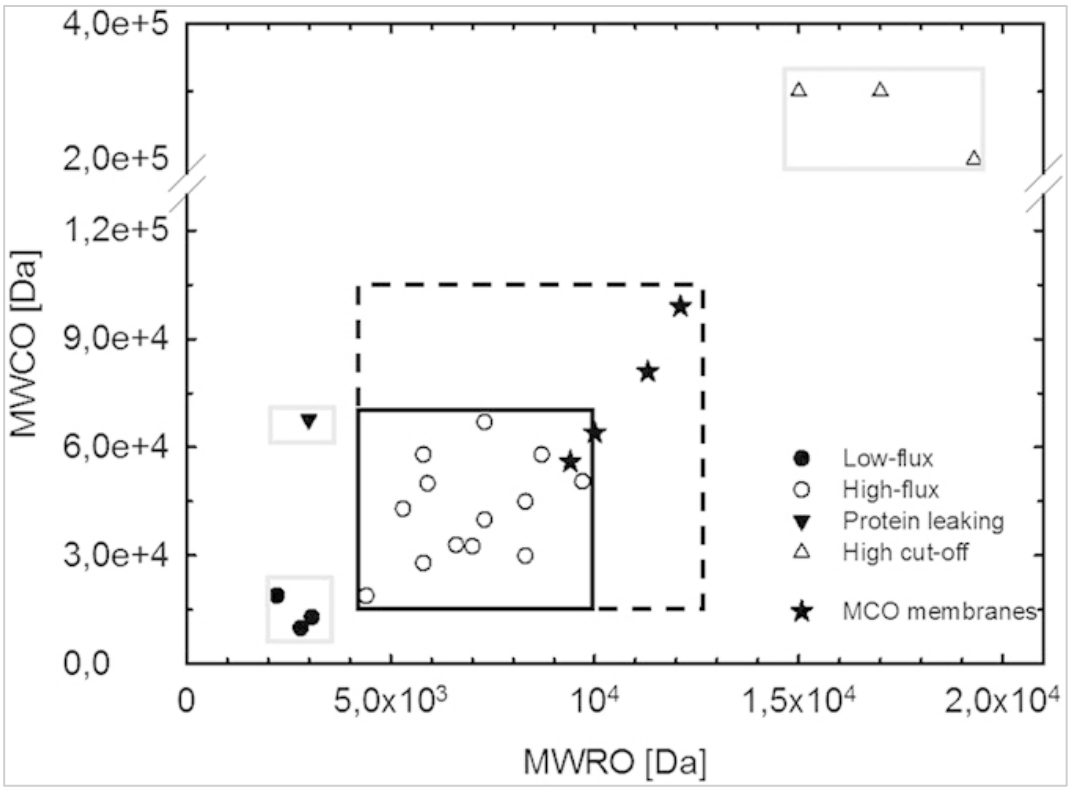

Figure 1-13: The molecular weight retention onset (MWRO) and molecular weight cut-off (MWCO) of different membranes within different membrane classes (low-flux, high-flux, protein leaking, high cut-off and medium cut-off (MCO). *The grey squares represent the boundaries of low-flux, high-flux and protein leaking membranes. The black square shows the boundaries of high-flux membranes and the dotted line shows the boundaries for medium cut-off membranes. Adapted from Boschettide-de-Fierro 2015* [333]

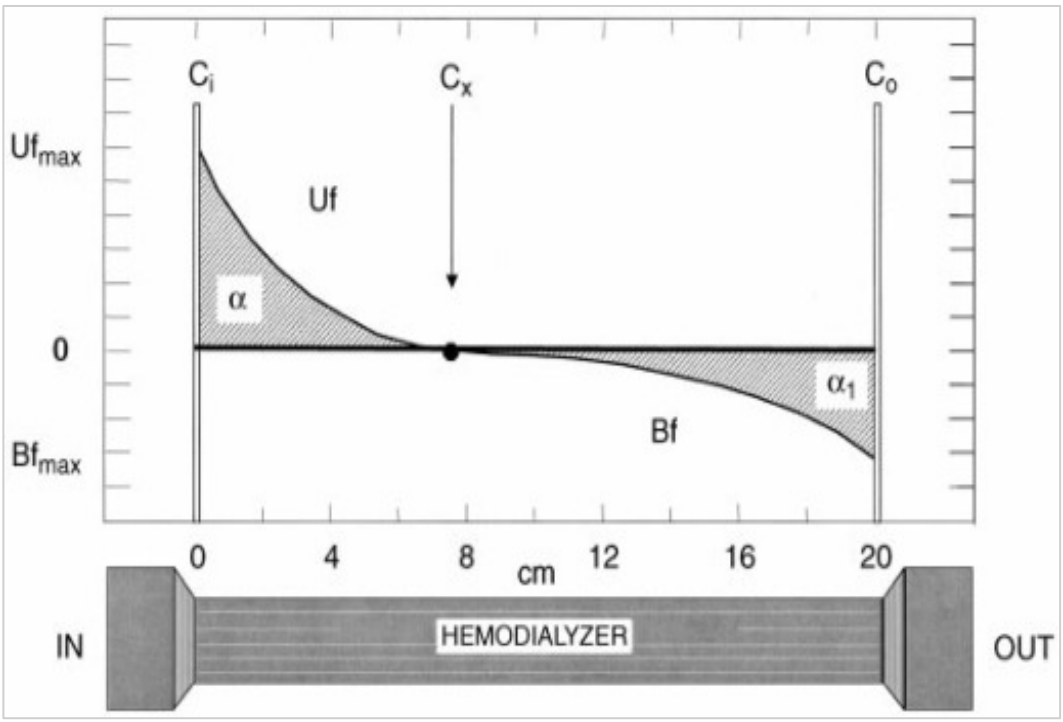

Figure 1-14:The process of "internal filtration" in high-flux haemodialysis membranes*. The ultrafiltration (Uf) and backfiltration (Bf) rates change across the length of the hollowfibre. The filtration volume (α) and backfiltration volume ($\alpha 1$) are equivalent resulting in zero net ultrafiltration. Adapted from Ronco et al 2000* [334].

1.24 Challenges in membrane design: Approximating the glomerular basement membrane

Current dialysis membranes that are in widespread use (high-flux) provide limited clearance of larger middle molecules (20-60kDa) and diffusive therapies also provide limited clearance of protein-bound toxins. This unmet need has focused research into developing technologies which can mimic the natural glomerular basement membrane more closely with the aim of providing toxin clearance that can match the kidney. High cut-off (HCO) membranes were designed with a larger pore radius (8-12nm vs 3.5-5.5nm in high-flux) [335]. The MWCO for the membrane is 50kDa however due to variation in pore size,

use of the membrane leads to significant albumin loss (up to 20g in a single 4-hour session[491]. Although treatment with this membrane provides effective clearance of larger molecules including cytokines [336] [337] and free light-chains[338], long term use is not feasible owing to the high protein loss [336]. Following on from this, a new class of medium-cut off (MCO) membranes has been developed. As depicted in figure 1-15, the MRWO and MWCO characteristics for the MCO membrane demonstrating enhanced clearance of larger MW molecules compared with high-flux but at values considerably lower than HCO membranes.

1.25 Medium Cut-Off Membranes

Medium cut-off (MCO) membranes have been developed to provide a MWCO close to that of albumin (65kDa) and with a narrow pore size distribution such that the MWRO and MWCO are close to each other and therefore the albumin loss is lowered whilst the clearance of larger molecules is maximised. The SC profile of the MCO membrane is demonstrated in figure 1-15 in red (labelled as HRO). Although the MWCO for the membrane is similar to a high-flux membrane, the MWRO is much higher at around 12kDa and this offers enhanced solute removal (in the range between the red and blue lines) the loss of albumin associated with HCO membranes (in green and with a MWCO around the size of albumin-68kDa).

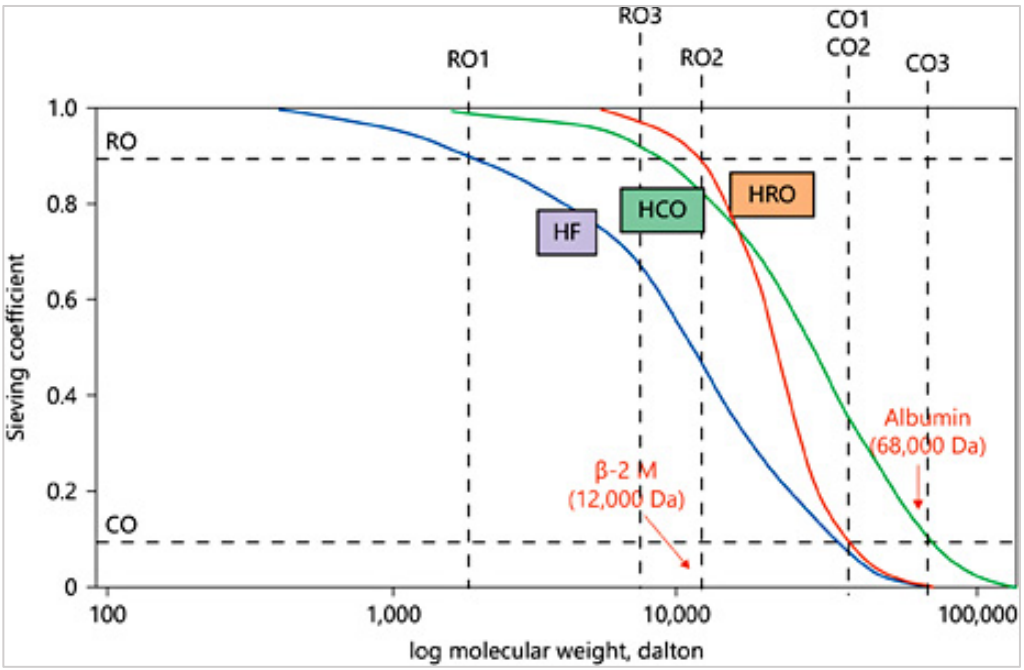

Figure 1-15: The sieving coefficient profiles of high flux (HF), medium cut-off (HRO) and high cut-off (HCO) membranes. *The molecular weight retention onset (MWRO) and molecular weight cut off (MWCO) for each membrane of a range of molecular weights is demonstrated. The profile for the HRO membrane is much steeper than then HF and HRO membrane. Image adapted from Ronco et al 2017*[339].

In terms of performance, MCO membranes out-perform high-flux membranes in terms of both reduction ratios and overall clearance for a number of larger middle molecules. They also out-perform HDF for molecules such as YKL-40 and α1-microglobulin. Performance details are outlined in figure 1-16 which shows the reduction ratios and overall clearances for a range of solutes with two types of MCO membranes (MCO AA and MCO) as well as HF and HDF.

Table 5. (A) Overall clearances and (B) reduction ratios of medium-sized and small molecules in treatments in study 2

	MCO AA HD	MCO BB HD	High-flux HD	HDF
(A) Overall clearance (mL/min)				
α1-microglobulin	3.3 (0.20)*	4.6 (0.20)*	0.4 (0.20)	1.3 (0.20)
Complement factor D	26.3 (1.13)*	32.8 (1.10)*	8.2 (1.10)	12.4 (1.10)
Myoglobin	58.7 (2.46)*	62.7 (2.39)*	19.9 (2.39)	35.6 (2.46)
β2-microglobulin	84.7 (3.18)**,***	84.3 (3.10)**,***	55.1 (3.10)	73.1 (3.18)
Creatinine	210.4 (8.73)	203.7 (8.31)	208.9 (8.31)	210.0 (8.31)
Phosphate	209.8 (11.29)	218.0 (10.71)	193.2 (10.71)	194.5 (10.71)
Urea	281.9 (11.97)	268.1 (11.32)	277.0 (11.32)	263.4 (11.32)
(B) Reduction ratio (%)				
YKL-40	63.6 (2.21)*	68.8 (2.21)*	29.8 (2.21)	44.8 (2.21)
α1-microglobulin	24.8 (8.97)***	30.1 (8.97)***	10.0 (8.97)	−8.9 (8.97)
Complement factor D	63.0 (1.73)*	66.7 (1.73)*	32.9 (1.73)	46.3 (1.73)
Myoglobin	67.9 (2.34)*	71.6 (2.34)*	37.2 (2.34)	59.3 (2.37)
β2-microglobulin	78.5 (1.32)**,****	78.9 (1.32)**	73.5 (1.32)	80.6 (1.33)
Creatinine	73.5 (1.45)	73.2 (1.45)	71.7 (1.45)	73.7 (1.45)
Phosphate	52.8 (2.13)	48.8 (2.13)	48.4 (2.13)	51.0 (2.13)
Urea	80.7 (1.33)*****	80.3 (1.33)****	79.4 (1.33)	81.6 (1.33)

Mean ± SD.
Comparisons are based on a mixed model with fixed effects of period and study dialyzer type, and the random effect of subject.
*P < 0.001 versus HD and HDF; **P < 0.001 versus HD; ***P < 0.01 versus HDF; ****P < 0.05 versus HDF; *****P < 0.05 versus HD.

Figure 1-16: Performance of two MCO membranes (MCO AA and MCO BB) compared with a high-flux membrane used in HD and a high-flux membrane used in HDF*. The treatment session was 4-5 hours long with a Qb of 400±50ml/min and a Qd of 500ml/min. HDF sessions were performed with a target convective volume of >23 litres and a Qd of 700ml/min. Table and study data from Kirsch et al 2016[40].*

Albumin loss was assessed in the same study [40] and was 2.9g (rang 1.9-3.5g) for MCO membrane AA (Theranova) compared with 0.2g for HDF (0.2-0.2). As a comparator, albumin losses of around 4g per 24 hours are seen in patients treated with continuous ambulatory peritoneal dialysis. Other studies have suggested a higher albumin loss in HDF[340] in the region of 3.99 +/- 1.81 g per session[341] in one study as an example.

Clinical studies investigating the effects of haemodialysis with an MCO membrane are in their infancy. In a crossover study of 48 patients, MCO treatment resulted in a significant reduction in the gene expression of TNF-α and IL-6 in peripheral blood mononuclear cells after 4 weeks [342].

A prospective crossover study involving 20 patients who switched from HFHD to MCO treatment for 3 months demonstrated a median reduction in serum albumin of -0.45g/l (IQR -0.575 to -0.05) however median serum albumin concentrations were maintained above 35g/l with no requirement for intravenous albumin administration. The same study saw a reduction in IL-6 levels in the MCO group but no change in TNF-α. A change in serum albumin of a similar magnitude has also been echoed in a much larger Colombian registry study with data for 638 patients[492].

A small retrospective study of 10 patients switched from HDF to MCO treatment showed no difference in the reduction ratio or clearances of myoglobin or ß2-microglobulin comparing during a 6-month period with a previous 6-month period [343]. There was also no difference in albumin concentration between the two modalities in this study.

A prospective randomised controlled-study with a total of 50 patients randomised to either remain on high-flux haemodialysis or switch to MCO showed no change in the quality of life SF-36 score at 12 weeks despite improved clearance of kappa and lambda light chains [344]. Whilst there was a separation between the two groups in two of the SF-36 domains (physical functioning and role-physical), the comparison between the score at 12 weeks and baseline within the MCO group is not reported. Multivariate linear regression suggested a membrane effect on physical functioning, role-physical, pruritis distribution and sleep disturbance.

In comparison to HDF, a study involving 22 patients demonstrated no difference in the reduction ratios of a wide range of solutes, including albumin suggesting similar efficacy in clearance between the two modalities [345].

With numerous further ongoing studies worldwide exploring the clinical effects of MCO haemodialysis, it is likely that we will determine whether this therapy can deliver improved clinical outcomes. With clearance that matches and possibly exceeds HDF but with ease of implementation and no requirement for the infusion of high volumes of ultrapure replacement fluid, this advancement in haemodialysis membrane technology appears promising.

Introduction Part D: Conclusion, Hypothesis & Aims

1.26 Conclusion

Outcomes for patients with advanced CKD remain poor with a considerable excess cardiovascular mortality. Patients experience a uraemic syndrome which is characterised by the accumulation of uraemic toxins, a high burden of inflammation and malnutrition. The vascular endothelium plays a central role in cardiovascular health. Endothelial activation, inflammation, uraemic toxins and cardiovascular outcomes are closely linked. Endothelial microvesicles have been identified as a biomarker of endothelial activation. Their concentrations correlate with cardiovascular outcomes in a range of inflammatory conditions including CKD and patients on haemodialysis. EMV's are too large to pass through dialysis membranes however their concentration is altered by dialysis technique, indicating a downstream effect of a change in membrane characteristics.

Haemodialysis offers life-sustaining treatment for patients with advanced CKD and technology is evolving. High-volume HDF enables significant clearance of a range of uraemic solutes but the benefit appears to be associated with the delivery of high convective volumes. HDF, alongside the current leading dialysis modality, high-flux haemodialysis, do not provide clearance of all uraemic toxins. Larger middle molecules (>15kDa) and protein-bound toxins are not removed. Medium cut-off dialysis membranes are a novel class of membrane with an increased pore size and reduced pore size variability compared with high-flux membranes. Clearance of larger middle molecules exceeds HFHD and matches or even exceeds (for some molecules) HDF.

Although the clearance characteristics of the treatment have been defined, the clinical benefits are only beginning to emerge.

1.27 Hypothesis

Treatment with medium cut-off haemodialysis will have a more favourable impact on markers of vascular endothelial health compared with haemodiafiltration, the current standard of care.

The clinical trial presented in this thesis has been designed as a randomised controlled study to compare MCO haemodialysis (a novel dialysis intervention), with haemodiafiltration.

Chapter 2: Methodology

2.1 Introduction

All methodology is described within this chapter and referred to in the results chapters. This study was designed and delivered in collaboration with a team at Manchester Metropolitan University (MMU). All laboratory work described here was performed by the MMU team. I have a good understanding of the laboratory methods used and also attended the laboratory to observe the techniques first hand.

2.2 Study Methods & Design

The MoDaL study (A Randomised Feasibility Study Investigating the Effect of Medium Cut-Off Haemodialysis on Markers of Vascular Health Compared With On-Line Haemodiafiltration) was a single-centre, open-label, interventional, randomised controlled feasibility study comparing haemodialysis treatment using a medium-cut off (MCO) membrane with haemodiafiltration over a 6-month period. The aim of the study was to explore the effects of expanded solute removal using the MCO haemodialysis membrane on a number of biomarkers, clinical outcome measures and patient-reported outcome measures. The study was funded by Baxter Healthcare through an Investigator Initiated (IIR) Research grant. The study was sponsored by Manchester NHS Foundation Trust (Manchester, UK) and took place within the same institution.

2.3 Objectives

To determine the effect of MCO HD treatment compared with HDF treatment on:

1. **Plasma endothelial microvesicle (EMV) concentration at 6 months**

2. **Markers of endothelial activation, inflammation and angiogenesis**

A panel of biomarkers were measured at 0, 12 and 24 weeks:

i. Von Willebrand factor (VWF)

ii. intracellular adhesion molecule (ICAM)

iii. vascular cell adhesion molecule-1 (VCAM-1)

iv. E-selectin

v. P-selectin

vi. Interleukin-6 (IL-6)

vii. Interleukin-8 (IL-8)

viii. Interleukein-10 (IL-10)

ix. Tumour Necrosis Factor-α (TNFα)

x. Vascular endothelial Growth Factor (VEGF)

xi. Vascular endothelial Growth Factor-C (VEGF-C)

xii. Vascular endothelial Growth Factor Receptor-1 (VEGFR-1)

3. **Middle molecule concentration**

The following serum middle molecule concentrations were measured at 0, 12 and 24 weeks:

I. α1-microglobulin (mg/l)

II. Leptin (ng/ml)

III. ß2-microglobulin(mg/l)

IV. YKL-40 (Chitinase-3-likeprotein 1) (ng/ml)

V. Pentraxin-3 (ng/ml)

4. **Serum albumin concentration**

    I. Measurement at 0 and 24 weeks

5. **Symptom burden**

    I. Palliative care Outcome Scale (POS-S) Renal score at 0 and 24 weeks

6. **Dialysis recover time**

    I. Self-reported dialysis recovery time at 0, 12 and 24 weeks (select from <2 hours, 2-6 hours, 7-12 hours or >12 hours)

7. **Self-reported fatigue**

    I. Chalder fatigue score at 0, 12 and 24 weeks

8. **Pulse wave analysis parameters**

    I. Measurement at 0 and 24 weeks

9. **Bioelectrical impedance parameters**

    I. Measurement at 0, 6, 12, 18 and 24 weeks

10. **In-vitro endothelial cell function**

    I. Angiogenesis

    II. Cell migration

    III. Wound healing

11. **Indirect effects: anaemia, residual renal function, bone mineral disorder parameters and ultrafiltration management**

    I. Measurement of routine dialysis blood tests at baseline and 24 weeks (Haemoglobin (Hb), phosphate (PO4), adjusted calcium

(aCA), intact parathyroid hormone (PTH) and dialysis adequacy (single pool Kt/V)

II. Measurement of ultrafiltration (UF) volume (average UF volume across 3 sessions in a single week each month of the study)

III. Measurement of urine volume and urea clearance at 0 and 24 weeks

12. All-cause mortality

13. Morbidity: hospitalisations

2.4 Inclusion Criteria

1. Patients aged 18 years or older with the ability to consent
2. Currently receiving thrice-weekly in-centre HDF
3. Established on treatment for a minimum of 12 weeks

2.5 Exclusion Criteria

1. Patients with a planned live donor transplant (within 6 months)
2. A planned switch in dialysis modality
3. A clinical prognosis of less than 6 months

2.6 Ethics, Consent & Safety

Full ethical approval for this study was received from the NHS Health Research Authority (REC Reference: 18/NW/0169) and approval was also received from the study and site sponsor. All participants provided written

consent prior to randomisation. A safety questionnaire was completed by all participants in the study every 6 weeks to record any hospitalisation episodes and any symptoms that could be attributed to the treatment.

2.7 Study Overview

An overview of the study is presented in table 2-1. Following screening and treatment optimisation, participants were randomised to either continue on HDF treatment or switch to haemodialysis using an MCO membrane. Study visits occurred at baseline and then every 6 weeks until the end of the 24-week study period.

Screening	Screening- patients at two haemodialysis units were screened for eligibility using inclusion and exclusion criteria. Eligible patients were approached.				
Optimisation	Patients who consented to take part in the study entered a treatment optimisation phase with target Kt/V >1.2 and target blood flow rate >300ml/min.				
Randomisation	Participants randomised 1:1 using a simple web-based randomisation tool				
STUDY VISITS	**BLOOD SAMPLES**	**PROM QUESTIONNAIRE**	**BCM MEASUREMENT**	**URINE COLLLECTION**	**PWV**
Baseline	✓	✓	✓	✓	✓
6 weeks			✓		
12 weeks	✓	✓	✓		
18 weeks			✓		
24 weeks	✓	✓	✓	✓	✓

Table 2-1: Outline of the MoDal Study. *Blood samples were collected pre-dialysis at baseline, 12 weeks and at 24 weeks. A Patient Reported Outcome Measure (PROM) questionnaire was completed by all participants at baseline, 12 weeks and 24 weeks. A Body Composition Monitor (BCM) bioimpedance measurement was taken at baseline and then every 6 weeks. A 24-hour urine collection was taken at the start and end of the study. A Pulse Wave Velocity (PWV) measurement was taken at the start and end of the study.*

2.8 Recruitment & Study Population

Participants were recruited from two haemodialysis units which were part of Manchester NHS Foundation Trust. All haemodialysis patients at the unit were treated with on-line haemodiafiltration. Full eligibility was assessed at

screening. Following recruitment and prior to randomisation, patients entered an optimisation phase to aim for a target blood flow rate greater than 300 ml/minute and a single pool Kt/V>1.2.

2.8.1 Healthy Controls

Healthy volunteers were recruited via posters and word-of-mouth via Manchester Metropolitan University. All volunteers were not known to have any significant health conditions including chronic kidney disease. A total of 16 healthy controls were recruited and a blood sample was collected at a single timepoint. Samples collected were analysed using the same techniques as described in section 2.12. Only a proportion of the samples (selected at random) were used as healthy controls in the experiments.

2.9 Randomisation

Participants were randomised to either continue HDF treatment or switch to haemodialysis with an MCO membrane for 6 months. A simple randomisation technique was used through an internet-based randomisation service (https://www.sealedenvelope.com). A permuted block technique was used to ensure equal numbers in both groups.

2.10 Sample Size

The study had a target sample size of 64 participants (32 in each group). This sample size was with the expectation of a 20% drop-out. A power calculation based on detecting a difference in EMV concentration between the two groups contributed to determining the sample size. This power calculation was taken with caution due to the significant differences between laboratories in measuring EMV and this study was designed as a feasibility study rather than a pilot study. The sample size was similar to other clinical studies investigating the MCO membranes at the time.

2.11 Dialysis Procedures

Patients received thrice weekly treatment using a Fresenius 5008 CorDiax® dialysis device (Fresenius Medical Care, Bad Homburg, Germany) with a minimum treatment time of 240 minutes. Patients who remained on HDF used a Fresenius FX CorDiax® HDF membrane (FX 600, 800 or 1000) either in pre-dilution or post-dilution (the majority) mode. Patients randomised to the MCO group used the Baxter Theranova 500 or 600 MCO membrane (Gambro Dialysatoren GmbH, Hechingen, Germany, a subsidiary of Baxter International Inc.) with the dialysis machine in haemodialysis mode.

2.12 Blood sampling

Blood samples were drawn from the vascular access of each participant prior to starting dialysis treatment and prior to administration of any anticoagulants.

All samples were obtained on the 2nd dialysis session of the week. Samples were collected into S-monovette® sample tubes (Sartedt AG, Nümbrecht, Germany) with some containing trisodium citrate and others containing a silicate clotting activator (20.7ml of blood in total at baseline, 12 weeks and 24 weeks).

2.13 Laboratory techniques

All of the laboratory work was performed by the team at Manchester Metropolitan University (MMU). The techniques that they used are described here.

2.13.1 Endothelial Microvesicle Analysis

Platelet-poor plasma (PPP) was obtained using two-step centrifugation where samples were centrifuged in a Sigma 3-16k centrifuge (11180 rotor) (Sigma-Aldrich) at 1,700g for 10 minutes at 4˚C, harvested and then centrifuged again in a Sigma 3-16K centrifuge (12131 H rotor) at 20,000g for 10 minutes at 4˚C. Samples were stored at -80 ˚C and all samples were analysed at the end of the study in a single run.

For analysis, counting beads 0.22 µm to 1.35µm (Spherotech Inc, Libertyville, Illinois, USA) in size were added to 20µl of PPP and incubated for 10 minutes with 1µl of PE-mouse anti-human CD-144 antibody (BD Pharmingen, UK). Flow cytometry was performed using a CyAn ADP flow cytometer

(Beckman Coulter, Brea, CA, USA) with Summit V4.3 software. Analysis was stopped once 1000 beads were counted. Gates were set to exclude artefact and beads. EMV events were defined as those positive for CD-144. Absolute EMV counts per microlitre (µl) of plasma were calculated.

2.13.2 Biomarkers (Middle Molecule, Endothelial Activation, Inflammation & Angiogenesis

Serum samples were centrifuged using a Sigma 3-16K centrifuge (11180 rotor) at 1,500g for 10 minutes at 4˚C. The serum was placed into Eppendorf's and stored at -80 ˚C until processing at the end of the study. A Human Magnetic Luminex® Assay was used (R&D Systems, USA) to quantify all of the biomarkers (α1-microglobulin, ß2-microglobulin, leptin, YKL-40 (Chitinase-3-likeprotein 1), pentraxin-3, von Willebrand factor, ICAM, VCAM-1, e-selectin, p-selectin, VEGF, VEGF-C, VEGF-R1, IL-6, IL-8, IL-10 & TNF-α).

2.13.3 Cell culture

Whilst there are several published studies that have assessed the effect of uraemic serum on cell viability [346] [347] [348] [349] [350] [351] [352] [353] [354] [355], to our knowledge, none have used AlamarBlue to assess viability (although a similar reagent, PrestoBlue® has been used [356]. AlamarBlue® has been used for over 20 years[357] and applied in numerous fields including cancer[358,359] and

drug development[360-362]. The assay is robust having been used in a variety of animal cell lines[363 364 365 366] and bacteria, yeast and fungal species too[367-369]. It is widely regarded as a reliable marker of cell viability. As the reagent used in the assay is non-toxic to cells, it has the advantage over other viability assays of allowing the re-use of cells and the assessment of cell viability over time. The assay does not measure cell death but measures cell metabolic activity and this must be considered when interpreting results.

Angiogenesis, the formation of new blood vessels from pre-existing vessels, is an essential physiological process. It is tightly regulate by a number of molecules including vascular endothelial growth factor (VEGF), transforming growth factors (TGF), tumour necrosis factor-α (TNF-α) and interleukins[370,371]. Given the central role of this process in wound repair, inflammation and cancer, a number of angiogenesis bioassays have been developed. Angiogenesis tube formation assays model the early reorganisation stage of angiogenesis and has been in use since 1988[372] in a wide range of settings. In CKD, it has been successfully used to assess angiogenesis and specifically the effect of uraemic toxins [373-375 376]. The assay is frequently performed alongside other markers of endothelial cell function such as a wound closure assay. This assay assessed cell migration by measuring the migration rate of cells in microscopy images after a scratch has been made in a monolayer of cells. The pro-migratory and anti-migratory effects of compounds can therefore be assessed. Like the other measures of endothelial cell function

used in this study, the technique has been well validated and used specifically in the setting of CKD and uraemic serum [377] [378] [379].

2.13.3.1 Cell Culture Technique

Human umbilical vein endothelial cells' (HUVEC's) were cultured in Endothelial Cell Growth Medium MV 2 (Promocell, UK) and supplemented with foetal calf serum (FCS) (0.05 ml / ml), Epidermal Growth Factor (5 ng / ml), Fibroblast Growth Factor (10 ng / ml), Insulin-like Growth Factor (20 ng / ml), Vascular Endothelial Growth Factor 165 (0.5 ng / ml), Ascorbic Acid (1 μg / ml) and Hydrocortisone (0.2 μg / ml) (Promocell, UK). Once 80-90% confluent, cells were split 1:2 or 1:3 via trypsinisation. HUVEC's were washed with phosphate-buffered saline (PBS) and then incubated with 4 mL trypsin for 5 minutes at 37 ^{o}C. Once fully detached, the cells were centrifuged at 400 x g for 7 minutes at room temperature. After removal of the supernatant, the cells were resuspended and seeded into culture flasks with media.

2.13.3.2 Cell Viability Assay (Alamar Blue)

Cell viability was assessed using an AlamarBlue® assay. AlamarBlue®, also known as resazurin, is a non-toxic, water soluble substance which is able to permeate cell membranes[357]. It changes colour from a non-fluorescent blue to a highly fluorescent red colour if the environment changes from an oxidised state to a reduced state (figure 2-1). As cells grow, innate metabolic activity

maintains a reduced environment whereas inhibition of cell growth maintains an oxidised environment. This change in fluorescence can be easily measured using a fluorescence microplate reader and assessed either as absorbance at 570nm and 600nm or as fluorescence at 530-560nm excitation wavelength and 590nm emission wavelength (see figure 2-2).

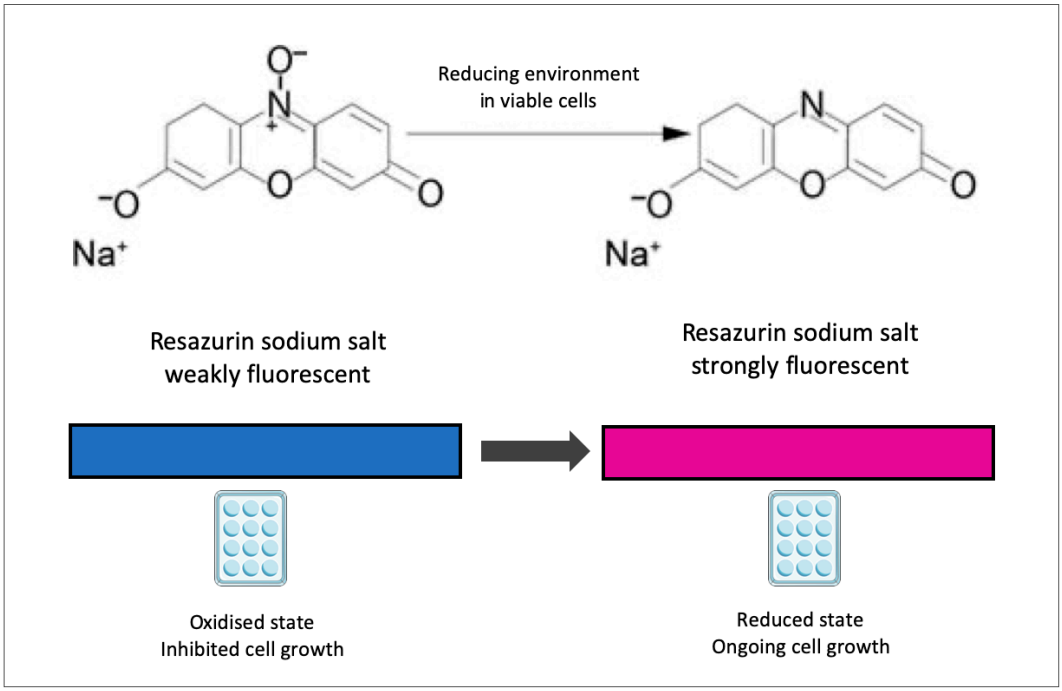

Figure 2-1: Overview of colour change associated with AlamarBlue® when added to cells

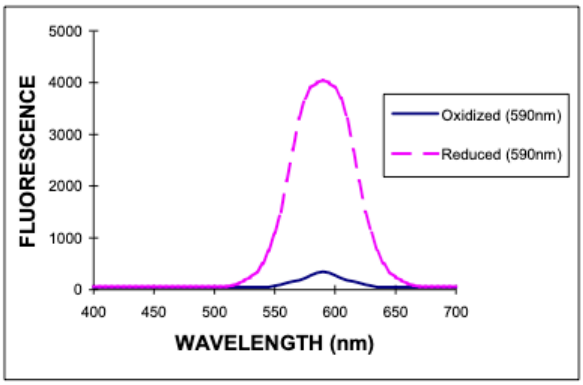

Figure 1a. alamarBlue® Fluorescence Emission Spectra

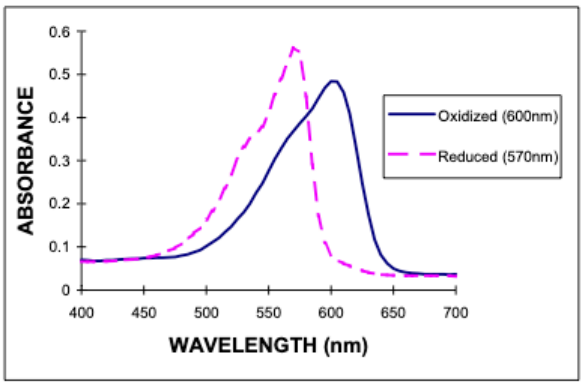

Figure 1b. alamarBlue® Absorbance

Figure 2-2: Fluorescence (above) and absorbance (below) profile of AlamarBlue in both oxidised (indicating inhibited cell growth) and reduced state (indicating ongoing cell growth). *Graphs adapted from ThermoFisher alamarBlue® manual*[β80].

HUVEC's were harvested from confluent T75 flasks, washed in PBS, trypsinised, resuspended in full media and plated at 1x10^4 cells/well, 100 μl/well on a 96 well plate, before being left to incubate at 37 ^{o}C for 24 hours to grow to confluency. Cells were then treated with 5% patient serum or 5% FCS (positive control) and 0.1% TritonX100 (negative control) in triplicate. Cells were incubated for 24 hours at 37^{o}C and 5% CO_2. 10 μl of Alamar Blue (Thermo Fischer Scientific) was added to the wells and the fluorescence at 530/590 nm was recorded at 4 and 8 hours on a Synergy HT micro-plate

reader (BioTek). Samples were run in triplicate and the average fluorescence value was taken.

2.13.3.3 Cell Migration Assay

HUVEC's were harvested from confluent T75 flasks, washed in PBS, trypsinised, resuspended in media and plated at $1x10^5$ cells/well, 1 mL/well on a 12 well plate. Cells were left to incubate at 37ºC 5% CO_2 until confluent, they were then be scratched with a sterile p1000 pipette tip, washed twice in PBS and incubated in their respective treatments (1 ml of 5% patient serum, healthy volunteer serum or FCS in triplicate). Scratches were imaged at 0, 4 and 24 hours at 4x magnification. Image analysis was by Image J Software (imagej.nih.gov). Samples were run in triplicate and the average scratch area value was taken.

