Thesis Title
Characterising haemodynamic responses to Conventional Haemodialysis

By

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Abstract

People with kidney failure treated with haemodialysis suffer from high rates of end-organ damage. This thesis describes research work focusing on understanding the haemodynamic stress during dialysis and its negative impact on end organs. Our results show that a higher frequency of blood pressure (BP) variation assessed using extrema point frequency analysis correlated with high levels of cardiac biomarkers, in keeping with the association of higher BP variation with adverse outcomes in the general population. We have explored this frequency of BP variation further by categorising patients into two groups based on the ratio of high and low-frequency BP changes. These two categories of patients demonstrated differing haemodynamic responses during dialysis. Understanding this haemodynamic behaviour might help us individualise therapies to address haemodynamic stress. We explored the extent of the impact of haemodynamic stress on the structure of the brain. We demonstrated a phenomenon of accelerated ageing when compared to age and sex-matched healthy population. Moreover, the observed association of reduction in the grey and white matter volumes with dialysis vintage emphasises the potential contribution of HD-specific processes in the pathogenesis of these changes. Finally, we explored the role of cool dialysis as an intervention to mitigate the risks of dialysis-induced haemodynamic stress using intradialytic magnetic resonance imaging of heart, brain and kidneys which enabled us to understand the significant structural and haemodynamic changes in these organs during dialysis.

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Declaration

Except where acknowledged, I declare that this thesis is entirely my work and is based upon research carried out in the Centre for Kidney Research and Innovation, Academic Unit for Translational Medicine, School of Medicine (Royal Derby Campus), University of Nottingham and Department of Renal Medicine, University Hospitals of Derby and Burton NHS Foundation Trust between 2017 and 2019.

All patients were recruited and randomised by me. MR image acquisition was performed by Eleanor Cox and Charlotte Buchannan, Sir Peter Mansfield Imaging centre team under the supervision of Sue Francis. I coordinated the patient's study visits to the University and provision of dialysis. I supervised all the study dialysis sessions and collected the data. I performed the image processing and analysis of the structural brain data of haemodialysis and healthy controls. MRI team helped with the image processing and analysis of the rest of the MRI data. I performed the statistical analysis of the clinical and the MRI parameters to present the final results.

I recruited all the patients to the observational study involving non-invasive monitoring during dialysis. Non-invasive monitoring was performed by myself and with the help of the other research colleagues in the Renal Unit. I processed and analysed the data. Prof Jill Stewart kindly helped me with the matlab code for extraction of the extrema points. I performed the statistical analysis, categorisation of the patients and explored the difference in the responses in the two categories to present the final results.

Venkata R Latha Gullapudi

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Publications and abstracts arising from this thesis

Peer-reviewed publications:

- **Latha Gullapudi VR**, Kazmi I, Selby N. Techniques to improve intradialytic haemodynamic stability 2018. 1 p.
- **Latha Gullapudi VR**, White K, Stewart J, Stewart P, Eldehni MT, Taal MW, et al. An Analysis of Frequency of Continuous Blood Pressure Variation and Haemodynamic Responses during Haemodialysis. Blood Purif. 2021:1-15.

Poster Presentations:

- **Latha Gullapudi**, Eleanor Cox, Charlotte Buchannan, Kelly White, Maarten W Taal, Nicholas M Selby, Susan Francis. Structural brain changes in Haemodialysis patients compared to Healthy Controls assessed using MRI. UK Kidney Week 2020
- **VRL Gullapudi**, E Cox, C Buchannan, S Coleman, K White, B Canaud, M Taal, S Francis, N Selby. A randomised cross-over trial using intradialytic MRI to compare the effects of standard versus cooled haemodialysis on cerebral blood flow and cardiac function. ASN Conference 2019
- **VRL Gullapudi**, J Stewart, K White, T Eldehni, P Stewart, T Walker, M W Taal, N M Selby. Characterisation of haemodynamic responses to haemodialysis using frequency analysis. ASN Conference 2019
- **VRL Gullapudi**, Jill Stewart, Kelly White, Tarek Eldehni, Paul Stewart, T Walker, Maarten Taal, Nicolas Selby. Frequency analysis reveals unique

haemodynamic responses to haemodialysis: baseline results from the iTrend study. ERA-EDTA conference 2019

Abstract:

- Eleanor F Cox, **Venkata R Latha Gullapudi**, Charlotte E Buchanan, Kelly White, Sebastian Coleman, Daniel Cocking, Isma Kazmi, Katrin Köhler, Bernard Canaud, Maarten W Taal, Nicholas M Selby and Susan T Francis. A multi-organ assessment of the acute effects of haemodialysis using intradialytic multiparametric magnetic resonance imaging. ISMRM 2022

Chapter 1 Introduction

Section 1.1 Role of Kidneys and prevalence of End-Stage Kidney failure

Kidneys regulate the fluid and electrolyte balance of the body. They also have a significant role in regulating the acid-base balance and performing endocrine functions, including renin production, vitamin D metabolism, and erythropoietin production. Filtering blood through millions of nephrons is the predominant process by which waste products of metabolism are excreted in the urine. Complete or nearly complete irreversible loss of kidney function is defined as end-stage kidney failure (ESKF). It is managed with chronic renal replacement therapy (RRT) or conservative care (medical management without RRT).

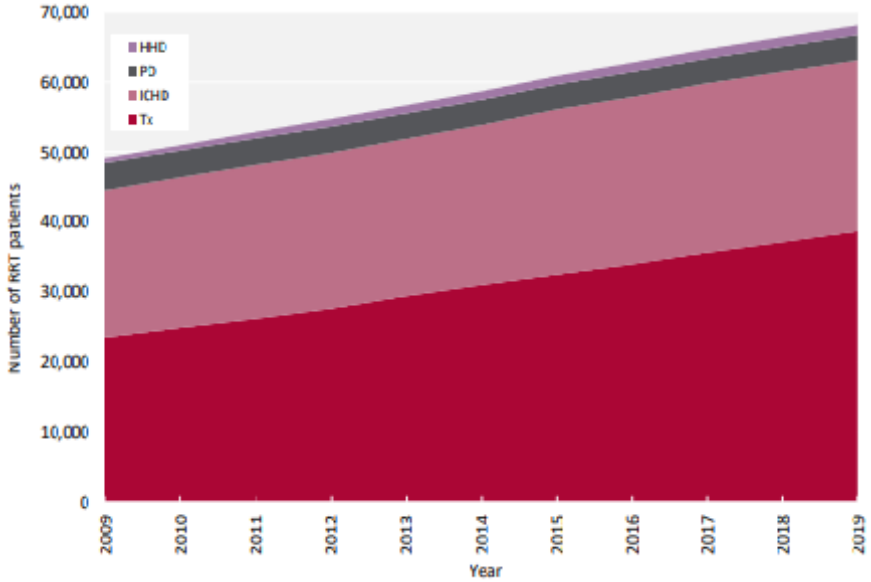


Figure 1-1: Growth in the prevalent patients by treatment modality between 2009-2019. Reproduced from UK renal registry 23rd annual report.

According to the 23rd UK renal registry annual report, there is a consistent growth in the number of adults receiving RRT in the UK (Figure 1-1). The prevalence of RRT

increased to 1293 pmp in 2019, an increase of 2.5% compared to 2018. In centre HD patients constitute 35.8 % of the prevalent RRT population (1).

Section 1.2 Renal Replacement therapy

Haemodialysis (HD), peritoneal dialysis (PD) and kidney transplantation are the various methods of providing RRT. Guidelines recommend holistic assessment of the patient as the renal clearance drops to eGFR≤15ml/min/1.73m^2 (estimated glomerular filtration rate, measure of level of kidney function) during the decision-making process around the commencement of RRT. This assessment should take into consideration of patient's symptoms and signs of renal failure, comorbidities, functional status, and psychological aspects. Careful discussion of the risk vs. benefits of RRT with the patient and their involvement in the decision-making process is crucial (2). Optimisation of fluid status, correction of anaemia, management of bone-mineral disease, provision of nutritional support, and in some instances palliative care involvement remain the key steps in the conservative management of patients where RRT is not appropriate.

Dialysis works primarily on the principle of diffusion, the movement of solutes depending on the concentration gradient across the semipermeable membrane. Additionally, a small amount of convective clearance of solutes happens during dialysis (clearance of dissolved solutes during fluid movement due to transmembrane pressure). This membrane can be an artificial filter as in HD or innate (peritoneal membrane) as in PD. Out of the available options for RRT, kidney transplantation is associated with the best clinical outcomes; however, limitations are donor organ availability and suitability of the patients. Emerging evidence suggests that home HD

offers comparable survival benefits to deceased donor kidney transplantation in selected groups of patients (3).

1.2.1 Haemodialysis

In HD, the blood and dialysis fluid continuously interact while flowing in opposite directions in two different compartments, separated by the dialyser (semipermeable) membrane. Dialysate fluid comprises of ultra-pure water mixed with various electrolytes, including sodium, calcium, magnesium, glucose. The concentration gradient of the waste products allows them to diffuse across the dialyser membrane into the dialysate compartment; this gradient is maintained continuously by counter-current flow in the two compartments. In addition to removing waste products, dialysis machines can be programmed to remove fluid by creating transmembrane hydrostatic pressure. This transmembrane hydrostatic pressure also leads to convective clearance during dialysis. The degree of clearance depends on multiple factors like blood flow rate, dialyser membrane size, sieving coefficient of the dialyser membrane, dialysate flow, and molecular characteristics of the solute.

Typically, patients dialyse for 3-4 hours, three times a week, and this regime is based on patient acceptability, logistics and costs of provision of such treatment. Thus, unlike the continuous clearance of the waste products and fluids by native kidneys, HD performs these excretory functions over a 3-4 hour period intermittently. This is followed by the slow accumulation of uraemic waste products and fluid during the inter-dialytic period (between HD sessions). This non-physiological process disturbs the body's equilibrium leading to multiple long-term health consequences. Furthermore, HD and PD do not replace the other functions of the kidneys like vitamin D metabolism and erythropoietin production. Some of these are addressed by medications.

Section 1.3 Long-term outcomes in haemodialysis patients:

The long-term prognosis of haemodialysis patients remains poor with high morbidity and mortality. The mortality rate in 2016 was 166 per 1,000 patient-years for HD patients. Most importantly, the patterns of mortality by time-since-dialysis-initiation have remained similar from 1997 to 2012, as reported in the USRDS 2018 annual report (4). According to the 20th UK renal registry and 2018 USRDS annual reports, cardiac disease remains the leading cause of death in the prevalent dialysis population (4, 5). These increased deaths were caused by arrhythmia and cardiac arrest, as shown in Figure 1-2 from the 2018 USRDS annual report (4). Moreover, evidence suggests that cardiovascular disease (CVD) is 10-20 times higher in ESKF patients when compared with the age-matched general population, as illustrated in Figure 1-3 (6).