2.13.3.4 Angiogenesis Assay

Well plates were coated with 30 μl of corning matrigel membrane matrix (ThermoFisher Scientific, Basingstoke, UK) and incubated for 30 minutes at 37°C. HUVEC's were resuspended in in 50 μl media treated with either 5% patient serum or 5% FBS (positive control) and plated at 9000 cells/well. Plates were incubated at 37°C and observed using a Zeiss PrimoVert microscope (Zeiss AG, Feldbach, Switzerland) after 5 and 24 hours. Image analysis was performed by Image J Software (imagej.nih.gov).

2.14 Bioelectrical Impedance Measurements

2.14.1 Background

Bioimpedance spectroscopy (BIS) provides a simple and non-invasive method of assessing body composition and devices such as the Body Composition Monitor (BCM, Fresenius Medical Care, Germany) have been validated in a large cohort of healthy volunteers[381,382] and haemodialysis patients[382-384]. BIS estimates body composition by passing and detecting alternating electrical currents through the body using two pairs of electrodes[385]. At low frequency, the current passes almost exclusively through the extracellular space and at a high frequency it passes though both the intracellular and extracellular spaces (due to the characteristics of the plasma membrane). Impedance is calculated from the sum of resistance and reactance, where resistance is the opposition of a conductor to the current and reactance is the opposition to the current from the storage effects of cell membranes, tissue interfaces and structural features[386,387]. The device uses 50 frequencies spaced between 5khz and 1MHz and uses a mixture of equations (the Cole-Cole plot and Hanai formulae[382]) to determine electrical resistance in the different body compartments. The device calculates the body composition as three compartments: overhydration, lean tissue and adipose tissue based on the calculated extracellular water (ECW) and intracellular water (ICW) (see figure 2-3).

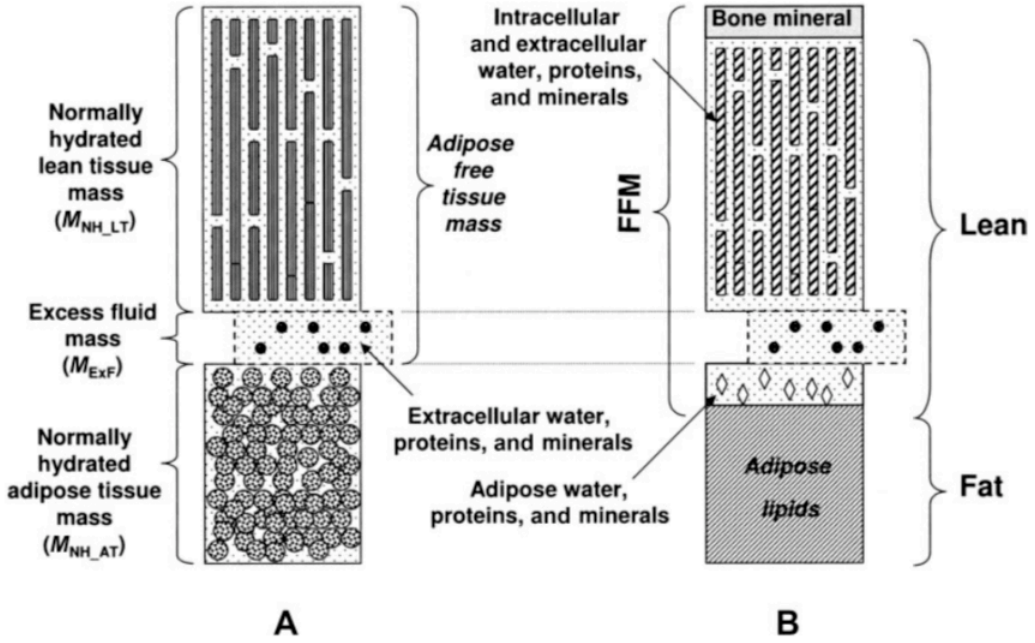

Figure 2-3: New 3-compartment mode of body composition*. 3 compartments (A) consist of normally hydrated adipose tissue mass, excel fluid mass and normally hydrated adipose tissue mass. Standard measures of body composition represented in B- lean mass, fat-free mass (FFM) and fat mass. Imaged adapted from Chamney et al* [388].

The device reports the total body water (TBW) with the proportion of water that is intracellular and extracellular. It also reports the estimated weight of fat tissue and lean tissue and these are reported as an index adjusted for body surface area. Studies that have utilised this device have demonstrated that Lean Tissue Index (LTI) predicts mortality in both CKD 4 and 5 (non-dialysis) [389] and in those on haemodialysis[390-394]. Low LTI is associated with a higher inflammatory burden (ie. association with CRP[391] and IL-6[391,395]. In a longitudinal study, a fall in LTI has been associated with older age, diabetes, male sex, high baseline LTI and low baseline FTI[159]. The same study has also demonstrated that despite an increase in BMI in the 12 months after commencing haemodialysis, there is a significant change in body composition

whereby FTI increases and LTI decreases [159] indicating poorer nutritional indices. Overhydration is associated with increased mortality[392,394,396] and is associated with a low Lean Tissue Index (LTI), atherosclerosis and a higher inflammatory burden[397].

2.14.2 BIS measurement technique

Bioimpedance Spectroscopy (BIS) took place at baseline and every 6 weeks during the study using the Body Composition Monitor(BCM) [388](Fresenius Medical Care, Germany). Participants completing the study had a total of 5 measurement. Measurements took place just before commencement of the 2nd dialysis session of the week (ie. a pre-dialysis measurement). Where possible, participants were placed in the supine position for 5 minutes and the skin was prepared using alcohol gel allowing before placing pairs of electrodes on the foot and wrist (avoiding the same side as any functioning AV fistulae). All jewellery was removed and care was taken to avoid any direct contact between the upper arms and the thorax. Measurements were repeated if any issues were noted with the on-screen Cole-Cole plot or measurement quality. Patients with lower limb amputations were excluded from BCM measurement. Ultrafiltration (UF) volume was recorded from the patient's haemodialysis record.

2.15 Pulse Wave Analysis

Pulse wave analysis measurements were performed at 0 and 24 weeks using a Mobil-O-Graph NG device (IEM, Stolberg, Germany). The device is an oscillometric ambulatory blood pressure (BP) monitor which has been validated[398,399] to provide an estimate of aortic and brachial BP, indices of wave reflection and pulse-wave velocity using specialist software. After measuring brachial BP, the device re-inflates (at diastolic blood pressure) and then records brachial pulse waves for 10 seconds using a high-fidelity pressure sensor.

2.16 Urine Collections

Inter-dialytic urine collections took place at the start and end of the study for participants who pass urine. A serum urea was taken at the start and end of the urine collection in order to calculate urea clearance and estimate residual kidney function.

2.17 Patient Reported Outcome Measures (PROMs)

A multiple-choice questionnaire was created to be completed during the mid-week dialysis session at baseline, 12 weeks and 24 weeks (study end). The questionnaire consisted of a symptom burden score (POS-S Renal), a fatigue questionnaire (Chalder Fatigue Score) and a question assessing dialysis recovery time (appendix 8) The paper questionnaire was self-completed by participants or completed with the assistance of a single research nurse during

their treatment session. In some cases, nursing staff verbally translated the questions into other languages.

The POS-S Renal consists of 17 symptoms and is composed of the 15 symptoms featured on the Palliative Outcome Scale (POS) [400] with the addition of two further symptoms common in CKD (itch and restless legs) [48]. The instrument (IPOS which is very similar to the POS-S) has been recently validated in a cohort of 81 CKD patients [401] (65 of these on haemodialysis) and it was shown to demonstrate both good validity and reliability. This questionnaire has been adopted by the UK Renal Registry and incorporated into a national survey (Your Health Survey) [402] to collect national PROM data in CKD patients.

The Chalder Fatigue Score [403] is an 11-question tool based on a 4-item Likert scale. It offers assessment of both physical and mental fatigue, it has good psychometric properties in HD patients [404] with good clinical validity [405] and internal consistency [404] [405] (how well items in a scale measure a single underlying dimension). CFS scores have been shown to correlate with both the Hospital Anxiety and Depression Scale (HADS) [406] and other fatigue measures such as the Modified Fatigue Impact Scale (MFIS) [406].

The question “How long does it take you to feel normal after a dialysis session?” with the response options being <2 hours, 2-6 hours, 7-12 hours or >12 hours was incorporated into the questionnaire. A very similar question

with identical response options has been validated[407] and used in 6040 haemodialysis patients in the Dialysis Outcomes and Practice Patterns Study (DOPPS) [408].

2.18 Statistical Analysis

Categorical and demographic data is presented as frequencies and percentages. All continuous data has been assessed for normality using a Shapiro-Wilk test. Normally distributed continuous data is presented as a mean with standard deviation (SD) and skewed data is presented as a median with an upper and lower quartile (Q1, Q3). Comparison between groups has been performed using a Pearson's Chi^2 Test for categorical data, a Student's t test for continuous data with a normal distribution or a Mann-Whitney U test for skewed continuous data. For comparison of 3 timepoints within a single group, a one-way ANOVA test has been used. For comparison of change at 24 weeks from baseline between the two groups for the primary and secondary outcome measures, a multiple regression model has been used. Correlation analysis has been performed using Pearson's correlation coefficient. Univariate and multiple regression have been used to further assess the relationship between variables. Statistical analysis was performed using IBM SPSS Statistics, Version 25 (IBM Corp., USA).

Chapter 3: Results: The Effect of Medium Cut-Off Haemodialysis on Biomarkers of Vascular Endothelial Health

3.1 Introduction

Medium cut-off (MCO) haemodialysis membranes are a recent advancement in dialysis technology. This treatment matches or even exceeds the middle molecule clearance of HDF[40,339] and performance is maintained at lower blood flow rates without the need for high convective volumes[39]. Several pro-inflammatory cytokines have been identified within the range of molecules [40,89,90], including TNF-α (17kDa) and IL-6 (24.5kDa) [409]. The clinical and biological benefit of MCO haemodialysis is largely unknown.

This chapter presents the results of the MoDal study, a feasibility study exploring effect of MCO treatment compared with HDF treatment, with a focus on markers of endothelial activation, middle molecules, inflammation and angiogenesis.

3.2 Methods

The full methodology and statistical techniques for this study are presented in chapter 2.

3.2.1 Patients

A total of 69 patients were screened and consented. Prior to randomisation, 6 patients were excluded (2 died, 1 was noted to have a treatment frequency

of 4x weekly, 1 due to participation in competing study, 1 transplanted, 1 moved to another HD unit).

63 patients were randomised using a simple web-based randomisation tool (www.sealedenvelope.com). 32 patients were randomised to switch their treatment from HDF to HD with an MCO haemodialysis membrane and the remaining 31 patients continued with HDF treatment. 50 patients completed the full 6-month study period. Reasons for drop-out are shown in figure 3-1.

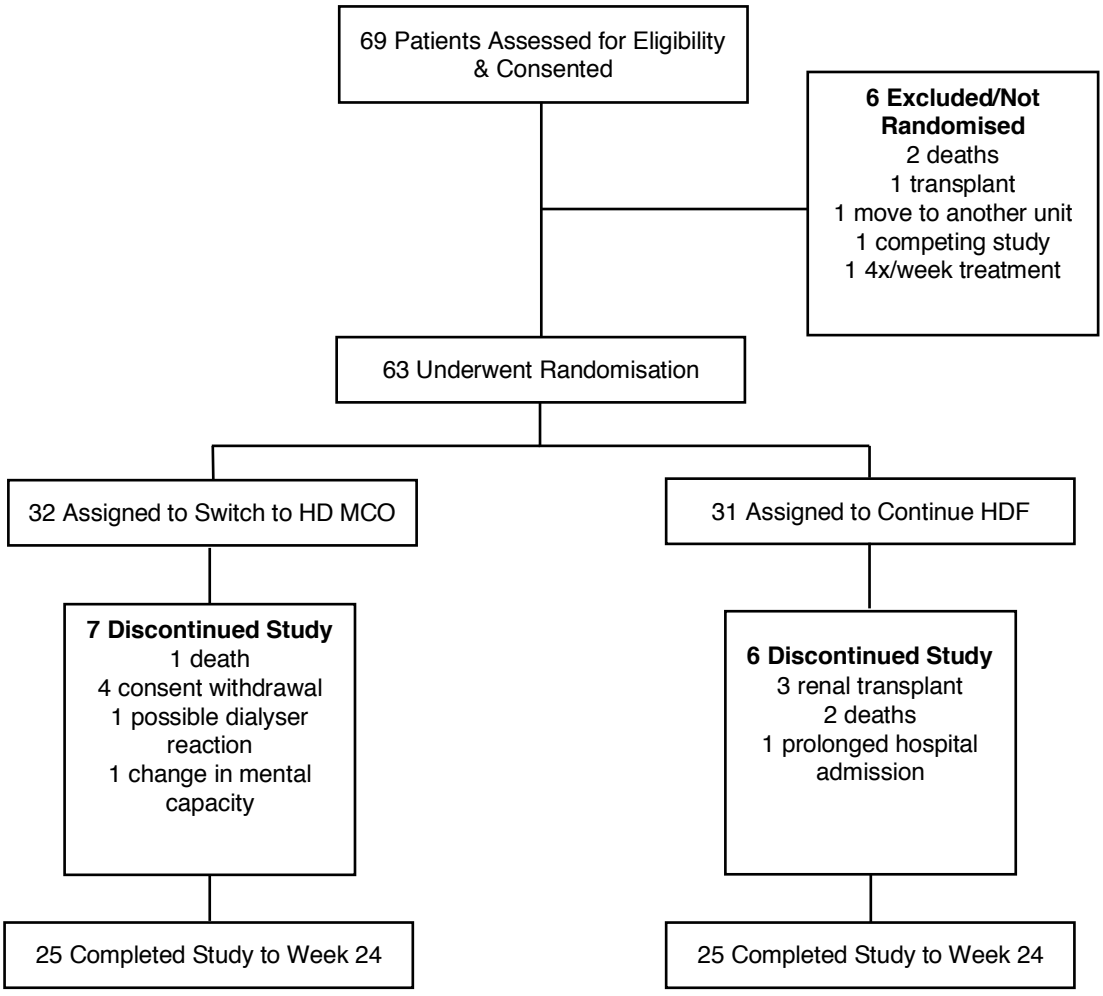

Figure 3-1: Flowchart of patient recruitment and drop-out during 24-week study period

3.2.2 Healthy Controls

A total of 16 health volunteers were recruited by the collaborative team at Manchester Metropolitan University. Blood samples were taken at the start of the study at a single timepoint. 7 of the samples were selected at random and the samples were analysed with the samples from the main MoDal study using the same techniques described in the methodology section (Chapter 2). The median age of healthy controls was 46 (34-53.5) and 4 (57%) were male.

3.2.3 Follow-up

During the 24-week study period, participants had a study visit at 0, 6, 12, 18 and 24 weeks. This is summarised in section 2.7 of chapter 2.

3.3 Results

3.3.1 Baseline Demographics

Baseline characteristics of all participants who were randomised are shown in table 3-1. Significance tests of baseline differences between the two groups was not performed in keeping with CONOSRT (CONsolidated Standards Of Reporting Trials) 2010 guidelines[410].

Variable	All (n = 50)	HDF (n = 25)	MCO (n = 25)
Age	65 (53.25, 75.75)	71 (60, 80)	59 (50, 72)
Male Sex	36 (72%)	17 (68%)	19 (76%)
Ethnicity			
Caucasian	30 (60%)	15 (60%)	15 (50%)
Asian	7 (14%)	3 (12%)	4 (16%)
African or Afro-Caribbean	13 (26%)	7 (28%)	6 (24%)
Weight (kg)	76.4 (64.8, 98.65)	72.1 (63, 77.3)	97.6 (70.3, 100.3)
BSA ($kg/m^{2)}$	1.90 (1.69, 2.15)	1.79 (1.66, 1.94)	2.13 (1.81, 2.29)
BMI	25.92 (23.70, 33.67)	24.59 (23.04, 29.49)	28.52 (24.61, 36.38)
Charlson Comorbidity Index	5 (4, 7)	6 (5,7)	5 (3, 6)
Diabetes	17 (34%)	9 (36%)	8 (32%)
HD Vintage	23.92 (12.83, 56.61)	38.50 (17.88, 67.82)	16.50 (10.45, 27.91)
Vascular Access			
AVF	31 (62%)	13 (52%)	18 (72%)
AVG	3 (6%)	3 (12%)	0 (0%)
Line	16 (32%)	9 (36%)	7 (28%)
Anuric (inter-dialytic urine volume <250ml)	32 (64%)	17 (68%)	15 (60%)
Urine Volume (ml)	1198.67 ± 895.95	1625 ± 1181	914 ± 552
Residual Urea Clearance (ml/min)	1.63 (1.31, 3.02)	2.50 (1.35, 3.42)	1.49 (1.35, 1.93)
HD Session Duration	240(240, 240)	240(240, 240)	240 (240, 240)
Kt/V	1.34 ± 0.29	1.48 ± 0.26	1.19 (0.97, 1.42)
Blood Flow Rate (ml/min)	280.7 ±43.67	281.32 ± 43.34	280.08 ± 44.89
Substitution Volume	20.25 (17.48, 22.6)	20.5 (17.4, 22.6)	19.5 (17.7, 22.6)
Hb (g/l)	114.82 ± 14.10	113.72 ± 13.92	115.92 ± 14.48
Adjusted Calcium	2.44 ± 0.18	2.46 ± 0.20	2.43 ± 0.18
Phosphate	1.55 (1.28, 1.89)	1.51 (1.31, 1.83)	1.68 (1.24, 1.93)
Albumin (g/l)	33.5 (19, 35)	32 (28, 35)	34 (31, 35)
CRP (mg/l)	8 (3.25, 14.75)	3 (3, 9)	11 (5, 25)
PTH	29.25 (10.63, 51.23)	26.9 (9.7, 48.3)	29.6 (11.9, 52.2)
BP Systolic	134 (120, 148.5)	137 (120.5, 150.25)	129 (120, 145)
BP Diastolic	82.17 (67.5, 96)	80 (67, 96.25)	80 (69.5, 95.5)

Table 3-1: Baseline characteristics and demographics of patients who completed the study. *Data presented as mean ± standard deviation (SD) for normally distributed data, median (lower quartile, upper quartile) for data with a skewed distribution and as frequencies and percentages for categorical data. *Result with statistical significance at level p<0.05.*

In the MCO group, participants did have a significantly lower HD vintage (p=0.0286), Kt/V was lower at baseline (median Kt/V 1.48 in HDF group vs 1.19 in the MCO group, p=0.0013) and median CRP was higher (CRP 3 vs 11, p = 0.012). Additionally, patients in the MCO group had a significantly higher median weight (97.6kg (70.3, 100.3) vs 72.1kg (63, 77.3) and other associated parameters (Body Surface Area and BMI).

3.3.2 Treatment

All 50 participants who completed the study remained on their designated treatment for the full 24-week study period. Throughout the study, treatment was monitored and mean blood flow and substitution volume for the two groups are demonstrated in table 3-2. There were no significant differences in these parameters between the two groups during the study period. Patients in the HDF group achieved mean substitution volumes consistent with high volume HDF throughout the study period.

	T0		T12		T24	
	BFR (ml/min)	**aSV (litres)**	**BFR (ml/min)**	**aSV (litres)**	**BFR (ml/min)**	**aSV (litres)**
HDF	281.3 ± 43.34	20.23 ± 5.74	285.96 ± 46.90	21.22 ± 6.01	300.72 ± 42.82	20.23 ± 2.84
MCO	280.08 ± 44.89	20.03 ± 3.64	288.8 ± 44.05	N/A	312.84 ± 34.23	N/A
Sig	0.9213	0.8825	0.8291		0.2748	

Table 3-2: Blood flow rate and substitution volume during study period in two study groups. *Data presented as mean± SD. BFR= Blood Flow Rate. aSV = Adjusted Substitution Volume (value adjusted if on pre-dilution HDF for session). Statistical analysis performed using unpaired t-test*

3.3.3 Clinical Results

3.3.3.1 EMV

At baseline, EMV count was higher in the MCO group (mean 2.63 ± 0.30 events/μl) compared with the HDF group (2.32 ± 0.41 events/μl, $p<0.05$). At the end of the 24-week study period, there was no significant difference between the two groups in mean plasma EMV concentration (2.60 ± 0.40 log EMV events/μl HDF and 2.49 ± 0.49 log EMV count/μl MCO, $p = 0.39$). Data presented in figure 3-2.

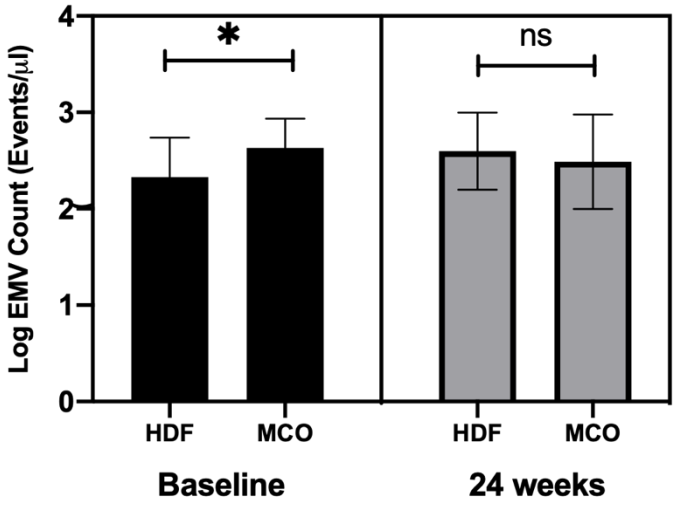

Figure 3-2: Endothelial microvesicle concentration (EMV) at baseline and at the end (24 weeks) of the study. *Statistical analysis performed using paired t-test, *p<0.05*

Change in EMV concentration within each group during the study period is demonstrated in figure 3-3. Over the 24-week study period, there was a rise in EMV (+0.27 ± 0.43 log EMV count/μl, p = 0.0091) in the HDF compared with a reduction in the MCO group (-0.14 ± 0.45 log EMV count/μl, p = 0.0247). In the MCO group, there was a sharp fall in EMV concentration followed by a small rise. In the HDF group, there was a progressive rise.

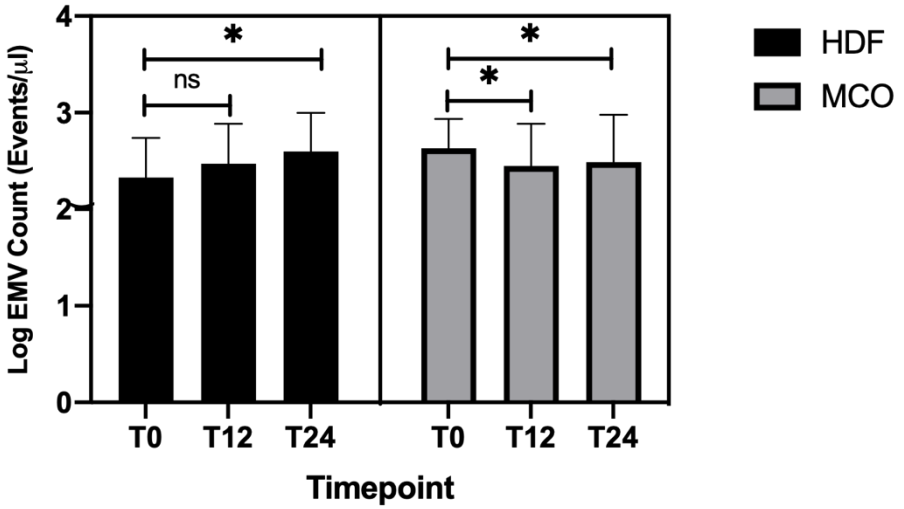

Figure 3-3:Change in EMV (log events/μl) at baseline (T0), 3 months (T12) and 6 months (T24) in the two study groups (HDF and MCO). Statistical analysis performed using paired t-test, * = p<0.05

3.3.3.2 Albumin

Mean change in serum albumin in the HDF group was 0.00g/l ± 1.89 and was -1.72g/l ±2.95 in the MCO group, p=0.0179 (unpaired t-test) during the study period. There was no significant difference in serum albumin concentration between the 2 groups at the end of the study, 32.5g/l (29, 35)) HDF vs 30g/l (29, 33) MCO (p = 0.35, Mann-Whitney test).

3.3.3.3 Middle molecule, endothelial activation, inflammation & angiogenesis panel

There was no statistically significant difference in change in concentration of any of the analytes during the study period when comparing the two study groups (table 3-4). Baseline data for the middle molecule, endothelial activation, inflammation and pro-inflammatory cytokine panel for the 50

participants that completed the study are presented in table 3-3 alongside data from healthy controls.

There were differences at baseline between the two groups for leptin, VEGF and IL-8 with leptin and VEGF higher in the MCO group and IL-8 higher in the HDF group (see supplementary data). The significantly higher leptin level in the patients in the MCO group is consistent with the findings of higher BMI, body weight and associated CRP and EMV levels.

Serum levels for all middle molecules were significantly lower in healthy controls compared with the study participants. This was similar for most of the remaining study markers with values significantly lower in 8 out of 12 markers.

		Study Participants	Healthy Controls
Total Participants Analysed	**Total number of samples**	**50**	**7**
MIDDLE MOLECULES			
α1-microglobulin (mg/l)	50	14.60± 1.97	6.70±
Leptin (ng/ml)	50	51.78 (14.58-176.8)	3.76±2.75
ß2-microglobulin(mg/l)	50	32.68 (20.03-44.11)	1.30 (1.22-1.41)
YKL-40 (Chitinase-3-likeprotein 1) (ng/ml)	49δ	89.83 (57.83-119.7)	13.84 (11.80-16.25)
Pentraxin-3 (ng/ml)	50	1.607 (1.015-2.447)	0.73±0.42
ENDOTHELIAL ACTIVATION			
Von Willebrand factor (pg/ml)	50	234.7(164.8-326.0)	67.63±47.64
Intercellular Adhesion Molecule (ICAM)(ng/ml)	50	356.7 (274.8-436.9)	209.18±72.77
Vascular Cell Adhesion Molecule-1 (VCAM)(ng/ml)	50	1536 (1204-2759)	1072.94±330.64
E-Selectin (ng/ml)	50	24.54 (16.32-33.05)	16.45±10.15
P-Selectin (ng/ml)	50	30.42± 12.15	31.02±12.57
INFLAMMATION			
Interleukin-6 (IL-6) (pg/ml)	50	7.700 (6.825-9.530)	4.07±0.82
Interleukin-8 (IL-8) (pg/ml)	50	12.34 (8.995-16.21)	8.75±3.20
Interleukin-10 (IL-10) (pg/ml)	39Δ	2.365 (1.783-2.843)	1.76±0.88
Tumour Necrosis Factor-α (TNFα) (pg/ml)	50	28.58± 5.50	19.31±4.80
ANGIOGENESIS			
Vascular endothelial Growth Factor (VEGF) (pg/ml)	50	94.96 (70.49-140.8)	55.24±38.02
Vascular endothelial Growth Factor-C (VEGF-C) (pg/ml)	50	0.912± 0.47	1.83±0.40
Vascular endothelial Growth Factor Receptor-1 (VEGFR) (pg/ml)	50	260.6± 53.37	223.32±68.04

Table 3-3: Study markers (middle molecule, endothelial activation, inflammation & angiogenesis) at baseline compared with healthy volunteers*. Data presented as mean± SD for normally distributed data and median (Q1, Q3) for data with skewed distribution. Statistical analysis performed using unpaired t-test for normally distributed data and Mann-Whitney U Test for skewed data. *Result with statistical significance at level p<0.05. Δ: 11 samples were under range of detection for IL-10 and not included in analysis. δ: sample for single patient above reportable range.*

	HDF		MCO		P Value
MIDDLE MOLECULES	**T0**	**T24**	**T0**	**T24**	
α1-microglobulin (mg/l)	15.15± 2.35	15.50± 2.44	14.60± 1.97	14.84± 1.59	0.497
Leptin (ng/ml)	7.11 (1.83-68.96)	12.6 (2.17-87.12)	51.78 (14.58-176.8)	41.23 (14.09-158.6)	0.454
ß2-microglobulin(mg/l)	26.54 (21.65-52.31)	26.78 (20.06-43.08)	32.68 (20.03-44.11)	28.25 (21.52-47.79)	0.595
YKL-40 (Chitinase-3-likeprotein 1) (ng/ml)	105.2 (57.28-174.5)	93.64 (62.79-186.5)	89.83 (57.83-119.7)	93.74 (60.81-122.3)	0.517
Pentraxin-3 (ng/ml)	1.836 (1.262-2.922)	1.475 (0.7694-2.702)	1.607 (1.015-2.447)	1.756 (1.150-2.543)	0.317
ENDOTHELIAL ACTIVATION					
Von Willebrand factor (pg/ml)	274.7 (133.0-348.3)	196.3 (102.1-358.9)	234.7(164.8-326.0)	266.0 (187.5-399.7)	0.261
Intercellular Adhesion Molecule (ICAM)(ng/ml)	359.5 (277.1-469.2)	362.0 (277.3-448.6)	356.7 (274.8-436.9)	339.7 (279.5-473.4)	0.283
Vascular Cell Adhesion Molecule-1 (VCAM)(ng/ml)	2100 (1525-2558)	1676 (1314-2706)	1536 (1204-2759)	1921 (1066-2287)	0.444
E-Selectin (ng/ml)	21.85 (15.95-27.61)	12.60 (2.17-87.02)	24.54 (16.32-33.05)	41.23 (14.09-158.6)	0.067
P-Selectin (ng/ml)	30.59± 10.37	28.96± 8.28	30.42± 12.15	29.27± 9.41	0.766
INFLAMMATION					
Interleukin-6 (IL-6) (pg/ml)	7.190 (5.94-9.260)	7.370 (5.520-8.755)	7.700 (6.825-9.530)	8.050 (6.545-11.23)	0.233
Interleukin-8 (IL-8) (pg/ml)	15.25 (11.79-23.09)	15.80 (10.96-19.87)	12.34 (8.995-16.21)	11.58 (8.535-19.76)	0.156
Interleukin-10 (IL-10) (pg/ml)	2.460 (1.880-3.365)	2.485 (1.953-3.213)	2.365 (1.783-2.843)	2.400 (1.818-3.000)	0.778
Tumour Necrosis Factor-α (TNFα) (pg/ml)	28.58± 5.50	28.82± 4.38	28.58± 5.50	28.82± 4.38	0.250
ANGIOGENESIS					
Vascular endothelial Growth Factor (VEGF) (pg/ml)	57.55 (39.19-115.6)	56.42 (34.22-109.5)	94.96 (70.49-140.8)	93.91 (60.67-134.7)	0.744
Vascular endothelial Growth Factor-C (VEGF-C) (pg/ml)	1.026± 0.45	0.910± 0.45	0.912± 0.47	0.910± 0.45	0.344
Vascular endothelial Growth Factor Receptor-1 (VEGFR) (pg/ml)	249.7± 74.35	241.1± 73.84	260.6± 53.37	265.1± 62.14	0.207
OTHER					
Serum Albumin (g/l)	32 (28, 35)	33 (29, 35)	34 (21, 35)	30 (29, 33)	**0.026***

Table 3-4: Study markers (middle molecule, endothelial activation, inflammation & angiogenesis) at baseline and end of study (T24, 6 months). *Data presented as mean± SD for normally distributed data and median (Q1, Q3) for data with skewed distribution. Difference in change between each group for each analyte assessed using a multiple regression model.*

Concentrations of all biomarkers at all 3 timepoints are presented in supplementary table in section 3.6.

3.3.4 Adverse events, hospital admissions, deaths and transplants

There were no adverse events (AE's) or serious adverse events (SAE's) that were attributable to the treatment or trial assessments during the study period. The number of patients with non-elective hospital admissions was not statistically different between the two groups (table 3-5), however, the total number of hospital admission episodes was much higher in the HDF group compared with the MCO group (17 vs 9). A&E attendances were higher in the MCO group although this did not quite reach statistical significance and none of these attendances could be attributed to the study. Rates of transplants and deaths were relatively similar between the two groups.

Event	HDF	MCO	Total	Sig
n	**31**	**32**	**63**	
Patients with ≥1 non-elective hospital admission	11 (35.5%)	7 (21.9%)	18	0.27
Non-elective hospital admission episodes	17	9	26	N/A
Patients with ≥1 ED attendance	1 (3.2%)	7 (21.9%)	8	0.05
ED attendance episodes	1	9	10	N/A
Transplant	2 (6.5%)	1 (3.1%)	3	>0.99
Death	1 (3.2%)	1 (3.1%)	2	>0.99

Table 3-5: Episodes of elective hospital admissions, non-elective admissions, A&E attendances, transplants and deaths during the 6-month study period. *Data presented as number and proportion within group as a percentage (n (%)). Statistical analysis performed using Fisher's exact test. ED= Emergency Department.*

3.3.5 Haemodynamic Indices

Analysis of intradialytic hypotension (IDH) episodes was not completed due to inconsistency in data capture and poor data quality. There was no significant change in systolic blood pressure, pulse wave velocity or augmentation index during the study period in either study group.

3.3.6 Correlation Analysis

Correlation analysis was performed using Pearson correlation coefficient to assess for any linear correlation between study results.

1. **EMV**

Baseline EMV correlated with baseline VEGF (p=0.039), TNF-α (p=0.036), IL-10 (p=0.017), Augmentation Index (p-0.042), extracellular to intracellular water ratio (E/I) (p= 0.005) and Fat Tissue Index (p=0.044). There was a trend towards significance for a correlation between baseline EMV and VCAM (p=0.058), YKL-40 (p=0.095), P-selectin (p=0.076) and baseline IPOS score (p=0.073). Change in EMV correlated with baseline VCAM (p=0.016).

2. **Remaining analytes**

There was a correlation between baseline serum albumin and Age (p=0.023), Charlson Comorbidity Index (CCI) (p=0.016), Pulse Wave Velocity (p=0.048), baseline haemoglobin, IL-6 (p=0.025), VCAM (p=0.010), E/I (p=0.019) and lean tissue index (p=0.038).

3.3.7 Residual Function

Analysis of data on residual function during the study was limited by the sample size and the completion of urine collections (table 3-6). Of those that passed urine, only 8 of the 15 participants (53.3%) had a urine collection performed both at the start and end of the study (3 collections missing at the start and 7 at the end of the study). Of these 8 participants, 2 (25%) participants became anuric by the end of the study. Both of these participants

were in the MCO group. Of the two participants, BCM data was present for a single patient and interestingly, this participant saw the biggest change in overhydration in the whole study (+3.9 litres at the start and -0.4 litres at the end). ß2-microglobulin however unexpectedly reduced significantly during the study period for this participant (28.99mg/l to 10.34mg/l). The other participant who became anuric during the study period saw rise in ß2-microglobulin (35.9mg/l to 41.58mg/l) as you may expect.

The change in residual kidney function in the MCO group did not reach statistical significance, urea clearance 1.48ml/min (1.22, 1.68) at the start and 0.95ml/min (0.00, 2.19) at the end, p = 0.2188, Wilcoxon test for the patients that had a collection at the start and end of the study. For the HDF group, there were not enough data pairs to perform an analysis (only 2 patients had a urine collection at the start and end of the study).

	All	HDF	MCO	Sig
n	**50**	**25**	**25**	
	STUDY START			
Anuric	32 (64%)	17 (68%)	15 (60%)	0.7688
Non-anuric	15 (30%)	6 (24%)	9 (36%)	
Missing collection	3 (6%)	2 (8%)	1 (4%)	
Urine volume (ml)	1198.67 ± 895.95	1625 ± 1181	914 ± 552	0.137
Residual Function (urea clearance (ml/min))	1.63 (1.31, 3.02)	2.50 (1.35, 3.42)	1.49 (1.35, 1.93)	0.388
	STUDY END			
Anuric	35 (70%)	18 (72%)	17 (68%)	>0.99
Non-anuric	8 (16%)	2 (8%)	6 (24%)	
Missing collection	7 (14%)	5 (20%)	2 (8%)	
Urine volume (ml)	960 ± 710	555 ± 7	988 ± 806	0.4980
Residual Function (urea clearance (ml/min))	1.42 (0.90, 2.38)	0.86 (0.78, 0.95)	2.00 (1.16, 2.76)	0.2857

Table 3-6: Residual kidney function at start and end of the study. *Data presented as mean± SD for normally distributed data and median (Q1, Q3) for data with skewed distribution. Statistical analysis performed using unpaired t-test for normally distributed data and Mann-Whitney U test for skewed data.*

Of the middle molecules, both α1-microglobulin (p= 0.024) and ß2-microglobulin (p = 0.048) predicted volume status (anuric vs non-anuric) at baseline (table 3-7).