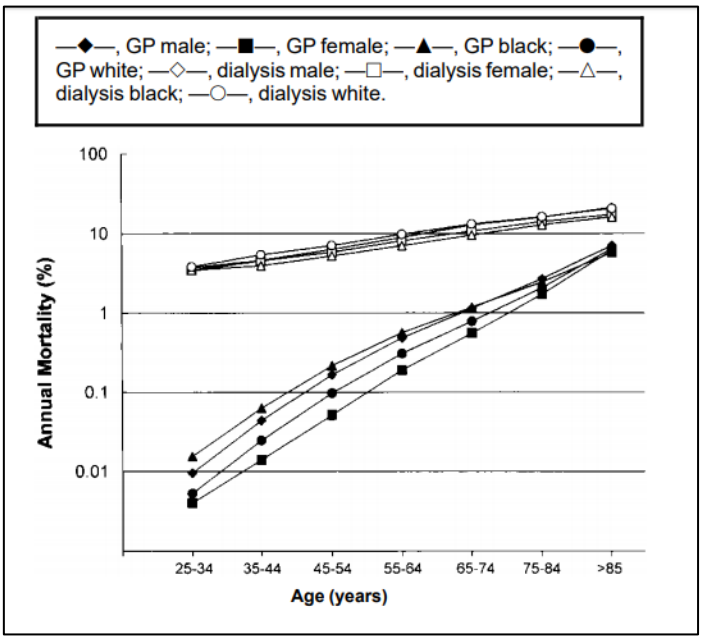


Figure 1-2: Cardiovascular disease mortality in the general population (GP) compared to ESRD treated by dialysis stratified by age, race, and gender. Reproduced with permission from (6)

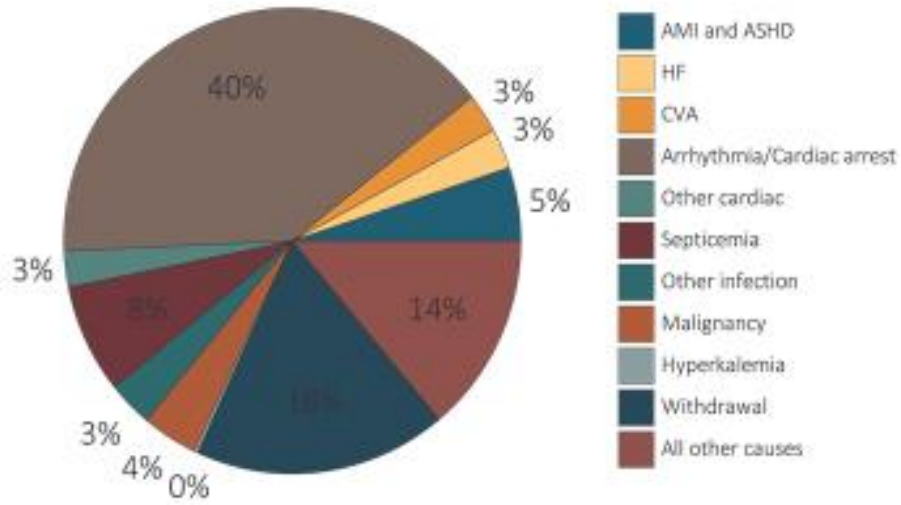


Figure 1-3: Reproduced from 2018 Annual report of USRDS: Unadjusted % in 2015 by cause among dialysis patients (excluding missing/unknown causes of death) (4)

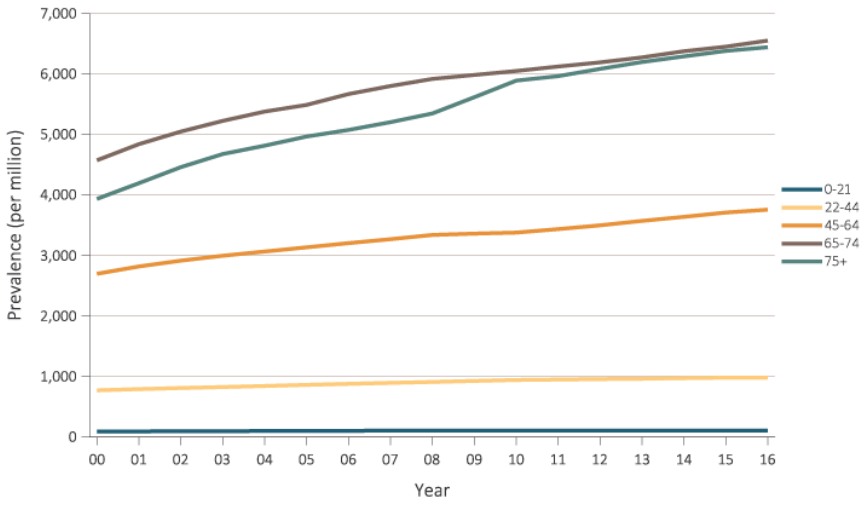


Figure 1-4: Reproduced from UDRDS 2018 Annual report: Trends in the standardised prevalence of ESKF, by age group, in the US population, 2000-2016 (4)

Furthermore, advances in dialysis technology and the wider availability of resources for the provision of RRT have led to a steeper rise in the prevalence of the older population with ESKF (4) (Figure 1-4), thus increasing the prevalence of associated comorbidities and traditional risk factors for cardiovascular disease among the ESKF patients.

Well-established treatment strategies to address the cardiovascular risks (hypertension, high cholesterol, smoking, and lifestyle) in the general population have not been beneficial to the same extent in ESKF patients. For example, Aspirin is regarded to be protective in a high-risk population prone to occlusive vascular events (7). However, such evidence is sparse in haemodialysis patients (especially for primary prevention), and no convincing trend towards reducing the overall risk of all-cause mortality or cardiovascular events was identified (8, 9). Similarly, the role of statins in significantly reducing the risk of the composite endpoint of death from CVD, non-fatal myocardial infarction or nonfatal stroke in haemodialysis patients is disputed (10, 11). Even interventions to address multiple risk factors simultaneously (focused on blood pressure control, hyperlipidaemia, homocysteinemia, anaemia, hyperphosphatemia, antiplatelet therapy, and smoking) were not beneficial in the reduction of cardiovascular risk (12). These studies suggest that the increased cardiovascular risk in ESKF patients is not just secondary to the traditional risk factors.

In addition to high cardiovascular risk, increased hospitalisation rate, high prevalence of malnutrition, psychological issues, and reduced quality of life are the other significant issues in HD patients contributing to adverse outcomes.

The pathophysiology of this increased cardiovascular morbidity is not fully understood. It is believed to be related to a complex interaction of the pre-existing traditional risk factors for cardiovascular disease, uremic-related (non-traditional) risk factors, and recurrent circulatory stress caused by HD (13). The non-traditional factors include chronic inflammation (C-reactive protein), oxidative stress, uremic cardiomyopathy, arterial calcification, bone-mineral disease, chronic volume overload, endotoxemia, malnutrition and more. The physiological effects of these factors are not just limited to

cardiovascular disease but affect other organ systems along with additional circulatory stress posed by HD therapy.

Section 1.4 Blood pressure

Prevalence of hypertension (HTN) in haemodialysis population is high (14, 15). In contrary to the linear association between BP and the cardiovascular mortality demonstrated in general population, U or J shaped association has been demonstrated HD population (16-18). Furthermore, this U shaped association seem to be true for peri-dialytic BP changes and not so for home readings (19). One of the factors that might be responsible for this observation is variation in the BP in dialysis patients depending on the dialysis schedule/sessions. Although the most practical approach in-centre HD population is to measure BP when they attend for dialysis sessions, poor agreement has been reported between pre and post diastolic BP with ambulatory BP (ABP) readings. A study by Agarwal et al, pre-dialysis BP readings seem to overestimate (agreement limits for SBP were 41.7 to −25.2 mmHg and for DBP 23.7 to −18.9 mmHg) and post-dialysis BP underestimate average ABP with wide agreement limits (SBP by 33.1 to −36.3 mmHg and DBP by 19.3 to −23.9 mmHg). Moreover, home BP (20, 21) and 44 hr interdialytic BP monitoring (21) were shown to be better predictors of all-cause mortality than the peri-dialytic BP's.

Several factors in HD patients influence the pathophysiology of systemic blood pressure regulation (Figure 1). The non-physiological way of removal of salt and water intermittently during 4 hrs of dialysis 3 times weekly of conventional HD undoubtedly contributes to the variation in the BP during dialysis and inter-dialytic period in HD patients. This is supported by the studies demonstrating progressive rise in the BP along with loss of nocturnal dip as the interdialytic period progresses with three times

weekly dialysis schedule (22) and progressive rise in pre-dialysis SBP by 1mmHG with every 1% rise in the interdialytic weight gain (23). Furthermore, impairment of natriuresis in patients with ESRF contributes to significant accumulation of salt. This accumulated salt exists in two forms: 1) Osmotically active extracellular sodium contributes to fluid retention and increased thirst, 2) Non- osmotically active tissue sodium was shown to be linked to inflammation and endothelial activation pathways (24), hence aggravating the issues with BP control. Given the progressive loss of residual renal function in this patient group, focus on reduction in the inter dialytic weight gain by reduction in the oral fluid/salt intake seem to be the effective ways of reducing BP, although much more difficult to achieve practically. This is supported by studies demonstrating significant improvement in BP and reduction in antihypertensive therapy requirements with more frequent dialysis by enabling careful management of fluid balance (25-27).

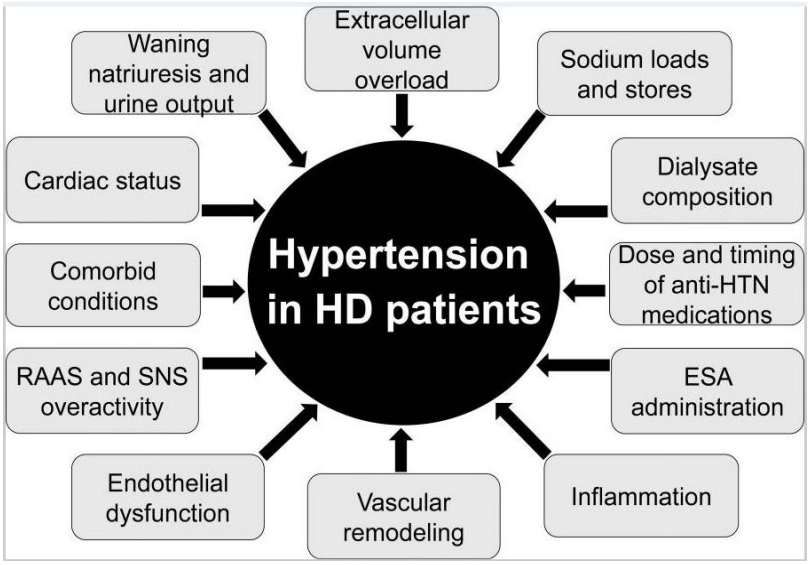


Figure 1-5: Pathophysiology of hypertension in haemodialysis. HD, haemodialysis; RAAS, renin-angiotensin-aldosterone system; SNS, sympathetic nervous system; ESA, erythropoietin-stimulating agent; anti-HTN, antihypertensive. Reproduced with permission from Flythe JE, Bansal N (28).

Elevated levels of serum ACE were observed in HD patients in several studies(29-31). Serum ACE plays a role in activation of angiotensin 1 (AG1) to its active form of angiotension2 (AG2) which leads to activation of pathways contributing to vasoconstriction, sodium/fluid retention by stimulating the release of aldosterone and thus has a significant role in BP regulation. Higher ACE levels are likely to be normal physiological response to the reduction in intravascular volume due to ultrafiltration and may also explain the post dialysis hypertension observed in certain HD patients. Furthermore, reduction in the density of the peritubular capillaries has been demonstrated in animal models with infusion of AG2 (32). This reduction in the capillary density combined with vasoconstrictor effect of AG2 leads to tissue hypoxia and activation of apoptotic process in renal tissue, increased oxidative stress and differentiation of tubular cells to myofibroblasts predisposing kidneys to fibrosis (33, 34). Perhaps these physiological processes may explain the observed association of preservation of residual renal function in HD patients on angiotensin converting enzyme inhibitors (ACEi) in limited studies (35, 36).

Kidney act as a source as well as target of sympathetic activity. Improvement of BP in patients with functional renal denervation in a small group of ESRF patients with resistant hypertension provides evidence to role of sympathetic activation in the pathogenesis of hypertension (37, 38). Elevated levels of muscle sympathetic nerve activity were reported in HD patients (39). Several mechanisms have been proposed for this increased sympathetic activity. These include 1) activation of chemoreceptors (40), 2) reduced availability of nitric oxide (NO) (mediated by several mechanism including reduced activity of nitric oxide synthetase) driving oxidative stress and increase in central sympathetic activity (41, 42) 3) Reduction and alternation in renalase (which normally metabolises the circulating catecholamines) activity in

patients with renal impairment (43). In addition to contribution to hypertension, increased sympathetic activity also leads to vascular remodelling by promoting growth of medial smooth muscle cells and adventitial fibroblasts (44).

Endothelial dysfunction is characterised by impairment of the endothelium- dependant vasodilation. It is a consequence of reduction in the bioavailability of vasodilator and/ or increase in the endothelial derived vasoconstrictor factors (45). In addition to impairment of endothelium- dependant vasodilatation, it also contributes to proinflammatory, proliferative and pro-coagulatory state thus enhancing atherogenesis (46). In HD patients, endothelial dysfunction is reported to be possibly mediated by oxidative stress (47) and linked to extracellular fluid overload (48) thus contributing to pathogenesis of hypertension.

Several other factors play a role in the pathogenesis of hypertension in HD patient. There is high prevalence of atherosclerosis (secondary to traditional risk factors) and arteriosclerosis (related to bone-mineral abnormalities) in HD population. Increased arterial stiffness due to these factors leads to higher pulse wave velocity in HD patients compared to general population (49). In a cross-sectional study involving 11 833 interdialytic BP measurements from 125 haemodialysis patients, log of PWV was shown to be linearly related to BP. In this study, SBP, DBP and PP was shown to increase by 18.8, 7.08 and 11.7 mmHg respectively with each log increase in PWV (50). Increasing pulse wave velocity was also associated with blunting of the circadian amplitude of systolic and pulse pressure. Dialysability of antihypertensive therapy can be important to consider when optimising BP control in dialysis patients. For example, a retrospective study showed higher mortality rates with the nondialyzable carvedilol versus the highly dialyzable metoprolol, which was attributed to a higher likelihood of

intradialytic hypotension with carvedilol (51). Erythropoietin which is commonly used in the treatment of renal anaemia is well reported to cause hypertension. Proposed mechanisms for this unintended effect of erythropoietin include hypersensitivity to angiotensin II and norepinephrine, as well as increased ET-1 activity (52).

Section 1.5 End organ damage

1.5.1 Cardiovascular system

A high prevalence of structural and functional changes in the heart exists among dialysis patients (53). As illustrated in Figure 1-6, the interaction of several pathophysiological mechanisms contribute to these changes, including (13):

- Vascular changes- atherosclerosis and arteriosclerosis
- Chronic pressure and volume overload
- Circulatory/haemodynamic stress induced by dialysis therapy
- Other factors include activation of renin-angiotensin-aldosterone system (RAAS), endothelial dysfunction, and oxidative stress.

High prevalence of atherosclerosis (focal & patchy intimal disease affecting medium-sized blood vessels) and arteriosclerosis (predominantly medial disease, a hallmark of arterial remodelling with diffuse calcification and is characteristic of vascular disease in renal failure population) are associated with increased all-cause and cardiovascular mortality (54, 55).

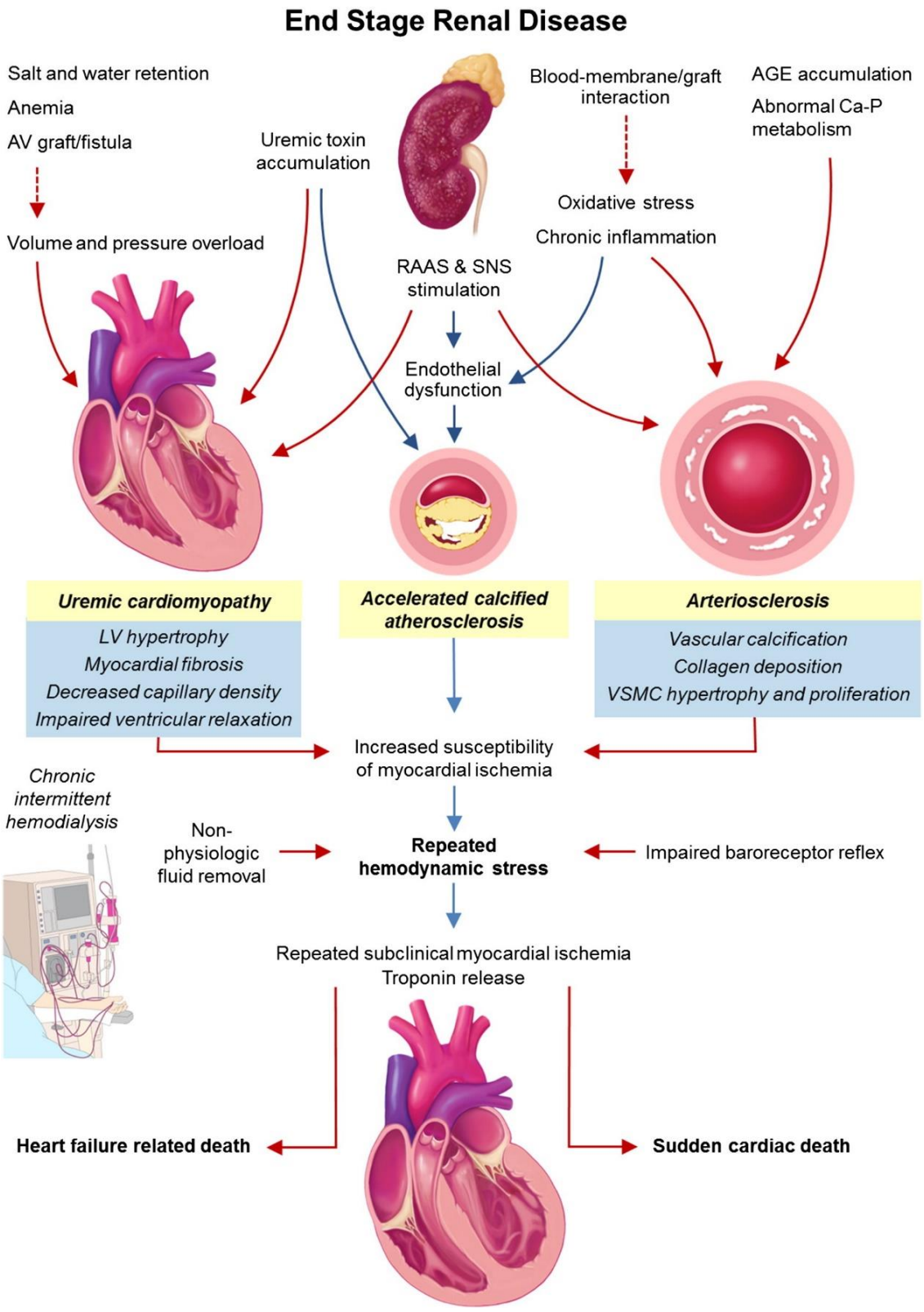


Figure 1-6: Pathophysiology of haemodialysis induced cardiomyopathy. Reproduced with permission (13)

Arteriosclerosis is characterised by decreased elasticity and an increase in the extracellular matrix leading to increased arterial stiffness, thus reduced cushioning effect. The consequences of these changes include 1) increased afterload causing left ventricular hypertrophy (LVH) and increased myocardial oxygen demand, 2) drop in diastolic pressure leading to a reduction in the coronary perfusion pressure and 3) increased central systolic pressure contributing to increased risk of stroke (56). These vascular changes are not limited to coronary vessels and aorta but are widespread in the vascular tree (57, 58).

The prevalence of LVH is as high as 74%-79% in the ESKF population and is reported to be present even in the advanced chronic kidney disease (CKD) population (59, 60). In the CRIC study, patients with advanced CKD (eGFR <20ml/min/1.73 cm^2) had an echocardiographic assessment at baseline and when they reached ESKF (mean follow-up period was 2 ± 1 years). In the study population, authors did not observe a change in the average left ventricular mass index at the follow-up compared to baseline; however, the proportion of patients with ejection fraction ≤50% increased from 29% to 48% when they reached ESKF (59), suggesting disease progression leading to organ dysfunction. The presence of LVH was demonstrated to be associated with poor survival (61) and with rhythm abnormalities (62). In addition to LVH, pre-existing structural abnormalities like mismatch in the myocardial-capillary densities (63) and diffuse calcification of the coronary vessels (64) can contribute to the pathogenesis of adverse cardiovascular outcomes.

It is plausible that combined effects of the reduced circulatory blood volume (leading to vasoconstriction, thereby increasing the afterload) during dialysis and impaired autonomic reflexes (reduced baroreceptor reflexes(65)) on the maladapted

myocardium can lead to a mismatch of supply-demand, thus triggering ischaemic injury. Evidence suggests that this ischaemic injury that happens recurrently during HD can lead to systolic dysfunction in ESKF patients (66). In addition, ischaemia triggers myocyte apoptotic and autophagy pathways. Activation of these pathways causes diffuse interstitial myocardial fibrosis leading to reduced ventricular compliance and diastolic dysfunction (67, 68).

Several studies reported the occurrence of silent myocardial ischaemia (SMI) during haemodialysis by using continuous electrocardiography (ECG) monitoring (69, 70). These findings did not correlate with the presence of coronary artery disease on angiography; hence their prognostic significance is disputed (70, 71). Although some of these findings in the ECG could be explained by the fluctuations in the electrolytes during dialysis, evidence from studies involving functional assessment of the heart supports the occurrence of myocardial ischaemia. A study demonstrating reversible regional wall motion abnormalities (RWMA) associated with IDH using echocardiography (ECHO) (72) provides such evidence. McIntyre et al. showed a global and regional reduction in the myocardial blood flow during HD in patients without angiographically significant coronary artery disease using cardiac PET. These authors also reported that areas with a regional intradialytic reduction in the myocardial blood flow by >30% compared to baseline (pre-dialysis) myocardial blood flow were associated with the development of RWMA's (73), thus supporting a phenomenon of recurrent silent myocardial ischaemic during dialysis.

In the non-CKD patients with coronary artery disease, in the event of transient myocardial ischaemia, a phenomenon of myocardial stunning is described. Essentially myocardial stunning is a reduction in ventricular function for several hours after an

ischaemic insult, despite the restoration of normal perfusion, which eventually recovers to normal. Repetitive insults lead to a cumulative effect and reduction in the function in the longer term (74). This phenomenon was demonstrated in HD patients by Burton et al. (75). Intradialytic echocardiography was performed at various time points (pre dialysis, 2hr and 4hrs into dialysis and 30minutes post-dialysis) at baseline and repeated the same assessments at 1 year. This study showed a high incidence of RWMA during dialysis and their persistence in the recovery period accounting to the description of myocardial stunning induced by HD. Magnitude of the myocardial stunning (described by the % drop in the shortening fraction of the myocardium, measure of reduced contractility on ECHO) correlated with the drop in the systolic BP, advancing age, high ultrafiltration volumes, and high troponin levels. There was no significant difference in the resting left ventricular function in patients with or without RWMA's. At follow-up, in patients with RWMA's at baseline, there was a reduction in the resting left ventricular ejection fraction (preserved in those without RWMAs) and a greater extent of reduction in systolic BP during dialysis (Figure 1-7). Most importantly, the presence of myocardial stunning was associated adversely with survival and cardiovascular events (75).

It is evident that HD therapy is associated with significant circulatory stress leading to a vicious cycle of cardiac injury (Figure 1-8) and has long-term implications. Even though some interventions to address the haemodynamic instability have shown promising results (76), high cardiovascular risk in HD patients remains a significant issue. Efforts should continue to tackle this problem with a multifaceted approach, given the complexity of the pathogenesis.