Middle Molecules	Significance
α1-microglobulin	0.024*
Leptin	0.577
ß2-microglobulin	0.048*
YKL-40 (Chitinase-3-likeprotein 1	0.216
Pentraxin-3	0.898

Table 3-7: Results of multiple regression analysis of concentration middle molecules at baseline as a predictor of urine volume status (anuric vs non-anuric) at baseline.

3.4 Discussion

This study has demonstrated that at 24 weeks after switching from HDF treatment to MCO treatment, there is no significant difference in plasma EMV concentration.

A difference in the mean baseline EMV concentration between the two study groups (EMV was higher in the MCO group at baseline) meant that there was a reduction in EMV concentration during the study period and a progressive rise in EMV concentration in the HDF group. This pattern of change was not seen in the other biomarkers measured in this study (middle molecule, endothelial activation, inflammation or angiogenesis). There is therefore no clear biological basis for the changes in EMV concentration seen and the change in plasma EMV could be explained by regression to the mean rather than reflecting a true effect from the treatment itself. Additionally, the baseline

difference in EMV could be related to covariate differences between the two groups, particularly where CRP and BMI were higher in the MCO group.

Expanded haemodialysis (HDx) enabled by a medium cut-off (MCO) haemodialysis membrane provides enhanced clearance of larger uraemic retention solutes compared with conventional haemodialysis[90,344,411-415] and provides similar clearance of larger middle molecules and in some cases exceeds[39,40]) haemodiafiltration (HDF) [40,90,343,345]. The majority of studies have assessed clearance through reduction ratios (RR's) from pre and post-dialysis samples, albeit in comparison with high flux treatments (HFHD). In this study, we opted to look at longitudinal change in biomarkers and reduction ratios were not assessed. In this study, there was a clear relationship between the serum concentration of both ß2-microglobulin and α1-microglobulin and residual kidney function (RKF). RKF may have masked any longitudinal change seen in some of the biomarkers. Measurement of reduction ratios may have provided a more accurate representation of membrane performance.

This study highlights the challenges in dialysis studies where there is considerable heterogeneity amongst patients and numerous factors such as residual kidney function, co-morbidity and obesity can influence study outcomes. Despite recruiting and randomising 63 patients into this study, there were significant baseline differences between the two groups. A crossover study may have been more suited to a feasibility study of this size. Crossover studies can have drawbacks such as a potentially reduced

exposure to the intervention and randomised studies are usually required to drive changes in practice.

MCO treatment was well tolerated during the course of this study and drop-out was close to the 20% initial projection. Although there was a significant reduction in serum albumin in the MCO treatment group, serum albumin was not significantly different between the two groups at the end of the study (median albumin ≥30g/l in both groups). The reduction is albumin in this study was of similar magnitude to that seen in the study by Krishnasamy et al (-0.7g/l) [416]. Several other groups have demonstrated similar findings with either no significant difference in albumin between the study groups or no change in serum albumin when MCO is compared with both HFHD[412,415,417,418]and HDF[343,344]. Data for albumin loss into the dialysate is conflicting with one study demonstrating a greater albumin loss associated with HDF compared with MCO[90] and another showing no difference between the two[345]. Albumin reduction ratios have been shown to be no different between MCO and HDF[345,414]. Overall, there appears to be a clear signal that any albumin loss associated with MCO treatment is relatively low with no safety concerns highlighted. The full consequences of albumin loss through dialysis treatment remains unknown. Albumin losses of around 4g per 24 hours are seen [419] and tolerated in CAPD patients with no known difference in survival between PD and HD [420]. Some may argue a benefit to protein loss during dialysis through removal of some protein-bound toxins which are otherwise difficult to remove

however randomised studies with clinical outcome data supporting this theory are lacking. The "optimal" dialysis protein loss remains undetermined.

HDx as a treatment modality was straightforward to implement during the study period with a minimal training requirement and no requirement for additional dialysis equipment. There were no membrane reactions identified in either study group and mortality and admission rates were similar in both groups. Maintaining target blood flow rates in both arms of the study was challenging (see table 3-2), highlighting the difficulties of implementing HDF with high convective volumes. Membrane performance in this study appeared similar to HDF even at a modest blood flow rate of 312.84ml/min $\pm$ 34.23 at the end of the study. This finding has been echoed elsewhere[39,421].

There were several strengths to this study. Firstly, this study is one of the first randomised studies comparing HDx with HDF in a clinical "real-world" setting. The study is also very relevant, given the current clinical equipoise surrounding the optimal dialysis modality (HD vs HDF). Secondly, incorporating several patient-reported outcome measures delivers context to researchers, clinicians and patients. The similarity of HD prescription in relation to blood flows and treatment times also provide a good clinical setting to study the effect of the membrane and the modality itself. The compliance in data returns throughout the study, low drop-out rates and relative tolerability of both treatment interventions are reassuring.

In addition to the strengths, there are several limitations to this study. Firstly, this was an open-label study which could have contributed to confounding, particularly with regards to the an unblinded dialyser treatment intervention. A blinded study would have been challenging to implement and would have raised some safety issues in implementing the treatment modality. Secondly, the sample size of the study was relatively small. Given the sample size, a crossover study may have provided more meaningful results. The study was designed in this manner to provide a 6-month treatment exposure and a crossover study would have required either a shorter exposure or long study duration which was not feasible. Thirdly, despite being a randomised study, there were differences between the two groups at baseline (BMI and associated parameters, HD vintage Kt/V and CRP). Although none of these variables (weight, height, BSA, BMI, HD vintage, Kt/V and CRP) were shown to significantly determine the main outcome measure in regression models, they may have contributed to confounding. Obesity is associated with higher levels inflammation and endothelial activation[422-426]. Kt/V tends to be underestimated in obese individuals due to an overestimation of total body water using Watson formula[427]. Interestingly, there was a correlation between baseline EMV and FTI such that a higher baseline FTI was associated with a higher baseline EMV. Baseline FTI was different between the two groups ($p = 0.039$) however no relationship was found between baseline FTI and change in EMV using a multiple regression model ($p=0.089$). Finally, it is possible that the clearance profile of uraemic toxins were overlapping in the 2 treatment

arms of high volume HDF and HDx. This may have blunted the difference in the spectrum of uremic toxins for a range of middle molecules.

3.5 Conclusion

There is no significant difference in plasma EMV concentration 6 months after switching from HDF to HDx treatment. Although the changes in EMV concentration in each of the treatment groups could indicate a treatment benefit in terms of optimising vascular endothelial health, there were no overall differences between the two treatment in any other study parameter measured. The overall study findings (in combination with EMV, pro inflammatory and procoagulant mediators of endothelial function) support non-inferiority of HDX using MCO membranes over high-volume HDF.

3.6 Supplementary Table

	MCO T0	MCO T12	MCO 24		HDF T0	HDF T12	HDF T24	
Total Participants Analysed	**25**	**25**	**25**		**25**	**25**	**25**	
Symptom	**Serum concentration (pre-dialysis)**			**p value$^{\Delta}$**	**Serum concentration (pre-dialysis)**			**p value$^{\Delta}$**
MIDDLE MOLECULES								
α1-microglobulin (mg/l)	14.60± 1.97	14.59± 1.96	14.84± 1.59	0.4068	15.15± 2.35	15.21± 2.60	15.50± 2.44	0.3184
Leptin (ng/ml)	51.78 (14.58-176.8)	35.08 (10.44-141.3)	41.23 (14.09-158.6)	**0.0049***	7.11 (1.83-68.96)	10.64 (2.95-60.03)	12.6 (2.17-87.12)	0.7558
ß2-microglobulin(mg/l)	32.68 (20.03-44.11)	30.45 (20.03-37.97)	28.25 (21.52-47.79)	0.6188	26.54 (21.65-52.31)	26.78 (20.06-43.08)	26.78 (20.06-43.08)	0.8869
YKL-40 (Chitinase-3-likeprotein 1) (ng/ml)	89.83 (57.83-119.7)	95.07 (60.93-123.0)	93.74 (60.81-122.3)	0.5682	105.2 (57.28-174.5)	84.11 (61.25-167.4)	93.64 (62.79-186.5)	0.6873
Pentraxin-3 (ng/ml)	1.607 (1.015-2.447)	1.691 (0.9874-2.430)	1.756 (1.150-2.543)	0.1409	1.836 (1.262-2.922)	1.379 (0.8283-2.041)	1.475 (0.7694-2.702)	**0.0263***
ENDOTHELIAL ACTIVATION								
Von Willebrand factor (pg/ml)	234.7(164.8-326.0)	210.4 (179.9-339.0)	266.0 (187.5-399.7)	0.4677	274.7 (133.0-348.3)	186.0 (135.4-275.5)	196.3 (102.1-358.9)	0.4531
Intercellular Adhesion Molecule (ICAM)(ng/ml)	356.7 (274.8-436.9)	348.8 (305.9-473.9)	339.7 (279.5-473.4)	0.3263	359.5 (277.1-469.2)	401.3 (301.8-440.6)	362.0 (277.3-448.6)	0.4677
Vascular Cell Adhesion Molecule-1 (VCAM)(ng/ml)	1536 (1204-2759)	1602 (1235-2801)	1921 (1066-2287)	>0.9999	2100 (1525-2558)	1794 (1343-3609)	1676 (1314-2706)	**0.0118***
E-Selectin (ng/ml)	24.54 (16.32-33.05)	35.08 (10.44-141.3)	41.23 (14.09-158.6)	**0.0478***	21.85 (15.95-27.61)	10.64 (2.95-60.03)	12.60 (2.17-87.02)	0.2894
P-Selectin (ng/ml)	30.42± 12.15	30.00± 9.36	29.27± 9.41	0.5225	30.59± 10.37	28.98± 9.53	28.96± 8.28	0.2791
INFLAMMATION								
Interleukin-6 (IL-6) (pg/ml)	7.700 (6.825-9.530)	8.840 (6.550-11.28)	8.050 (6.545-11.23)	0.6237	7.190 (5.94-9.260)	7.390 (5.655-8.265)	7.370 (5.520-8.755)	0.6188
Interleukin-8 (IL-8) (pg/ml)	12.34 (8.995-16.21)	12.85 (8.37-15.86)	11.58 (8.535-19.76)	0.3533	15.25 (11.79-23.09)	12.72 (10.35-20.17)	15.80 (10.96-19.87)	**0.0093***
Interleukin-10 (IL-10) (pg/ml)	2.365 (1.783-2.843)	2.500 (1.918-3.010)	2.400 (1.818-3.000)	0.9061	2.460 (1.880-3.365)	2.360 (1.930-3.185)	2.485 (1.953-3.213)	0.4860
Tumour Necrosis Factor-α (TNFα) (pg/ml)	28.58± 5.50	29.37± 5.98	28.82± 4.38	0.3683	29.26± 6.94	28.69± 7.60	28.43± 7.64	0.3420
Interleukin-6 (IL-6) (pg/ml)	7.700 (6.825-9.530)	8.840 (6.550-11.28)	8.050 (6.545-11.23)	0.6237	7.190 (5.94-9.260)	7.390 (5.655-8.265)	7.370 (5.520-8.755)	0.6188
ANGIOGENESIS								
Vascular endothelial Growth Factor (VEGF) (pg/ml)	94.96 (70.49-140.8)	98.49 (63.27-138.7)	93.91 (60.67-134.7)	0.6977	57.55 (39.19-115.6)	56.33 (35.78-104.7)	56.42 (34.22-109.5)	0.4677
Vascular endothelial Growth Factor-C (VEGF-C) (pg/ml)	0.912± 0.47	0.957± 0.46	0.910± 0.45	0.1766	1.026± 0.45	1.053± 0.97	0.910± 0.45	0.0581
Vascular endothelial Growth Factor Receptor-1 (VEGFR) (pg/ml)	260.6± 53.37	284.0± 68.14	265.1± 62.14	0.1500	249.7± 74.35	248.4± 79.22	241.1± 73.84	0.4265

Table 3-8: Study markers (middle molecule, endothelial activation, inflammation & angiogenesis) at baseline, 3 months (T12) end of study (T24, 6 months). *Data*

presented as mean± SD for normally distributed data and median (Q1, Q3) for data with skewed distribution. Concentration at 3 timepoints compared within each group using one-way ANOVA for normally distributed data and Friedman's test for data with skewed distribution.

Chapter 4: Results: The Impact of Medium Cut-Off Haemodialysis on *In Vitro* Cell Viability, Angiogenesis and Wound Healing

4.1 Introduction

The uraemic syndrome in advanced chronic kidney disease (CKD) is characterised by a persistent inflammatory state, malnutrition and a significant burden of cardiovascular disease. For decades, there has been an awareness of the significant cardiovascular mortality observed in dialysis patients, however, to date, the impact of innovation in technology and targeted interventions have been minimal. Endothelial dysfunction and activation have been identified as key early players in the process of the accelerated cardiovascular disease seen in patients with CKD. Knowledge is still evolving in our understanding of the complex relationship between the vascular endothelium, inflammation and uraemic toxins.

Over 100 separate uraemic toxins have been identified[62,64-66] and current dialysis techniques do not adequately remove all of these toxins, particularly those that are bound to protein and are larger than 15kDa in size (so called larger "middle molecules"). Serum from uraemic patients and toxins, such as indoxyl-sufate, have been shown to adversely impact on endothelial cell function[346 347 348 349 350 351 352 353 354 355], promote endothelial activation[428-430] and trigger the release of endothelial microvesicles (EMV) [18,19]. Toxin removal from a single haemodialysis session appears to have a favourable impact on measures of *in vitro* endothelial function[377] adding weight to the notion that improving toxin clearance could lead the way to improving patient outcomes.

A novel haemodialysis membrane has been recently developed to improve the removal of larger middle molecules. This medium cut-off (MCO) membrane out-performs high-flux haemodialysis (HFHD) in terms of clearance of middle molecules and matches or even exceeds the clearance of haemodiafiltration (HDF) [40,339,431].

The aim of this study was to determine the effect of switching treatment from HDF to MCO for 6-months on in-vitro measures of endothelial cell function; cell viability, angiogenesis and wound healing. The study also sought to determine factors that impact on endothelial cell function, angiogenesis and cell viability in patients on haemodialysis.

4.2 Methodology

4.2.1 Study Design

The full methodology and techniques for this study are presented in Chapter 2. All the laboratory work was performed by a collaborative team at Manchester Metropolitan University. I collected all study samples and performed all data analysis.

4.2.2 Patients

At the start of the main MoDal study, 8 of the 25 patients from each arm of the study were recruited to this sub study where extra blood samples were taken

for cell culture experiments. Samples were taken at the start and end of the MoDal study using the same methods described in Chapter 2. Samples from 4 of the 16 healthy controls (selected at random) were also included for use in this sub study. The aim of this sub study was to assess the effect of switching from HDF treatment to MCO treatment on endothelial cell function *in vitro*. Specifically, cell viability, angiogenesis and wound healing (cell migration).

4.3 Results

4.3.1 Demographics

Baseline demographic data and inflammatory markers for the participants in this study are presented in table 4-1.

Variable	MCO (n = 8)	HDF (n = 8)	Healthy Controls (n = 4)
Median Age (Years)	56 (Range 35-82)	60 (Range 23-86)	50.5 (Range 36-61)
Sex (Male)	7 (87.5%)	5 (62.5%)	3 (75%)
Charlson Comorbidity Index (CCI)	4.5 (2.75, 5.25)	5 (3.5-6.5)	γ
Diabetes	3 (37.5%)	3 (37.5%)	γ
Weight (Kg)	105.8 (96.2, 179.0)	72.1 (60.2, 76.7)	γ
Body Mass Index (BMI)	36.49 (28.20, 54.89)	26.30 (21.67, 29.43)	γ
HD Vintage (months)	16.08 (13.59, 20.37)	17.56 (8.43, 58.93)	γ
Anuric	4 (50%)	5 (62.5%)	γ
C-Reactive Protein (CRP) (mg/L)	10.83 (7.0, 29.0)	4.00 (2.5, 9.0)	γ
Tumour Necrosis Factor-α (TNFα) (pg/ml)	25.96 ± 3.77	26.64 ± 6.54	γ
P-Selectin (ng/ml)	35.14 ± 14.27	29.81 ± 13.46	γ

Table 4-1: Baseline demographic data and inflammatory parameters for participants in study. *Data presented as mean ± standard deviation (SD) for normally distributed data, median (lower quartile, upper quartile) for data with a skewed distribution and as frequencies and percentages for categorical data. γno data available.*

4.3.2 Cell Viability

Cell viability was measured in fluorescence arbitrary units and expressed as a percentage of the experimental control (5% foetal calf serum). There was no difference in 4-hour cell viability after 6 months MCO treatment (baseline median viability 84.77% (84.27, 100.1) vs 6-month 81.85% (79.94, 92.48), p = 0.5469 (Wilcoxon signed-rank test) (Figure 4-1). A similar result was observed cell viability at 8 hours (baseline mean viability 83.86% ± 5.91 vs 6-month mean 82.19% ± 10.42, p = 0.7253, paired t-test). There was also no significant

change in 4-hour cell viability for the patients who remained on HDF for the study period (baseline median viability 111.24% (99.36, 125.49) vs 6-month 111.97% (105.37, 121.66), p = 0.95, Wilcoxon signed-rank test), data presented in figure 4-1.

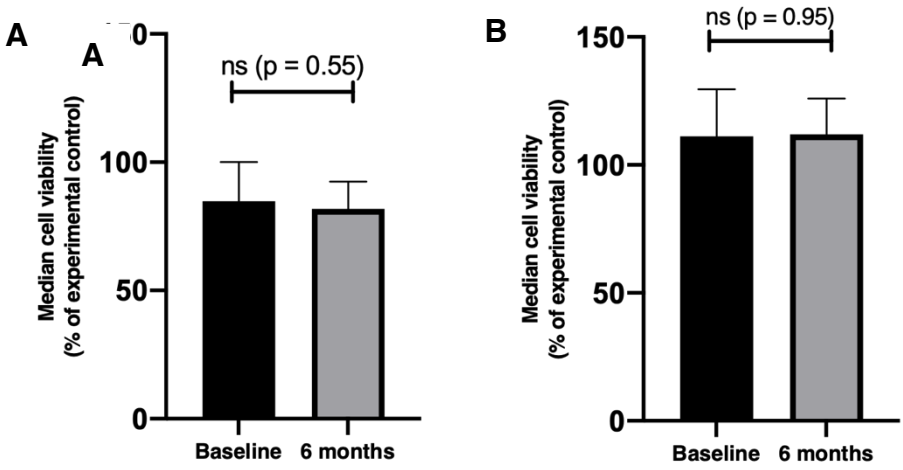

Figure 4-1: Cell viability study. *Median cell viability (measured in fluorescence arbitrary units (FLU)) expressed as a percentage (%) of the experimental control following 4 hours incubation with AlamarBlue® with serum of patients following 6-months MCO treatment (A) and serum of those remaining on HDF treatment for 6 months (B). 5% foetal calf serum used as experimental control. Error bars represent interquartile range of data. Statistical analysis performed using Wilcoxon signed-rank test comparing fluorescence of samples at baseline and 6 months*

There was a reduction in 4-hour cell viability in 6 out of the 8 (75%) patients after 6-months MCO treatment. For patients remaining on HDF, 4 out of 8 (50%) patients saw a reduction in cell viability. The overall combined change in cell viability did not reach statistical significance in this relatively small sample size.

4.3.3 Cell viability: correlations

Cell viability at baseline correlated with BMI (p = 0.0498) and VCAM (p = 0.045) (figure 4-2).

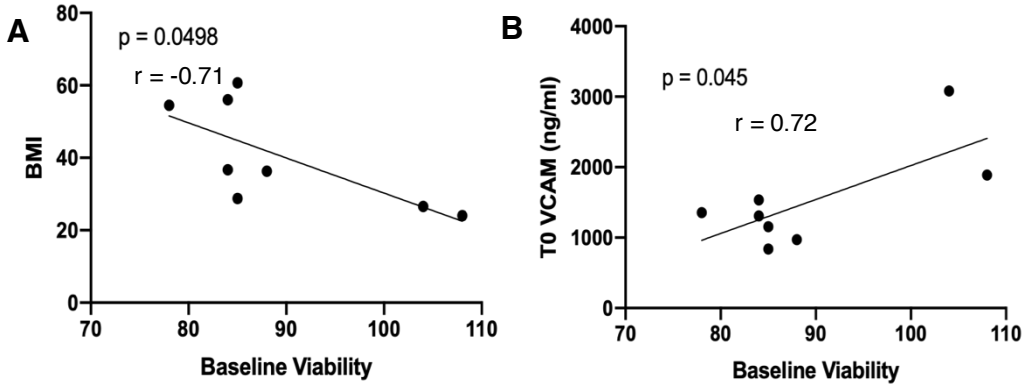

Figure 4-2: Correlation analysis with wound healing. *Correlation analysis was performed using Pearson's correlation coefficient. There was a significant correlation (p<0.05) between cell viability as a percentage (%) of the experimental control and body mass index (BMI)(A) and vascular cell adhesion molecule (VCAM) (B).*

4.3.4 Wound healing (endothelial cell migration)

There was no significant difference in the change in scratch area at 4 hours in the cells treated with serum from samples at the start of the study and after 6 months MCO treatment. The mean percentage change in scratch area was -51.93% ± 10.69 vs -51.73 ± 14.11, p = 0.9719, paired t-test. Data presented in figure 4-3, with representative images shown in figure 4-4.

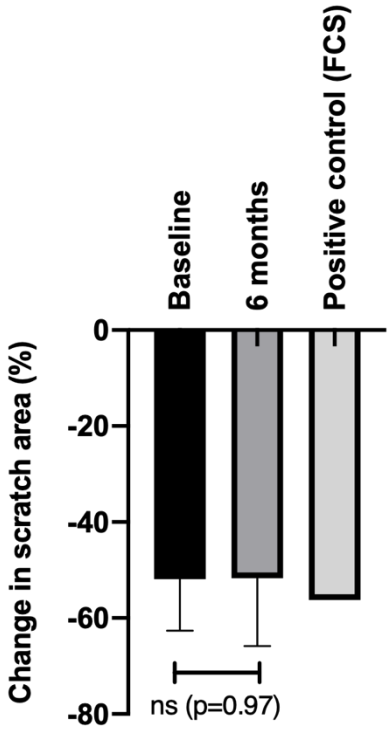

Figure 4-3: Scratch assay analysis. *Mean values taken from technical triplicate of 3. Data presented as mean percentage (%) change at 4 hours with standard deviation (error bars). Human umbilical vein endothelial cells (HUVECs) incubated with serum from patients at the start of the study and repeated after 6 months treatment with MCO. HUVEC's incubated with 5% foetal calf serum (FCS) for positive control. Statistical analysis performed using paired t-test.*

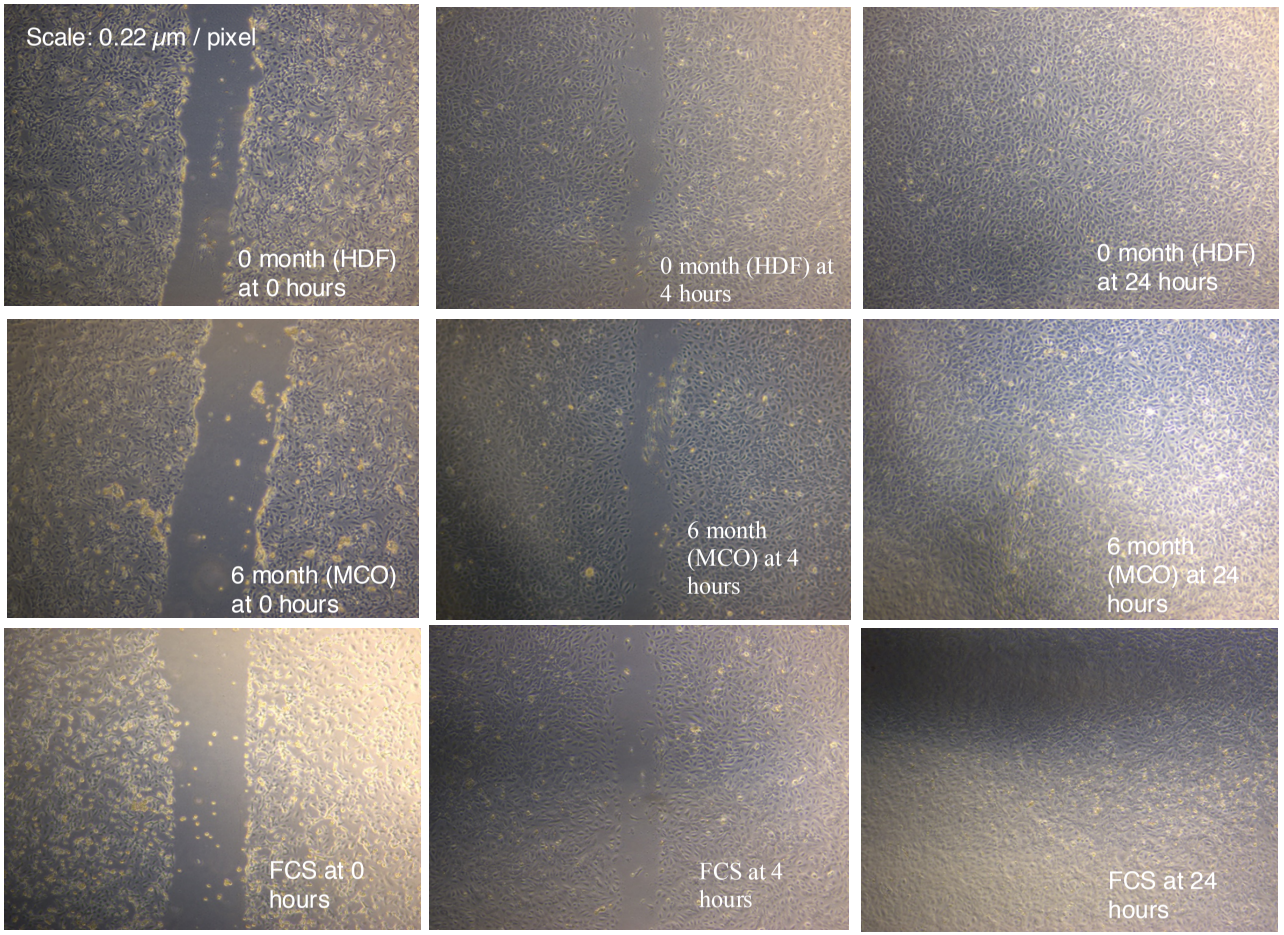

Figure 4-4: Scratch assay wound closure. *Representative images of scratch creation and closure taken at 4x magnification. Scratch was created on a confluent monolayer of HUVEC cells using a P1000 pipette tip and then treated in 5% serum in serum-free media. Micrographs were taken at 0,4 and 24 hours after scratch wound creation. Technical repeats were in triplicate.*

4.3.5 Endothelial migration (wound healing): correlations

Wound healing correlated with levels of ICAM (p = 0.048), p-selectin (p = 0.048), IL-8 (p = 0.05) and residual kidney function (Figure 4-5).

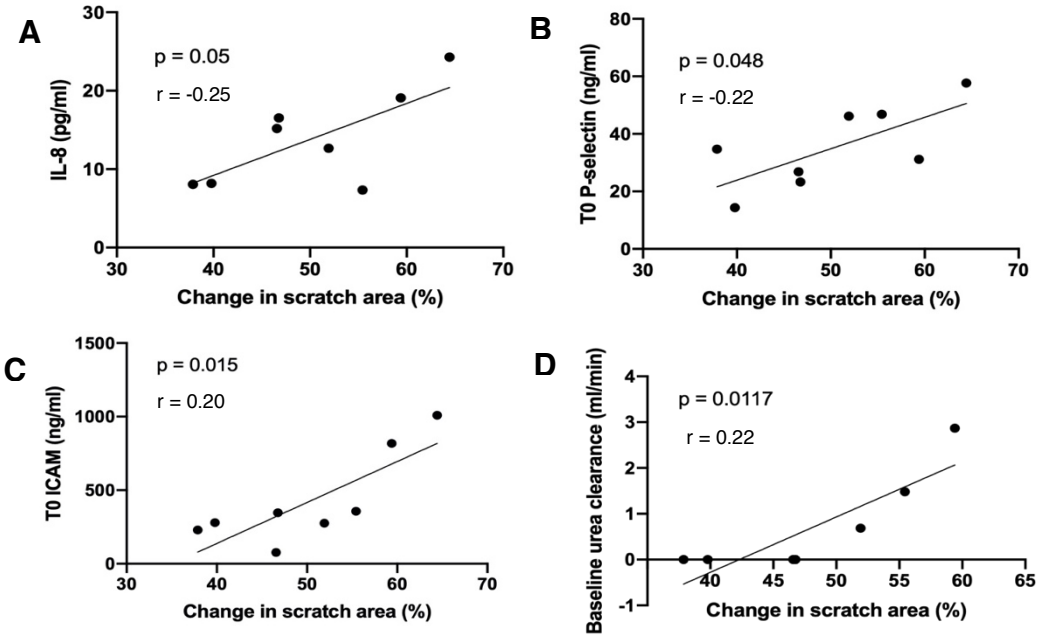

Figure 4-5: Correlation analysis with wound healing. *Correlation analysis was performed using Pearson's correlation coefficient. There was a significant correlation ($p<0.05$) between % change in scratch area at 5 hours and IL-8 concentration at baseline (A), P-selectin concentration at baseline (B), ICAM concentration at baseline (C) and residual kidney function (urea clearance) (D).*

There was no correlation between any of the markers of endothelial cell function and age, albumin or HD vintage.

4.3.6 Angiogenesis

There was no significant difference in mean tube number or mean tube length at 5 hours between cells treated with serum from patients at baseline and after 6 months of MCO treatment (tube number 73.5 ± 18.5 baseline vs 80.9 ± 24.6 at 6 months, p=0.341, paired t-test and mean tube length 1902.4±496.5μm at baseline vs 2138.3±670.9μm at 6 months, p=0.209, paired t-test). Data at 5 hours presented in figure 4-6 with representative images presented in 4-7. There was also no significant difference in tube length or number at 24-hours in samples treated with serum taken at baseline and 6 months.

Although the mean tube length and tube number were lower in patients with diabetes compared to those without diabetes at baseline at 5 hours, this difference was not statistically significant (mean tube number 62.9 ± 11.2 diabetics at 5 hours vs 79.8 ± 20.1 non-diabetics, p= 0.24 and mean tube length 1695.9±282.1μm diabetics vs 2026.3±583.5μm non-diabetics, p=0.40). There was no statistically significant difference at 6 months between diabetics and non-diabetics at 6 months in tube length and number and there was no significant difference between these two groups in change from baseline at 6 months.

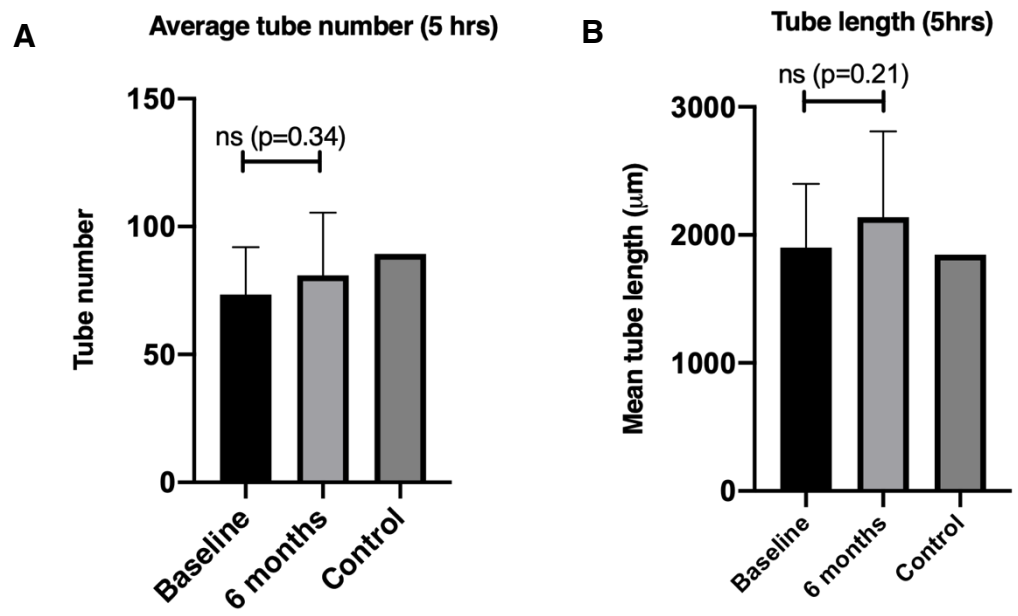

Figure 4-6: Effect of patient serum on angiogenesis. *Angiogenesis was assessed using the Matrigel® tube forming assay where tube number (A) and length (B) were measured at 5 hours and 24 hours. Serum from patients at the start of the study and after 6-months MCO treatment incubated with human umbilical vein endothelial cells (HUVECs) and plated with matrigel membrane matrix. Experimental control incubated with 5% foetal calf serum (FCS). Statistical analysis performed using unpaired t-test.*

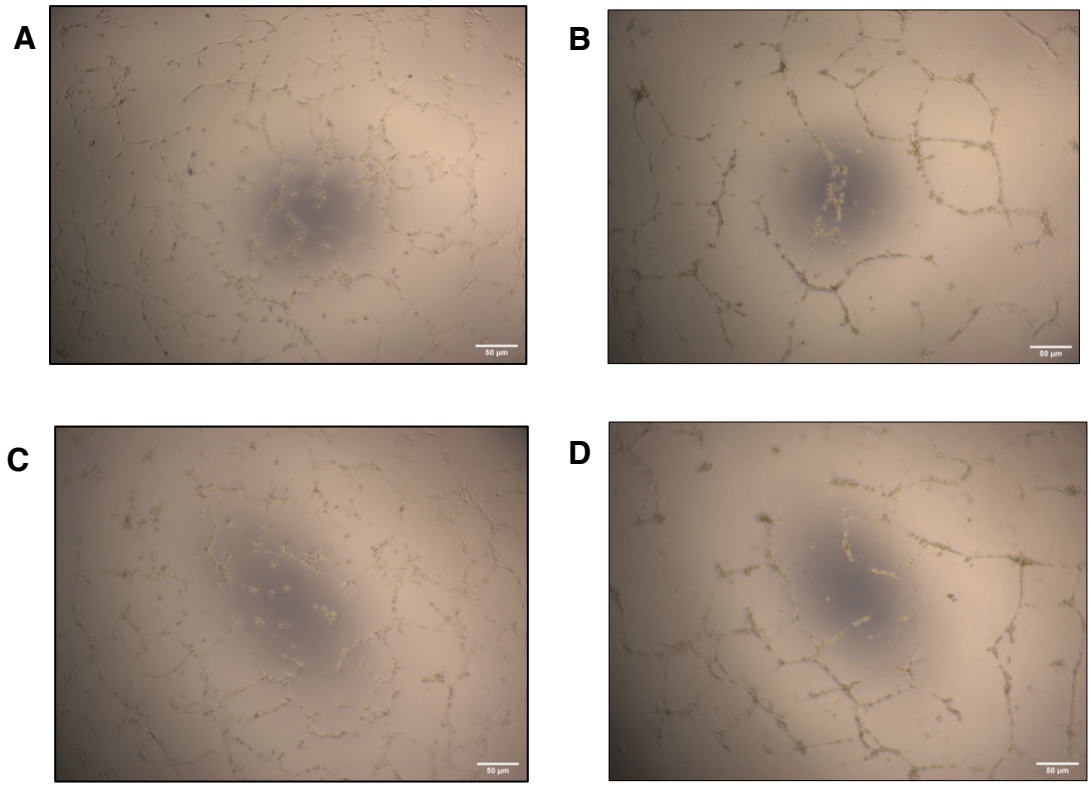

Figure 4-7: Representative images from angiogenesis study. *HUVEC's were suspended with either patient serum or 5% FCS as the experimental control and plated onto well plates containing matrigel membrane matrix. Images acquired at 5-hours (A) and 24-hours (B) with a repeat of the study with serum after 6-months treatment with MCO- 5-hours (C) and 24-hours (D)*

4.3.7 Angiogenesis: Correlations

Tube length at 5 hours correlated with concentrations of VEGF-C (r=0.81, p= 0.015), pentraxin-3 (r= 0.80, p = 0.018) and p-selectin (r= 0.80, p=0.018) at baseline (figure 4-8). A significant correlation was also found for the same three biomarkers with 5-hour tube number at baseline.