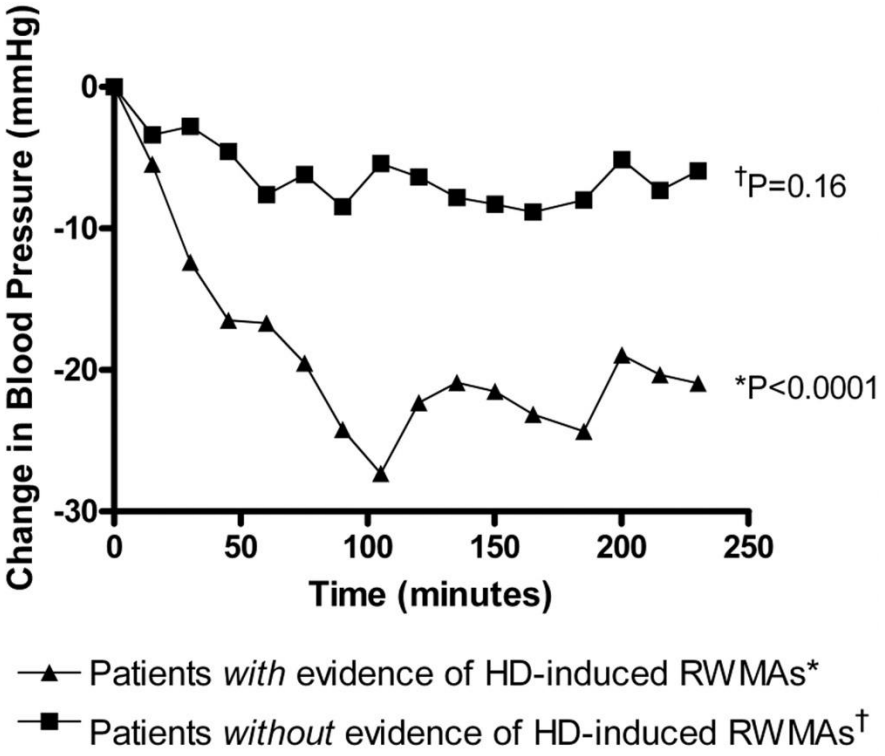


Figure 1-7: Trend of change in systolic BP during dialysis in HD patients with and without RWMA's. Reproduced with permission from (66, 75)

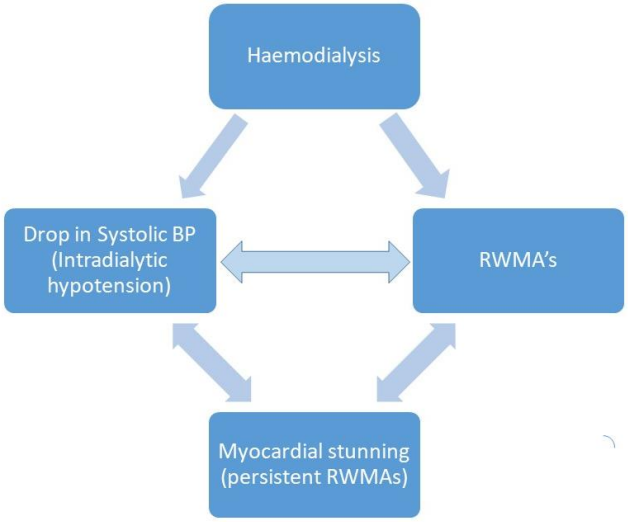


Figure 1-8: Vicious cycle of cardiac injury and circulatory stress induced by Haemodialysis

1.5.1.1 Cardiac imaging in Dialysis patients

Cardiac dysfunction and morphology have been extensively studied in dialysis patients using various imaging techniques as outlined in previous section. Pros and cons of some of these imaging techniques imaging are discussed in this section.

Echocardiography (ECHO) is a widely available, cost effective, non-invasive cardiac imaging technique used in assessment of function, morphology, and valve abnormalities without need for radiation. Assessment of left ventricular ejection fraction (77) and LVMI (61, 78, 79) has prognostic value in HD patients. There are several limitations of 2D-ECHO in assessing these accurately. This method is dependent on operator experience and image quality which can be influenced by the body habitus of the patients. In addition to these general limitations, there are well described specific issues in using these ECHO in HD population. Some of the calculations are based on end diastolic volume measurement which is dependent on the fluid status of the patient, so prone to variability depending on when the patients had dialysis (80). Likewise, the volume status of the patients can also influence the classification of the LVH, for example concentric LVH can be misreported as eccentric when the patient is fluid overloaded. This is relevant as response to treatment may vary depending on the type of hypertrophy (81) and so is the prognosis (eccentric LVH patients with greater risk of sudden cardiac death) (82). Post dialysis or non-dialysis day assessments are considered to better compared to the pre-dialysis ECHO. In addition, assumptions are made about the symmetry of LV hypertrophy during some of the calculations. Evidence suggests that types of LV hypertrophy vary in HD patients and hence these assumptions about the symmetry may lead to misinterpretation (83). Lack of need to make these assumptions is the advantage in 3-D ECHO, which is

based on the reconstructed images from multi-planar 2D (84). However, it can be time consuming to obtain great volume of good quality images and requires breath holding for image acquisition which can be challenging task for some of the HD patients with dyspneoa. Assessment of diastolic dysfunction can be challenging as well with 2D ECHO due to increased prevalence of volume overload in this population affecting the flow velocities across mitral valve in various diastolic phases. Techniques like tissues doppler imaging (TDI) and speckled echocardiography (sECHO) may be better in assessing diastolic dysfunction and have advantage in their ability to assess myocardial strain. Myocardial strain is a percentage change in the length of myocardium from relaxed state to contracted state. This is assessed in longitudinal, circumferential, and radial spatial directions providing more comprehensive assessment of the myocardial function. Myocardial strain is proven to have better prognostic value when compared to ejection fraction (85, 86). Both techniques have been used in HD patients to study strain (87, 88). Dependence on high-quality images obtained over at least 3 regular consecutive heart beats at a frame rate around 40-60/ sec means sECHO cannot be used in patients with tachycardia (89). Similarly, angle-dependency and inability to differentiate active vs passive shortening of myocardium is reported to be a limitation in TDI (90). Computed tomography has limited utility in ESRF patients due to the need for contrast agents and extensive vascular calcification limits the assessment of coronaries.

Cardiac magnetic resonance imaging (MRI) is considered to be gold standard imaging technique (91) to provide comprehensive assessment of the cardiac function, structure, ventricular volume, fibrosis, coronary and myocardial perfusion. MRI uses strong magnetic field and radiofrequency energy to provide detailed tissue structure and composition maps with the help of series of complex algorithms (92). Lack of need

for ionising radiation enables to use this imaging modality repeatedly. Improvement in the equipment, data acquisition algorithms, usage of respiratory and ECG gating helped to improve the resolution of the MR imaging. Multiple novel MRI methods (Figure 1-9) have been successfully used in cardiac assessment of ESRF patients.

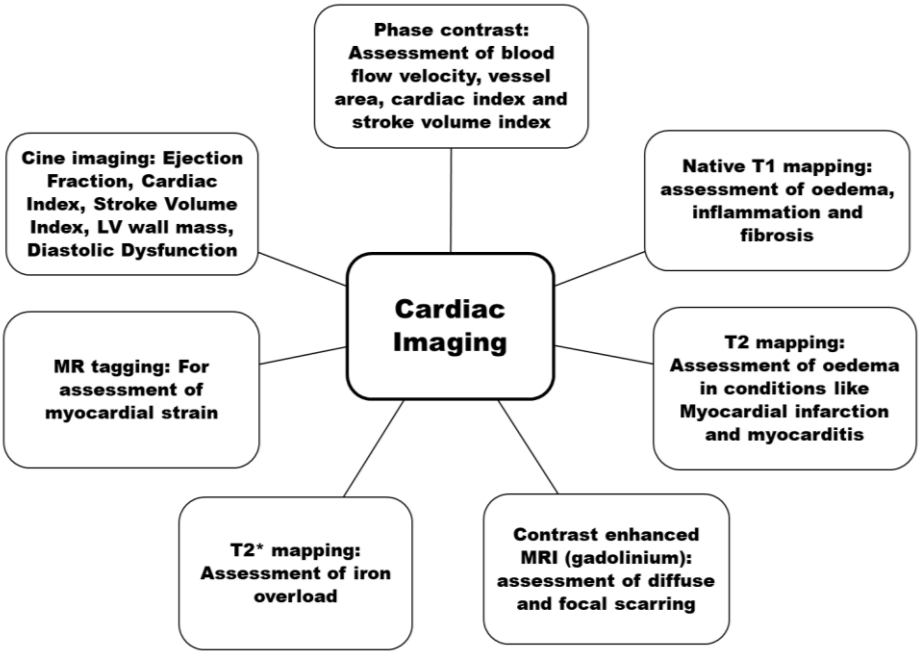


Figure 1-9: Cardiac Magnetic Resonance Imaging techniques

The ability to characterise cardiac tissue without the need for administration of gadolinium contrast (associated with concerns of nephrogenic systemic fibrosis in patients with advanced kidney disease) (93) is another advantage they offer. Some of the limitations of this modality include long acquisition times, claustrophobic patients tend to tolerate it poorly although more latest scanners are wide bored, and inability use in patients who have ferro-magnetic particles/devices in situ.

1.5.2 Neurological disorders

The haemodialysis population suffers from a significant neurological disease burden, both of the central (stroke, cognitive decline) and peripheral nervous systems

(autonomic dysfunction and peripheral neuropathy). The reported prevalence of cognitive impairment ranges from 6.6% to 87%, depending on the type of assessments (elaborative or simple) used (94-96). The risk of stroke in the ESKF population is substantially higher when compared to the general population (97, 98). A recent systematic review and meta-analysis by Masson et al. concluded that the risk of stroke increases by 7% with every 10ml/min/1.73m² decline in eGFR and by 10% with every 25 mg/mmol increase in albumin-creatinine ratio. Both eGFR and albuminuria were shown to be independently associated with an increased risk of stroke in this systematic review (99). Moreover, the presence of CKD in patients affected by stroke is associated with poor outcomes in the form of neurological deterioration, in-hospital mortality, and poor functional outcomes (100).

In the ESKF population, several pathological changes are described in the brain

1. Cerebral atrophy (measured by sulcal prominence, ventricular enlargement and hippocampal size) is more pronounced in the ESKF population than the healthy controls (101, 102).

2. Cerebral microbleeds (CMBs) are a consequence of blood leakage from the damaged, fragile small vessels in the brain white matter and are reported to be highly prevalent in the HD group when compared with healthy controls (103). In a meta-analysis by Charidimou et al., CMBs were shown to be associated with increased risk of ischaemic stroke (adj-HR: 2.09; 95% CI: 1.71-2.57), increase in future intracerebral haemorrhage risk (adj-HR: 3.93; 95% CI: 2.71-5.69), an independent predictor of all-cause mortality (adj-HR: 1.36; 95% CI: 1.24-1.48) and independently associated with incident dementia (adj-HR: 1.35; 95% CI: 1.00-1.82) (104). In a longitudinal study on HD patients conducted by Chao

Chai et al., the prevalence of CMBs at baseline was 54% (33 out of 61 patients), and 50% of the followed up patients developed new CMBs in a mean MRI imaging interval of 25±5 months(105). CMBs located in the basal ganglia and brainstem were associated with the presence of cognitive impairment (105).

3. Cerebral white matter changes (CWMC) are determined by measurement of fractional anisotropy (FA) and other measures like mean diffusivity in diffusion tensor magnetic resonance imaging. FA is a measure of the directionality of the diffusion of water in the tissues and depends on the integrity/ organisation of the microstructure of the tissues. (106). Normal white matter is associated with high FA values as the fibres/tracts are well organised. While lower FA values indicate loss of white matter integrity that is in keeping with the reported negative correlation with age (107). The presence of CWMC was shown to be positively correlated with dialysis vintage, which suggests that HD may be contributing to the white matter injury (108). Moreover, the prevalence of these changes was reported to be higher in HD patients than ESKF patients managed conservatively (51), which raises the possibility of additional risk factors specific to the process of HD in the earlier group.

4. Cerebral autoregulation is a physiological phenomenon that maintains constant cerebral blood flow (CBF) even with significant fluctuations in the mean arterial pressure (MAP), and this process seems to be impaired in the HD population. This is supported by a previous study demonstrating a moderate association of intradialytic hypotensive episodes with fluctuation in cognitive performance (109) suggesting possibility of cerebral hypoperfusion. In normal individuals, CBF was shown to be lower in individuals with white matter lesions than those

without white matter lesions (110). This finding leads us to the studies assessing CBF changes in the HD population. Previous studies using transcranial doppler monitoring to assess CBF changes in chronic HD patients (111, 112) did not demonstrate significant change. On the contrary, a recent study with intradialytic [15O]H2O positron emission tomography–computed tomography (PET-CT), which is considered to be the gold standard for assessment of the CBF, 10% ± 15% reduction in the cerebral blood flow was demonstrated in a group of elderly dialysis patients (113). A more recent study involving 97 chronic HD patients supports these findings, which showed a decline in the mean cerebral artery flow velocity during HD and linked these changes to cognitive decline in the long term (114).