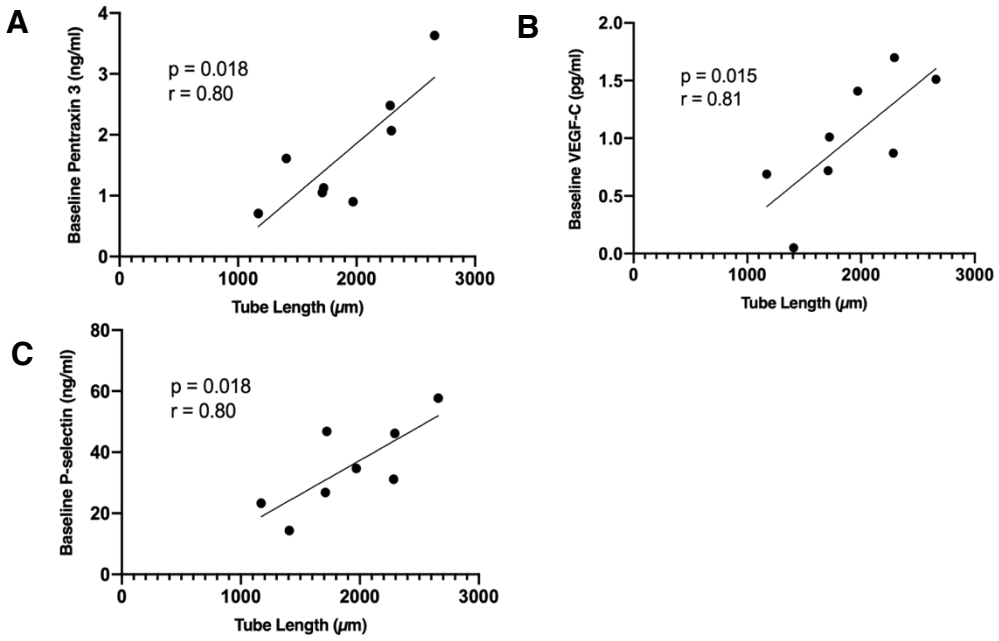

Figure 4-8: Correlation analysis with angiogenesis (tube length). *Correlation analysis was performed using Pearson's correlation coefficient. There was a significant correlation ($p<0.05$) between tube length at baseline in the matrigel assay and baseline serum pentraxin-3 concentration (ng/ml)(A), serum VEGF-C concentration (pg/ml) at baseline (B) and p-selectin concentration (ng/ml) at baseline (C).*

4.4 Discussion

Uraemic toxins are known to contribute to endothelial dysfunction and serum concentration of many of these toxins are linked with declining eGFR and increasing cardiovascular risk. Advanced dialysis modalities with superior clearances and enhanced blood purification might offer greater cardiovascular protection and improved endothelial function. On-line haemodiafiltration (OL-HDF) allows patients to receive the benefits of both a convective and diffusive therapy thereby providing enhanced solute clearance compared with standard high-flux haemodialysis [39-43]. Haemodialysis with a medium cut-off (MCO) membrane provides a novel 3rd treatment option for patients. MCO matches the enhanced clearance of HDF[40], it is simple to implement, patients are not exposed to high volumes of substitution fluid and therapy performance is maintained, even at low blood flow rates[39]. Whether these attempts to achieve clearance closer to the glomerular basement membrane equate to improved clinical outcomes are yet to be determined.

In this sub study, we have demonstrated that high volume HDF and MCO treatment provided a similar impact on in *vitro* endothelial cell function over a period of 6 months in the domains of cell viability, wound healing and angiogenesis. These findings are in keeping with the main MoDal study where there was no difference was identified between HDF and MCO treatment with respect to a panel of biomarkers assessing inflammation, angiogenesis, endothelial activation and concentration of larger middle molecules at 6

months. Patients remaining on HDF also had no change in endothelial cell markers at 6 months.

In the viability study, cell viability at 4 hours was greater than the experimental control for some of the patient samples. Cell viability as assessed using AlamarBlue® reflects cell metabolic activity rather than cell death. It may well be that solutes in the serum of study participants increased cell metabolic activity above that of the control (FCS). Utilisation of a cell viability which is not reliant on metabolic activity may have given a more reliable result. Resazurin-based assays are however in wide use and highly regarded as a robust measure of cell viability.

Endothelial cell markers were selected as it has been demonstrated by others that uraemic toxins directly impact on endothelial cell function [19,52,53]. This study has demonstrated that switching to HDF does not impact significantly on markers of endothelial cell function. There are, however, several other cell lines that are actively involved in the process of inflammatory cardiovascular disease in CKD including monocytes[432,433] and platelets[433-435]. Platelet-derived microvesicles (pMVs) for example, are the most abundant microvesicles in circulation and have a central role in inflammation, angiogenesis and cardiovascular disease[436]. An assessment of monocyte and platelet activity in this study would have been of interest and may have shown differences between the two arms of the study. This will be investigated in future studies.

High BMI is associated with lower cell viability (metabolic activity) at 4-hours for the patients in this study- this is a novel finding and somewhat in contrast to the obesity paradox recognised in HD patients. Once again, it is possible that the serum of obese patients induced reduced metabolic activity in cells rather than cell death. Higher BMI is the MCO group samples may have influenced and acted as a major confounder to assessing the effect of the MCO membrane on the endothelial parameters.

The correlation between endothelial cell migration (wound healing) and concentrations of adhesion molecules and cytokines (IL-8) are interesting. These findings highlight the important physiological role that these markers play in endothelial cell function- higher levels of all 3 markers were associated with faster *in vitro* wound healing in this study at 4 hours. The strong correlation of endothelial function to adhesion molecules may indicate that studying cell types (platelets and monocyte subsets) may provide more specificity to assessing endothelial dysfunction in dialysis. Although a high burden of inflammation and endothelial activation are associated with poorer cardiovascular outcomes, it is important to note the important physiological and protective roles that these markers also have. Amongst the enhanced spectrum of solutes removed by new dialysis technologies, it is plausible that alongside removal of toxins, solutes which have a protective effect may also be removed. The correlation between residual kidney function and wound

healing once again highlights the importance of residual function and the relationship it has with patient outcome measures.

The findings of the angiogenesis matrigel assay are in keeping with main MoDal study where there was no significant change in markers of angiogenesis (VEGF, VEGF-C and VEGF-R1). Although VEGF (42kDa) [437] is within the spectrum of solutes that may be removed by MCO treatment, only a single study has demonstrated a reduction in serum VEGF in MCO treatment[413]. VEGF-C and pentraxin-3 correlated with both tube length and tube number in this study and therefore it is feasible that improving clearance could impact on angiogenesis. HDF has been demonstrated to increase clearance of VEGF[438]. It is possible that the high volume HDF and the MCO treatments did not sufficiently differ in the terms of the spectrum of clearance of these toxins to impact on endothelial markers, which could explain the lack of significant differential between the two treatments in the angiogenesis assay.

4.5 Study Limitations

There are some limitations to this analysis. Firstly, there is a lack of published data on the cell culture methods used in this study in haemodialysis patients. There is a lack of sufficient data on the performance of these tests in this patient group which makes a comparison of the two performance groups in this study challenging. Studies in low-flux and high-flux dialysis could have added to the interpretation. Inclusion of a third comparator group in the study,

such as pre-dialysis patients, may also have added value to the interpretation of the results. Secondly, there were limitations in the measures of endothelial cell function used in this study. In the viability study, there were unexpected results whereby cell viability appeared to be better preserved in those with CKD compared with health volunteers. The test used in this study represents metabolic the activity of cells rather than representing cell death. Selecting an alternative measure of endothelial cell viability may have provided better discrimination between the two treatment modalities. It is also possible that tests focussing on specific cell subtypes like platelets or monocytes may be important. A comprehensive analysis or review of the performance of these tests and their applicability might help select a battery of measures in this context. Thirdly, there were significant differences between the two study groups such as BMI. BMI was significantly high in the MCO group and appears to be linked with cell viability and function. Adjusting for these confounders in future studies would be important

4.6 Conclusion

This study demonstrated a significant relationship between large middle molecules (VEGF, cytokines IL8, P-Selectin, adhesion molecules and Pentraxin-3) and clinical factors such as BMI and residual renal function impact on endothelial function. Both the treatment modalities of HvHDF and MCO dialysis showed a similar impact on in vitro endothelial function over a period of 6 months. Studies designed to provide a greater differential in clearance of relevant uremic toxins adjusted for clinical confounders would

help define the best prescription of dialytic clearance to improve endothelial function and cardiovascular protection in ESRD.

Chapter 5: Results: Exploring the Effects of Dialysis Membrane Modality on Markers of Protein Energy Wasting

5.1 Introduction

Volume status and malnutrition status are important determinants of outcome measures in haemodialysis patients. Volume overload is associated with hypertension[439-442], a higher mortality rate[443-446] and a number of symptoms including fatigue [447] and shortness of breath. Underhydration has the potential to lead to a faster loss of residual kidney function (RKF) and therefore the loss of benefits associated ongoing RKF (clearance of larger middle molecules, more liberal fluid restrictions and a favourable life expectancy[34]). Nutritional parameters such as albumin[448,449] and handgrip strength[450] correlate with outcome measures in haemodialysis patients. Protein-energy malnutrition is common and a well-recognised syndrome in patients with advanced CKD [451] [452] [453]. It is associated with the high burden of inflammation seen in this patient group [160] [161]. Measures of hydration and nutritional status as well as interventions to optimise these parameters could lead to improvements in patient care.

At present, clinical methods of assessing hydration and malnutrition status can be unreliable or operator-dependent.

Haemodialysis using a medium cut-off (MCO) membrane (HDx therapy) provides enhanced clearance of larger middle molecules compared with high-flux haemodialysis (HFHD) [342] [90] [333] [40]. It provides similar and, in some cases, improved clearance compared with HDF [90] [40]. Enhanced clearance of inflammatory cytokines such as IL-6 and TNF-α through HDx [342] could

potentially reduce the burden of protein-energy wasting. At present, the effect of HDX therapy on hydration and nutritional parameters as measured by BIS is unknown. As such, we sought to assess the effect of HDx therapy, as compared to HDF therapy on nutritional and hydration parameters as measured by BIS.

5.2 Methods

5.2.1 Ethics statement

Ethical approval for this study was received from the NHS Health Research Authority Research Ethics Committee North West-Preston (18/NW/0169). All patients provided informed written consent. This study was registered on ClinicalTrials.gov, Trial Reference: NCT03510520.

5.2.2 Study Population

Data was collected through the main MoDal study. Full details of the methodology of this study are presented in chapter 2. Briefly, this was an interventional, single-centre, open-label randomised controlled study comparing haemodiafiltration (HDF) to haemodialysis with a medium cut-off haemodialysis membrane (HDx). Patients were recruited from 2 haemodialysis units within a single renal centre (Manchester NHS Foundation Trust, UK). 63 patients were recruited with 31 patients remaining on HDF therapy and 32 patients switching to HDx therapy for 24 weeks. Exclusion

criteria were planned live donor transplant within 6 months, planned switch in dialysis modality within 6 months or clinical prognosis predicted as less than 6 months.

5.2.3 Measurement of Body Composition

This is detailed in section 2.14.2 in Chapter 2.

5.2.4 Statistical Methods

An intention-to-treat analysis was carried out such that all participant data was analysed, regardless of whether they completed the full study protocol. Data normality was assessed by the D'Agostino & Pearson test. Normally distributed continuous data are presented as a mean with standard deviation (SD) and skewed data are presented as median with an upper and lower quartile (Q1, Q3). Comparison between groups was performed using a Pearson's Chi^2 Test, Cochran-Armitage test or Fisher's exact test. Student's t test was used to compare continuous data with a normal distribution and a Mann-Whitney U test was used for skewed continuous data.

For missing data, a last observation carried forward (LOCF) technique was utilised.

Where 3 timepoints were compared within a single group, a one-way ANOVA test was used. Correlations between data groups were analysed using Pearson's test. GraphPad Prism v 8 (GraphPad Software, San Diego, California, USA) was used to conduct all statistical analysis.

5.3 Results

5.3.1 Patients

A total of 55 patients were included in this study. Of the 63 patients at the start of the main MoDal Study, 8 patients were excluded from the study analysis due to a significantly raised BMI (3 patients in total, BMI 56.0, 60.7 and 54.5), poor skin contact or the presence of dressings (3 patients) or lower limb amputation (2 patients) making reliable BCM measurements challenging.

The BCM device has not been validated in very high BMI's and there is uncertainty around the accuracy of results in this setting[454]. A single study has compared the utility of the BCM device in very high BMI's (range 35-51 kg/m^2), comparing the overhydration (OH) data obtained pre- and post-dialysis with relative blood volume monitoring data[455]. Whilst this study did not show evidence of systemic bias in BCM measured OH in obese subjects, there are no studies that have validated the device in BMI's greater than 51 kg/m^2. Very high BMI's (>50 kg/m^2) were excluded. For clarity, details of participants from

the main study whose BCM data was excluded form analysis are presented in figure 5-1.

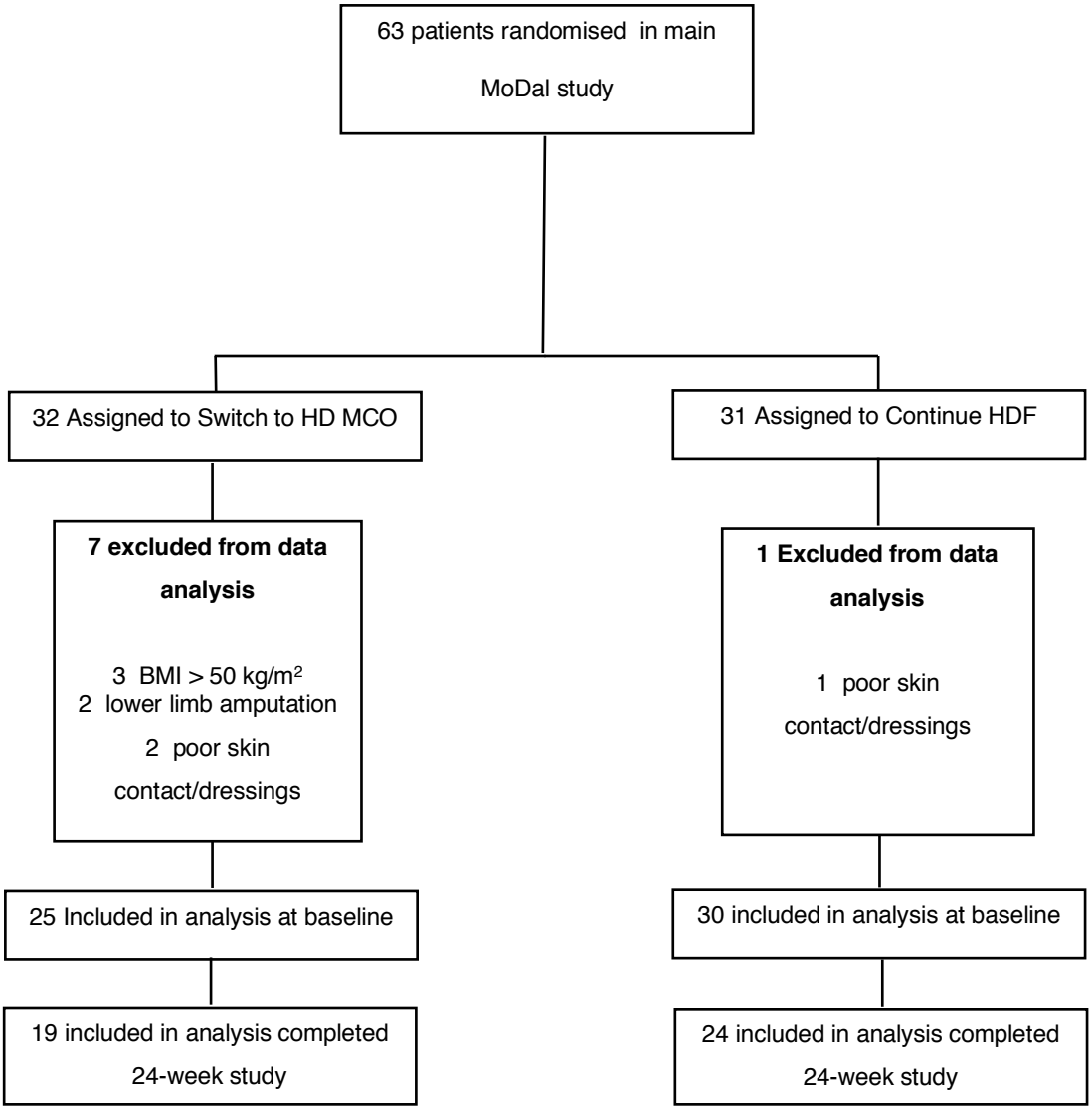

Figure 5-1:Consort diagram outlining participant data excluded from analysis of Body Composition Monitor data

Of the remaining patients included in this study, further single measurements were excluded if there was uncertainty regarding the reliability of the result. These were very few measurements in total. In general, results were reviewed when they were greater than two standard deviations above or below the

previous result for the same participant or study group. Some participants did not have a BCM measurement at every timepoint within the study however the numbers of missed measurements were low and are detailed in Table 5-1. Missing or excluded measurements accounted for <4% of measurements in the analysis and analysis was completed with the last observation carried forward.

	T0	T6	T12	T18	T24
Total in main MoDal study	63	56	55	51	50
Patients excluded for this study	8	8	8	7	7
Measurement excluded at timepoint or not completed	2	6	2	0	0
Total measurements included in analysis	53	42	45	44	43

Table 5-1: Overview of data excluded from study analysis. *A total of 8 patients from the main MoDal study were not included in this study (see figure 5-1). Measurements were also excluded at some of the time points if there were technical issues or a suspected spurious result.*

5.3.2 Baseline Characteristics

At baseline, there were differences between the two groups where the MCO group had a higher weight and Body Surface Area (BSA). Kt/V and HD vintage was lower in the MCO group (data presented in table 5-2).

	All	HDF	MCO
n	55	30	25
Age	66 (50, 74)	69.5 (53.75, 77.0)	59 (39.5, 70.0)
Male Sex	40 (72.7%)	21 (70%)	19 (76%)
Ethnicity			
Caucasian	33 (60%)	19 (63.3%)	14 (56%)
Asian	11 (20%)	4 (13.3%)	7 (28%)
African or Afro-Caribbean	11 (20%)	7 (23.3%)	4 (16%)
Weight	75.69±17.96	70.81±13.96	81.55±20.61
BMI	25.37 (22.07, 31.44)	24.49 (21.55, 28.86)	25.57 (23.45, 32.83)
BSA	1.87±0.25	1.80±0.20	1.95±0.29
Diabetes	27 (49.1%)	10 (33.3%)	7 (28%)
HD Vintage	20.22 (12.26, 51.12)	31.63 (16.55, 65.09)	16.50 (9.22, 30.84)
Vascular Access			
AVF/G	40 (72.7%)	21 (70%)	19 (76%)
Line	15 (27.3%)	9 (30%)	6 (24%)
Anuric	32 (58.2%)	20 (66.7%)	12 (48%)
Urine Volume (ml)	730 (537.5, 1900.0)	860 (580.0, 2330.0)	600 (485.0, 1270.0)
Residual Urea Clearance (ml/min)	1.65 (1.23, 11.45)	1.79 (1.26, 3.44)	1.49 (0.96, 2.40)
spKt/V	1.33±0.29	1.40±0.30	1.24±0.24
Substitution Volume (L)	21.87±9.05	21.49± 7.44	22.34±10.91
Hb (g/l)	114.02±19.28	114.5±23.27	113.4±13.29
Adjusted Calcium (mg/l)	2.46±0.18	2.48±0.18	2.42±0.19
Phosphate (mg/l)	1.72±0.43	1.68±0.53	1.78±0.46
Albumin (g/l)	34.50 (29.5, 35.0)	32.5 (28.8, 35.0)	34.5 (29.5, 35)
CRP (mg/l)	8.50 (3.25, 23.0)	5.0 (2.0, 11.5)	8.5 (3.3, 23.0)
PTH (pmol/l)	29.75 (10.13, 115.3)	30.9 (9.43, 49.9)	36.9 (15.1, 101.0)
BP Systolic (mmHg)	130.91±23.10	128.1±22.31	134.3±24.05
BP Diastolic (mmHg)	79.34±16.21	80.21± 17.30	78.29±15.07

Table 5-2: Baseline characteristics *of all patients in whom BCM measurement analysis has been performed with subgroup analysis. Data presented as mean ± standard deviation, as total number (%) or as median (IQR). Means compared using unpaired t-test (normally distributed) or Mann-Whitney test for skewed data. Categorical data compared using Fisher's exact test. *denotes p<0.05.*

At baseline, the MCO group had a higher FTI (11.86±6.11 kg/m^2 vs 16.07±7.40 kg/m^2 in the MCO group, p = 0.0307). As expected, there were also associated differences between the two groups in other measures of fat composition (data presented in table 5-3). The MCO group had a lower relative lean tissue mass (LTM) (p = 0.04) with a higher relative fat (p= 0.03), fat mass (p = 0.005) and adipose tissue mass (p = 0.0048).

There was no significant difference between the two groups in LTI and although the HDF group had a higher proportion of patients with a high LTI compared with the MCO group, this did not reach statistical significance (20% HDF vs 4% MCO, p = 0.11).

	All	HDF	MCO	p value
n	55	30	25	N/A
LTI (kg/m^2)	12.49±2.88	12.91±2.71	12.02±3.05	0.2728
LTI Reference Groups				
Low LTI	13	7 (23.3%)	6 (24%)	
Normal LTI	35	17 (56.6%)	18 (72%)	
High LTI	7	6 (20%)	1 (4%)	0.1117$^{\Delta}$
FTI (kg/m^2)	13.84±7.01	11.86±6.11	16.07±7.40	**0.0307***
FTI Reference Groups				
Low FTI	5	4 (13.3%)	1 (4%)	
Normal FTI	33	20 (66.6%)	13 (52%)	
High FTI	17	6 (20%)	11 (44%)	0.0798$^{\Delta}$
Pre-Dialysis Overhydration (L)	0.81±1.58	0.72±1.69	0.91±1.48	0.6725
Relative Overhydration (%)	4.54±9.74	3.98±10.99	5.17±8.31	0.6606
TBW (L)	35.23±6.74	34.33±6.43	36.23±7.08	0.3106
ECW (L)	16.72±3.23	16.07±3.94	17.46±3.44	0.1185
ECW/TBW	0.48±0.04	0.47±0.04	0.48±0.04	0.3959
ICW (L)	18.50±4.05	18.26±4.08	18.78±4.09	0.6463
E/I	0.92±0.14	0.89±0.14	0.95±0.15	0.1728
LTM (Kg)	35.39±9.74	36.31±9.93	34.78±9.67	0.5804
Rel LTM (%)	48.73±16.3	53.14±15.86	43.77±15.63	**0.0389***
Fat (Kg)	29.1±14.63	23.79±11.55	35.08±15.62	**0.0048***
Rel Fat (%)	36.18±12.38	32.67±12.14	40.14±11.65	**0.0299***
ATM (Kg)	39.59±19.9	32.37±19.77	47.72±21.24	**0.0048***

Table 5-3: Baseline BCM measurements *for all participants with subgroup analysis. Data presented as mean ± standard deviation. Means compared using unpaired t-test. $^{\Delta}$Fisher's exact test comparing proportion of "high" vs "low + normal" in each group". *denotes p<0.05. Abbreviations: Lean Tissue Index (LTI), Total Body Water (TBW), Extracellular Water (ECW), Intracellular Water (ICW), Fat Tissue Index (FTI), Ratio of Extracellular Water to Intracellular Water (E/I), Overhydration (OH), Relative Overhydration (OH/ECW), Ratio of Extracellular Water to Total Body Water (ECW/TBW), Lean Tissue Mass (LTM), Adipose Tissue Mass (ATM).*

5.3.3 Treatment with MCO may be associated with preservation of LTI and FTI compared with HDF over 24 weeks

There was a fall in HDF in LTI in the HDF group that was close to reaching statistical significance. LTI change was -0.81 kg/m^2 ±1.90 (p = 0.058) compared with MCO 0.28 kg/m^2 ±2.10 (p = 0.58). There was a rise in FTI close to significance of 0.94 kg/m^2 ±2.14 (p = 0.052) compared with MCO where change was 0.13 kg/m^2 ±5.41(p = 0.91). Data presented in figure 5-2.

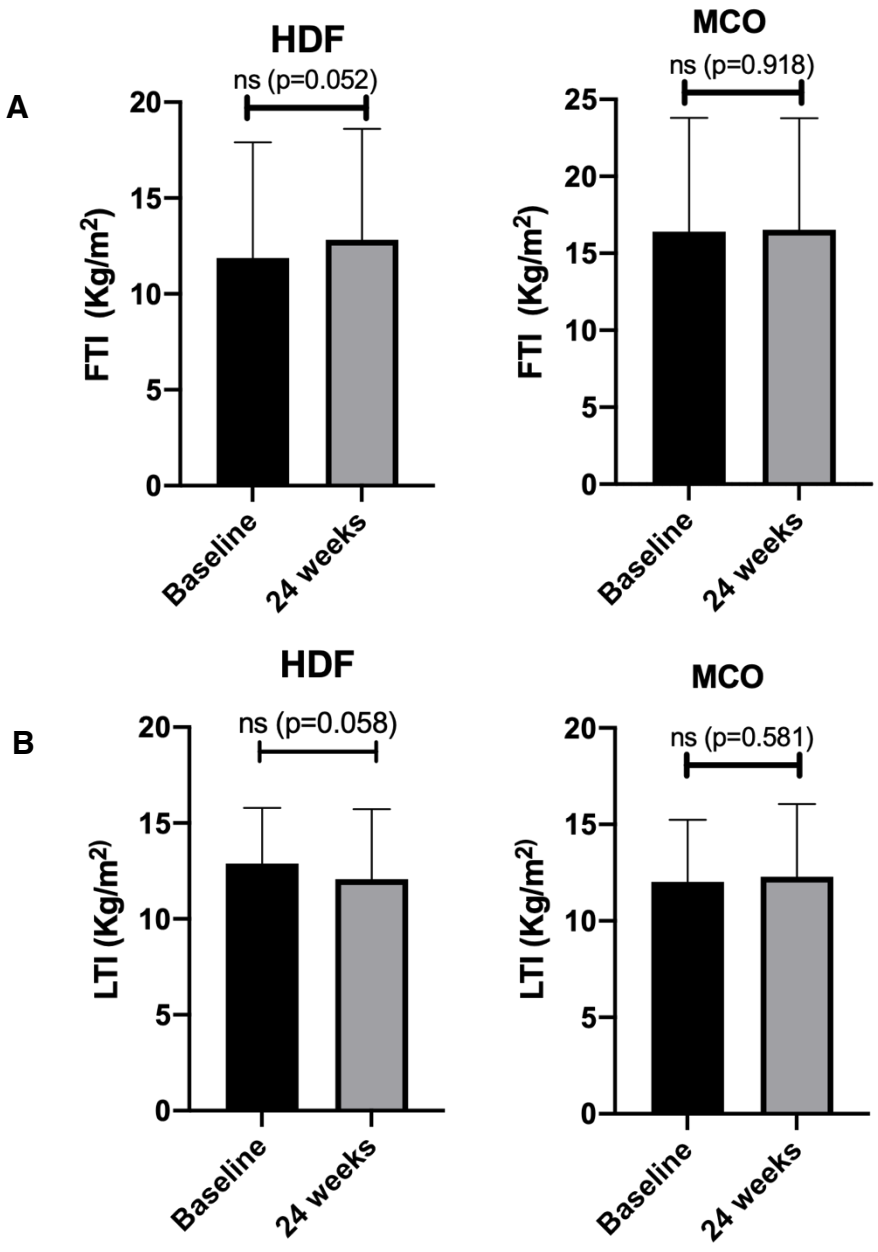

Figure 5-2:Change in fat tissue index (FTI) and lean tissue index (LTI) during study period. *Mean FTI (A) and LTI (B) with standard deviation (error bars) at start and end of the study. There was no significant change in either parameter in either group during the study period.*

	HDF (n = 22)			
	Study Start	**Study End**	**Change**	**p**
OH (Litres)	0.80 ±1.42	0.80±1.26	0.00±0.99	>0.9999
Rel OH (%)	4.88±9.22	5.24±8.43	0.36±5.58	0.7658
E/I	0.91±0.12	0.95±0.14	0.04±0.10	0.0514
ECW/TBW	0.47±0.04	0.49±0.04	0.006±0.02	0.2921
LTI (kg/m^2)	12.9±2.91	12.1±3.65	-0.81±1.90	0.0578
FTI (kg/m^2)	11.9±6.03	12.8±5.79	0.94±2.14	0.0520
Total Body Weight	69.28±13.80	69.75±14.40	0.48±2.12	0.9112
	MCO (n = 19)			
OH (Litres)	0.91±1.64	0.74±1.70	-0.17±1.95	0.7109
Rel OH (%)	4.85±9.03	3.89±9.17	-0.95±9.74	0.6748
E/I	0.95±0.16	0.96±0.16	0.004±0.16	0.9212
ECW/TBW	0.48±0.04	0.49±0.04	0.001±0.04	0.9027
LTI (kg/m^2)	12.0±3.22	12.3±3.77	0.28±2.10	0.5813
FTI (kg/m^2)	16.4±7.39	16.5±7.24	0.13±5.41	0.9180
Total Body Weight	84.71±21.40	84.55±18.93	-0.16±7.41	0.9809

Table 5-4: Change in body composition parameters between start and end of the study *(24 weeks total). Only patients who had a BCM measurement at the first and last visit were included for this analysis (n = 41). Data presented as mean ± standard deviation. Means compared using paired students t-test.*

Abbreviations: Lean Tissue Index (LTI), Fat Tissue Index (FTI), Ratio of Extracellular Water to Intracellular Water (E/I), Overhydration (OH), Relative Overhydration (OH/ECW), Ratio of Extracellular Water to Total Body Water (ECW/TBW)

5.3.4 Lean tissue index correlates with serum albumin, VEGF, Kt/V and other body composition parameters

There were significant linear correlations between LTI at baseline and the following baseline values: serum albumin, Kt/V, VEGF, FTI and E/I (as well as ECW/TBW). Data presented in figure 5-3. The correlation between EMV and LTI was close to significance ($r = -0.248$, $p = 0.079$). There was no correlation with any patient reported outcome measures (PROM'S). The PROM results are presented in chapter 6.

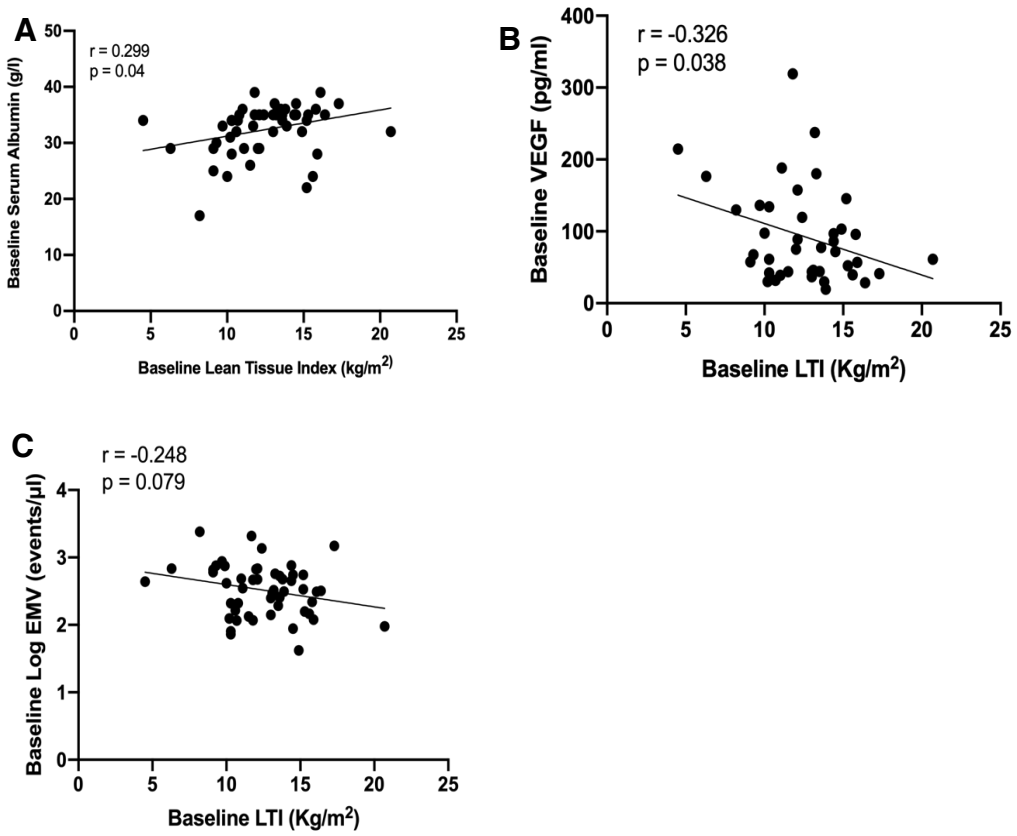

Figure 5-3:Correlation analysis with lean tissue index (LTI). *Correlation analysis performed using Pearson's correlation coefficient. There was a significant correlation ($p<0.05$) between LTI at baseline and serum albumin (A) and serum vascular endothelial growth factor (VEGF) (B). The correlation between LTI and EMV (C) was close to significance ($p = 0.07$). There was also a significant correlation between LTI and fat tissue index (FTI) and extracellular to intracellular fluid (E/I) ratio (graphs not presented here).*

5.3.5 Fat tissue index correlates with serum albumin, markers of endothelial activation, leptin other body composition parameters

Baseline FTI correlated with baseline values of EMV, leptin, vascular endothelial growth factor (VEGF), LTI, and E/I. Correlations presented in figure 5-4.

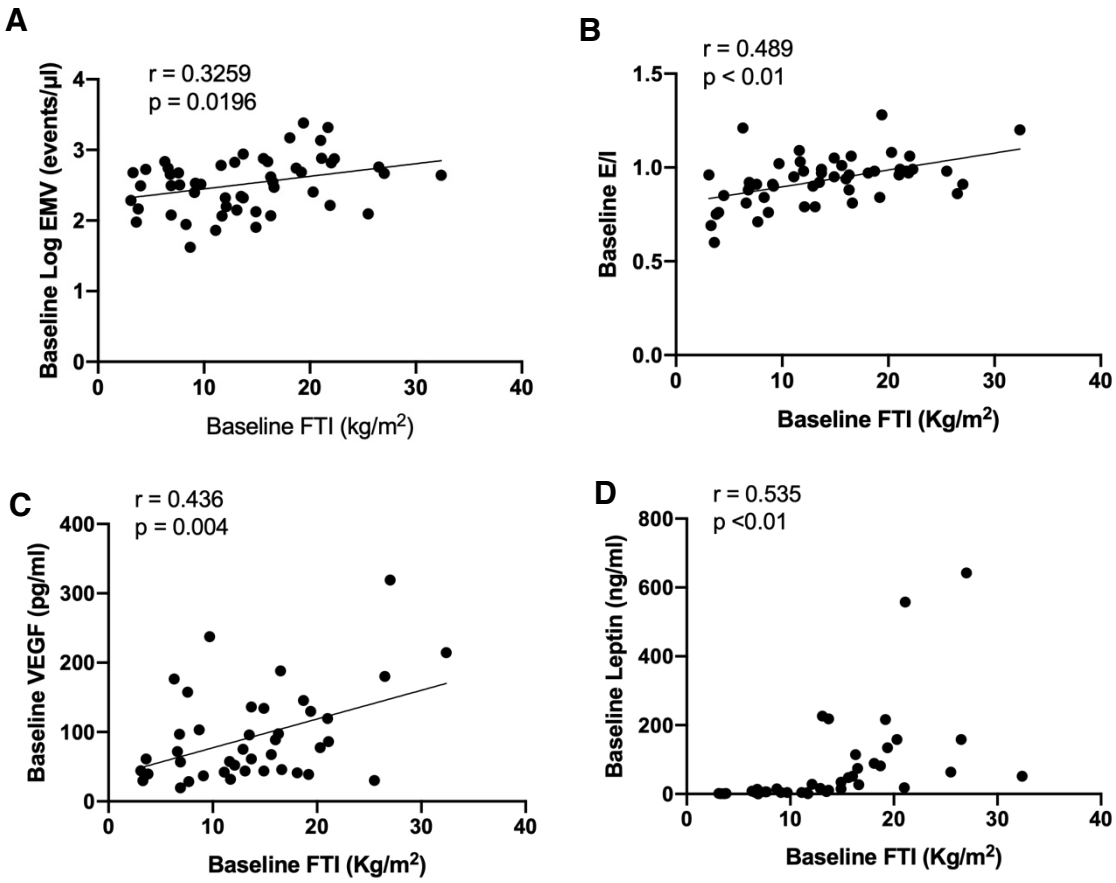

Figure 5-4: Correlation analysis with fat tissue index (FTI). *Correlation analysis performed using Pearson's correlation coefficient. There was a significant correlation ($p<0.05$) between FTI at baseline and baseline endothelial microvesicle (EMV) count (A), extracellular water to intracellular water ratio (E/I)(B), serum vascular endothelial growth factor (VEGF) concentration (C) and serum leptin concentration (D).*

5.3.6 Treatment with MCO is not associated with any change in hydration status over 24 weeks

There were no significant changes in hydration status during the study period. In the MCO group, both mean overhydration (-0.17 ± 1.95 litres) and mean relative overhydration -0.95% ±9.74 decreased slightly during the 24-week

study period but this did not reach statistical significance (p = 0.7109 and 0.6748 respectively). Data presented in figure 5-5. In the HDF group, mean overhydration was unchanged during the study period and mean relative overhydration increased by 0.36% ± 5.58 (p = 0.7658). The ratio of extracellular to intracellular water (E/I) changed very little during the study period in both groups (0.004±0.16 MCO and 0.04±0.10 in HDF, P>0.05 for both of these changes).