All the above adverse pathological changes are of prognostic significance in the general population and HD population. However, there is minimal evidence of specific interventional studies targeting to reduce these changes in HD patients. A study by Eldehni et al. demonstrated the usage of cool dialysate (which has been well documented to be beneficial in lowering the hemodynamic instability during HD) to be protective against adverse white matter changes (115). In an observational study by Gupta et al., cognition and brain white matter changes improved after kidney transplantation in ESKF patients suggesting the potential for at least partial reversibility of these changes (116). Hence, there is a further scope and need to explore interventions to prevent such changes from developing in this high-risk HD population.

1.5.3 Kidneys

In the literature, the term residual renal function (RRF) is variably used to refer to the renal clearance of small solutes and/or urinary volume. Traditional ways of measuring

renal clearance using eGFR are not reliable in HD patients because of the significant fluctuation in serum levels of the small solutes due to the intermittent nature of HD. Thus, interdialytic urine collection remains the mainstay for measuring RRF in HD patients.

European best practice guidelines for HD suggest collecting urine during the interdialytic period to remove the effects of dialysis. They recommend using the mean blood urea & creatinine of the pre and post HD samples (post-HD sample collected immediately after dialysis and the pre-HD blood sample immediately before the following dialysis session) to calculate renal clearance (117).

RRF not only helps with small solute clearance and fluid balance but also contributes to other functions like better clearance of middle molecules, improved phosphate control, anaemia, reduced inflammation, and reduced cardiovascular risk (less LVH and vascular calcification) and hence has prognostic significance.

The presence of RRF (defined as urine volume >100ml/day) in HD patients was shown to be protective against mortality (odds ratio for death, 0.44; 95% confidence interval, 0.24 to 0.81; P = 0.008), even after adjustment for risk factors like duration of dialysis treatment, age, smoking, presence of diabetes, presence of cardiovascular disease, serum albumin level, and urea reduction rate (118). In the Netherlands Cooperative Study on the Adequacy of Dialysis (NECOSAD)-2, a prospective longitudinal study involving 740 incident HD patients, for each 1-unit increase in renal Kt/Vurea, a 66% decrease in relative risk of death was observed. Furthermore, in patients with preserved RRF, no consistent trend toward improved patient outcomes with increasing dialysis dose was found (119). Similarly, Obi et al. reported that in the presence of RRF, survival was not affected in patients with lower single pooled Kt/V (120). The

above findings suggest that the preservation of RRF has a vital role in the survival outcomes of HD patients.

Renal association clinical practice guidelines (121) recommend the following minimum therapy strategies to preserve RRF:

- Maintenance of haemodynamic stability on HD (i.e., avoid IDH, hypovolemia, or excess ultrafiltration)
- Avoid using nephrotoxins whenever possible
- Reduction in infective complications
- Use of ultrapure water for HD

However, it is important to note that these recommendations were based on evidence from smaller studies or projection of available evidence from PD patients and is graded as 2B evidence (121). Ultrapure dialysis water and biocompatible membranes reduce inflammation and improve haemodynamic stability; hence may be protective in maintaining the RRF (122-124). It is reasonable to avoid nephrotoxic drugs like NSAIDS, aminoglycosides and radiocontrast agents from the available evidence in CKD patients.

Angiotensin-converting enzyme inhibitors (ACEi) and angiotensin receptor blockers (ARBs) were shown to preserve RRF in PD patients with hypertension (125, 126). In a study by Moist et al., patients receiving PD had a 65% lower risk of RRF loss than HD patients. They have also demonstrated that ACEi and calcium channel blockers (CCBs) were independently associated with decreased risk of RRF loss. In the HD population, the effect of ACEi and CCBs was not significant, but the magnitude and direction of risk were generally similar to PD patients (36). There is further evidence

that Enalapril is protective of RRF in HD patients in the first year after initiation of HD therapy (35). Even though these small studies support the use of such therapies, in practice, a significant risk of hyperkalaemia and high prevalence of haemodynamic instability limit their usage in at least in a subgroup of HD patients.

Fluid status seems to be important in the preservation of RRF in the dialysis population, including the HD group. In a study by Jensen et al., intradialytic hypotension in HD patients and episodes of dehydration in PD patients contributed to the loss of RRF (127). Moist et al. showed that higher post-dialysis MAP significantly correlated with a lower risk of RRF loss (36), and speculated that lower BP might be the reason for the increased loss of RRF. There is also evidence that persistent fluid overload can cause a rapid decline of RRF in PD patients (128). Hence, maintaining euvolemia and avoiding haemodynamic instability (thus avoiding circulatory stress) are important in the preservation of RRF, although can be challenging to achieve with standard HD. Advances in understanding the pathogenesis of haemodynamic instability might help explore effective interventions for the preservation of RRF. A recent study demonstrated an acute decline in renal perfusion during dialysis, and dialysate cooling helped reduce this decline, although did not reach significance (129). These findings support the potential role of dialysis-based interventions in preserving RRF and should be explored further.

1.5.4 Other organ systems:

The hemodynamic effects of HD are not just limited to the above-described organ systems but are widespread systemically.

In a post-mortem study of 78 HD patients, Vaziri et al. described the presence of widespread inflammation in the gastrointestinal tract (GIT) like oesophagitis, gastritis, duodenitis, enteritis, and colitis. In addition, they have also reported the prevalence of ischaemic phenomena like thrombosis, embolism, and infarction of various segments of GIT (130). In normal individuals, translocation of endotoxins (a component of the bacterial cell wall) from the normal gut microbiome is controlled by the intestinal barrier (made up of mucosal layer, intestinal epithelial cell, and tight junctions) and only leak at low levels into the blood. Several studies have reported increased levels of endotoxins in HD patients that might indicate that there is disruption of this intestinal barrier, as summarised in review by March et al. (131). Disruption of the intestinal barrier has been demonstrated in animal experiments following ischaemic/reperfusion injury (132). In acute HD, splanchnic hypo-perfusion and increased oxygen extraction were demonstrated despite stable systemic haemodynamic parameters, indicating a possible role of ischaemic injury (133). High ultrafiltration rates seem to contribute to non-occlusive mesenteric ischaemia (134) and high levels of circulating endotoxins (135). This is supported by the presence of high levels of endotoxins in conventional HD than the modalities like frequent or nocturnal HD with lower ultrafiltration volumes (135). These endotoxins are pro-inflammatory and trigger the release of cytokines like Interleukin-6, interferon α, and tumour necrosis factor α, which lead to adverse cardiovascular effects, including reduced left ventricular function (136, 137). Thus, intradialytic haemodynamic instability, intestinal barrier disruption, endotoxemia are interlinked and contribute to adverse outcomes.

Skin is also adversely affected by vascular calcification and leads to complications like calciphylaxis (associated with skin ulceration and necrosis) associated with high

mortality (138). There is emerging evidence regarding the effects of HD on skeletal muscles.

Section 1.6 Summary

HD is a life-sustaining therapy for those with end-stage kidney disease (ESKD); however, it is an imperfect treatment, and patients are subject to hugely elevated cardiovascular mortality rates and dramatic reductions in quality of life. There is substantial recent evidence to demonstrate how the haemodynamic stress of HD directly contributes to these poor outcomes by driving recurrent ischaemic injury in vulnerable vascular beds. Although this evidence predominantly comes from studies on the heart and some from the brain, it is plausible that given the generalised nature of the physiological changes like vascular stiffness and the haemodynamic instability of dialysis, such changes are likely to happen in other organs systems leading to a multisystem organ dysfunction. Interventions to reduce haemodynamic instability might help minimise the subclinical ischaemia in these organs and prevent long-term adverse outcomes.

Chapter 2 Aims of Thesis:

This thesis aims to examine the following hypothesis

"*The process of haemodialysis contributes to significant haemodynamic instability and plays a role in the pathogenesis of long-term end-organ damage. A better understanding of the impact of haemodialysis on end-organs along with the improved characterisation of the haemodynamic changes during dialysis might provide additional insights in understanding the role of dialysis-based interventions in alleviating this risk*."

To do so:

A literature review was planned to understand the extent of the clinical problem caused by haemodynamic instability during dialysis and outline the currently available interventions. Followed by this work, various research studies were planned to answer the below research questions:

- *Can we improve the characterisation of the haemodynamic changes during haemodialysis using analysis of blood pressure variation.*
- *Examine the impact of haemodialysis on the structure of the brain to understand the adverse effects of haemodialysis on end-organs.*
- *Examine the impact of cool dialysis, a well-established dialysis-based intervention, on the haemodynamic changes in the vital organs during dialysis and explore its role in mitigating the risk of dialysis-induced haemodynamic stress.*

Chapter 3 Intradialytic Hypotension

Section 3.1 Structured abstract

3.1.1 Purpose of review

Haemodynamic instability during dialysis, most commonly manifested as intra-dialytic hypotension (IDH), remains a significant problem for patients undergoing chronic haemodialysis. IDH causes symptoms that degrade patients' experience, compromises dialysis delivery and is strongly associated with adverse patient outcomes. A greater understanding of the link between IDH and dialysis-induced ischaemia in the heart and brain has characterized mechanistic pathways, with repeated episodes of ischaemia shown to result in organ dysfunction. This review provides the most recent updates from published evidence across the range of potential interventions for IDH.

3.1.2 Recent findings

A literature search was undertaken to identify articles published in peer review journals between January 2016 and April 2018 using terms' intradialytic hypotension,' 'haemodynamic instability,' 'ESKF,' 'renal replacement therapy,' 'dialysis' in Medline and EMBASE and identified 58 references from which 15 articles were included in this review. Interventions included: cooling the dialysate; sodium profiling; convective therapies; strategies to minimize inter-dialytic weight gain (IDWG) and improve the accuracy of target weight assessment; review of antihypertensive medications; and carnitine supplementation.

3.1.3 Summary

IDH remains a significant clinical problem, with cooling the dialysate and reducing IDWG the cornerstones of management. Further understanding of the mechanistic process of the protective effects of cool dialysate will guide us in developing further interventions to address this clinical problem.

Section 3.2 Introduction

Haemodynamic instability, most commonly manifested as intradialytic hypotension (IDH), is one of the most frequent complications of haemodialysis (HD). IDH is generally accepted to complicate 10-20% of HD sessions, but the reported prevalence rate ranges between 17% and 40% of HD sessions and varies depending on patient characteristics and which definition of IDH is used (139, 140). IDH has clear associations with adverse outcomes; for example, in a US-based cohort of 112,013 patients, IDH occurring in more than 40% of dialysis sessions had a hazard ratio for mortality of 1.49 (1.42-1.57) (141), and in a landmark study by Shoji et al. the adjusted odds ratio for death was 1.5 (95% CI 0.90-2.48) with fall in systolic blood pressure (SBP) during HD by 40mmHg or greater (142). Several factors underlie the relationship between IDH and adverse outcomes, but one potential causal link has been the demonstration of subclinical ischaemia during dialysis occurring in various organs, including the heart and brain, with repeated episodes of ischaemia resulting in chronic organ dysfunction (113, 115). Repeated subclinical ischaemia during dialysis sessions is believed to lead to a cycle of repeated injury, ultimately leading to fixed permanent defects in the organ function as described in the reduction of myocardial function (66). IDH also leads to unpleasant symptoms, including abdominal discomfort, yawning, sighing, nausea, vomiting, muscle cramps, restlessness, dizziness or fainting, and anxiety (15). Dialysis time may be cut short as a consequence of these symptoms, with failure to achieve adequate solute or fluid removal.