Of interest, there appeared to be a rise in relative overhydration in both groups during the study period, peaking during the middle of the study and then falling again (see figure 5-5). The change in relative overhydration over the course of the study was not statistically significant in a mixed effects model (MCO p = 0.16, HDF, p = 0.61).

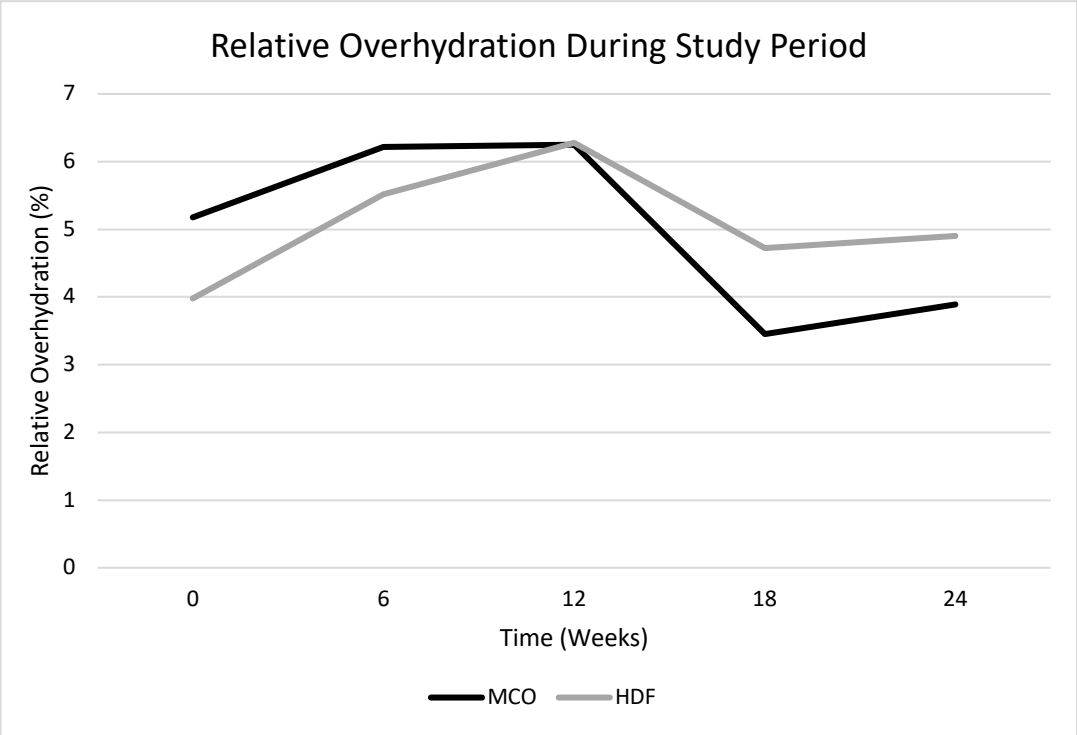

Figure 5-5:Relative overhydration (%) during study period. *Bioimpedance measurements were performed at the start of the study and every six weeks until the end of the study (24 weeks). There was no significant change in relative overhydration during the study period in either group in a mixed effects model (MCO p = 0.16, HDF, p = 0.61).*

There was no significant change in mean UF volume in either group during the study period, MCO p= 0.5052 and HDF p = 0.4665, mixed effects model. Data presented in figure 5-6. Data capture for episodes of intra-dialytic hypotension was incomplete and unreliable therefore analysis was not undertaken.

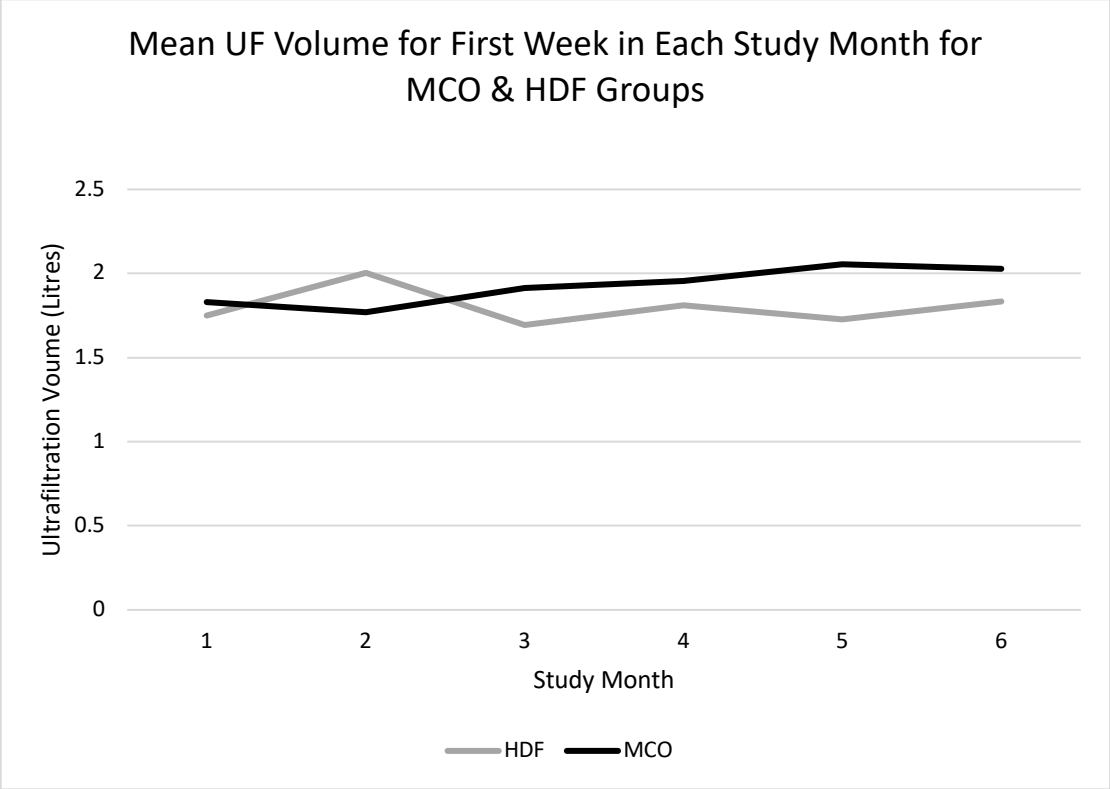

Figure 5-6:Average ultrafiltration (UF) volume *for the first week in each study month for participants in the HDF & MCO groups. There was no significant change within either group in ultrafiltration volume during the study period (MCO p= 0.5052 and HDF p = 0.4665, mixed effects model).*

5.3.7 Indices of overhydration correlate with serum albumin, age, co-morbidity and markers of endothelial activation (VEGF and VCAM)

There was a significant correlation between the ratio of extracellular water and total body water (ECW/TBW) and the following parameters: Charlson comorbidity index (CCI), age, BMI, serum albumin, VCAM and VEGF. There was also a significant correlation between ECW/TBW and other BCM parameters- OH, E/I, LTI and FTI. Again, correlation with baseline EMV was close to significance ($r = 0.237$, $p = 0.081$). There was no linear correlation

between any of the remaining inflammatory markers (CRP, IL-6, IL-8, IL-10, TNF-α) and parameters of hydration status (ECW and ECW/TBW).

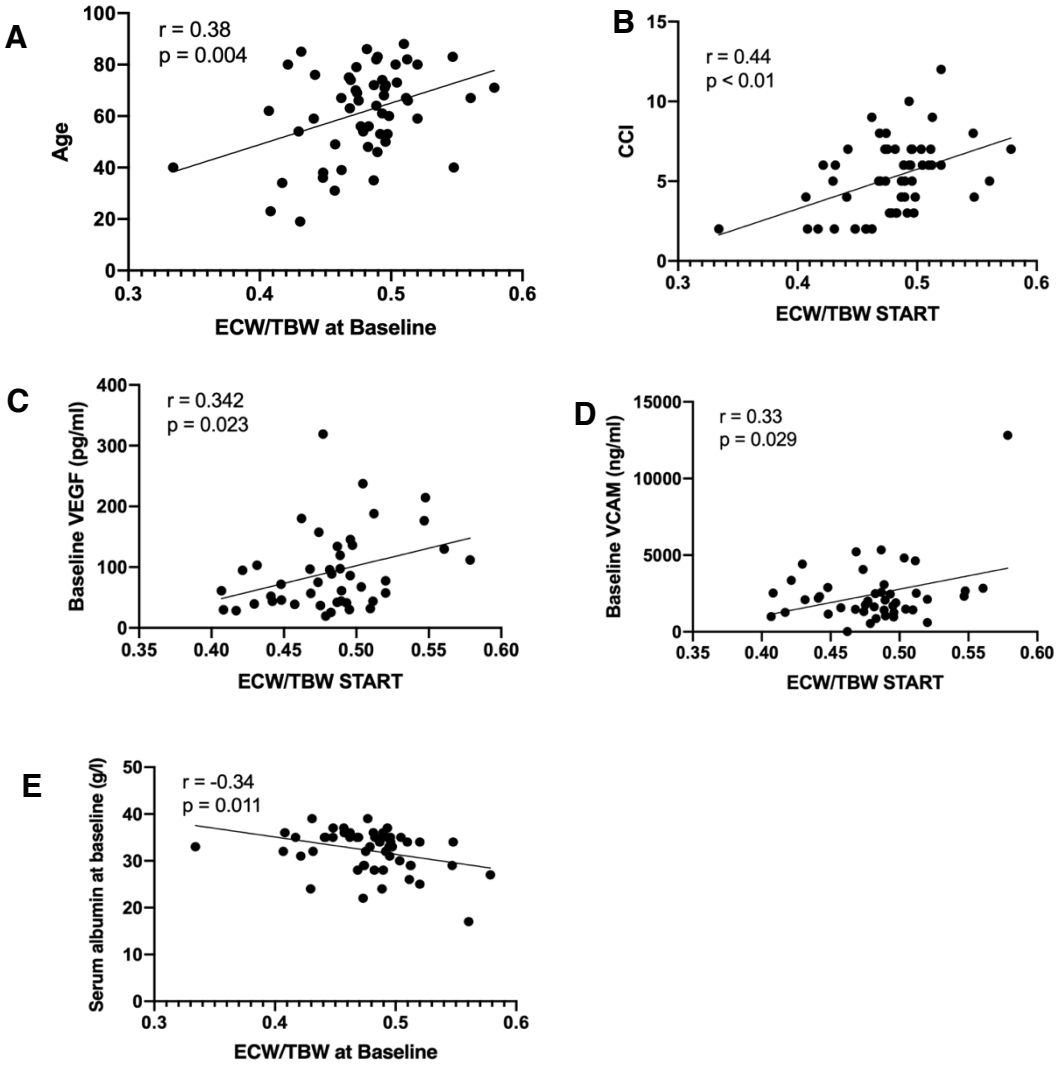

Figure 5-7: Correlation analysis with fat tissue index (FTI). *Correlation analysis performed using Pearson's correlation coefficient. There was a significant correlation ($p<0.05$) between the ratio of extracellular water and total body water (ECW/TBW) and age (A), Charlson comorbidity index (CCI) (B), vascular endothelial growth factor (VEGF) (C), vascular cell adhesion protein (VCAM) (D) and serum albumin (E).*

5.4 Discussion

5.4.1 Treatment with MCO could be associated with preserving LTI and FTI over a 6-month period compared with HDF

Although none of the changes in body composition during the study period reached statistical significance, there was a signal of change in the HDF group (a fall in LTI and rise in FTI) that was not seen in the MCO group. Preserving LTI could be of benefit to patients given the poorer outcomes associated with a low LTI. Several studies have demonstrated the association with a low LTI with poorer outcomes and demonstrated LTI as a predictor of mortality[389-394]. There are limited longitudinal studies that have assessed the changes in LTI and FTI in serial BCM measurements in patients treated with HD or HDF and none of these studies have assessed whether a change in LTI or FTI impacts on outcome. In a large study (n = 8,227) by Marcelli et al[159], LTI fell by 0.4kg/m^2 during a 2 year follow-up period with a mean of 319 days between the first and last BCM measurements indicating that a change in these parameters are likely to be over years.

In this study, the MCO group did have a higher FTI at baseline and it is possible that the signal of change here is confounded by the baseline differences between the two groups in terms of body composition. It is feasible, however, that medium cut-off dialysis could impact on nutritional indices through enhanced solute clearance (this could impact on inflammation) and this link should be explored further in future studies.

5.4.2 A low LTI or high FTI are associated with markers of endothelial activation and increased serum leptin

The link between markers of endothelial activation (VEGF and EMV) highlight the important link between inflammation, endothelial activation and nutritional status[456] [457,458]. Higher concentrations of EMV were seen in patients with a lower LTI and higher FTI suggesting lower endothelial activation in patients with more optimal nutritional parameters. In this study there was no difference in EMV concentration between the two groups at months. Other studies, however, have shown that dialysis treatment modality can impact on plasma EMV concentration[301,303,311] and it is plausible that this could translate into changes in nutritional parameters. A longer, study powered to assess for this change would be of interest.

The link between leptin and FTI in this study is in keeping with leptin production being predominantly in adipose tissue[459]. Several studies have identified a correlation between BMI and leptin such that higher leptin levels are seen in obesity [460-462] and although it is associated with anorexia, leptin "resistance" may exist. The results of studies assessing the impact of improving leptin clearance appear to be mixed[114,117,463-465]. Although improving clearance of leptin could help modulate nutritional parameters and inflammation, it does not address leptin production and therefore could be of limited utility.

5.4.3 MCO treatment is not associated with any change in hydration status compared with HDF over 6 months

Hydration status remained unchanged during the study period in both groups. These findings suggest that changing membrane flux and modality does not impact on hydration status. Interestingly, volume overload was not common in the patients in this study with a mean pre-dialysis overhydration of just 0.8 litres ± 1.42 and a relative overhydration of 4.54% ± 9.7. Extracellular fluid overload is associated with microinflammation and endothelial dysfunction[466]. Expansion of extracellular fluid above 15% is linked with poorer outcomes[396,443,467]. The fairly modest overhydration seen in patients in this study could have masked any effect of the membrane. Additionally, there was no significant change in this study in other markers of endothelial activation, angiogenesis and middle molecules to account for changes in hydration status that you may see as a result.

5.4.4 Overhydration is associated with markers of endothelial activation

Markers of endothelial activation in this study (VCAM and VEGF) were associated with overhydration in this study. A link with EMV was close to significance. These findings are similar to other studies in demonstrating a relationship between volume status and inflammation[463,465,466]. Despite these known links, several markers of inflammation such as CRP, IL-6 and TNF-α in this study did not correlate with hydration parameters suggesting a more

complex relationship. This link between endothelial activation, microinflammation and fluid redistribution requires further exploration.

5.5 Study Limitations

There are a number of limitations to this study that should be considered. Firstly, data on episodes of intradialytic hypotension would have been interesting and was not reliably captured in this study. Given that multiple studies have demonstrated a reduction in IDH episodes with HDF[468-471], the impact of membrane treatment on this modality would have been of interest as well as exploring the link between overhydration and IDH episodes in this context. Secondly, the 8 participants not included in the analysis for this study (12.7% of participants due to poor quality results, lower limb dressings or amputation) may have introduced a study bias as patients where reliable BCM data capture is challenging often have high co-morbidity. Thirdly, the clear baseline differences between the 2 groups (the MCO group had a higher relative fat and FTI) may have influenced the study findings for example, a lower baseline FTI is associated with a reduction in LTI over time [159].

5.6 Conclusions

In conclusion, HDx therapy may be associated with preservation of nutritional parameters (LTI and FTI) over a 24-week period compared with HDF treatment and this finding warrants further exploration in future studies. This study has demonstrated a relationship between markers of endothelial

activation and body composition parameters (LTI, FTI and hydration status). Given the close link between inflammation, hydration, nutritional parameters and outcomes in CKD patients, further studies exploring the impact of dialysis modalities and membranes on body composition would be of interest.

Chapter 6: Results: The Impact of Medium Cut-Off Haemodialysis on Patient-Reported Outcome Measures

6.1 Introduction

Medium cut-off (MCO) haemodialysis (HDx) is a significant advancement in haemodialysis membrane technology. It offers a significant improvement in the clearance of larger "middle" molecules such as ß2-micrglobulin, kappa free light chains and YKL-40 in comparison to HFHD[342 90 333 40]. It provides similar and in the case of some uraemic retention solutes, improved clearance, compared with HDF[40]. Although MCO membranes are in clinical use, studies assessing their clinical efficacy are lacking and there is only a single clinical study published comparing HDx treatment with HDF treatment[344]. The MoDal Study (A Randomised Feasibility Study Investigating the Effect of Medium Cut-Off Haemodialysis on Markers of Vascular Health Compared with On-Line Haemodiafiltration) was designed to compare the effect of HDx therapy on markers of vascular health in comparison to HDF.

In this study, we sought to assess the effect of switching patients from HDF to HDx therapy on patient-reported outcome measures (symptom burden, fatigue and dialysis recovery time).

6.2 Methodology

The full methodology for this study is presented in Chapter 2.

6.2.1 Study Population

Full details of the methodology of this study are published in chapter 2. Briefly, this was an interventional, single-centre, open-label randomised controlled study comparing haemodiafiltration (HDF) to haemodialysis with a medium cut-off haemodialysis membrane (HDx). Patients were recruited from 2 haemodialysis units within a single renal centre (Manchester NHS Foundation Trust, UK). 63 patients were recruited and randomised with 31 patients remaining on HDF therapy and 32 patients switching to HDx therapy for 24 weeks. Exclusion criteria were planned live donor transplant within 6 months, planned switch in dialysis modality within 6 months or clinical prognosis predicted as less than 6 months.

6.2.2 Statistical Methods

An intention-to-treat analysis was carried out such that all participant data was analysed, regardless of whether they completed the full study protocol. Data are presented as mean ± standard deviation or as median with an upper and lower quartile (Q1-Q3). Data normality was assessed by the D'Agostino & Pearson test. The Chi squared test, Cochran-Armitage test or Fisher's exact test were used to analyse categorical data. A t-test or Mann Whitney U test were used to compare means depending on the normality of data distribution. One-Way ANOVA was used to compare means across the 3 timepoints. Correlations between data groups were analysed using Pearson's test.

GraphPad Prism v 8 (GraphPad Software, San Diego, California, USA) was used to conduct all statistical analysis. Mean imputation was utilised for any missing data.

6.3 Results

6.3.1 Descriptive Analysis

Details of randomisation, participant withdrawal as well as the demographics and baseline clinical characteristics of participants are reported in chapter 3.

6.3.2 Data collection and completion

Questionnaire completion rates are presented in table 6-1 and was 98.4% (62 out of 63) at baseline, 94.6% at 3 months (53 out of 56) and 96% at 6 months (48 out of 50). There were, in some questionnaires, the occasional answer or section left blank. For the purposes of analysis, blank responses were never scored as 0, mean imputation was used.

	Baseline	12 Weeks	24 Weeks
Participants	63	55	50
Questionnaire Completion	62 (98.4%)	52 (94.5%)	48 (94.6%)
Blank Responses	2.2% (23 out of 1054)	0.7% (6 out of 901)	0.7% (6 out of 816)

Table 6-1: Rates of data completion for POS-S Renal (symptom burden) *during the course of the study. For blank responses, the total number of blank responses has been demonstrated and presented as a total number and percentage of maximum potential number of responses).*

6.3.3 Treatment with medium cut-off haemodialysis does not result in a change in overall symptom burden

There was no significant difference in total POS-S score between the two treatment groups at 6 months. Data presented in figure 6-1.

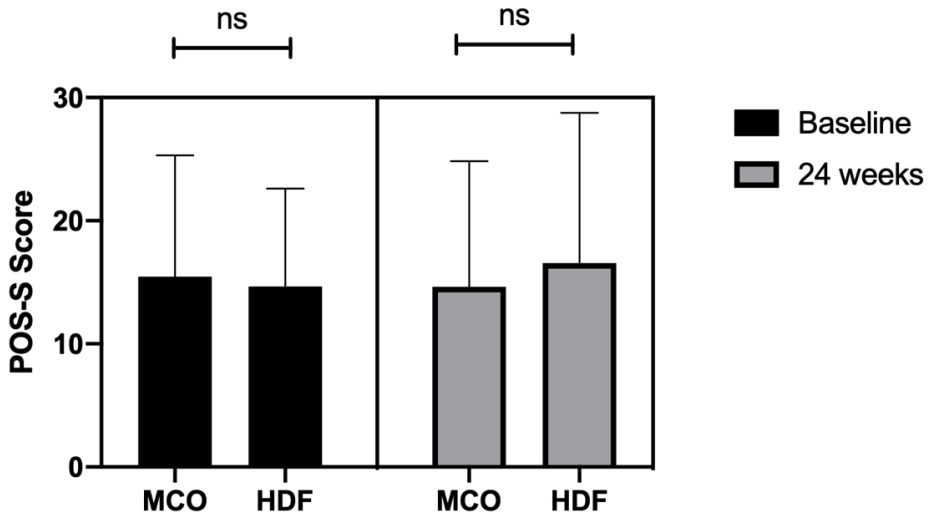

Figure 6-1: POS-S Renal score at baseline and 24 weeks in both treatment groups. *There was no significant difference in the POS-S Renal score between the two groups at baseline or at 6 months. Data presented as mean and standard deviation (error bars). Statistical analysis performed using an unpaired t-test.*

6.3.4 There is consistency in the most severe symptoms experienced by dialysis patients

Out of the 17 symptoms assessed in the IPOS score, the top 5 symptoms in terms of severity were fairly consistent at each timepoint during the study period in both groups (presented in table 6-2).

	HDF		MCO	
Rank	**T0**	**T24**	**T0**	**T24**
1	Poor mobility	Poor mobility	Poor mobility	Difficulty sleeping
2	Weakness or lack of energy	Pain	Pain	Weakness or lack of energy
3	Pain	Weakness or lack of energy	Weakness or lack of energy	Poor mobility
4	Itching	Itching	Difficulty sleeping	Pain
5	Difficulty sleeping	Difficulty sleeping	Drowsiness	Itching

Table 6-2: Most severe symptoms in both study groups at start and end of study period. *Symptoms ranked in order of highest mean IPOS score.*

6.3.5 Treatment with medium cut-off haemodialysis is not associated with a change in the proportion of moderate to severe symptoms over a 6-month period

The proportion of patients scoring each symptom as either moderate, overwhelming or severe was analysed separately within each treatment group. The results of this analysis for each symptom are outlined in table 6-3. Although there were changes in the severity of each symptom during the study period, none of the changes seen reached statistical significance (Cochran-Armitage test) for any of the 17 symptoms.

	HDF T0	HDF T12	HDF T24			MCO T0	MCO T12	MCO 24		
Total Participants	**30**	**27**	**23**	**HDF Change in % T0 to T24**	**Sig**	**32**	**25**	**25**	**MCO Change In % T0 to T24**	**Sig**
Symptom	**% patients with moderate to overwhelming symptoms (total number)**					**% patients with moderate to overwhelming symptoms (total number)**				
Poor mobility	50.00 (15)	62.96 (17)	52.17 (12)	2.27	0.8134	53.13 (17)	48.00 (12)	48.00 (12)	-5.13	0.6896
Weakness or lack of energy	43.33 (13)	51.85 (14)	47.83 (11)	4.49	0.7139	53.13 (17)	40.00 (10)	48.00 (12)	-5.13	0.6560
Itching	40.00 (12)	25.93 (7)	30.43 (7)	-9.57	0.4215	18.75 (6)	36.00 (9)	24.00 (6)	5.25	0.5845
Pain	33.33 (10)	37.04 (10)	47.83 (11)	14.49	0.2924	40.63 (13)	32.00 (8)	44.00 (11)	3.37	0.8417
Difficulty sleeping	30.00 (9)	37.04 (10)	26.09 (6)	-3.91	0.8086	46.88 (15)	48.00 (12)	52.00 (13)	5.12	0.7063
Drowsiness	30.00 (9)	37.04 (10)	30.43 (7)	0.43	0.9353	31.25 (10)	20.00 (5)	20.00 (5)	-11.25	0.3073
Changes in skin	26.67 (8)	25.93 (7)	26.09 (6)	-0.58	0.9593	18.75 (6)	36.00 (9)	28.00 (7)	9.25	0.8871
Restless legs or difficulty keeping legs still	26.67 (8)	25.93 (7)	26.09 (6)	-0.58	0.9593	21.88 (7)	20.00 (5)	24.00 (6)	2.12	0.8630
Feeling depressed	23.33 (7)	29.63 (8)	39.13 (9)	15.8	0.2164	31.25 (10)	24.00 (6)	20.00 (5)	-11.25	0.3279
Shortness of breath	23.33 (7)	44.44 (12)	34.78 (8)	11.45	0.3259	28.13 (9)	28.00 (7)	28.00 (7)	-0.13	0.9913
Feeling anxious or worried about your illness or treatment	20.00 (6)	18.52 (5)	26.09 (6)	6.09	0.6154	25.00 (8)	28.00 (7)	28.00 (7)	3	0.7919
Poor appetite	16.67 (5)	33.33 (9)	17.39 (4)	0.72	0.8491	28.13 (9)	28.00 (7)	24.00 (6)	-4.13	0.7360
Sore or dry mouth	16.67 (5)	22.22 (6)	21.74 (5)	5.07	0.6287	21.88 (7)	16.00 (4)	16.00 (4)	-5.88	0.5537
Constipation	13.33 (4)	18.52 (5)	26.09 (6)	12.75	0.2410	12.50 (4)	8.00 (2)	12.00 (3)	-0.5	0.9214
Diarrhoea	10.00 (3)	7.41 (2)	13.04 (3)	3.04	0.7472	6.25 (2)	8.00 (2)	0.00 (0)	-6.25	0.3053
Nausea (feeling like you are going to be sick)	6.67 (2)	14.81 (4)	21.74 (5)	15.07	0.1119	21.88 (7)	8.00 (2)	12.00 (30	-9.88	0.2623
Vomiting (being sick)	6.67 (2)	7.41 (2)	8.7 (2)	2.03	0.7830	9.38 (3)	8.00 (2)	12.00 (3)	2.62	0.7593

Table 6-3: Table illustrating prevalence of moderate symptoms in each group at baseline (T0), 12 weeks (T12) and 24 weeks (T24). *Data presented as % of participants within each group who rated each symptom as affecting them either moderately, severely or overwhelmingly over the previous week. Total numbers are in brackets. Statistical analysis performed using chi squared test for trend (Cochran-Armitage test for trend).*

6.3.6 Symptom burden does not correlate with middle molecules, markers of inflammation, endothelial activation or indices of body composition

There was no significant linear correlation (Pearson's correlation coefficient) between IPOS score and any of the study biomarkers or body composition measurements.

6.3.7 Chalder Fatigue Score

6.3.7.1 Data Completion

Data completion for this section of the questionnaire was high and remained above 90% at all 3 timepoints (see table 6-4). The number of blank responses remained at <1.2% throughout.

	Baseline	**12 Weeks**	**24 Weeks**
Participants	63	55	50
Questionnaire Completion	62 (98.4%)	50 (90.9%)	48 (96%)
Blank Responses	7 (1.1%)	1 (0.2%)	6 (1.1%)

***Table 6-4 Data completion for both groups combined at baseline, 12 weeks & 24 weeks**. For blank responses, the total number of blank responses has been demonstrated and presented as a total number and percentage of maximum potential number of responses (ie. a percentage of 11 multiplied by the total number of questionnaires completed).*

6.3.7.2 Treatment with medium cut-off haemodialysis is not associated with a difference in Chalder fatigue score compared with HDF at 6 months

Median Chalder Fatigue Score (CFS) fell in both groups during the study period and there was no significant difference in two groups at 6 months. Data presented in figure 6-3.

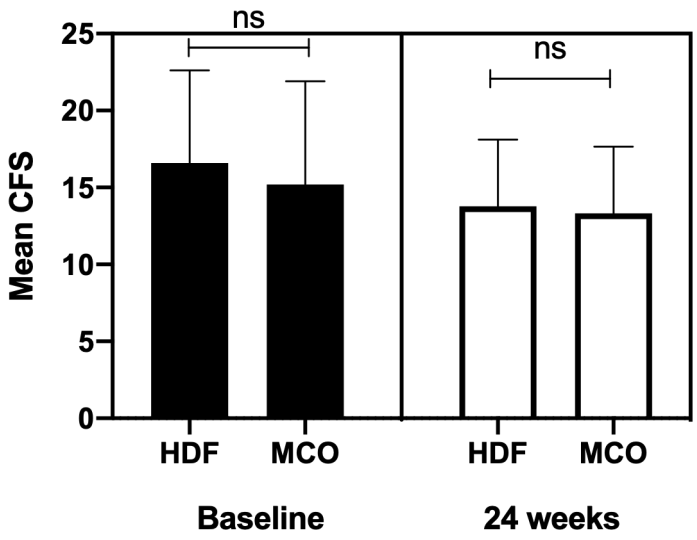

Figure 6-2: Mean bimodal Chalder fatigue score in both groups at baseline and at 24 weeks. *Data presented as mean with standard deviation (error bars). Statistical analysis performed using an unpaired t-test.*

The mean change in CFS score was -3.04 ± 4.15 in the HDF compared with -2.64±5.60 in the MCO group. The difference in change was not significant (p=0.77, unpaired t-test).

6.3.7.3 Chalder fatigue score does not correlate with middle molecules, markers of inflammation, endothelial activation or indices of body composition

There was a significant linear correlation (Pearson's correlation coefficient) between CFS and pentraxin-3 at baseline (r = 0.28, p = 0.05) (figure 6-5). There was no significant relationship between these two variables at both 12 weeks and 24 weeks. There was no other significant correlation between CFS and other middle molecules, markers of inflammation, endothelial activation or indices of body composition.

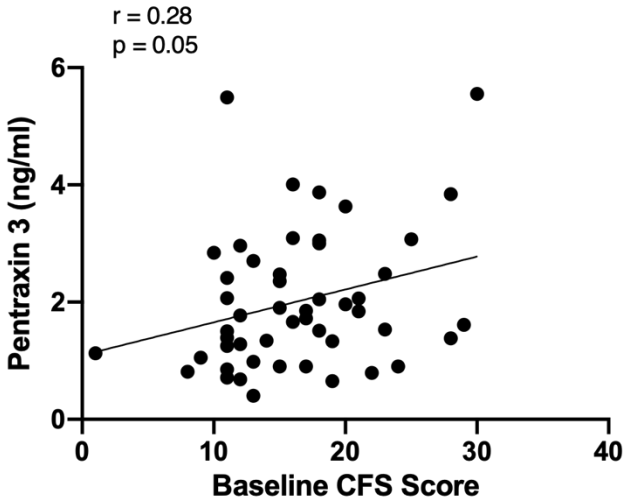

***Figure 6-3: Correlation between Chalder Fatigue Score (CFS) and pentraxin-3 at baseline**. Statistical analysis performed using Pearson's correlation coefficient.*

6.3.8 Medium cut-off haemodialysis may be associated with a change in dialysis recovery time

Dialysis recovery time was self-reported by each patient at baseline (T0), 12 weeks (T12) and 24 weeks (T24), the data is presented in figure 6.4. There were 2 blank responses at T12 (one in each group) and no other blank responses in the study for this section.

Treatment modality (HDF vs MCO) did not predict dialysis recovery time at 24 weeks ($p = 0.583$, multiple regression analysis).

The change in the proportion of patients reporting a dialysis recovery time <6 hours in both groups between T0 and T24 was close to significance in the MCO group but not the HDF group (58% to 84% MCO, $p = 0.05$ vs 58% to 66% HDF, $p = 0.51$). The results were also analysed after converting each of the 4 responses into a numerical value between 0 and 3 and analysing with a one-way ANOVA. There was no significant change in either group (MCO $p = 0.9826$, HDF $p = 0.9716$).

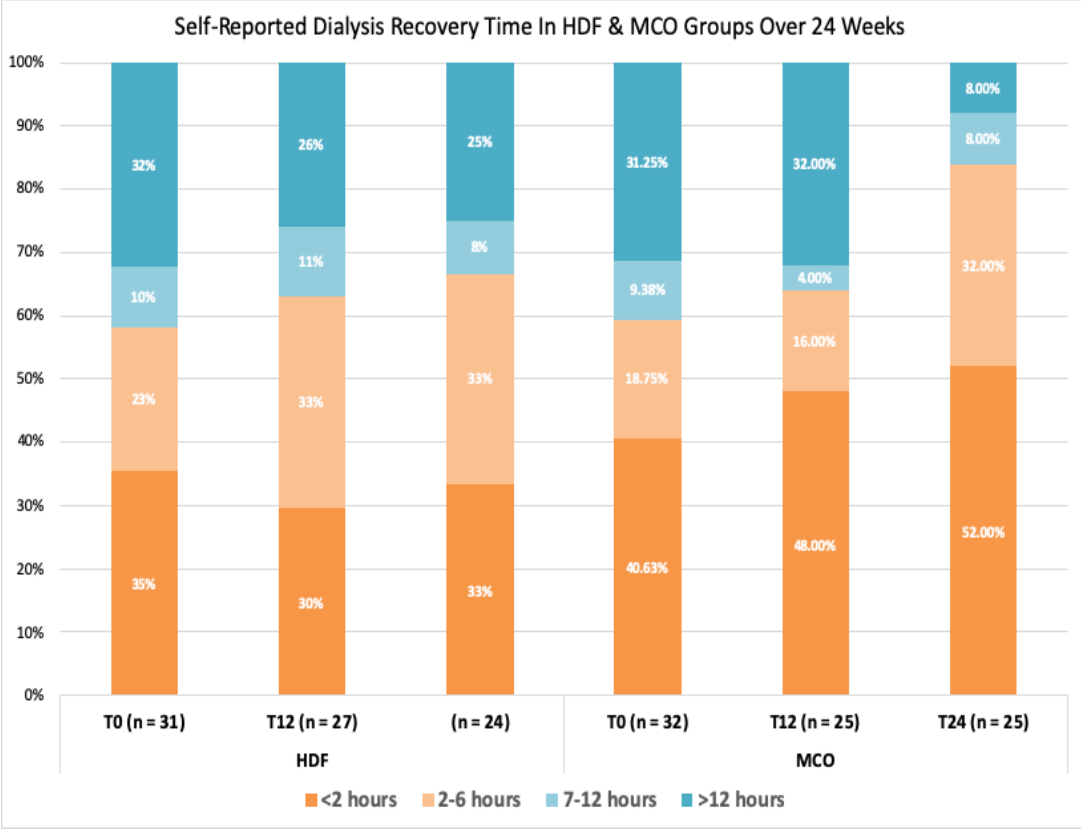

Figure 6-4: Self-reported dialysis recovery time during study period. *There was an increase in the proportion of patients with a dialysis recovery time<6 hours throughout the study both groups. The change was close to statistical significance in the MCO group (HDF p = 0.51, MCO p = 0.05). Statistical analysis performed using chi squared test for trend across three timepoints.*

6.3.9 Dialysis recovery time correlates with symptom burden and change body composition parameters

Dialysis recovery time at baseline correlated with IPOS Score (R= 0.390, p = 0.004). There was also a significant correlation change in LTI during the study period (R = -0.419, p = 0.033) and change in FTI (R = 0.448, p = 0.004).

Patients reporting a dialysis recovery-time of <2 hours at the start of the study had, on average, had a rise in their lean tissue index (LTI) during the 24-week

study period of 1.16kg/m2. This is in contrast to the mean change in LTI seen in patients who reported a dialysis recovery time>2 hours- these patients had a mean fall in their LTI over the study period (figure 6-7).

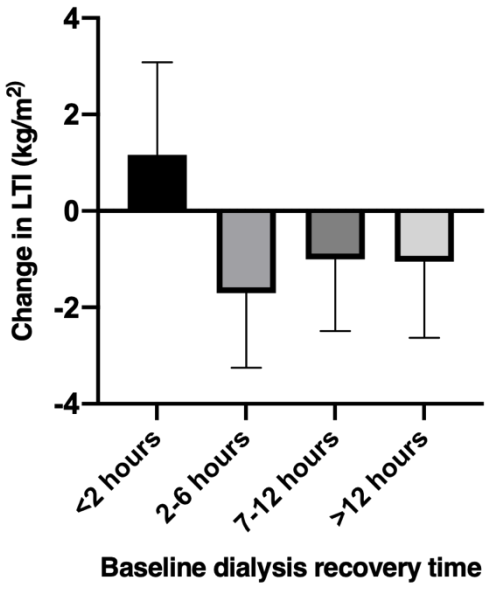

***Figure 6-5: Mean Change in Lean Tissue Index (LTI)* over 24-week study period by baseline self-reported dialysis recovery time.** Change in LTI between groups significant (p = 0.0008, one-way ANOVA)

6.4 Discussion

This study has demonstrated that a switch from HDF treatment to HDx treatment is not associated with a significant change in patient symptom burden. Although there was a reduction in self-reported fatigue, this was seen in treatment groups and there was no significant difference in median bimodal Chalder fatigue score at the end of the study. MCO treatment may be associated with an improvement in dialysis recovery time where the proportion

of patients reporting a recovery time less than 6 hours increased in the MCO group (p = 0.05).

Symptom burden and severity in this study remained very consistent in both study groups and the most prevalent symptoms reported by patients in this study are similar to those reported in other studies in haemodialysis patients [402 472]. There appeared to be no impact on the severity of symptoms. This is in contrast to the study by Alarcorn et al[473] which demonstrated a reduction in symptom severity when patients were switched from HFHD to HDx. This study had a longer follow-up period with a different comparator group to this study (HFHD rather than HDF) and the change in symptom severity was fairly modest at 12 months (Dialysis Symptom Index change from 30.7±22.3 to 28.5±21.7). The randomised comparing HFHD with HDx showed no impact on symptoms[344].

Interestingly, the change in Chalder Fatigue Score (CFS) in both groups exceeded the reported minimal important difference (MID) for improvement in CFS (0.7-1.4) [474]. Given that the change in both groups was of similar magnitude and there was no significant difference between the two study groups after 24 weeks, these changes cannot be attributed to the treatment modality.

Interestingly, CFS did correlate with pentraxin-3 concentration at baseline but this relationship was not maintained at the 12 weeks or 24 weeks. Pentraxin-

3 (PTX-3) is clearly an important potential target for MCO membranes at 42kDa. Both HDF and HFHD do not impact on concentrations of and in this study, there was no significant change in PTX-3 concentration during the study period (data presented in chapter 3). However, in high volume HDF the clearance of middle molecules is enhanced and it is possible that there was overlap between the groups in terms of PTX-3 clearance. The measurement of a reduction ratio might have helped assess any differential clearance between the 2 modalities. The main area of interest in PTX-3 as a uraemic toxin is related to atherogenesis[140,141] and endothelial dysfunction[138,475]. There are no studies that have explored this biomarker in relation to fatigue. Whether a reduction in cardiovascular toxicity can improve fatigue is not well understood however a link between cardiovascular burden and fatigability is plausible. It is interesting that fatigue fell in both groups and could indicate that engagement with patients through the study has an impact on fatigue.

Whilst the changes in Dialysis Recovery Time (DRT) during the study period did not reach statistical significance in either group, the rise in patients reporting a recovery time less than 6 hours in the MCO group (59% of patients at the start of the study and 84% at the end of the study) was close to reaching statistical significance ($p = 0.0524$). This is an important signal as a prolonged dialysis recovery time is associated with high mortality, time to first hospitalization and a higher symptom burden [408]. Dialysis recovery time has been identified as one of the most important symptoms to address in trials by patients[61]. No published studies have assessed the association of dialysis

recovery time with concentrations of middle molecules and markers of inflammation. Volume parameters such as ultrafiltration rate do seem to influence dialysis recovery time [408] [476]. In this study, we did not find a relationship between volume status and DRT or a relationship between DRT and other study biomarkers (inflammation, endothelial activation and middle molecules). The study did show the ability of DRT to predict changes in both LTI and FTI. The observed stabilization of LTI over a 6-month period in HDX group is also consistent with the improvements in dialysis recovery time. Whilst several studies have demonstrated the increased risk of mortality associated with a low LTI in both pre-dialysis CKD[389] and in haemodialysis patients[390-394], there are very few longitudinal studies of BIS in haemodialysis patients and none of these studies have incorporated PROM's. Given the ease of implementation of this PROM, DRT could be a useful screening tool in dialysis units to help stratify more enhanced care for patients. The change in DRT in this study appears to mirror that of the change in fatigue in the MCO group.

We are in a new era of clinical studies. Through initiatives like the Standardised Outcomes in Nephrology (SONG) [60], we are beginning to re-think the design of studies and study design is becoming much more aligned with improving outcomes that matter to both patients and clinicians. Whilst this is a huge step forwards, our knowledge of the relationship between patient-reported outcome measures and more traditional outcome measures and biomarkers is in its infancy. The experience of patients changes

constantly and numerous factors can influence symptoms. Traditional study designs where data is captured at very specific timepoints may not be the optimal way of capturing meaningful PROM's. Additionally, the hospital setting where patients receive their treatment but spend the least amount of their time in may not be the optimal place to capture this data.

Although higher levels of inflammation and markers of poor nutritional status have also been linked with PROM's [477] [478] [479], we are yet to demonstrate that improving clearance results in improvements of quality of life. Although HDF provides increased clearance of middle molecules compared with HD[39-43,480], a recent meta-analysis, incorporating data from 7 RCT's and 1334 patients demonstrated that HDF has no effect on quality of life [481]. Only three studies have been published assessing the impact of HDx therapy on PROM's and results are mixed. The largest of the studies by Alarcon et al [473] suggests a benefit to both restless leg symptoms and some domains of the Kidney Disease Quality of Life 36-Item Short Form Survey (KDQoL-SF36), the prevalence of restless legs syndrome and the severity of symptoms in the dialysis symptoms index (DSI). The study was large (992 patients in total) and had a 12-month follow-up period but was an observational study with no comparator group. Krishnasamy et al[416] showed no impact of switching to HDx from HFHD on 6-minute walk score, restless legs, malnutrition inflammation score and quality of life at 24 weeks in a crossover study (89 participants). Lastly, Lim et al[344] compared HDx with HFHD in a 12 week RCT and demonstrated a reduction in 2 out of 12 domains of the KDQoL-SF36 and in

some domains of a pruritis score. Moving forwards we need reliable and consistent ways of capturing PROM's in studies in order to draw clear conclusions.

6.5 Study Strengths & Limitations

This study has several strengths. Firstly, this is only the second published study assessing the effect of HDx therapy on several patient-reported outcome measures in comparison to HDF therapy. Secondly, this is one of very few studies to incorporate PROM's with BIS measurements in dialysis patients thereby exploring the link between PROM's and measures of hydration and nutritional status. Completion rates for the PROM questionnaires were high.

There was variation in the way that PROM's were collected during the study. In some cases, staff-assistance was utilised. The influence of staff-assistance as well as the location of where the questionnaire is completed (home vs dialysis unit) is unknown and could have influenced the results. There appeared to be a "study effect" where the fatigue scores for patients in both groups reduced during the study period- this ought to be considered when utilising PROM's in future studies. A measure of patient activation would have been interesting to assess if changes in PROM's correlated with any change in patient activation. Whilst a focus on patient-reported symptoms and quality of life in future studies is potentially of huge benefit to our patients, the heterogenicity and interplay of the underlying causes of these symptoms pose

new challenges to trial design. Overcoming these challenges may deliver rewarding and meaningful improvements to the future care of our patients.

6.6 Conclusions

Switching from HDF to HDx therapy may be associated with an improvement in dialysis recovery time. The treatment modality does not appear to have a significant impact on symptom burden or fatigue at 24 weeks. We are yet to find a target biomarker that is clearly linked to fatigue. Although the relationship between pentraxin-3, a large middle molecule and baseline fatigue in dialysis patients was apparent here, it was not consistent at other timepoints. Further exploration, however, would be of interest. Future studies should seek to gain further understanding of the link between dialysis clearance and patient-reported symptoms as well as explore the optimal method of collecting PROM's and incorporating them into clinical trials.

Chapter 7: Conclusion

7.1 Key Findings

The key findings from each results chapter in this thesis will now be summarised in turn.

7.1.1 Chapter 3: Results: Medium Cut-Off Haemodialysis Versus Haemodiafiltration: Comparison of the Effect on Biomarkers of Vascular Endothelial Health

The aim of this study was to evaluate the effect of medium-cut off haemodialysis (MCO) on markers of endothelial activation. This was a randomised controlled study and patients in the control group remained on High Volume Haemodiafiltration (HvHDF). The study showed a significant reduction in plasma EMV concentration within 12 weeks of starting treatment in the MCO group and this reduction was sustained for the 24-week study period. Patients in the control group who remained on HDF, saw a rise in EMV concentration during the same time period. At 24 weeks, however, there was no significant difference between the two groups in plasma EMV concentration. The biological basis for the reduction in EMV in the study is unclear- treatment group did not influence the concentration of any of the uremic toxin biomarkers at the end of the study period in comparison to HvHDF (middle molecules, angiogenesis, endothelial activation and inflammation) in a regression analysis. There were significant differences between the two study groups at baseline (including HD vintage, BMI and CRP) and although these variables were not shown to be predictor of EMV concentration at the

end of the study, they may have contributed to confounding. It is possible that the effect of MCO is more pronounced in the presence of higher inflammatory states as seen in the treatment group. Alternatively, the changes in EMV concentration seen could represent regression to the mean. EMV did appear to be a robust biomarker in this study such where EMV concentration correlated with several biomarkers, nutritional parameters and vascular stiffness.

MCO treatment was associated with a reduction in serum albumin however median serum albumin concentration was 30g/l at the end of the study and several studies have indicated that MCO treatment is safe with a modest albumin loss[90,343-345,412,415-418]. Again, there was no significant difference between the two groups in serum albumin concentration at the end of the study.

This study has highlighted the challenges in performing randomised controlled trials in dialysis patients where there is significant heterogeneity amongst patients. The unblinded intervention, randomisation bias and the intrinsic clinical heterogeneity poses challenges to study the effect of a single intervention. A large number of trials examining single interventions have proven to be largely negative in differentiating an outcome measure despite proven benefits in the non-dialysis population (such as the 4D trial[482] and EVOLVE study[483]).

7.1.2 Chapter 4: The Impact of Medium Cut-Off Haemodialysis on In-Vitro Cell Viability, Angiogenesis and Wound Healing

The aim of this study was to evaluate the effect of MCO treatment on *in-vitro* measures of endothelial function in a small subset of patients. The study showed that there was no effect from switching from HDF to MCO treatment on cell viability, cell migration and angiogenesis at 6 months. Despite the small numbers in the study, the study demonstrated strong correlations between concentrations of middle molecules such as pentraxin-3 and angiogenesis. There were also clear correlations between wound healing and markers of endothelial activation and inflammation. Clinical factors such as residual kidney function and BMI were shown to be closely linked with endothelial cell function. This study has demonstrated the importance and relevance of these biomarkers in relation to endothelial cell function. Overall, switching from HDF to HDx therapy is not associated with a change in endothelial cell function at 6 months.

7.1.3 Chapter 5: Results: Exploring the Effects of Dialysis Membrane Modality on Markers of Protein Energy Wasting

The aim of this study was to assess the impact of this study was to assess the impact of MCO treatment on hydration and nutritional parameters as measured by bioimpedance spectroscopy (BIS). This study demonstrated a preserved lean tissue index (LTI) and fat tissue index (FTI) during the 6-month

study period in the MCO group. This was in contrast to a trend towards a reduction in LTI and increase in FTI in the HDF group which was close to statistical significance. There was a significant difference between the two study groups in terms of body fat composition at baseline which could have been a major confounder. MCO treatment showed no effect on hydration status. Markers of endothelial activation were shown to be associated with both FTI and with measures of overhydration highlighting once again the important link between inflammation and malnutrition. Overall this study has demonstrated a signal that MCO treatment could be associated with improved nutritional parameters compared with HDF. Further studies with a primary focus of assessing the impact of improving dialytic clearance on longitudinal nutritional parameters stratified for baseline nutritional status should be considered.

7.1.4 Chapter 6: The Impact of Medium Cut-Off Haemodialysis on Patient-Reported Outcome Measures

The aim of this study was to assess the impact MCO treatment on patient-reported outcome measures (PROMs). This study showed no impact on symptom burden (POS-S Renal). There was a reduction in Chalder Fatigue Score (CFS) in both study groups with no significant difference between the two treatment groups in CFS at 24 weeks. Fatigue correlated with pentraxin-3 (PTX-3) concentration in this study at a single timepoint and this could represent an important biomarker for fatigue.

MCO treatment resulted in an improvement in dialysis recovery time that was close to reaching statistical significance. DRT was shown to be a predictor of change in LTI during the study period indicating and this PROM could therefore be a useful screening tool. The link between muscle mass and dialysis recovery time should also be explored. Overall, this study has demonstrated that MCO treatment may be associated with improvements in fatigue and dialysis recovery time.

7.2 Strengths and limitations

This strengths and limitations of each part of the study are discussed in each of the results chapters of this thesis. This section provides a single combined summary.

7.2.1 Strengths

This significant prospective study is unique in several ways. To my knowledge, there is the only published randomised controlled study comparing HDx treatment with HDF treatment, the current gold standard. There are no published studies evaluating HDx treatment that have incorporated BIS measurements or incorporated the significant range of techniques included in this study (biomarkers, PROMs, BIS measurements and cell culture). This study has provided further novel insights- there is little published data on the

relationship between PROMs and body composition parameters and few studies that have assessed longitudinal change in body composition.

The completion rate for PROM questionnaires in this study was high (over 97% of questionnaires were completed during the study). Incorporating PROMs into the study with such high completion rates provides context to the study results and is highly valuable to clinicians and commissioners.

Although blood flow rates during the study period were modest (final mean blood flow 312.84 $\pm$ 34.23 MCO group and 300.72 $\pm$ 42.82 in the HDF group), consistency between the two groups was maintained throughout the study and importantly, a high convective volume was delivered in the HDF group (mean substitution volume 20.23 $\pm$ 2.84 litres). There is a suggestion that the benefit associated with HDF could be related to the convective volume (sum of the substitution volume and ultrafiltration volume) with convective volumes greater than 23 litres/1.73m^2 are associated with the best outcomes[45]. The control group in this study was therefore very relevant and meaningful.

7.2.2 Limitations

In addition to the strengths of this study, there were some limitations. Firstly, there were key baseline differences between the two study groups. The differences were mainly related to body fat. The MCO group had a higher body mass index (BMI), body surface area, weight and fat tissue index. Kt/V

was lower in the MCO group however given the similarity in lean tissue index between the two groups, this difference is likely due to differences in urea volume distribution rather than dialysis clearance. HD vintage was lower in the MCO group and there were also differences in PTH and CRP. There is a significant relationship between obesity and inflammation[422-426]. Although none of these baseline covariates predicted a change in the main study outcome measure (EMV) they may have contributed to cofounding. HD vintage often correlates with residual kidney function which has a significant impact on clearance of solutes and patient survival[35,484]. Although there was no significant difference between the two groups in terms of residual kidney function or the proportion of anuric patients, the difference in HD vintage once again could have resulted in confounding. These baseline differences highlight the challenges in dialysis studies where there is significant heterogeneity between patients, adjusting for baseline covariates may be required in future studies. The design of this study meant that the intervention was not blinded to the participants or investigators. Double-blinded randomised controlled studies are considered the gold standard of clinical trials. Unblinding in this study may have introduced bias, particularly in relation to PROMs where data capture is subjective. Blinding the participants in this study would have been a challenge due to the nature of the setup of a dialysis machine. Additionally, for the dialysis staff, blinding would have introduced a safety issue as the setup of the machine is different for the two treatments (HDF vs HD mode). Use of the MCO dialysis membrane in HDF mode rather than HD mode results in unacceptably high albumin loss.

An alternative trial design (ie. a crossover study) would have overcome many of the issues faced in this study. A crossover study, where participants acted as their own controls would allow a clearer evaluation of the membrane effects.

Whilst EMV was shown to be a clinically relevant biomarker in this study, for wider use in future clinical studies and clinical practice, there are a lot of barriers to overcome. Firstly, there is significant variation between laboratories in terms of the methodology used to measure EMV. There is significant variation between laboratories in the values obtained. Processing of samples is currently labour-intensive and costly.

There was variation in the way PROM's were collected during the study- for some participants, assistance from the research nurse or a dialysis nurse was required. This variation in data capture could have influenced the results. At present, the optimal way of collecting PROM data is unknown. The symptoms that patients experience constantly change and therefore a snapshot assessment at only a few study timepoints may not be meaningful. It was interesting that there was a trend towards improvement in two out of three of the PROMs indicating a possible Hawthorne effect. Incorporating other measures such as patient-activation may help differentiate changes due to the intervention from changes in patient engagement. PROM's, particularly self-reported dialysis recovery time, with a relationship with lean tissue mass in this study, may represent a promising primary outcome measure for future

interventional studies. It is simple to administer, reproducible [408] and offers much more meaning to patients over biomarkers. The data from this study could be used to design and power a future with dialysis recovery time as a primary outcome measure in a crossover trial design.

There is considerable overlap in the clearance characteristics of the two treatments in this study, particularly when high volumes of convection are achieved in HDF in comparison with medium cut-off membranes. As a result, it is likely that there would be few differences in outcomes between the two treatments that are due to a change in clearance parameters. A third arm in the study (for example, pre-dialysis patients or standard high flux dialysis) may have provided a clearer insight into the findings of this study.

BIS measurements were not accurately captured in all study participants due to technical issues (approximately 12% of results excluded) which may have introduced bias. Additionally, data on episodes of intradialytic hypotension was not captured, this would have provided a very useful insight given that the control group (HDF) has been shown to reduce IDH episodes[468-471].

Lastly, some of the techniques used in this study (the cell culture experiments) have not been widely reported in haemodialysis patients. With lack of sufficient data on the performance of these test in HD patients (for example in low-flux and high-flux HD), comparison of two groups with overlapping clearance performance is challenging.

7.3 Implications for future research and clinical practice

The results of the clinical trial presented in this thesis have provided a novel insight into outcomes associated with HDx treatment, a new dialysis membrane technology. The introduction of MCO membranes provides an opportunity to enhance blood purification in routine dialysis treatments to achieve the broadest spectrum of detoxification in uraemia, with baseline performances at least equivalent to high dose convective treatments. Alongside closing gaps in our knowledge about the clinical effect of this treatment on endothelial activation, endothelial function, nutritional parameters and patient-reported outcome measures, it has cast light on unanswered questions that should be the focus of future research.

Protein-bound toxins and larger middle molecules are poorly removed by current therapies. A single target molecule representative of these toxin groups would be helpful in developing future studies with more meaningful outcome measures. Dialysis studies with hard outcome measures such as mortality and cardiovascular events are challenging and costly. Urea clearance, whilst routinely measured, only assesses small water-soluble solute clearance. β-2 microglobulin at approximately 12 kDa does not differentiate the clearance of larger middle molecules between 15kDa and 65kDa which newer emerging technologies provide some clearance of. Target molecules should not only be representative of larger or protein-bound toxins but they should also have a meaningful connection with clinical outcomes such

as cardio-protection. In this study, we assessed the concentration of several biomarkers during the study period, including a panel of 5 middle molecules. Of these, pentraxin-3 (PTX-3) holds some promise and should be evaluated further in future studies. At approximately 40kDa in size it is representative of larger middle molecules. We have shown concentrations of PTX-3 to correlate with both self-reported fatigue and markers of *in vitro* endothelial cell function (angiogenesis). It has been shown to predict cardiovascular mortality in advanced CKD[137,138], it is associated with endothelial dysfunction[137,138] and it is likely to have a role in atherosclerosis[140,141]. At present it remains unknown whether enhancing clearance of PTX-3 improves outcomes in CKD and this should be evaluated. A global initiative to standardise membrane studies in dialysis with agreed outcome measures could potentially improve resource allocation and deliver meaningful outcomes to help drive practice changes. Large registry-linked multi-site prospective studies (similar to the design of the H4RT study) may be the best way to gain clearer insights into the effects of future dialysis interventions.

PROMs are being increasingly utilised in clinical studies and producing research with outcomes meaningful to both patients and clinicians has been highlighted by the SONG initiative[60]. Traditional research design involves collection of data at discrete timepoints throughout the study. Patient symptoms are subjective and are constantly changing- current research methodology is not reflective of this. Future research should look to investigate the best way of collection PROM data in dialysis- should this be on

a daily basis, on a dialysis day or non-dialysis day, before or after the dialysis session. How can PROMs be collected and how does the method influence the outcome- should smart devices be used or should PROMs be completed with an assistant to avoid misinterpretation? We should seek to determine the influence of clinician-assistance in completing PROM data- does assistance lead to bias in results? Can this be adjusted for? At present, we do not know which PROMs are the most sensitive or specific to the symptoms that patients experience- although tools have been developed that are specific to CKD, determining a "gold standard" range of PROMs and a set of standards for their collection would allow effective comparison of studies and pooling of data.

MCO dialysis is associated with albumin loss and this study, and several others [343,344,412,415-418,485] have demonstrated the safety of this. At present, dialysis therapies make a minimal impact on the removal of protein-bound toxins. Whilst high-cut off dialysis was associated with an unacceptably high protein loss to make long-term use feasible, there has been a suggestion that protein loss could may stimulate protein production[486]. We are yet to determine how much protein loss is acceptable in dialysis and is there such a thing as an "optimal" protein loss. Future research should evaluate the combination of both increasing larger solute clearance combined with removal of protein-bound toxins through methods such as absorption. Health economic appraisals of the benefits that these advances in membrane technology should also be carried out.

We have demonstrated a potential signal that improving solute clearance through HDx may be associated with improved nutritional parameters (preservation of LTI and FTI). Malnutrition in dialysis is strongly linked with poor outcomes[478]. Interventions aimed at improving malnutrition have had limited effect on outcomes in dialysis patients. As technologies such as BIS are increasingly being utilised at the bedside, larger studies evaluating longitudinal changes in body composition and the relevance of this should be undertaken. The link between uraemic toxins and LTI should be further explored as well as the link between PROM data and BIS. We have shown a relationship between dialysis recovery time and preservation in LTI. Further research should be undertaken on the role of muscle mass in patient symptoms.

7.4 Conclusion

There remains unmet need in the spectrum of toxins that current dialysis therapies are able to remove, in particular, larger middle molecules and protein-bound toxins are poorly removed. Dialysis technology is evolving and the developing of medium-cut off membranes is an important step forwards in improving toxin clearance. I have demonstrated in this thesis that enhanced solute clearance enabled through HDx appears non-inferior to the current gold-standard dialysis treatment, HDF in a range of biomarkers of vascular endothelial activation, angiogenesis and inflammation. Additionally, I have demonstrated a possible benefit in terms of patient-reported fatigue, dialysis

recovery time and preservation of nutritional parameters. In my experience, HDx was simple implement, it was safe and I have shown good performance even at low blood flow rates. Overall, HDx therapy appears to offer a promising advance and an alternative to HDF treatment without a requirement for delivery of high convective volumes. Larger multi-site prospective studies in MCO dialysis are now required with and dialysis recovery time should be considered as a key outcome measure.

Chapter 8: Appendix

8.1 Appendix 1: Commentary on the NICE Guideline on Renal Replacement Therapy and Conservative Management

Submitted for publication in peer-reviewed journal.

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Commentary on the NICE Guideline on Renal Replacement Therapy and Conservative Management

Final version: March 2020
Review date: TBC

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Method used to arrive at a recommendation

The recommendations for the first draft of this guideline resulted from a collective decision reached by informal discussion by the authors and, whenever necessary, with input from the Chair of the Clinical Practice Guidelines Committee. If no agreement had been reached on the appropriate grading of a recommendation, a vote would have been held and the majority opinion carried. However this was not necessary for this guideline.

Conflicts of Interest Statement

All authors made declarations of interest in line with the policy in the Renal Association Clinical Practice Guidelines Development Manual. Further details can be obtained on request from the Renal Association.

Contents

(Removed for purposes of thesis)

Introduction

NICE Guideline NG107, "Renal replacement therapy and conservative management" [1] was published in October 2018 and replaced the existing NICE guideline CG125, "Chronic Kidney Disease (Stage 5): peritoneal dialysis"[2] and NICE Technology Appraisal TA48, "Guidance on home compared with hospital haemodialysis for patients with end-stage renal failure"[3]. The aim of the NICE guideline (NG107) was to provide guidance on renal replacement therapy (RRT), including dialysis, transplant and conservative care, for adults and children with CKD Stages 4 and 5. The guideline is extremely welcomed by the Renal Association and it offers huge value to patients, clinicians, commissioners and key stakeholders. It overlaps and enhances current guidance published by the Renal Association including "Haemodialysis"[4] which was updated in 2019 after the publication of the NICE guideline, "Peritoneal Dialysis in Adults and Children"[5] and "Planning, Initiation & withdrawal of Renal Replacement Therapy"[6] (at present there are no plans to update this guideline).

There are several strengths to NICE guideline NG107 and we agree with and support the vast majority of recommendation statements in the guideline. This summary from the Renal Association discusses some of the key highlights, controversies, gaps in knowledge and challenges in implementation. Where there is disagreement with a NICE guideline statement, we have highlighted this and a new suggested statement has been written.

Summary of recommendations

1.1: Indications for starting dialysis

1.1 Indications for starting dialysis
1.1.1 Follow the recommendations on referral criteria in NICE's guideline on chronic kidney disease in adults 1.1.2 Consider starting dialysis when indicated by the impact of symptoms of uraemia on daily living, or biochemical measures or uncontrollable fluid overload, or at an estimated glomerular filtration rate (eGFR of around 5 to 7 ml/min/1.732 if there are no symptoms. 1.1.3 Ensure the decision to start dialysis is made jointly by the person (or, where appropriate, their family members or carers) and their healthcare team.

1.1.4 Before starting dialysis in response to symptoms, be aware that symptoms may be caused by non-renal conditions

We fully support and endorse this section of the guidance. Symptoms and eGFR should be taken into account but with some caution if waiting to start until someone is very symptomatic as this could impact on patient wellbeing, education and training for self-care or shared-care. Systems and tools such as patient-reported outcome measures (PROMs) could be used to collect and monitor the severity of symptoms reported by patients for an optimal start of RRT.

In infants and children there are no data to support starting dialysis on the basis of eGFR alone[7]. Decisions to start dialysis should be on the basis of symptoms which include those listed in the NICE guidance but also include poor growth and nutrition which are critical in this early stage of life[8]. Using eGFR to decide when to initiate dialysis is particularly challenging in infants and children under 2 years of age, where rapid growth and ongoing renal maturation make it difficult to estimate GFR.

1.2: Preparing for renal replacement therapy or conservative management

1.2. Preparing for renal replacement therapy or conservative management

1.2.1 Start assessment for renal replacement therapy (RRT) or conservative management 1 year before therapy is likely to be needed, including for those with a failing transplant
 	
1.2.2 Involve the person and their family members or carers (as appropriate) in shared decision-making over the course of assessment to include:
 	
• Clinical preparation
• Psychosocial evaluation, preparation and support
• The individuals preferences for type of RRT and when to start
• How decisions are likely to affect daily life
 	
1.2.3 Consider further assessment by a clinical psychologist or psychiatrist for:
 	
• All children and young people being considered for a transplant, and
• Adults being considered for a transplant if risk factors for poor outcomes have been identified; these may include:
  ○ lack of social support
  ○ neurocognitive illness
  ○ non-adherence (medicines, diet, hospital appointments)

○ poor understanding of process and complexities of treatment ○ poorly controlled mental health conditions or severe mental illness ○ substance misuse or dependence

We feel strongly that decisions regarding RRT modality or conservative care should be fully individualised and should take into consideration all of the factors mentioned in this section of the NICE guideline. All treatment options (dialysis, transplant and conservative care) should be discussed with patients (and families or carers for those under 18), including home dialysis. Patient autonomy, involvement and choice have been associated with favourable outcomes on RRT[9]. It remains unclear how clinical factors, demographics (age) and patient functional status impact on the choice and outcomes of RRT and conservative care.

1.3: Choosing modalities of renal replacement therapy or conservative management

1.3 (A) Choosing modalities of renal replacement therapy or conservative management
1.3.1 Offer a choice of RRT or conservative management to people who are likely to need RRT *1.3.2 Ensure that decisions about RRT modalities or conservative management are made jointly with the person (or with their family members or carers for children or adults lacking capacity) and healthcare team, taking into account:* • *Predicted quality of life* • *Predicted life expectancy* • *The person's preferences (see recommendations in section 1.8)* • *Other factors such as co-existing conditions* *1.3.3 Offer people (and their family) members or carers, as appropriate) regular opportunities:* • *To review the decision regarding RRT modalities or conservative management*

- *To discuss any concerns or changes in their preferences*

1.3 (B) Transplantation

Transplantation

1.3.4 Discuss the individual factors that affect the risks and benefits of transplantation with all people who are likely to need RRT, and their family members or carers (as appropriate)

1.3.5 Include living donor transplantation in the full informed discussion of options for RRT

1.3.6 Offer pre-emptive living donor transplant (where there is a suitable living donor) or pre-emptive listing for deceased donor transplantation to people considered eligible after a full assessment

1.3.7 Do not exclude people from receiving a transplant based on BMI alone

1.3 (C) Choice of dialysis modalities

1.3.8 Offer a choice of dialysis modalities at home or in centre ensuring that the decision is informed by clinical considerations and patient preferences (see recommendation 3.2)

1.3.9 (NICE) Offer all people who choose peritoneal dialysis a choice of continuous ambulatory peritoneal dialysis (CAPD) or automated peritoneal dialysis (APD), if this is medically appropriate.

1.3.9 (RA) We recommend that adults who have opted for PD be offered APD or CAPD according to their preference, if clinically feasible. We suggest that assisted PD be made available as a viable option, for those who cannot undergo self-care PD.

1.3.10 Consider peritoneal dialysis as the first choice for children 2 years or under

1.3.11 (NICE) Consider HDF rather than HD in centre (hospital or satellite)
Consider HDF or HD at home, taking into account the suitability of the space and facilities

1.3.11 (RA) We recommend that either high flux HD or HDF can be offered as an RRT modality both in-centre or at home, taking into account the local infrastructure and technology available.

i) Peritoneal Dialysis

The flexibility offered by APD during daytime hours has led to an expansion in its use over time[10], with 59% of the UK PD population on APD[11]. The current clinical evidence comparing outcomes between continuous ambulatory peritoneal dialysis (CAPD) and automated peritoneal dialysis (APD) is of low grade and is largely based on observational studies that are limited by confounding and bias and may not always be relevant to the NHS population. The randomised studies[12,13] were invariably underpowered to detect significant differences between CAPD and APD. Consequently, one modality was not found to be consistently superior to the other in terms of patient survival, technique survival or health-related quality of life. These small RCTs from the 1990s reported lower peritonitis rates with APD as compared to CAPD. In the more recent, albeit observational studies, the reported outcomes are inconsistent. The NICE guidance is consistent with the ISPD update on peritonitis in 2016 in suggesting that the risk of peritonitis should not determine PD modality choice[14]. Clinical outcomes therefore no longer drive the choice of modality (CAPD vs APD) in adults opting for peritoneal dialysis, with patient preference being the principal determining factor.

The studies included in the NICE guidance evidence review did not include patients on staff assisted PD (aPD). However, aPD is increasingly used to facilitate dialysis at home in patients, often older and frail, who are not capable of self-care PD. Observational studies have found that aPD is associated with comparable clinical outcomes when compared in-centre haemodialysis in older people[15, 16]. In comparison to self-care PD, aPD has been shown to have similar quality of life outcomes[17] and a lower risk of technique failure[18]. A recent retrospective study of 6,167 patients from the French PD registry found that there is no difference in technique survival and peritonitis risk between assisted APD and assisted CAPD[19].

There are cost implications associated with the utilization of aPD, with very limited evidence on its cost effectiveness. Future research should include evaluation of the health economic impact of aPD in comparison to other renal replacement modalities. A cost effectiveness analysis of the various aPD delivery models (assisted APD and assisted CAPD) would add value to the current body of evidence.

We agree with the NICE guidance that clinicians should consider PD as the first choice for children 2 years or under. PD is the commonest dialysis modality in children, accounting for 45% of patients starting RRT in the UK in 2016[20]. Clinical, patient and family factors, as well as age predominantly determine the choice of dialysis modality in the paediatric population, with PD being predominant in those aged 5 years or below[21]. The perceived benefits of PD in this age group are the flexibility of dialysing at home as well

as preserving vascular access. There is, however, a lack of evidence on comparative outcomes between HD and PD in this cohort.

ii) Home Haemodialysis

Home haemodialysis(HHD) remains an under-utilised modality in the UK[22] despite it being a therapy associated with lower costs compared with in-centre haemodialysis[23- 29]. HHD allows greater flexibility in treatment, a considerable reduction in travel to hospital and enables more extended and frequent prescriptions which are associated with several clinical benefits including a reduction in LV mass[30, 31], improved blood pressure control[32, 30, 33], improved phosphate clearance[34,35] and lower ultrafiltration rates. Several observational studies have demonstrated a significant survival advantage associated with HHD[36,37,38] although prospective randomised studies supporting this finding are lacking.

There have been no randomised trials comparing home to hospital dialysis outcomes. Patients choosing HHD however, go through steps of clinical selection, education and rigorous training on initiation of RRT. The impact of such interventions, hitherto untested in dialysis clinical trials, might provide insight into reported improvements in patient outcomes in HHD when compared to in-centre HD[38]. It is clear that patients frequently do not always start on their chosen RRT modality[39,40] and that this can be a key barrier to the uptake of home therapies. Effective tool and strategies should therefore be put in place to minimise these barriers in order to increase the proportion of patients starting on home modalities.

Like PD, the choice of HHD is largely determined by patient choice and training. The home setting, flexible scheduling, dialysis intensity, lower pill burden and freedom with diet and fluids offer major advantages to those who choose HHD. Although many of the benefits observed with HHD may be attributed to patient selection and preparation, given the numerous benefits reported in the literature, we feel that this treatment modality should be considered and offered to all patients deemed suitable.