Despite this, there remains uncertainty around optimal preventative strategies for an individual. One problem is that the optimal definition of IDH for use in clinical practice

or research studies is not well established, and definitional variation permeates the current evidence base. European Best Practice Guidelines (EBPG) define IDH as a decrease in systolic blood pressure (SBP) of ≥20 mmHg or a decrease in mean arterial pressure (MAP) by 10 mmHg associated with clinical events and the need for nursing interventions (143). Kidney Diseases Outcomes Quality Initiative (KDOQI) recommends the same blood pressure changes but in association with symptoms (15). However, concern remains that smaller or asymptomatic changes in blood pressure (BP) may still be clinically relevant. Recently Flythe et al. have studied the association of eight commonly used IDH definitions with mortality in two large US dialysis cohorts (144). Their results showed that nadir BP during dialysis retained the strongest association with mortality (nadir intradialytic BP <90mmHg for those with a pre-dialysis BP of <160mmHg; nadir of <100mmHg for those with a pre-dialysis BP of ≥160mmHg). To build on this, further work to define BP thresholds based on patient outcomes, symptoms and pathophysiological consequences of IDH is needed.

The fundamental process that leads to IDH is a reduction in circulating blood volume that exceeds plasma refill rate and cardiovascular compensatory mechanisms; these have been discussed in multiple previous reviews (145, 146). Established preventative strategies for IDH recommended in current guidelines include regular review of target weight; minimising intradialytic weight gain (by dietary fluid and salt restriction); optimising dialysate composition (use of bicarbonate buffer; avoidance of low calcium dialysis concentration); cooling the dialysate; review of anti-hypertensive medications; and longer/more frequent dialysis schedules. However, even with these interventions, some patients remain prone to IDH.

This review was undertaken in May 2018 with an aim to provide a summary of new and emerging evidence in the management of IDH (Haemodynamic instability) from the preceding two-year period so that up-to-date literature in this area is covered. A literature search was undertaken to identify papers published in peer-reviewed journals between January 2016 and April 2018. We used the terms ' intradialytic hypotension', 'haemodynamic instability', 'ESKF', 'renal replacement therapy', 'dialysis' in Medline and EMBASE for performing the search. Inclusion criteria included original published research articles in full-text case series, cohort studies, randomised controlled trials, and systematic reviews involving adult (≥ 18 years) haemodialysis patients. We identified 58 references from which 15 publications were selected for inclusion.

Section 3.3 Reducing Dialysate Temperature

Standard dialysis leads to a rise in the core body temperature, and the reasons behind this are not fully understood. Possible mechanisms include ultrafiltration driven vasoconstriction leading to accumulation of thermal energy, gain in thermal energy from the extracorporeal circuit and potential inflammatory response triggered by dialysis membranes. When this rise in core body temperature reaches a certain level, it triggers reflex peripheral vasodilatation and may contribute to IDH. Cooling the dialysate helps achieve net negative thermal balance and thus avoids reaching the trigger point. This leads to improvement in haemodynamic stability (147) by increasing the total peripheral resistance (148) and improving left ventricular contractility (149). It is one of the more widely studied interventions to reduce intradialytic hypotension and is a first-line recommendation in the EBPG guidelines (143). However, it remains under-utilised in clinical practice because of concerns about unpleasant symptoms like

cold and shivering. Concerns about a potential theoretical risk of reduced dialysis clearance with peripheral sequestration of uraemic toxins due to increased vasoconstriction caused by cool dialysis were raised; however, this was no evidence to support such concern (150).

Cooling can be achieved by standard dialysate temperature reduction or biofeedback systems like blood temperature monitoring (BTM) devices. BTM devices measure the temperatures of arterial and venous blood lines of the HD machine and calculate the corresponding fistula and core body temperatures. During the calculation of core body temperature, factors like thermal energy loss from the blood lines, ambient room temperature, access recirculation, and distance between the patient and the BTM sensor are taken into consideration. By repeating these measurements continuously throughout the dialysis session, the BTM device can maintain a preset core target body temperature or can be used to achieve a fixed reduction in the dialysate temperature to prevent a rise in core body temperature.

A 2016 meta-analysis combined results from 26 randomised or crossover trials, consisting of 484 patients (151). Of note, only five of these studies had been published in the last 10 years since the previous systematic review (150). The authors confirmed previous results, showing that cooled dialysis reduces the rate of IDH by as much as 70% (95% CI: 49%-89%) and that intra-dialytic blood pressure is significantly higher. Importantly, the intervention is widely available on all HD machines, confers no cost and does not affect dialysis adequacy (pooled Kt/V mean difference compared with standard dialysis of -0.05 (95% CI, -0.09 to 0.01)) (151). The improved intradialytic haemodynamics with cooled dialysate have also been shown to translate into amelioration of dialysis-induced ischaemia in the heart (as measured by a reduction

in left ventricular regional wall motion abnormalities (76)) and brain (prevention of ischaemic cortical white matter changes as detected by magnetic resonance imaging (MRI) (115, 152)). Although entirely safe, there are some patients who do not tolerate a reduction of dialysate temperature; Mustafa et al. reported that uncomfortable thermal symptoms were approximately three times more likely (151). One potential way to address this may be to manually individualise dialysate temperature manually (97) or use isothermic dialysis (153).

Therefore, reducing dialysate temperature is a safe and effective way to prevent IDH and may lessen end-organ injury; the stage would appear to be set for clinical trials to determine whether this approach also translates into improved patient outcomes. This is further supported by a recent Cochrane systematic review by Tsujimoto et al. (154)

Section 3.4 Sodium Profiling

It remains controversial whether sodium profiling provides an overall benefit in the setting of IDH. The concept evolved from an attempt to combat the faster reduction in plasma osmolality and subsequent reduction in the plasma refill rate that can occur at the start of dialysis when the plasma dialysate ratio of uraemic toxins is highest. Several studies have shown improvement in haemodynamic stability in the short term (155, 156). However, this may come at the expense of increased thirst, larger interdialytic weight gains (IDWG) and higher pre-dialysis systolic BP (156, 157).

A 2017 study from Iran reported favourable results with sodium modelling (158). The study had a complex crossover design in which 80 patients received four different combinations of sodium profiling and/or dialysate temperature reduction in different orders. Dialysate temperatures of 35^0C and 37^0C were compared as were a fixed

dialysate sodium of 138 mmol/l and sodium profiling (linear sodium profiling, reducing from 150 mmol/l to 138 mmol/L by the third hour of HD). The authors reported that IDH was least frequent in the two treatment schedules that included sodium profiling, that there was no additive effect of dialysate cooling, but cooling was still protective as compared with standard dialysis. However, patients only experienced each dialysis modification for one week (three treatments) and the change in IDWG was not reported, so potential adverse consequences of sodium profiling were not adequately assessed. In addition, a one-week washout period between study sessions is likely to be too short to exclude carry-over effects, particularly related to changes in dialysate sodium.

Some of the arguments around relative benefits and harms of sodium profiling originate from the many different methods that have been employed (e.g. linear, step-wise or exponential reductions) and whether differences in application explain conflicting results. This was seen in a recent meta-analysis that included ten crossover studies; whilst the author concluded that IDH was less with sodium profiling (but only with a stepwise regimes), the significant heterogeneity and small size of studies make it difficult to drawn any firm conclusions (159).

In summary, it remains controversial as to whether sodium profiling does reduce IDH and in view of potential negative effects in terms of increased thirst and IDWG, its use is not currently recommended (143). Recent data do not justify a change in this position.

Section 3.5 Haemodiafiltration (HDF)

Convective therapies have long been suggested by researchers to improve haemodynamic stability. The suggested mechanisms by which HDF helps to reduce IDH include 1) by causing positive sodium balance supported by reported higher pre-dialysis BP in previous studies (160, 161), 2) extracorporeal cooling (162), 3) reduced inflammation reported in the form of reduction of interleukin-6 levels (163) and 4) more clearance of middle molecules and thus reducing the incidence of dialysis-related amyloidosis (164). Despite these proposed mechanisms, previous studies that investigated the impact of haemodiafiltration (HDF) on symptomatic IDH have yielded conflicting results. Unfortunately, recent studies have not dispelled this.

The 'FRENCHIE' study was a prospective, multi-centre randomised controlled trial that looked specifically at tolerability of HD and HDF in the elderly (165). 381 patients aged >65 were randomised to either high-flux haemodialysis (HF-HD) or online haemodiafiltration (OL-HDF). OL-HDF treatments were mainly performed in post-dilution mode, but pre-dilution was allowed; convection volumes in the HDF arm were reasonably high, between 19.32 ± 4.46L and 22.53 ± 6.76L over the course of the study. Over 80% of patients had at least one adverse event (asymptomatic hypotension, symptomatic hypotension, headache, muscle cramps, nausea, vomiting, fever, chest pain, arrhythmia, others) with no significant difference between study arms (OR 0.94, 95% CI 0.51–1.76, p=0.85). Furthermore, there were no significant differences in health-related quality of life, morbidity or mortality. An exploratory analysis, from which results have to be interpreted with caution, compared all 11,981 treatment sessions as the unit of analysis (as opposed to the patient) and found a

lower rate of asymptomatic IDH was lower with HDF (20.6% of sessions versus 18.4%, p=0.002), although there was no difference in symptomatic IDH.

In contrast, Koda et al. reported more positive outcomes with intermittent back-filtration infusion HDF (I-HDF) when compared to standard HD (166). I-HDF utilises on-line-quality ultrapure dialysate that is intermittently reinfused (back-filtered) automatically, which theoretically helps to preserve blood volume and blood pressure. A total of 74 hypotension prone patients were exposed to both treatments for four weeks each in a crossover study design. From 816 sessions in both groups, the total number of interventions for symptomatic IDH was less with I-HDF (668 compared to 819, p= 0.003), which resulted in a lower median IDH frequency, 3.0 times per person per month versus 4.5 with HD (p=0.003). They reported no change in dry weight, or pre- and post-body weight throughout the study.

Smith et al. reported a paradoxical increase in IDH with HDF (167). In a randomised crossover study, 100 patients received eight weeks of both HD and HDF (mean convection volume for HDF treatments was 20.6L). Ultrafiltration volumes were similar in both groups. Symptomatic hypotension occurred in 8% of HDF treatments as compared to only 5.3% of HD sessions (RR, 1.52; 95% CI 1.2-1.9; p=0.001) although 80% of IDH episodes were 'mild', managed only with temporary cessation of ultrafiltration.

Finally, Buchanan et al used intra-dialytic functional MRI to compare effects of HD and HDF on cardiac function and perfusion in a randomised crossover study. Whilst significant reductions in left ventricular systolic function and myocardial perfusion were observed during dialysis, these changes did not differ between HD and HDF (168).

Therefore, whilst the evidence remains conflicted, HDF is not a first line option in the prevention of IDH, although convective techniques may be trialled in patients who have not responded to simpler, first line manoeuvres. It should be noted that two large, multicentre randomised controlled trials of high volume HDF are currently underway that may provide definitive answers as to whether HDF reduces IDH and more importantly improves patient outcomes (H4RT trial, ISRCTN10997319; CONVINCE trial, Netherlands Trial Registry NTR7138).

Table 3-1: Summary of trials included in this review

Study	Purpose	Method	Sample size and follow up	Outcome measures	Relevant findings
Mustafa RA, Bdair F, Akl EA, et al. Effect of lowering the dialysate temperature in chronic hemodialysis: a systematic review and meta-analysis. Clin J Am Soc Nephrol 2016; 11:442–457.	Comparison of standard temperature dialysis with any method of cool dialysis	Systematic review and metanalysis	Included 26 trials in final analysis from January 1946 to April 2015 Total 484 patients from 24 trials, 95 and 128 respectively from the other 2 trials	To review mortality, hospitalisations, access failure, cardiovascular events, bleeding and system clotting, quality of life and intradialytic hypotension	Rate of IDH reduced by 70% (95% CI: 49%-89%) without reducing the dialysis adequacy compared with standard dialysis with no difference in dialysis clearance Symptoms of discomfort occurred 2.95 times more frequently in reduced temperature group compared to standard dialysate temperature group
Ebrahimi H, Safavi M, Saeidi Marzieh H, Emamian Mohammad H. Effects of sodium concentration and dialysate temperature changes on blood pressure in hemodialysis patients: a randomized, triple-blind crossover clinical trial. Ther Apher Dial 2017; 21:117–125.	To study the effects of different temperatures and sodium dialysate concentration on BP	Randomised, triple blind crossover clinical trial	80 prevalent dialysis patients from a single centre were divided into 4 groups: Mode A (dialysate temperature: 37°C; std sodium concentration: 138 mmol/L); Mode B (dialysate temperature: 35°C; std sodium concentration: 138 mmol/L); Mode C (dialysate temperature: 37°C; linear sodium profiling every hr, beginning of HD was 150 mmol/L,to 138 mmol/L in the last hour, decreased by 1mmol very 15min to 138mmol/l by end of 3 hrs of HD); Mode D (dialysate temperature:35°C; linear sodium profiling, beginning of HD was 150 mmol/L to 138 mmol/L in the last hour, decreased by 1mmol very 15min to 138mmol/l by end of 3 hrs of HD). 80 dialysis sessions were carried out for each group, and the results of 320 dialysis sessions were analysed	Incidence of intradialytic hypotension	The mean incidence of intradialytic hypotension in all haemodialysis sessions and in different modes of treatment for each patient was 7.16 (with a range from 0 to 12). The mean incidence of intradialytic hypotension in modes A, B, C and D was 9.97, 8.42, 5.49, and 4.74, respectively ($P < 0.001$) Conclusion: Mode C and D are better than A or B in reducing incidence of IDH

Dunne N. A meta-analysis of sodium profiling techniques and the impact on intradialytic hypotension. Hemodial Int 2016; 21:312–322	Comparison of conventional dialysis with sodium profiling techniques	Systematic review and metanalysis	Included 10 cross over designed studies consisting of total of 3660 dialysis sessions (1847 control sessions and 1813 intervention sessions); however, four different profile were used in all these studies labelled as Other, alternative, stepwise and linear by the authors	Incidence of intradialytic hypotension events	The “other” profile group had a pooled odds ratio of 0.48 (95% CI: 0.30 to 0.78), the stepwise group had pooled odds ratio of 0.58 (95% CI: 0.44 to 0.76), the linear group had pooled odds ratio of 1.01 (95% CI: 0.77 to 1.32), and alternate 0.67 (95% CI: 0.25 to 1.74) when compared to conventional dialysis
Morena M, Jaussent A, Chalabi L, et al., FRENCHIE Study Investigators. Treatment tolerance and patient-reported outcomes favor online hemodiafiltration compared to hemodialysis in the elderly. Kidney Int 2017; 91:1495–51509. 4	Comparison of online haemodialfiltration with haemodialysis	Prospective, open label randomised controlled trial	381 elderly prevalent HD patients (over age 65) were randomly assigned in a one-to-one ratio to either high-flux haemodialysis (HFHD) or online haemodiafiltration (OLHDF) and followed over 24 months.	Primary outcome was intradialytic tolerance (from day 30–day 120 after enrolment) Secondary outcomes included health-related quality of life, cardiovascular risk biomarkers, morbidity, and mortality	84.9% and 84.1% of patients in the HFHD and OLHDF arms, respectively, experienced at least 1 adverse event, with no significant difference between groups (odds ratio [OR] of 0.94, confidence interval [CI] 95% [0.51–1.76], P: 0.85) No differences in the secondary outcomes were noted.
Koda Y, Aoike I, Hasegawa S, et al. Feasibility of intermittent back-filtrate infusion hemodiafiltration to reduce intradialytic hypotension in patients with cardiovascular instability: a pilot study. Clin Exp Nephrol 2017; 21:324–332.	Intermittent back-filtrate infusion haemodiafiltration (I-HDF) versus haemodialysis (HD)	Open, non-controlled, prospective, crossover, multicentre trial	77 patients were enrolled and spent four weeks in each intervention group. 74 were included in the final analysis	Incidence of IDH (defined as systolic BP drop over 20 mmHg from baseline) or presentation of symptoms associated with hypotension requiring any medical/nursing interventions	18.4 % lower need for interventions in I-HDF group compared to HD group (P = 0.003). Intradialytic BP was higher by 4mmHG in I-HDF group (P=0.006)
Smith JR, Zimmer N, Bell E, et al. A randomized, single-blind, crossover trial of recovery time in high-flux hemodialysis and hemodiafiltration. Am J Kidney Dis 2017; 69:762–770	Online post dilution Haemodiafiltration (HDF) versus haemodilalysis (HD)	Randomized patient-blinded crossover trial.	100 patients were enrolled. They were allocated at 1:1 ratio to have eight weeks of HD to cross over to HDF and vice versa.	Post treatment recovery time, symptomatic IDH events, dialysis circuit clotting events, and biochemical parameters.	HDF was associated with a significantly higher chance of immediate recovery (P 5 0.01), but resulted in significantly longer recovery time than HD for those who had delayed recovery (P , 0.001); higher rates of IDH (8.0% in HDF vs 5.3% in HD; P= 0.001), higher intradialytic tendency to clotting (1.8% in HDF vs 0.7% in 7HD; RR 2.7; P= 0.002) and lower serum albumin levels (3.2 vs 3.3 g/dL; P , 0.001) with no differences in the Health related QoL measures.

Kosmadakis G, Correia EDC, Albaret J, et al. Comparison of the hemodynamic tolerance and the biological parameters of four acetate-free hemodialysis methods. Nephrol Ther 2017; 13:532–536.	Comparison of Acetate-free biofiltration with potassium profiling (AFKB) and 3 methods with citrate buffer-conventional HD, online HDF, online haemofiltration (HF)	Single centre, prospective cross over study	14 stable HD patients enrolled and each of them randomly spent 2 weeks (six 4hr dialysis sessions) in each of the four studied techniques	Incidence of IDH, comparison of BP and biologic parameters (haemoglobin, baseline serum sodium, potassium, calcium, phosphate urea, alkaline reserve, creatinine, uric acid, beta-2-macroglobulin and magnesium concentrations)	Incidence of IDH in AFKB (1 in 84 sessions, 1/84), HD (29/84), HDF (22/82), HF (14/78) were significantly different (P< 0.001) Hourly intradialytic systolic BP were higher in AFKB when compared to HDF (P = 0.001) or HF (P = 0.035), but not when we compared the AFBK with HD. AFKB group had net higher UF (P< 0.001) compared to others Potassium levels were reported to be higher only at 1st hour of AFKB (P < 0.001)
Viegas M, Caˆndido C, Felgueiras J, et al. Dialysate bicarbonate variation in maintenance hemodiafiltration patients: impact on serum bicarbonate, intradialytic hypotension and interdialytic weight gain. Hemodial Int 2016; 21:385–392.	Comparison of the effects of two different dialysate bicarbonate (DB) concentrations (34 mmol/L vs. 30 mmol/L)	Prospective randomized study	After an initial run-in period with DB of 34 mmol/L, 93 prevalent HDF patients were randomised 1:1 to DB of 34 mmol/L (n=46) and DB of 30 mmol/L (n= 47) with 9 months follow up period	Differences in pre and post dialysis serum bicarbonate (SB) Differences in the rate of IDH and IDWG.	34 mmol/L DB group had high pre (22.7 mmol/L vs. 21.1 mmol/L, P<0.001) and post SB levels (28.0 mmol/L vs. 25.3 mmol/L, P<0.001) when compared to the other group. No impact on IDH and IDWG was observed.
Pirkle James L, Comeau Mary E, Langefeld Carl D, et al. Effects of weightbased ultrafiltration rate limits on intradialytic hypotension in hemodialysis. Hemodial Int 2017; 22:270–278.	Comparison of maximum ultrafiltration rate (UFR) ≤13 mL/kg/hr to standard ultrafiltration practice	Retrospective cohort study	Out of the 135 patients who were in that single centre, only 123 patient's data was used for analysis as other 12 patients had short dialysis vintage of <8 weeks Extra fluid was removed by prolonging the dialysis sessions or offering extra sessions. Baseline: 8 weeks baseline period immediately prior to the study period served as its own historical controls.	Incidence of IDH, lowest recorded intradialytic BP, hospitalization for all causes and hospitalisation for CHF or fluid overload, and IDWG.	UFR limit resulted in a lowered delivered weight-based UFR for the entire cohort compared to baseline (mean rate 7.90+/-4.45 mL/kg/h vs.8.92+/-5.64 mL/kg/h; P=0.0005) and were contributed by subgroup of patients that had a high UF at baseline (n=72) (mean rate 9.42+/-4.26 mL/kg/h vs. 11.25+/-5.88 mL/kg/h; P<0.0001) Risk of having nadir SBP drop to <90mmHg was also lower (event rate per treatment 0.0284 vs. 0.0440, OR 0.65[95% CI 0.46–0.91]; P=0.0116) and this was attributed to patients with higher UF rates. Significant reduction in the pre-dialysis weights was noted with UFR limit but no change in the hospitalisation rates

Colson A, Brinkley A, Braconnier P, et al. Impact of salt reduction in meals consumed during hemodialysis sessions on interdialytic weight gain and hemodynamic stability. Hemodial Int 2018.	Impact of dietary salt restriction	Prospective, single centre, cross over study	76 patients enrolled but 40 were included in the final analysis, rest excluded due to change in the RRT status, hospitalisations, deaths or moved out of the centre One sandwich with 2.4 grams of salt per session was served for 2 months, followed by sandwich with 1.4 grams of salt for 4 months	Mean values of IDWG, BP, and dry weight	Low salt sandwiches led to significantly decreased IDWG (2.17 ± 0.98 to 2.03 ± 1 kg, P = 0.001) and number of symptomatic IDH were reduced from 6.1% to 3.25% (P =0.04)
Huan-Sheng C, Yeong-Chang C, Ming-Hsing H, et al. Application of bioimpedance spectroscopy in Asian dialysis patients (ABISAD-III): a randomized controlled trial for clinical outcomes. Int Urol Nephrol 2016; 48:1897–1909.	Role of Bioimpedance spectroscopy (BCM-BIS) in setting post dialysis target weight (PDTW) compared to standard clinical assessment guided PDTW.	Prospective randomised multicentre centre study	298 prevalent HD patients aged >18 yrs were randomised to control (n=150) and study group (n=148) stratified by DM for 1 year.	Primary outcome: All cause hospitalisation rates Secondary: Incidence of hypertension, acute fluid overload (AFO) and CV-related events, incidence of intradialytic complication (both subjective and objective)	Hospitalisations were not different but incidence of AFO+CV related events were lower in the study group (0.10 vs 0.21, p=0.03; HR: 0.57 (0.26-1.25) which resulted mainly from non-diabetics (0.059 vs 0.18; p=0.03; HR: 0.29 (0.08-1.07). Lower rate of intradialytic complications (subjective and objective) happened during PDTW adjustment in study group (11.76% vs 30.88%, P=<0.001) Incidence of IDH was significantly lower in study group (6.10 vs 6.62%, P<0.05). Longitudinal analysis showed complications significantly decreased since 6th month when compared to 1st month.
Leung KCW, Quinn RR, Ravani P, et al. Randomized crossover trial of blood volume monitoring–guided ultrafiltration biofeedback to reduce intradialytic hypotensive episodes with hemodialysis. Clin J Am Soc Nephrol 2017; 12:1831–1840.	Comparison of Blood volume guided ultrafiltration biofeedback versus best clinical practice	Randomised, single blinded, cross over, multicentre trial	Had 35 patients in the run-in period (4 weeks) when dialysis prescriptions were standardised and dry weights were optimised, then randomised 32 patients to control (n=16) vs biofeedback HD (n=16) for 8 weeks, followed by 2 weeks of wash over period and then crossed over to other arm for 8 weeks.	Primary outcomes: rate of symptomatic IDH (IDH episodes/duration of HD in hours).	Reduction in the rate of symptomatic IDH from run-in to the eighth week was noted in the biofeedback arm (0.15 to 0.07; P=0.01) but there was no difference in the incidence of IDH between the two arms at 8 weeks (P=0.41).
Ibarra-Sifuentes H, Del Cueto-Aguilera A´ , Gallegos-Arguijo DA, et al. Levocarnitine decreases intradialytic hypotension episodes: a randomized controlled trial. Ther Apher Dial 2017; 21:459–464.	To study if Levocarnitine (LC) reduced the incidence of IDH	Single-centre, prospective, randomized, placebo-controlled quadruple-blinded trial	33 prevalent HD patients were randomised to LC (n=18) and placebo (n=15)	Rates of IDH	Risk of IDH was reduced in the LC group with absolute risk reduction of 23.9% (CI 0.213 to 0.366, P < 0.001) when compared to placebo. Numbers needed to treat was 4.2.