Several centres in the UK report the use of HDF in the home setting[39] where there is local provision and technical feasibility for offering this therapy. Offering the same HD modality in-centre and in the home setting allows for continuity of care and facilitates smoother transition from hospital to home dialysis. There is very little published literature on the safety of HDF in the home setting[40]. There are no data to suggest that HDF is unsafe in the home setting provided HDF devices are installed, maintained and used as instructed and that feed water is monitored at least every 6 months for chemical and microbial quality[41]. Whilst there is no clear reason for the benefits of either HD or HDF to be any different at home compared with in-centre , the effects of more frequent and extended prescriptions using high volume HDF are largely unknown and under researched. We agree that at

present there is insufficient evidence to recommend one modality over the other in the home setting and that either HD or HDF can be considered.

iii) In-Centre Haemodialysis

High flux haemodialysis is predominantly a diffusive treatment combined with limited volumes of convective clearances. Haemodiafiltration (HDF), combines both diffusive and high dose convective therapy. Newer technology has enabled ultrapure replacement solution to be generated and delivered by the device (on-line HDF), allowing higher convective volumes and easier delivery of this therapy to patients. As a result, there has been a growth in the use of HDF as a treatment modality[42], particularly in Europe. However, there is considerable geographic variation in uptake[43]. There is also increasing use of HDF in children and adolescents[44].

There have been several recent prospective randomised clinical trials comparing HDF with HD treatment. Of the 4 large recent studies (CONTRAST study[45], ESHOL study[46], the FRENCHIE study[47] and the Turkish study[48]), only a single trial (ESHOL) has demonstrated a benefit of HDF over HD in terms of the primary outcome measure. Evidence from this trial needs to be interpreted with caution, however, as discussed below.

NICE's conclusion that HDF was associated with an 18% reduction in mortality (relative risk (RR) 0.82, 95% confidence interval (95% CI) 0.72-0.94) was unexpected, as at least three systematic reviews had recently reported that there was no evidence of superiority of HDF over HD[45-47]. An investigation reproducing their analyses found that the explanation was two-fold.

First, NICE had used a fixed effects model. Such an approach assumes that the effect of an intervention is in the same direction and of similar magnitude in all the studies being included in the meta-analysis. This was not the case for trials of HDF vs HD and a more appropriate approach to minimise type 1 error and inappropriately narrow confidence intervals would have been a random effects model [49, 50]. When this was applied, the effect of HDF became non-significant (RR 0.84, 95% CI 0.64-1.10). Second, they took no account of biases within some of the trials that were driving the effect. Specifically, two trials[46, 48] removed about ten percent of patients after randomisation from the HDF arm due to the inability to achieve high volumes of HDF in these patients (patients with similar blood flow issues in the HD arm were not removed). The key determinants of high convective volumes (filtration fraction, blood flow and treatment time) favour patients with more optimal vascular access and less comorbidity in whom outcomes may already be superior. This is reflected by the imbalance of age, diabetes and catheter use in the ESHOL study[46]. Combining biased studies in a meta-analysis amplifies the bias, with no way to weight biased studies differently and reduce their influence on the observed effect. Instead, therefore, it is

recommended that sensitivity analyses are done excluding the biased trials, to see how much they are driving the effect. Excluding the two trials that reported excluding patients post randomisation from the HDF arm[46,48] from the NICE meta-analysis resulted in complete loss of any evidence of a benefit of HDF over HD (RR 0.94, 95% CI 0.53-1.66).

Recognising this, feedback from the Renal Association, British Renal Society and Cochrane Renal challenged the draft NICE recommendation and NICE changed its recommendation to "consider HDF". The authors feel the current NICE recommendation to consider HDF over high flux HD in-centre is not supported by credible evidence and that further evidence is needed. There are currently two large randomised controlled trials underway (H4RT (https://doi.org/10.1186/ISRCTN10997319) and the CONVINCE study (https://www.trialregister.nl/trial/6942)) which have been designed to compare HD with HDF with a target convective volume of 21+ litres. Additionally, the MoTHER HDx study (https://clinicaltrials.gov/ct2/show/NCT03714386), comparing medium cut-off haemodialysis with HDF is also underway. Awaiting the results of these large and significant studies (target recruitment 3,350 participants combined for HDF vs HD) will allow a much more informed recommendation.

The field of dialysis is rapidly advancing with trials in new technology, medium cut-off membranes, miniaturised devices and alternative modalities (incremental, alternate day and nocturnal dialysis) Further technical guidance is available in the Renal Association guideline, "Haemodialysis"[4] including comprehensive evidence-based practice guideline on haemodialysis prescribing including scheduling time and frequency to improve patient outcomes.

iv) Transplantion

1.3 (B) Transplantation
Transplantation *1.3.12 Discuss the individual factors that affect the risks and benefits of transplantation with all people who are likely to need RRT, and their family members or carers (as appropriate)* *1.3.13 Include living donor transplantation in the full informed discussion of options for RRT* *1.3.14 Offer pre-emptive living donor transplant (where there is a suitable living donor) or pre-emptive listing for deceased donor transplantation to people considered eligible after a full assessment* *1.3.15 Do not exclude people from receiving a transplant based on BMI alone*

We fully support and endorse this section of the guidance. We agree that robust evidence is needed in determining the optimal timing for renal transplantation. Pre-emptive renal transplantation (a mode of transplantation that lends itself to pre-planning) is considered to be the preferred initial option for RRT in eligible patients. We suggest, however, that the existing evidence does not support earlier pre-emptive kidney transplant on the basis of GFR. A matched cohort study from the Australian and New Zealand Dialysis and Transplant (ANZDATA) registry, did not find a statistically significant difference in survival between pre-emptive (median GFR of 9.6ml/min/1.73m2) and non-pre-emptive live kidney transplant recipients with up to 6 months HD vintage (median GFR of 6.9 ml/min/1.73m2) , even when lead time bias was considered[51]. An earlier cohort study of 19,471 pre-emptive transplant recipients reported to United Network of Organ Sharing (UNOS) found no association between the GFR at the time of transplantation and patient or graft survival[52].

In the absence of evidence to the contrary, listing potential recipients for transplantation 6 months prior to anticipated start of RRT appears to be a sensible approach.

v) Conservative management

There is considerable variability in the uptake of conservative management both within the UK[53] and globally[54,55] and national registry data is lacking for this modality is most countries. When considering the options of conservative management and dialysis, decision-making can be difficult and there are no randomised studies comparing outcomes between patients choosing conservative management and dialysis. Data from observational studies[56] suggest comparable survival in older patients[57] and those with significant co-morbiditie[58] or poor performance status. Given the nature of these studies, there is risk of significant bias as well as confounding factors which makes their interpretation difficult and may in part explain the variability seen in current clinical practice. In addition to survival, the influence of treatment modality choice on other factors such as measures of quality of life, the number of hospital-free days, symptom burden, travel and the effect on family and carers should be considered and discussed. Once again, high quality data in this area is lacking and should be a focus for future research. There is currently one large randomised controlled trial examining this topic in the UK (The Prepare for Kidney Care Study https://doi.org/10.1186/ISRCTN17133653).

We fully support the NICE NG107 guideline in offering conservative management as a treatment option alongside RRT modalities and that decision-making should be made in conjunction with the patient and carers or family members where appropriate. Further technical guidance within this

field is available in the Renal Association guideline, “Planning, Initiating and Withdrawal of Renal Replacement Therapy”[6].

1.4: Planning dialysis access formation

1.4 Planning dialysis access formation
1.4.1 Discuss with the person, their family members and carers (as appropriate) the risk and benefits of the different types of dialysis access, for example, fistula, graft, central venous or peritoneal dialysis catheter 1.4.2 (NICE) When peritoneal dialysis is planned via a catheter placed by an open surgical technique, aim to create the access around 2 weeks before the anticipated start of dialysis. **1.4.2 (RA) We recommend a break in period of at least 2 weeks after PD catheter insertion, taking into consideration patient preference and local clinical pathways to avoid the need for temporary HD. We suggest that low volume APD be used in the setting of acute start PD.** 1.4.3 When HDF or HD is planned via an arteriovenous fistula, aim to create the fistula around 6 months before the anticipated start of dialysis to allow for maturation. When deciding to timing, take into account the possibility of the first fistula failing or needing further interventions before use 1.4.4 Offer ultrasound scanning to determine vascular access sites for creating arteriovenous fistulae for HDF or HD

The NICE recommendations on access planning are broadly supported by the authors. They highlight optimal timing of access placement to avoid unplanned RRT initiation by temporary vascular access, which is associated with adverse clinical outcomes.

Whilst observational studies suggest better outcomes in terms of access patency and mortality with early as against late arteriovenous fistula formation in potential HD patients, the optimal time for access placement differs depending on the outcome measure evaluated. Pragmatically, it is difficult to predict with certainty the timeframe for HD initiation due to unpredictable clinical events and non-linear GFR decline. It is therefore unsurprising that the recommendations on timing of AVF placement differ among the various national bodies[59], with some opting for GFR based criteria as against time. In the absence of robust evidence, the NICE recommendation for access formation at about 6 months prior to intended use, seems reasonable taking local clinical pathways into consideration.

Peritoneal access placement is a key part of the pathway for preparing a person for PD. There is significant variation in catheter insertion methods across renal units, based on local facilities and expertise. A recent systematic review involving 7 cohort studies found that the advanced laparoscopic insertion technique was associated with clinical superior outcomes when compared to open surgical insertion, including catheter migration, survival and leaks[60]. The NICE recommendation on timing of catheter insertion is predominantly based on a single randomised study of 122 participants which found a higher rate of leaks in those starting PD one week post insertion compared to two and four weeks post insertion. All catheters were inserted using the open surgical technique and thus the findings may not be applicable to other insertion methods[61]. The study may also be statistically underpowered as it was stopped early. The recommendation is thus based on moderate grade evidence. Nevertheless, the recently published ISPD guidance on optimal PD access recommends a break-in period of at least 2 weeks, regardless of the catheter insertion method[62]. This recognises the need to factor in patient convenience, training duration and availability into care pathway for establishing PD access.

On the other hand, there is a role for acute start PD in unplanned starters who would like home dialysis in the long term, avoiding the need for temporary HD. Several observational studies have reported a higher risk of mechanical complications (malfunction, leaks etc.) with urgent start PD (generally less than 2 weeks post insertion) compared to planned start PD. These complications are generally conservatively managed with no impact on patient or technique survival[63,64]. Low grade observational evidence suggests that clinical outcomes are at least similar when acute start PD is compared to acute start HD[65,66]. An important modifier of outcomes relating to acute start PD is the use of low volume APD to reduce the risk of leaks. This is a grade 1C recommendation by the ISPD[62].

The optimal break-in period post insertion may vary depending on the method of insertion used. Studies that compare the various insertion methods particularly in acute start PD would be beneficial.

1.5: Indications for switching or stopping renal replacement therapy

1.5 Indications for switching or stopping renal replacement therapy
1.5.1 Offer information on all medically appropriate treatment options when discussing switching RRT modality. 1.5.2 Consider switching treatment modality or stopping RRT if medically indicated or if the person (or, where appropriate, their family members or carers) asks.

1.5.3 Plan switching treatment modality or stopping RRT in advance wherever possible.

1.5.4 Do not routinely switch people on peritoneal dialysis to a different treatment modality in anticipation of potential future complications such as encapsulating peritoneal sclerosis. However, monitor risk factors, such as loss of ultrafiltration.

1.5.5 Seek specialist advice on the need for switching treatment modality when women become pregnant or wish to become pregnant.

We are in support of the guidance on switching treatment modalities or stopping renal replacement therapy. The recommendation not to electively swap patients on PD to other modalities in anticipation of encapsulating peritoneal sclerosis (EPS) is very much consistent with consensus view as per the ISPD position paper. Whilst longer PD vintage is associated with a higher risk of EPS, evidence of that elective transition from PD is preventative is lacking.

RRT patients are likely to utilise different modalities at different time points of their disease. It is therefore important to consider treatment pathways rather than individual RRT techniques Perspectives of patients, caregivers, and health professionals on the process of transitioning are even less well documented. Available literature suggests that at present, transition between the different modalities is poorly coordinated, causing significant morbidity and mortality[67]. While predictors of PD technique failure and transition to HD have been assessed in some studies, clinical outcomes following transfer from PD to in-centre HD are lacking. HD-to-PD transition, has been associated with an increased risk of death and technique failure[68]. Given that more than one-third of patients will experience a transition to another RRT modality, particularly to facility-based conventional HD within the first 3 years on PD[68], a better understanding of morbidity and mortality associated with this transition is critically important for the care of patients with ESKD.

A key transition point is during hospitalisation and readmissions for both RRT and conservative care patients. Systems for improving communication between the hospital and nephrologist about patient care are needed[69]. Transition considerations as outlined in the guideline are key to address such high-risk periods in patient lives on RRT. Robust policies informed by ongoing research will be required for implementation[68].

1.6, 1.7 & 1.8: Recognising symptoms, diet and fluids and information, education and support

1.6 Recognising Symptoms

1.6.1 Recognise that people on RRT or receiving conservative management may have the symptoms in table 1 and that these may affect their day-to-day life.

1.6.2 Throughout the course of RRT and conservative management:

- Ask people about any symptoms they have.
- Explore whether symptoms are due to the renal condition, treatment or another cause.
- Explain the likely cause of the symptoms and how well treatment may be expected to control them.

1.7 Diet and fluids

1.7.1 Offer a full dietary assessment by a specialist renal dietitian to people starting dialysis or conservative management. This should include:

- weight history
- fluid intake
- sodium
- potassium
- phosphate
- protein
- calories
- micronutrients (vitamin and minerals)

1.7.2 After transplantation, offer dietary advice from a healthcare professional with training and skills in this area.

1.7.3 Re-assess dietary management and fluid allowance when: a person's circumstances change (for example, when switching RRT modality), or biochemical measures or body composition measures (for example, unintentional weight loss) indicate, or the person (or, where appropriate, their family members or carers) asks.

1.7.4 Provide individualised information, advice and ongoing support on dietary management and fluid allowance to the person and their family members or carers (as appropriate). The information should be in an accessible format and be sensitive to the person's cultural needs and beliefs.

1.7.5 Follow the recommendations on dietary management and phosphate binders in NICE's guideline on chronic kidney disease (stage 4 or 5): management of hyperphosphataemia.

1.8 Information, education and support

1.8.1 To enable people, and their families and carers (as appropriate), to make informed decisions, offer balanced and accurate information about: all treatments available to them (including RRT modalities and conservative management), and how the treatments may affect their lives.

1.8.2 Recognise the psychological impact of a person being offered RRT or conservative management and discuss what psychological support may be available to help with decision-making.

1.8.3 Discuss with people which treatment options are available to them and explain why any options may be inappropriate or not advised.

1.8.4 Offer oral and written information and support early enough to allow time for people to fully understand their treatment options and make informed decisions. Information should be in an accessible format.

1.8.5 Direct people to other sources of information and support (for example, online resources, pre-dialysis classes and peer support).

1.8.6 Remember that some decisions must be made months before RRT is needed (for example, a fistula is created at least 6 months before starting dialysis).

1.8.7 Be prepared to discuss the information provided both before and after decisionsar e made, in line with the person's wishes.

1.8.8 Take into account information the person has obtained from other sources (suchas family members and carers) and how this information has influenced their decision.

1.8.9 Ensure that healthcare professionals offering information have specialist knowledge about late stage chronic kidney disease and the skills to support shared decision-making (for example, presenting information in a form suitable for developmental stage).

1.8.10 Offer people who have presented late, or who started dialysis in an unplanned way, the same information as people who present at an earlier stage.

1.8.11 Follow the recommendations on enabling patients to actively participate in their care in NICE's guideline on patient experience in adult NHS services and on information and education in NICE's guideline on chronic kidney disease in adults.

We fully support the guidance on recognising symptoms during the course of RRT, providing adequate nutritional support and developing resources and systems to provide adequate information, education and support to patients, carers and family members. Growth failure can be an important

manifestation of CKD in the younger population; clinicians should be aware of this and monitor it not only through weight history but also through charting of height and weight on age-appropriate growth charts.

1.9: Coordinating care

1.9 Coordinating care
1.9.1 Provide the person with the contact details of the healthcare professional responsible for their overall renal care: • before they start RRT or conservative management • when they switch from one modality to another. 1.9.2 Coordinate care to reduce its effect on day-to-day life and wellbeing (treatment burden). For example, take account of people's preferences and avoid scheduling appointments on non-dialysis days for people on hospital dialysis wherever possible. 1.9.3 Follow the recommendations on: • delivering an approach to care that takes account of multimorbidity in NICE's guideline on multimorbidity, and • continuity of care and relationships, and enabling patients to actively participate in their care in NICE's guideline on patient experience in adult NHS services.

The emphasis on coordination of care in Guidance 1.9 highlights the complex medical needs of this diverse, high-risk patient population. Its implementation is critically dependent on the interface between care pathways and multiprofessional stakeholders (ie. dieticians, specialist nurses providing education, psychologists, diabetes specialists and other key specialists). Care fragmentation in dialysis patients between nephrology units and primary care providers is well recognised and can result in : a) duplication of care leading to overuse, medication errors and scheduling errors, b) uncoordinated care with lack of communication and c) delayed or undelivered care resulting in delays or missed opportunities[70]. Several gaps in care delivery of RRT patients such as vaccination , cancer screening, HbA1c, foot care and eye testing, could be improved through better coordination between primary and secondary care. Considerable growth in the ESKD prevalent population with increasing age, diabetes and multimorbidity and a shrinking nephrology and primary care workforce is predicted[70]. Coordination of primary and secondary care therefore remains a major area of concern. A future dialysis care model will require innovative pathways designed through collaboration of dialysis clinics, nephrologists,

GP practices and other secondary care providers to address the needs of this unique patient group. This could deliver major transformation in care through improvements in patient experience, clinical outcomes and efficiency.

Renal replacement therapy for children continues to be co-ordinated through the 13 paediatric nephrology centres in the UK. Shared care arrangements with secondary paediatric services are variable across the regions of the UK, and improved network working is likely to improve patient experience and may improve clinical outcomes. This is particularly important for adolescents approaching transition to adult services, where good co-ordination between paediatric and adult nephrology units is key to ensuring an effective individualised approach[71].

Conclusion

The NICE guidance on RRT focusses on the entire life course of the patient with ESKD. The focus is on integrated, multidisciplinary and holistic care improvements to meet the needs of this unique, complex and high-risk patient group. Many of the aspects such as care coordination and transition are unique in the guidance. Implementation of these recommendations will require comprehensive review of policies, practice, care pathways and infrastructure, which can be potentially challenging within the constraints of current health care systems. There are several areas of controversy where definitive trial evidence is lacking. Much of our clinical current practice and current guidance is based on expert opinion and data largely obtained from observational studies. High quality prospective randomised studies are needed to answer many questions raised within the areas covered by the NICE guideline. Several of these are already underway, led by the UK kidney community, but further broadening of attitudes towards recognising uncertainty and offering randomisation could transform our ability to generate robust evidence to inform shared decision making. Other NICE research recommendations aimed at improving the gaps in evidence base, will need to be supported by kidney research consortiums and funding bodies. Ongoing trials and recommended research in RRT aim to improve the evidence of best practice in ESRD care and determine the future need for reaffirmation or reappraisal of the NICE RRT guidance.

Audit Measures

1. Percentage of patients commencing RRT referred <3months and <12months before date of starting RRT

2. Percentage of incident RRT patients followed up for >3 months in dedicated pre- dialysis or low clearance clinic
3. Proportion of incident patients on UK transplant waiting list at RRT initiation
4. Proportion of incident RRT patients transplanted pre-emptively from living donors and deceased donors
5. Proportion of incident patients commencing peritoneal or home haemodialysis
6. Proportion of incident children under 2 years of age commencing peritoneal or haemodialysis
7. Proportion of patients who have undergone a formal education programme prior to initiation of RRT
8. Proportion of incident RRT patients who report that they have been offered a choice of RRT modality
9. Proportion of patients remaining on initial treatment modality 3 and 12 months post initiation of RRT
10. Proportion of patients recording satisfaction with initial RRT decision at 3 and 12 months post initiation of RRT
11. Proportion of patients who have initiated dialysis in an unplanned fashion who have undergone formal education by 3 months.
12. Evidence of formal continuing education programme for patients on dialysis
13. Proportion of planned initiations with established access or pre-emptive transplantation
14. Inpatient/outpatient status of planned initiations
15. eGFR at start of renal replacement therapy
16. The number of patients with Stage 5 CKD who are undergoing conservative kidney management - as a proportion of all patients with Stage 5 CKD
17. The number of patients withdrawing from dialysis as a proportion of all deaths on dialysis
18. Morbidity and mortality associated with transition from Home to Hospital modalities and between modaities in RRT patients
19. Hospitalisation and Readmission rates in RRT
20. Vaccination rates in RRT patients
21. Coordination of care in diabetes in RRT patients
22. Coordination of care - Management of ischemic heart disease in Dialysis

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8.2 Appendix 2: Published book chapter: Effects of Expanded Haemodialysis on Clinical Outcomes

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Effects of Expanded Hemodialysis Therapy on Clinical Outcomes

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Abstract

The invention of dialysis has been a phenomenal advance in the treatment of kidney fail- ure. The introduction of artificial kidneys in clinical care remains one of the most success- ful lifesaving interventions in modern medicine. Its glory, however, has been tempered by poor long-term outcomes and a negative qualitative impact on the lives of patients who suffer from an extremely complex, burdensome, and restricted life on dialysis. There remains a huge gap in patient well-being and outcomes between artificial kidney treat- ments and kidney transplantation. The inadequacy of dialysis, at least in part, is due to the chronic accumulation of organic retention solutes of middle and large molecules in chron- ic kidney disease, which are poorly removed by current dialytic treatment modalities. In- cremental benefits observed through alternative strategies such as high volume hemodi- afiltration, high frequency, and expanded hemodialysis (HD) schedules have had limited success, due to a host of organizational, complex technology need and human factor barriers. Expanded HD (HDx) therapy offers a novel blood purification technology, with the use of high retention onset (HRO) membranes designed to achieve a superior spec- trum of solute waste removal in uremia. Limited studies have demonstrated the potential benefit of HRO membranes in reducing cardiovascular risk, vascular calcification, and in- flammation commonly associated with the "residual uremic syndrome" and patient symp- tom burden. Robust and efficient clinical trials are now required to establish the rationale and impact of HDx therapy in driving improvements in both physician- and patient-di- rected clinical goals and outcomes in dialysis.

Introduction

The landscape of hemodialysis (HD) provision has changed considerably over recent years. A change in practice has been driven largely by the ongoing poor outcomes that are observed in patients. Adverse cardiovascular outcomes and apersistent inflammatory state predominate, with cardiovascular and infective causes being the leading causes of deaths among this patient group. As men- tioned elsewhere in this publication, the importance of "middle molecules" has been increasingly recognized as key in the uremic syndrome. With the use of high-flux membranes now widespread in clinical practice, we appear to have reached a hiatus in terms of improving the care and clinical outcomes for our patients.

Clinical Outcomes in HD: What Progress Have We Made?

The provision of renal replacement therapy has to adapt to increasing demand year after year. There has been a 1.7-fold increase in the global prevalence of end-stage renal disease over a 20-year period (1990–2010), and its incidence has more than doubled in the same time period [1]. Whilst the delivery of HD in the 1960s was in the form of long and slow treatment delivered to a few and often in the home setting, today we see the treatment scheduled for most patients at 4 h thrice weekly and predominantly in the hospital setting. The early National Co- operative Dialysis Study [2] partly paved the way to shorter dialysis hours. Later, the much larger HEMO study further shaped practice by demonstrating that increasing the dialysis dose above a urea Kt/V of 1.3 made no difference to out- come [3]. Interestingly, this study also demonstrated no benefit from high-flux HD (HFHD) membranes compared with low-flux (defined as B2M clearances of <10 mL/min for low flux and >20 mL/min for high flux). HEMO may not have been sufficiently powered to detect a significant difference between the two membranes [4], and the reuse of dialysis membranes was also permitted which could account for no difference being seen. The European MPO study which was later conducted demonstrated a benefit associated with the use of high-flux membranes in diabetics and those with a serum albumin of less than 40 g/L [5]. High-flux membranes are now in widespread use; however, despite the in- creased clearance that they offer, improvements in clinical outcomes for HD patients remain fairly static. Whilst HFHD can clear larger molecules than low- flux HD, large middle molecules greater than 20 kDa are not effectively removed. Molecules within the spectrum between 20 and 60 kDa (a similar size to albu- min) accumulate and we are only at the beginning of understanding their

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toxicity. Further understanding and therapies within this area could well be the key in improving clinical outcomes for our patients.

Hemodiafiltration (HDF), which combines both diffusive and convective therapies, have gained increasing use in recent years, particularly in Europe. Technology allowing the online production of ultrapure dialysate fluid has made this a feasible treatment for maintenance dialysis patients. Whilst HDF provides enhanced clearance of larger molecules compared with HFHD (20–25 kDa rath- er than 15–20 kDa [6]), data from 3 randomized control trials are conflicting. The ESHOL study demonstrated a significant reduction in all-cause, cardiovas- cular and infection-related mortality associated with HDF, whilst the CON- TRAST study and a Turkish study showed no benefit compared with HFHD. On further analysis, the convective volumes used in the ESHOL study were found to be significantly higher compared with the other 2 studies, and a post-hoc analysis of all 3 studies suggests a benefit from HDF over HFHD when higher convective volumes are used. In the case of ESHOL, this was both in the 23.1–25.5 L/treatment cohort and in the >25.4 L/treatment cohort [7]. In CON- TRAST, benefit was seen in the >21.95 L/treatment cohort [8] and in the Turk- ish study >19.5 L/treatment [9]. The true benefit delivered by HDF remains unclear as caution must be taken when interpreting data from such analysis. Of interest in the ESHOL study, the incidence of intradialytic hypotension was sig- nificantly lower in the HDF group compared with the HD group (679 episodes per 100 patient-years vs. 938 per 100 patient-years). HDF with a replacement fluid at 2.5 L/h at room temperature shows a similar effect on hemodynamic stability to HD with a dialysate temperature of 35.5 [10]. A definitive random- ized trial of high volume HDF with HFHD is lacking but clearly needed.

The benefit of OL-HDF could perhaps be related to both energy transfer rate and enhanced middle molecule clearance. The latter is strongly determined by convective volumes. Higher convective volumes require ideal conditions of high blood flow rates, optimum vascular access and can be difficult to achieve in clin- ical practice. Moreover, a sharp rise in blood viscosity has been noted during HDF employing very high ultrafiltration rates, which may primarily be related to baseline hematocrit and plasma protein concentrations. This may have an adverse effect on the microcirculatory bed and organ perfusion, and pose a car- diovascular risk [11]. Whether high retention onset (HRO) membranes with limited internal convection can lead to a more favorable viscosity profile during HDx therapy need to be studied in comparison to high volume HDF.

Poor long-term outcomes are also likely to be due to the scheduling and fre- quency of dialysis. Current scheduling, with a 2-day inter-dialytic gap for the majority of patients is associated with harm. There is an increase in mortality and cardiovascular events after the 2-day inter-dialytic interval [12]. The period

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of transition to dialysis also appears to be harmful with a heightened period of risk to patients within the first 4 months [13], which again could be related to scheduling or frequency. Associated outcomes are considerably favorable for patients on home HD, where dialysis regimes are more extended or more fre- quent [14, 15]. However, uptake of such alternative modalities remains extreme- ly low despite incentivization, physician and patient's willingness due to major limitations in technology, infrastructure, and care delivery. Improvement in uremic symptoms, as well as improved energy levels, physical performance, and mental health seen in quotidian dialysis (with limited patient reach) need to be replicated universally in all HD patients using advanced dialysis technology.

Combining the benefits of alternative HD strategies of high volume convec- tive therapy, additional dialysis scheduling and frequent HD in achieving an improved solute clearance, within a thrice weekly schedule through the use of a novel dialysis technology, is potentially an attractive proposition for the provid- ers, physicians, and patients alike.

Clearances on Extracorporeal Treatments: Addressing Unmet Need in Uremia

The syndrome of uremia is a process that starts well before the initiation of renal replacement therapy. It is characterized by the retention of numerous com- pounds, collectively known as uremic retention solutes, which continue to build as the renal function progressively worsens. Their retention may also interfere with normal biological functions and in this setting they are generally referred to as uremic toxins. As glomerular filtration rate declines, cardiovascular risk increases such that the hazard ratio for death for CKD stage 3A is 1.2 compared with a hazard ratio of 1.8 for stage 3B, 3.2 for stage 4, and 5.9 for stage 5 [16]. Clearly, early changes, which may well be related to the impaired excretory func- tion of the kidney, manifest with early clinical sequelae which progressively worsen. In 2003, the European Uremic Toxin Work Group published a review of uremic toxins and proposed a classification system [17]. Molecules are classi- fied as low molecular weight (<500 Da), protein-bound or middle molecules (≥500 Da and <60 kDa) based on their size and whether they have known or likely protein binding. Molecules too large to be filtered by the glomerular base- ment membrane (>60 kDa) were not included. This original publication classi- fied 90 uremic retention solutes with the majority (68 out of 90) being small molecules. Since then, at least 25 further molecules have been identified [18]. All molecules (other than oxalate) which may have a cardiovascular impact (i.e., an effect on leukocytes, endothelial cells, smooth muscle cells or thrombocytes) are

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Table 1. Uremia retention solutes inadequately cleared by current hemo- dialysis techniques [42]

Solute	MW, Da		Action/effect
β-2M	12,000		Amyloidosis CTS
Leptin	16,000	Middle*	Malnutrition
Myoglobin	17,000		Organ damage
κ-FLC	23,000		Toxicity
Prolactin	23,000		Infertility
Interleukin-6	25,000	Large*	Inflammation
Hepcidin	27,000		Anemia
Bound p-cresol	33,500		CV toxicity
Pentraxin-3	43,000		Acute phase protein
λ-FLC	45,000		CV toxicity
TNF-α (trim)	51,000		Inflammation

* Value referred to as the molecular weight interval between urea and al- bumin. β-2M, β2-microglobulin; κ-FLC, kappa free light chains; λ-FLC, lambda free light chains.

middle molecules or protein-bound and these are cleared least by current HD strategies. Additionally, many inflammatory mediators such as interleukin (IL)- 6, IL-18, and tumor necrosis factor-α are also within the range of large middle molecules. Conventional renal replacement therapies cannot remove biologi- cally relevant retention solutes in the large middle molecule spectrum, and there appears to be wide interindividual variability in the retention factors of these solutes [17]. Expanded HDx promises to address this unmet need in blood pu- rification in the treatment of uremia.

Clinical Consequences of Uremic Solute Toxicity

Conventional dialysis strategies, based on urea and other water-soluble small molecules, fail to slow or prevent cardiovascular damage effectively. Studies eval- uating different solutes show that most compounds suggested to play a role in cardiovascular toxicity, show a dialytic behavior which is different from that of urea, such as advanced glycation end products, advanced oxidation protein prod- ucts, homocysteine, phosphate, asymmetric dimethylarginine, and cytokines [19]. A list of putative retention solutes in the middle molecules linked with clin- ical effects are described in Table 1. β-2 Microglobulin (β2M) which has a mo- lecular weight of around 11,800 Da is now well known both as a potential mark- er for middle molecule accumulation and as a molecule with direct pathological consequences in the case of dialysis-associated amyloidosis [20]. Improving the

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clearance of β2M through the use of a high-flux membrane has been shown to have beneficial consequences in postponing the onset of amyloidosis [21]. Fur- thermore, pre-dialysis serum β2M levels predict mortality in HD patients even after adjustment for residual kidney urea clearance [22]. This association is like- ly to reflect the effect of many larger middle molecules and therefore whilst at- tempting to improve clearance strategies of middle molecules, we must also gain further understanding of how their accumulation can manifest with clinical con- sequences. Although this work is underway, our understanding remains very limited. Additionally, dinucleoside polyphosphates are a group of molecules that are made up of either adenosine, guanidine or uridine interconnected by a poly- phosphate chain. They are released from various cell types including platelets, chromaffin cells [23], and renal tubular cells [24]. Their levels are increased in platelets of uremic patients compared with healthy subjects [25]. They appear to have a role in renal vascular regulation [26] and are also associated with an in- creased left ventricular mass [27]. Optimizing clearance of these molecules could therefore lead to improved cardiovascular outcomes. Cardiovascular damage in dialysis is linked with inflammation and is in part due to insufficient removal of pro-inflammatory ILs. It has been shown that serum and dialysates from treat- ments with higher cut-off membranes affect the immunomodulation of cellular apoptosis and expression of inflammation-associated genes. Anti-inflammatory effects of high cut-off (HCO) serum and efficient removal of mediators decreas- ing cellular viability by HCO-haemodialysis create a solid base for future im- provements in the development of membranes with an increased nominal cut-off point [30]. The putative toxins implicated in inflammatory burden and oxidative stress typically fall into the category of large middle molecules and are therefore likely to be reduced by HRO membranes. The combined approach of ultrapure dialysis fluids and higher removal of middle-sized inflammatory toxins (erythro- poietic inhibitor, IL-6) can lead to anti-inflammatory effect and reduction in pro- inflammatory cells. Whether such a strategy can lead to an improvement in sep-sis and inflammatory status remains unproven.

Potential Benefits of HRO Membranes in HDx

In an attempt to improve clinical outcomes through the increased clearance of larger middle molecules (those greater than 20 kDa), but avoid albumin losses, HRO membranes offer a promising technology. These membranes have pore sizes which allow the clearance of solutes of up to 45 kDa [31], and whilst albu- min loss is greater than HFHD (median 2.9 vs. 0.2 g) and HDF, they are at levels lower than those seen in peritoneal dialysis [32]. The pore size variance is around

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half that of HRO membranes [33], allowing more selectivity. Following expo- sure to blood, the membrane is less permeable than the glomerular basement membrane [33]. HD with a HRO membrane gives a greater clearance of λFLC (45 kDa in size) when compared with both HFHD and HDF [31]. In addition to this, treatment in HD with an HRO membrane appears to downregulate the ex- pression of both IL-6 and tumor necrosis factor-α mRNA when compared with HFHD. HRO membranes modulate inflammation in chronic HD patients com- pared to high-flux dialyzers. Transcription of pro-inflammatory cytokines in peripheral leukocytes is markedly reduced and removal of soluble mediators is enhanced after 12 weeks of expanded HDx with no significant adverse events [34]. However, it is expected to have a limited effect on protein-bound toxins. Certainly this advance in membrane technology is promising and clinical out- comes may well be improved through the clearance of this expanded range of uremic retention solute clearance. In addition to this, the use of this membrane in existing hemodialysis facilities is potentially simple, avoiding the need for more complex installations that are required to provide ultrapure substitution fluids for HDF.

The use of HRO membranes in clinical treatments have been shown to mod- ulate calcification of human vascular smooth muscle cell (VSMC). In vitro cal- cification, apoptosis, and expression of calcification markers are reduced during dialysis with HRO and the effects regress on switching to high-flux dialyzers. HRO dialyzers are a promising tool to modify the pro-calcifying effects of ure- mic serum. The impact on clinical endpoints needs further investigation. In vi- tro studies also demonstrate that HRO-treated serum after 4 weeks can demon- strate significantly improved endothelial function when compared with high- flux dialyzers in a randomized controlled first-in-man trial [34].

Expanded HDx offers a unique opportunity and hopes to address a spectrum of uremic burden above and beyond the capabilities of current blood purifica- tion technology. As a result of the ability to reduce the time averaged concentra- tions of solutes in the large middle molecule range, a host of clinical consequenc- es associated with its accumulation and described in Section "Clinical Conse- quences of Uremic Solute Toxicity", may be reduced and this needs to be substantiated in larger clinical trials.

Patient-Centered Dialysis Outcomes – An Unmet Need

Recently, it has come to light that as a clinical and research community, our pri- orities for outcomes in dialysis studies are not completely aligned with those of our patients and caregivers. Despite the focus by us on mortality in many HD

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Table2. The10topranked outcomemeasures inhemodi- alysis from patients and caregivers [38]

1	Fatigue
2	Survival (resilience andcoping)
3	Ability to travel
4	Dialysis-free time
5	Impact on family
6	Ability to work
7	Sleep
8	Anxiety and stress
9	Blood pressure control
10	Anorexia

trials, few interventions have resulted in a significant improvement. Over a 10- year period in the UK, incident one-year survival for hemodialysis patients has shown only a small rise from 85.3 to 89.8% [35] (adjusted 1 year after 90-day survival from 2004 to 2013) despite numerous studies within the field. Five-year survival remains at just 42% for US incident hemodialysis patients [36]. In ad- dition to mortality, other common outcome measures for studies focus on bio- chemical parameters and not validated surrogate markers. When patients and caregivers are asked to identify outcome measures important to them, the dif- ference between their priorities and those of clinicians delivering research is quite striking. The top 10 priorities for outcome measures in HD studies as iden- tified and ranked by patients and caregivers are listed in Table 2. It is clear from this list that dialysis patients value the quality of their lives and interestingly mortality did not even make the top 10.

A recent initiative known as the Standardized Outcomes in Nephrology-He- modialysis collaboration has established a core set of outcomes to be consistent- ly measured and reported in hemodialysis trials [37] (Fig. 1). These core out- comes have been established through a validated process consisting of system- atic reviews, focus groups, semi-structured interviews, Delphi surveys, and a consensus workshop. The outcomes align the priorities of patients with health- care professionals, which through the process have been demonstrated as differ- ent.

It will be important for future studies investigating the benefits of HDx ther- apy to incorporate these clinical outcome measures that matter to patients. Of interest, fatigue features within the core outcomes for this project. Cytokines, including IL-6 appear to be linked with fatigue among several chronic diseases. Serum IL-6, for example, in hemodialysis patients is associated with an increase of energy expenditure [39] and may manifest as fatigue. Self-reported fatigue has

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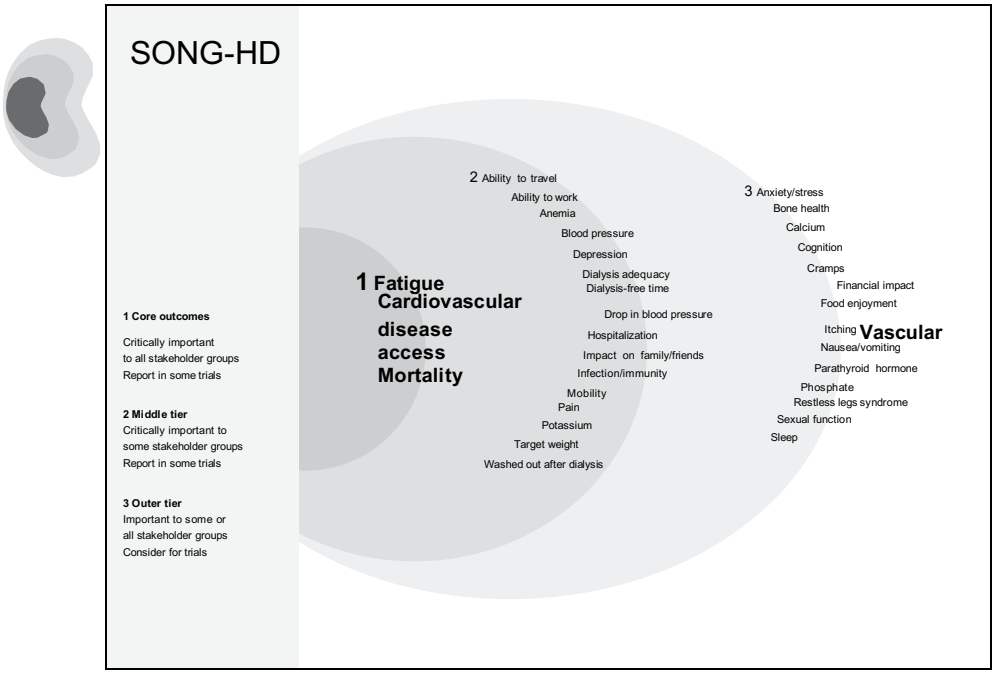

Fig. 1. Clinical outcome measures established by the Standardized Outcomes in Nephrol- ogy-Hemodialysis Group (adapted from http://songinitiative.org/projects/song-hd/).

been correlated to levels of human leptin [40], which is a 16-kDa retention solute identified in uremia and deserves further investigation with novel dialytic strat- egies.

The benefit afforded by renal transplantation provides the strongest evidence that reduction in solute levels would benefit patients. Transplantationimproves the quality of life and also enhances physiological functions, such as sleep, cog- nition, exercise capacity, and growth in children [41]. The goal of HDx with HRO membranes, that approximate more closely the membrane cut-offs in na- tive kidneys, should be to demonstrate such clinical improvements and well- being that matters most to patients.

Moving forward, great importance must be placed on listening to our pa- tients and moving the focus to improving their daily lives. Concentrating on mortality end points in clinical studies may cause us to miss opportunities for improving the quality of life. We should become more attuned and comfortable with the fact that improving the quality of life can be far more beneficial to our patients than merely prolonging the duration of life. The SONG initiative calls for a new approach to measuring dialysis outcomes and its future success is now in the hands of the Nephrology community.

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Future Developments

HDx and HRO membranes utilize the advanced knowledge in uremic toxicity and offer an opportunity to improve solute removal strategies in the treatment of uremic syndrome. There is a need to develop the clinical evidence on the val- ue of such a superior blood purification technology (HDx therapy), hitherto not achieved in dialysis, in improving both physician- and patient-directed clinical outcome goals. Interventional clinical trials that are efficient in design and allow generalizability to all those patients who might benefit are needed to provide clinical proof of benefits that matter most to ourpatients.

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8.3 Appendix 3: Academic Outputs During Study Period

Academic outputs during the M.D. period are listed below:

Submitted Manuscripts

Kharbanda K, Iyasere O, Caskey F, Marlais, M, Mitra M. Commentary on the NICE Guideline on Renal Replacement Therapy and Conservative Management. Submission pending via UK Renal Association https://renal.org/sites/renal.org/files/Commentary-on-the-NICE-Guideline-on-RRT-and-conservative-management.pdf (Accessed 10th December 2020)

Book chapters

Home Haemodialysis and Haemodiafiltration (Chapter)
Sandip Mitra and Kunaal Kharbanda (2016).
Advances in Hemodiafiltration, Dr. Ayman Karkar (Ed.), InTech, DOI: 10.5772/64095.
Available from: https://www.intechopen.com/books/advances-in-hemodiafiltration/home-haemodialysis-and-haemodiafiltration

Effects of HDx therapy on clinical outcomes (Chapter)
Sandip Mitra and Kunaal Kharbanda (2017).
Expanded Haemodialysis- Innovative Clinical Approach in Dialysis. Contrib Nephrol. Basel, Karger, 2017, vol 191, pp 188-199
https://doi.org/10.1159/000479267

Home Haemodialysis (Chapter)
Sandip Mitra and Kunaal Kharbanda (2017)
Oxford Desktop Reference: Nephrology 2nd Edition, Jonathan Barratt, Kevin Harris & Peter Topham (Ed.), Oxford University Press.

Grants

2016	**£18,682 awarded from Kidney Patient Research** Partnership (British Renal Society & British Kidney Patient Association) for Assist-HHD Study
2016	**£12,935 awarded from Kidneys for Life research grant** for "A feasibility study into developing a care delivery model for limited rate ultrafiltration in in-centre thrice weekly haemodialysis"
2017	**$243,415 awarded from Baxter Healthcare** for application to Investigator Initiated Research grant scheme for MoDal Study

Oral Presentations

Growing & Maintaining a Home Haemodialysis Programme- Invited Speaker
Renal SpR Club Spring 2018 Meeting, Leicester

"3 Minute Hero" Session: Assist-HHD Study (Selected as "Highlight of the Day")
UK Kidney Week, Brighton, June 2019

Recent Data on the Evaluation of the Efficacy of a Medium Cut-Off Membrane: MRI Experience- Invited Speaker
Baxter HDx Symposium, Brighton, October 2019

Feasibility of Home HD in the UK- Invited Speaker
12th Annual Home Therapies Conference, Manchester, 2019

Invited speaker but cancelled due to Covid-19:

"Membrane design how far can we go and what does this mean for our patient health?"- Invited Speaker
Dialysis Academy Spring 2020 Meeting, London, March 2020

"Staff assisted Home Hemodialysis (aHHD) as an alternative modality in patients on in-centre Haemodialysis: a feasibility study (BRS/KCUK Research Forum)"- Invited Speaker
UK Kidney Week, Birmingham, June 2020

Poster Presentations

A Single Centre 3 Year Experience of Implementing On-line Haemodiafiltration At Home (PO-166)
Kunaal Kharbanda, Gillian Dutton, John Woods & Sandip Mitra
UK Kidney Week (Renal Association) , Liverpool, 2017

Variability in Dialysate Temperature and Sodium Prescription Practices: A Survey of Renal Units in England, Scotland & Wales (PO-162)
Kunaal Kharbanda, Aghogho Odudu, Indranil Dasgupta, Sandip Mitra
UK Kidney Week (Renal Association), Liverpool, 2017

Patient Reported Treatment Burden and Wellbeing- Balancing Trade-Offs In Home Haemodialysis
Teresa Jeronimo, Kunaal Kharbanda & Sandip Mitra
ERA-EDTA Congress, Copenhagen, May 2018

A Randomised Pilot Study Investigating the Effect of Medium Cut-Off Haemodialysis On Markers of Vascular Health Compared With On-Line Haemodiafiltration: Study Design, Methodology & Rationale
Kunaal Kharbanda , Yvonne Alexander & Sandip Mitra
Cardiovascular Science Showcase, University of Manchester, 2018

Virtual Home Haemodialysis Review Tool (VH2RT): A Framework For Managing a Large Home Haemodialysis Programme (P365)
Kunaal Kharbanda & Sandip Mitra
UK Kidney Week, Brighton, June 2019

Assist-HHD Study: A feasibility study Into staff-assisted Home Hemodialysis (aHHD) as an alternative dialysis modality (P364)
Kunaal Kharbanda, John Woods, Victoria Jackson & Sandip Mitra
UK Kidney Week, Brighton, June 2019

A Randomised Study Investigating the Effect of Medium Cut-Off Haemodialysis On Markers of Vascular Health Compared With On-Line Haemodiafiltration (MoDal Study) (PO497)
Kunaal Kharbanda, Annie Herring, Fiona Wilkinson Yvonne Alexander and Sandip Mitra
American Society of Nephrology, Washington DC, October 2019

8.4 Appendix 4: Image Gallery

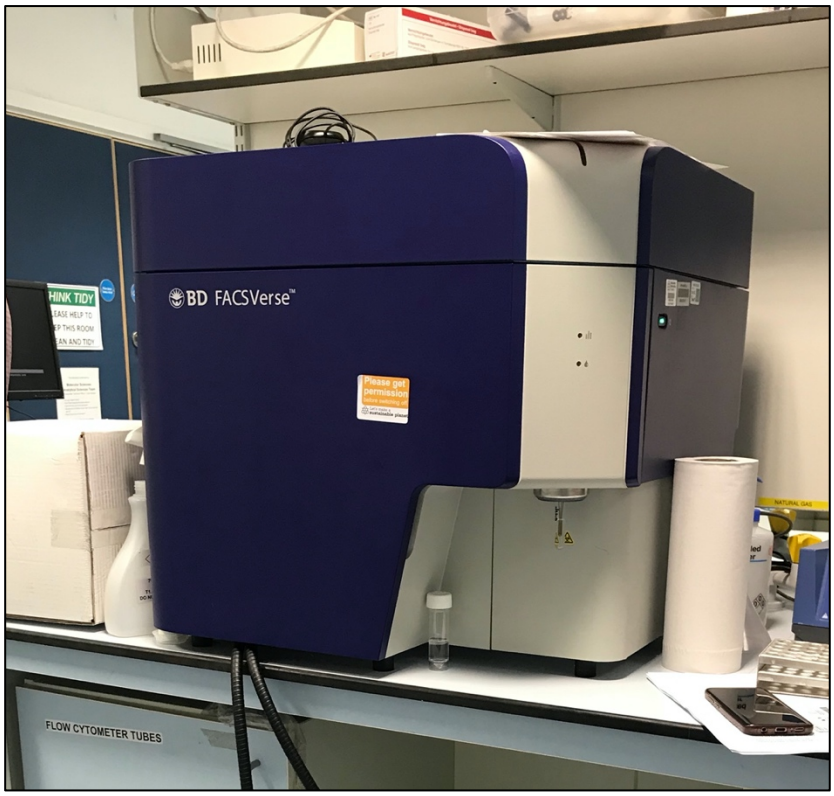


***Image 8-1**: Flow cytometer used for study samples in laboratory at Manchester Metropolitan University (BD Biosciences).*

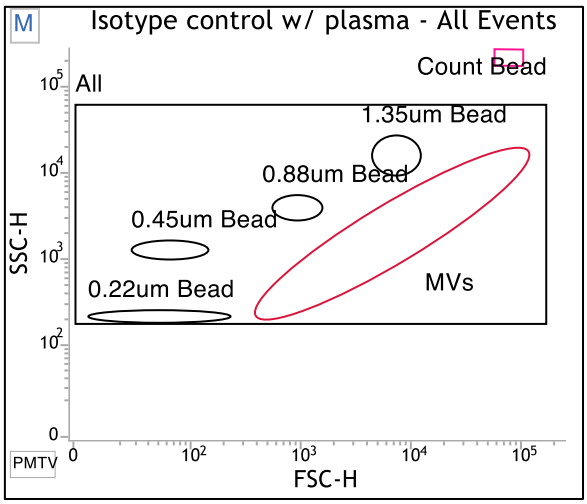
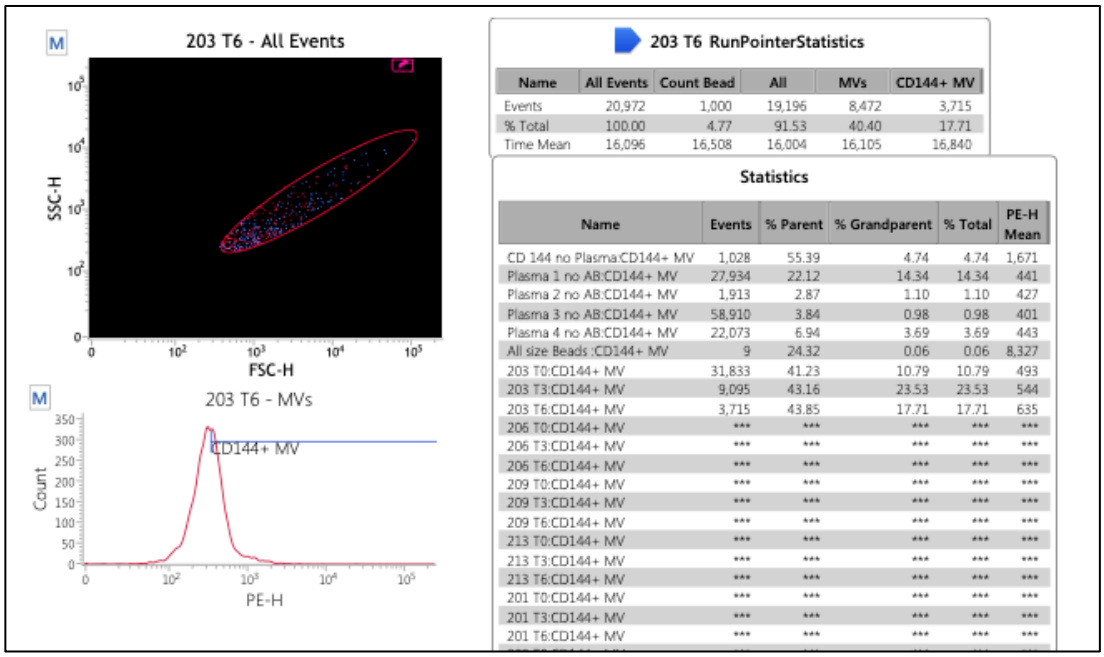


***Image 8-2**: Example of gating used in flow cytometry for EMV detection*



203 T6 RunPointerStatistics

Name	All Events	Count Bead	All	MVs	CD144+ MV
Events	20,972	1,000	19,196	8,472	3,715
% Total	100.00	4.77	91.53	40.40	17.71
Time Mean	16,096	16,508	16,004	16,105	16,840

Statistics

Name	Events	% Parent	% Grandparent	% Total	PE-H Mean
CD 144 no Plasma:CD144+ MV	1,028	55.39	4.74	4.74	1,671
Plasma 1 no AB:CD144+ MV	27,934	22.12	14.34	14.34	441
Plasma 2 no AB:CD144+ MV	1,913	2.87	1.10	1.10	427
Plasma 3 no AB:CD144+ MV	58,910	3.84	0.98	0.98	401
Plasma 4 no AB:CD144+ MV	22,073	6.94	3.69	3.69	443
All size Beads :CD144+ MV	9	24.32	0.06	0.06	8,327
203 T0:CD144+ MV	31,833	41.23	10.79	10.79	493
203 T3:CD144+ MV	9,095	43.16	23.53	23.53	544
203 T6:CD144+ MV	3,715	43.85	17.71	17.71	635
206 T0:CD144+ MV	***	***	***	***	***
206 T3:CD144+ MV	***	***	***	***	***
206 T6:CD144+ MV	***	***	***	***	***
209 T0:CD144+ MV	***	***	***	***	***
209 T3:CD144+ MV	***	***	***	***	***
209 T6:CD144+ MV	***	***	***	***	***
213 T0:CD144+ MV	***	***	***	***	***
213 T3:CD144+ MV	***	***	***	***	***
213 T6:CD144+ MV	***	***	***	***	***
201 T0:CD144+ MV	***	***	***	***	***
201 T3:CD144+ MV	***	***	***	***	***
201 T6:CD144+ MV	***	***	***	***	***

Image 8-3:*Example of output from flow cytometry machine for EMV detection*

Image 8-4:*The Theranova® dialyser (MCO Membrane) in use in one of the first patients in the study*

Image8-5: *Fresenius 5008® device used in the study. Setup shown with the MCO dialyser connected. The same machine was also used for HDF treatment.*

Image 8-6: *Victoria Jackson (research nurse) and I at the start of the clinical trial*

8.5 Appendix 5: Grant Award Letter

Baxter

November 13, 2017

Dr. Sandip Mitra
Central Manchester University Hopsital
Manchester Institute of Nephrology & Transplantation
Manchester Royal Infirmary
Grafton Street
Manchester M13 9WL
United Kingdom

Subject: Grant Application for Baxter In-Center Hemodialysis

Dear Dr.Mitra:

Congratulations!

On behalf of Dr. Angelito Bernardo, I am delighted to inform you that the Global Scientific Review Council has recommended that your grant application, "*A randomised pilot study investigating the effect of HDx therapy (Theranova) on markers of vascular health compared with on-line haemodiafiltration"*, be funded.

Total funding award to be provided by Baxter in the amount of $243,415.00 USD. In addition, study product (2,304 Theranova dialyzers) will be provided with an estimated commercial value not to exceed $35,000.00 USD.

In the near future, our contract associate will forward a Research Agreement including grant payment schedule for review and approval. The negotiation of the Research Agreement must be completed within 90 days from the Institution's receipt of the initial draft Agreement. Failure to meet this deadline may result in withdrawal by Baxter of the grant award. Also, please provide a copy of your protocol as a Word document to my attention at kelly_machak@baxter.com.

Thank you for submitting your application to our research grant program and again, congratulations on your grant award. Please do not hesitate to contact me if you have any questions.

Sincerely,

Kelly A. Machak
Senior Grants Program Manager
Life Sciences & Operations
Baxter Healthcare Corporation

Cc: Angelito Bernardo
Lars-Goran Nilsson
Markku Asola
Bernhard Kaumanns

8.6 Appendix 6: Ethics Approval Letter

North West - Preston Research Ethics Committee
Barlow House
3rd Floor
4 Minshull Street
Manchester
M1 3DZ

Telephone: 0207 104 8019

19 April 2018

Dr Sandip Mitra
Department of Nephrology, Manchester Royal Infirmary
Oxford Road
Manchester
M13 9WL

Dear Dr Mitra

Study Title:	**A Randomised Pilot Study Investigating the Effect of Medium Cut-Off Haemodialysis On Markers of Vascular Health Compared With On-Line Haemodiafiltration**
REC reference:	**18/NW/0169**
IRAS project ID:	**239423**

The Research Ethics Committee reviewed the above application at the meeting held on 23 March 2018.

Provisional opinion

The Committee would be content to give a favourable ethical opinion of the research, subject to receiving a complete response to the request for further information set out below.

Authority to consider your response and to confirm the Committee's final opinion has been delegated to none.

Further information or clarification required

1- The Committee requested the following changes to the Patient information Sheet:

i) Highlight that blood and urine samples will be discarded after 12 months.

ii) A sentence in regards to indemnity was added in.

iii) On page 1 you changed the word "death" to "complications".

iv) On paragraph 3 page 7 the word "data" was included.

2- The Committee requested the following changes were to the consent form:

i) Separate consent form was created for the sub-study.

8.7 Appendix 7: Participant Information Sheet

MoDal Study: Participant Information Sheet

"A Randomised Pilot Study Investigating the Effect of Medium Cut-Off Haemodialysis On Markers of Vascular Health Compared With On-Line Haemodiafiltration"

You have been invited to take part in a research study. Before you take part, it is important that you understand why the research is being carried out and what will be involved if you take part. Please take the time to read through the information provided in this leaflet and if needed, discuss it with your friends, family or GP. If anything is unclear or if you have any questions, please get in touch with us- the details are provided at the end of this leaflet.

The key information regarding this study will now be presented as a series of questions with corresponding answers:

What is the Purpose of the Study?

The main purpose of this study is to investigate the potential benefits of a new type of dialyser with enhanced clearance of toxins.

Patients who are on haemodialysis have a reduced life expectancy compared with those without kidney disease. The leading cause of complications in haemodialysis patients is cardiovascular disease (such as heart attacks and strokes). Reasons for this are still not yet fully understood however it may be, in part, due to the poor removal of certain toxins.

Haemodialysis treatment passes blood through an "artificial kidney" or membrane which is often referred to as a dialyser. The dialyser is made up of thousands of thin tubes which contain small pores and are surrounded by dialysis fluid. Toxins which are removed through haemodialysis treatment are able to pass from the blood and through the pores into the dialysis fluid. At present, due the size of the pores, some toxins are poorly removed and they build up in the body which can cause harm. A new type of dialyser has been developed called the Theranova dialyser. This product has a larger pore size and has been shown to provide improved removal of the larger sized toxins compared with current treatment. This product has been fully tested for safety in patients and carries a European CE safety mark. Whilst this product has been demonstrated as safe to use and more effective at removing larger toxins, the benefit to patients from the removal of these toxins is yet to be explored.

The purpose of this study is to investigate the potential patient benefits from treatment with a new haemodialysis membrane (the Theranova membrane) and compare it with the existing treatment that we provide (Haemodiafiltration). This study will focus on the following potential benefits:

1. The effect on how you feel such as itching, fatigue and the time it takes you to recover from your dialysis treatment
1.
2. The effect on the health of blood vessels
3. The effect on inflammation in the body (this is also linked to the health of blood vessels)

Why have I been invited?

We are inviting all patients in your current haemodialysis unit to take part in this study. All patients in your dialysis unit who have been on treatment for at least 12 weeks are eligible for this study.

Do I have to take part?

You do not have to take part in this study. If you decide not to take part, there will be no change to the care that you receive. If you would like to take part in the study you will be asked to sign a written consent form which you will be given a copy of.

What will happen if I take part?

If you decide to take part in the study, there is a 50% chance that your dialyser will change from your existing dialyser to the new Theranova dialyser. You may or may not receive the new dialyser if you take part in this study. Participants who are allocated to the new Theranova dialyser will be chosen completely at random by computer software. If you are selected to receive the Theranova dialyser you will receive treatment with the dialyser for 6 months in total and then switch back to your usual dialyser.

There are a number of different aspects to this study which you will be involved in (regardless of whether your dialyser changes) and these have been summarised in the table overleaf. For the purposes of the study, we will describe each time that you have any direct involvement as a “Episode”. You will however still be attending for your usual dialysis treatment 3 times per week. The table lists what will happen at each visit and these are described in more detail below the table. An estimate of

the extra time that these events will take you, over and above your usual treatment, is listed.

We will not require you to make an extra trips to the hospital for the purposes of this study but we will ask you to attend the hospital 30 minutes early on 2 occasions in the 6 month period during this study. On these occasions, we will pay for a taxi for you to the unit if you would find this helpful. In addition to this, we will also take consent for your participation in this study and record some medical information- this can take place when you attend for your usual treatment (either when you are connected to the machine or before or after your treatment depending on your preference).

This study also has an additional component which is completely optional. This is known as the "Sub Study" and details are provided in a separate information sheet.

Episode	Point in Study	Events	Extra Time Taken
1	Start of study	Consent (this will take 30 minutes but will take place during your dialysis treatment)	0 minutes
		Recording of medical details & medications (this will take 20 minutes but will take place during your dialysis treatment)	0 minutes
2	Within first 8 weeks	Treatment Optimisation	0 minutes
Randomisation Phase			
3	Within 4 months of consent This timepoint will now be called "Treatment Start"	Treatment with Theranova Starts (Half of the participants in the study only)	0 minutes
		Cardiovascular Measurements	20 minutes
		Questionnaire (Completed during your treatment, will take 15 minutes)	0 minutes
		Blood tests	0 minutes
		Urine Collection	48 hours
4	6 weeks after Treatment Start	Safety check (Completed during your treatment, will take 5 minutes)	0 minutes
5		Blood Tests	0 minutes

	12 weeks after Treatment Start	Questionnaire (Completed during your treatment, will take 15 minutes)	0 minutes
		Safety check (Completed during your treatment, will take 5 minutes)	0 minutes
6	18 weeks after Treatment Start	Safety check (Completed during your treatment, will take 5 minutes)	0 minutes
7	24 weeks after Treatment Start	Blood tests	0 minutes
		Cardiovascular measurements	20 minutes
		Questionnaire (Completed during your treatment, will take 15 minutes)	0 minutes
		Urine Collection	48 hours
		Safety check (Completed during your treatment, will take 5 minutes)	0 minutes
		Treatment for participants treated with Theranova ends and usual treatment resumes	0 minutes
	End of Study		

Event Details

Consent & Recording of Medical Details & Medications

After the study has been explained to you and we have answered any questions that you have, we will ask you to sign a written consent form if you would like to proceed with the study. At the same time, we will also ask for your medical history and your medications as this may influence some of the results of the study.

Treatment Optimisation

In order to make a comparison between the Theranova dialyser and existing treatment, it may be necessary for us to optimise your current treatment slightly. We will look at the data for the last 2 weeks of your dialysis treatment and make a decision based on this information. If you are currently having your treatment with a low blood flow rate (less than 300ml/min), we will need to optimise your treatment so that you can continue taking part in the study by entering the "optimisation phase". During the optimisation phase we will gradually increase

your blood flow rate to improve the efficiency of your treatment and reach a target dialysis dose. We may also discuss with you the option of changing the size of the dialysis needles that you use if you are using a fistula for your dialysis. If we are able to increase the dose of your treatment and you are happy to continue with the higher blood flow rate for the duration of the study, we will then proceed with the study and you will enter the "Randomisation" phase of the study. If we are not able to increase the dose of your treatment or you feel that you do not want to keep the dialysis prescription changes that are made, you will leave the study and continue with your usual treatment.

Randomisation

If your current dialysis treatment is at the required dose or you are able reach the required dose in the optimisation phase, you will then proceed with the study. Half of the participants in the study will be chosen at random to switch their existing haemodiafiltration treatment to haemodialysis treatment with the new Theranova dialyser for 6 months and then return to their normal treatment after. The other half of the participants who do not switch their treatment will keep their treatment exactly the same as before.

Vascular Health Measurements

At 2 points during the study (6 months apart) we will ask you to attend your dialysis session 30 minutes early so that we can make some measurements. 3 types of measurement will be taken each time and will take around 20 minutes to complete. All of these measurements will be taken in a private room and we will only need to expose your arms and the lower part of your legs. We will measure the following:

1. Pulse Wave Analysis- this will involve having a cuff (similar to a blood pressure cuff) inflated a few times on your upper arm (if you have a dialysis fistula we will use the opposite arm). This measurement will give us an idea of how stiff your blood vessels are.

2. Body Composition Monitoring- this will involve placing some small electrode stickers on your arms and legs (similar to if you have had an ECG before). This measurement will give us an idea of how much fluid you have in different compartments of your body.

3. Advanced Glycation Endproducts- this will involve placing your fingertip or arm onto a machine which will then shine a specialist light for less than 30 seconds. The reading will help give us an idea of your risk of cardiovascular risk.

Blood Tests

Blood tests for the purposes of this study will be taken every 12 weeks in addition to your usual monthly dialysis blood tests. These blood tests will be taken a total of 4 times during the study and will be taken when you are connected (we will not need to use a needle). A total of 30ml of blood (equivalent to around 5 teaspoons) will be taken each time. All blood samples will be discarded within 36 months of the end of the study.

Urine Collection

At 2 points during this study we will ask you to perform a urine collection for 48 hours. We will give you a urine collection bottle when you attend for your usual dialysis treatment (the 2nd session of the week) and ask you to collect all the urine that your pass until your next treatment session (the 3rd treatment of the week). We will measure the amount of urine that you pass and also test the urine to calculate how much your own kidneys are still working. If you do not pass any urine at all you will of course not be required to perform a collection. All urine samples will be discarded within 12 months of the end of the study.

Safety Check

If at any time during the study you notice any changes in your health that you are worried about then please let your dialysis nurse or the research nurse know immediately. In addition to this, if you are in the group of participants that have changed your treatment to the Theranova dialyser, we will ask you to list any more minor changes that you have noticed (whether these are positive or negative).

How will my treatment change if I take part in this study?

If you are one of the patients who switches to haemodialysis treatment the Theranova dialyser, the machine that you use for your treatment will remain exactly the same (Fresenius 5008). The duration of your dialysis treatment will also remain unchanged. The only thing that you will notice if your treatment changes is a different dialyser being used in your machine. We will also change a setting on the machine to switch the treatment from haemodiafiltration to Theranova enhanced haemodialysis. You may not feel any different if your treatment changes, however you may feel a difference and we will routinely record any changes that you do notice as part of the study.

Are there any risks from taking part?

The new Theranova dialyser that you may receive treatment with during the study has been fully tested for safety and compatibility in patients and has received a European safety CE mark. We do not expect any harm to occur as a result of switching your treatment to this product. Given that this product is fairly new to the market, we cannot completely eliminate a rare side effect of the product that was not detected during testing (however this is unlikely). This product is currently already routinely in use in some dialysis units across the UK and Europe.

During the studies that were performed on the Theranova dialyser, an increased loss of protein (albumin) was noted compared with existing treatment. The amount of albumin loss is similar to the loss seen in peritoneal dialysis (which has not been associated with harm) and we therefore feel that the protein loss is not harmful.

The vascular measurements that will be performed are not associated with causing any harm. The pulse wave analysis measurement may cause slight discomfort when the cuff inflates on your arm (in exactly the same way as a blood pressure cuff).

What will happen if I wish to withdraw from the study?

You are free to withdraw from the study at any time. This will not affect the care that you receive. Please let a member of the research team know if you wish to withdraw from the study.

What will happen if my mental capacity changes?

If during the course of the study it has been identified that you no longer have the mental capacity to give ongoing consent for participation in the study, we will withdraw you from the study. Any data that we have collected for the study until that point will be used however no further information will be collected.

What will happen at the end of the study- can I continue treatment on the new dialyser?

At the end of the study, if your treatment was changed to the new dialyser, your treatment will change back to your usual dialyser. At present, we do not intend to routinely use the new dialyser in our units after the study. This may however

change depending on the results of this study. At present, we cannot continue treating you with the new dialyser once the study has finished.

What will happen to the results of this study?

We aim to publish the results of the study in a medical journal once the study is complete. We also aim to present the findings at a medical conference and share the findings with other renal units. We will publish the results in our local patient newsletter. A copy of the results will be made available for participants.

What will happen to the data that you collect about me during the study?

All the data collected about you during the course of the study will be held securely and will only be accessible by authorised members of the research team. The questionnaire that you complete on paper will be kept securely in a locked area. This data will also be held electronically and will be stored on a secure password-protected database.

We may share anonymised data with other researchers which allows our research to be evaluated by others in the same field and may also support the development of future research by other research groups. We will also make anonymised data available if required for the purposes of any inspections to ensure we are carrying research to the required standards.

The hospital Trust (Manchester University NHS Foundation Trust) will have legal responsibility for your data that we hold. The data custodian for this study will be Dr Sandip Mitra.

We aim to publish data from the study in a medical journal and share the findings of the study at medical conferences. All of this data will be anonymous. All of your personal data from the study and your blood samples will be destroyed within 2 years of the end of the study. All other study data stored (other than anonymous data that we present or publish) will be destroyed within 5 years of the end of the study. Data from this study may form part of a university postgraduate research thesis.

Who is funding the study?

This study is being funded by Baxter Healthcare. Baxter Healthcare are the manufacturers of the Theranova dialyser. Whilst the study is funded by Baxter healthcare, the study was independently designed by and will be conducted by

clinicians from Manchester University NHS Foundation Trust and adopted by the National Institute of Health Research (NIHR).

Who has reviewed this study?

This study has been approved by the Research Ethics Committee (North West - Preston Research Ethics Committee). Manchester University Hospitals NHS Foundation Trust (MFT) Research and Development department will ensure that the research is being conducted to a high standard.

Will I be paid for taking part in this study?

You will not be paid any money for taking part in this study however we will pay for your travel to your dialysis unit on the 2 occasions you are required to attend 30 minutes early for this study.

Who should I contact for further information?

If you would like to know any more information about this study or if you have any questions then please contact any of the investigators listed here:

Researcher: Dr Kunaal Kharbanda
Email: Kunaal.Kharbanda@mft.nhs.uk
Telephone: 0161 276 7915

Researcher: Professor Sandip Mitra
Email: Sandip.Mitra@mft.nhs.uk
Telephone: 0161 276 6509

If you have any concerns and/or complaints about this study and wish to contact somebody independent from the research team, please contact the Patient Advisory Liaison Service (PALS). Appropriate insurance/indemnity is in place for this study (provided by NHS indemnity) in the event that any harm is caused due to somebody's negligence.

PALS can be contacted in the following ways:

Email: pals@mft.nhs.uk
Telephone: 0161 701 1999
Post: PALS, MFT Headquarters, Cobbett House, Oxford Road, Manchester, M13 9WL

8.8 Appendix 8: Participant Questionnaire

Participant ID: 001

MoDal Participant Questionnaire

"A Randomised Pilot Study Investigating the Effect of Medium Cut-Off Haemodialysis On Markers of Vascular Health Compared With On-Line Haemodiafiltration"

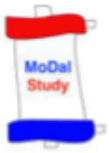
Thank you for taking time to complete this survey.

Please complete the questionnaire using a black pen and shade in the entire circle next to the answer you wish to select. Please do not use a cross or write in the circles.

The circle should look like this once you have shaded it in: ●

Once you have completed the survey please place it back in the envelope provided and hand it back to a member of staff.

On the next page is a list of symptoms, which you may or may not have experienced. For each symptom, please select the answer that best described how it has affected you over the past week.



Participant ID: 001

YOUR SYMPTOMS					
	Not at all	Slightly	Moderately	Severely	Overwhelmingly
Pain	⊙	⊙	⊙	⊙	⊙
Shortness of breath	⊙	⊙	⊙	⊙	⊙
Weakness or lack of energy	⊙	⊙	⊙	⊙	⊙
Nausea (feeling like you are going to be sick)	⊙	⊙	⊙	⊙	⊙
Vomiting (being sick)	⊙	⊙	⊙	⊙	⊙
Poor appetite	⊙	⊙	⊙	⊙	⊙
Constipation	⊙	⊙	⊙	⊙	⊙
Sore or dry mouth	⊙	⊙	⊙	⊙	⊙
Drowsiness	⊙	⊙	⊙	⊙	⊙
Poor mobility	⊙	⊙	⊙	⊙	⊙
Itching	⊙	⊙	⊙	⊙	⊙
Difficulty sleeping	⊙	⊙	⊙	⊙	⊙
Restless legs or difficulty keeping legs still	⊙	⊙	⊙	⊙	⊙
Changes in skin	⊙	⊙	⊙	⊙	⊙
Diarrhoea	⊙	⊙	⊙	⊙	⊙
Feeling anxious or worried about your illness or treatment	⊙	⊙	⊙	⊙	⊙
Feeling depressed	⊙	⊙	⊙	⊙	⊙

2. How long does it take you to feel back to normal after a dialysis session?

- ⦾ <2 hours
- ⦾ 2-6 hours
- ⦾ 7-12 hours
- ⦾ >12 hours

3. Have you been admitted to hospital for any reason over the past 3 months?

- ⦾ Yes
- ⦾ No

4. Have you had any infections over the past 3 months that have required treatment with antibiotics?

- ⦾ Yes
- ⦾ No

5. Have you had a heart attack or stroke over the past 3 months?

- ⦾ Yes
- ⦾ No

Thank you for your time. The survey is now complete. If you have answered "Yes" to any of the last 3 questions we may ask you for further details. Please place this questionnaire back in the envelope provided and hand it back to a member of staff.

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