Assimon MM, Brookhart MA, Fine JP, et al. A comparative study of carvedilol versus metoprolol initiation and 1-year mortality among individuals receiving maintenance hemodialysis. American Journal of Kidney Diseases 2018.	Comparison of Carvediolol and Metoprolol effects	Retrospective cohort study using an active comparator new-user design (described as an observational analogue to a head-to-head randomized controlled trial)	A total of 27,064 individuals receiving maintenance HD were included in the study: 9,558 (35.3%) carvedilol initiators and 17,506 (64.7%) metoprolol initiators. Average durations of follow-up were 7,219 vs 13,644 person-years for carvedilol and metoprolol initiators respectively.	Primary: 1 year all cause and cardiovascular mortality Secondary: Hospitalisation rates- all cause and cardiovascular; incidence of IDH	Higher rate of all-cause mortality was observed in Carvediolol arm (225.1 vs 195.8 events/ 1,000 person-years; adjusted HR, 1.08 [95% CI, 1.02-1.16]) and cardiovascular mortality (108.3 vs 85.1 events/1,000 person-years; adjusted HR, 1.18 [95% CI, 1.08-1.29]) All-cause hospitalisations were similar (2,383.8 vs 2,270.3 events/ 1,000 person-years; adjusted IRR, 1.00 [95% CI, 0.97-1.04]) but higher rates of cardiovascular hospitalizations (827.1 vs 726.5 events/1,000 person-years; adjusted IRR, 1.06 [95% CI, 1.01-1.12]) were reported in Carvediolol arm. IDH incidence were higher in Carvedilol arm (57.5 vs 55.2 episodes/1,000 person-treatments. adjusted IRR, 1.10 [95% CI, 1.09-1.11]).
Roberts MA, Pilmore HL, Ierino FL, et al. The b-Blocker to Lower Cardiovascular Dialysis Events (BLOCADE) feasibility study: a randomized controlled trial. Am J Kidney Dis 2016; 67:902–911.	Carvedilol tolerability in HD population	Multicentre, pilot randomised controlled trial	26 were randomised to carvedilol group and 23 to placebo (although the intention was to recruit 150 in total)	Tolerability of carvedilol and Incidence of IDH	4 participants withdrew from intervention group in view of bradycardia and hypotension When compared Carvedilol to placebo groups, 7 versus 2 IDH events per 100 haemodialysis sessions were noted (P = 0.1) in the 2 weeks immediately following a dose increase and 4 versus 3 IDH events per 100 HD sessions after no dose increase (P = 0.7)

AFKB- Acetate-free biofiltration with profiled potassium, AFO- acute fluid overload, BCM-BIS Bioimpedance spectroscopy, BP- blood pressure, CI- confidence interval, CHF- congestive heart failure, CV- cardiovascular, DB- dialysate bicarbonate, DM- diabetes mellitus, HF- haemofiltration, HR- Hazard ratio, HD- haemodialysis, HDF- Haemodiafiltration, HFHD- high flux haemodialysis, IDH- Intradialytic Hypotension, IDWG- Interdialytic weight gains, LC- Levocarnitine, OLHDF- online haemodiafiltration, PDTW- post dialytic target weight, SB- serum bicarbonate, SBP- systolic blood pressure, UFR- ultrafiltration rate

Section 3.6 Buffer composition

Historically, acetate was a major cause of IDH (169). Adverse effects of acetate are attributed to vasodilatation secondary to increased production of nitric oxide (170) and increased oxygen consumption leading to hypoxemia (171). Whilst modern dialysis uses bicarbonate buffer, a small amount of acetate is still present in dialysate (to prevent calcium precipitation), and whilst the concentration is low, the absence of acetate in the blood creates a dialysate: blood concentration gradient. Therefore, acetate-free techniques have been developed to eliminate acetate transfer completely in an attempt to reduce IDH due to this cause. A recent single-centre crossover study reported a large reduction in IDH with a combined acetate free biofiltration and potassium profiling technique as compared to acetate free HD and HDF (172); however such positive results are tempered by the small study size (only 14 patients) and practical aspects that may limit generalisability.

Viegas et al. (173) reported a randomised trial in which 93 patients received dialysis with either standard dialysate bicarbonate concentration (34mmol/L) or lower bicarbonate (30mmol/L). Over a follow-up period of 9 months, no differences in IDH or IDWGs were observed.

Section 3.7 Reducing ultrafiltration volume/rate/ intense dialysis

Reducing IDWG and Ultrafiltration (UF) volume is a key strategy in the prevention of IDH, although often challenging to achieve in practice. Higher ultrafiltration rates (UFR expressed in millilitres/hour/kg body weight, ml/h/kg BW) were shown to be associated

with adverse outcomes. In a cohort of 1,846 prevalent HD patients, Flythe et al. compared patients with UFR ≤10 mL/h/kg BW with UFR >13 mL/h/kg BW and reported the latter to be associated with a higher risk of all-cause and CV mortality, with adjusted hazard ratios of 1.59 and 1.71, respectively (174). Kim et al. studied similar findings in incident HD patients in a large cohort of 110,880 patients to find a linear association between UFR vs. all-cause and CV mortality. These authors also demonstrated when compared to UFR 6–<8 mL/h/kg BW, UFR ≥10 mL/h/kg BW conferred the highest risk in both unadjusted (HR 1.15 [95% CI 1.10–1.19]) and adjusted models (HR 1.23 [95% CI 1.16–1.31]) (175). Likewise, Saran et al. demonstrated modest association of all-cause mortality with UFR of >10ml/hr/Kg BW in DOPPS study (176). These findings are also supported by studies reporting association of higher UFR with end organ ischaemia (75, 115, 129, 135, 168). Potential contributory mechanisms could be haemodynamic instability due to lack of timely vascular refill. Studies reporting improvement in the haemodynamic stability using ultrafiltration profiling with decreasing UFR as dialysis progresses is supportive of this mechanism (177). However, pathogenesis of IDH and effects on organ perfusion cannot be attributed to UFR alone. Assa et al, compared the effects of isolated ultrafiltration to isovolumic HD in a cross over design using ^{13}N-NH_3 positron emission tomography and echocardiography in a cohort of 8 patients. Despite the UFR of 13.6±3.9 ml/hr/kg BW in isolated ultrafiltration group, they did not find change in the course of global or regional myocardial perfusion in both groups. They also reported regional LV dysfunction in 1 patient in isolated ultrafiltration group and 3 patients in isovolumic group (178). These findings are supported by Dasselaar et al. study. In a cohort of 7 patients, they demonstrated a reduction in the myocardial perfusion using ^{13}N-NH_3 positron emission tomography very early after commencement of dialysis,

prior to significant amount of fluid removal (179). But they also reported progressive decline in the myocardial perfusion in the repeat scan at 220 min of HD which correlated with UFR, supporting role of UFR in the complex pathogenesis of adverse effects of HD.

Whilst usually attempts for reducing UF rate are done on an individual patient basis, Pirkle et al. (180) reported an organisational approach by applying a UF limit of 13ml/kg/hr within a single US facility. There was a small reduction in IDH after implementation (event rate per treatment 0.06 vs. 0.07; OR 0.78, 95% CI 0.62-1.00, p = 0.05). Time was extended in 62 treatments and 28 additional treatments were performed to achieve ultrafiltration needs. Pre and post dialysis weights and UF volumes were lower with the intervention, raising the possibility that improvements may have resulted from a Hawthorne effect; however, it was reassuring that there were no signals around increases in fluid overload. Whilst this may not be definitive evidence, the principle of applying quality improvement approaches to the dialysis unit should be lauded and may offer a practical way to effect change. Findings from this study also support the previous evidence suggesting that more intense dialysis, either in the form of increased duration or frequency of HD sessions, reduces the incidence of IDH by predominantly reducing the ultrafiltration volume (181) and leading to improvement in the overall mortality (182).

Reducing dietary salt intake is also crucial to reducing IDWG and UF volumes. Colson et al. assessed the impact of reducing the salt content in sandwiches provided by the dialysis unit from 2.4g to 1.4g. Over a four month period, IDWG decreased significantly and the number of treatments with symptomatic IDH episodes were reduced from 6.1% to 3.3% (p=0.004) (183). Whilst study design makes it difficult to be certain about

causal relationships in this study, as it is possible that additional unmeasured factors were present (e.g. raised awareness of dietary sodium in general), the simple, practical approach described here has merit.

Section 3.8 Target weight assessment

Regular assessment of target weight is part of the essential care of patients on dialysis, and with respect to IDH aims to avoid excessive UF. Whilst historically this is based on clinical examination, a number of objective measures exist including bioimpedance, lung ultrasonography and measurement of inferior vena cava diameter. Previous studies have suggested that the use of bioimpedance is associated with improved BP control, left ventricular mass and pulse wave velocity (184). However, the use of bioimpedance in clinical practice is not universal. In a recent randomised multicentre centre study from Taiwan, Huan-Sheng et al compared the use of bioimpedance to standard care in 298 prevalent HD patients (185). Bioimpedance was used once per month to determine a post dialysis target weight based on correcting the degree of measured 'fluid overload' according to an algorithm. After six months, blood pressure control improved in the bioimpedance group as compared with baseline; this was achieved with fewer antihypertensive medications and a reduction in IDH (intervention vs control: 6.10 vs 6.62%, $P<0.05$) was observed. Further studies are required to validate these results and the use of the specific bioimpedance algorithm in non-Asian populations, although the approach to define thresholds for use of bioimpedance in clinical practice is important.

Section 3.9 Relative blood volume and biofeedback systems

Online feedback systems in the dialysis machines using relative blood volume (RBV) changes have long been used to modify the UF volumes in an attempt to prevent IDH. Although a number of studies have suggested their effectiveness, technical complexity has prevented clinical adoption. Leung et al. have recently reported a randomised crossover study in 32 patients (26 completed the study) comparing HD to blood volume based biofeedback dialysis over 16 weeks (eight weeks per modality) (186). Critical RBV was set on a weekly basis in the intervention arm; however, this process also included a weekly review of patients' target weight. Although there was a reduction in the rate of symptomatic IDH from run-in to the eighth week in the biofeedback period (0.15 episodes/hr to 0.07 episodes/hr; p=0.01), there was no difference between the two arms at 8 weeks. A number of other endpoints were unchanged by biofeedback dialysis. These results that suggest a lack of effectiveness in combination with technical complexity may reduce enthusiasm for this approach, although it should be noted that patients included in this study had fairly modest average UF volumes.

Section 3.10 Pharmacological Interventions

Surprisingly, relatively little is known about the effects of anti-hypertensive medications during dialysis. EBPG guidelines (143) suggest in patients prone to IDH that '*antihypertensive agents should be given with caution prior to dialysis depending on pharmacodynamics, but should not be routinely withheld on the day of haemodialysis treatment*', a statement supported by only grade III evidence. A recent retrospective

study using a clinical database of a large US dialysis organisation examined IDH rates in patients receiving the two most commonly prescribed beta-blockers in the US, metoprolol and carvedilol (51). An active comparator new-user study design was used (this compares the drug of interest to another commonly used agent for the same indication, rather than a 'non-user' group (187)). In 27,064 HD patients over a one-year follow up, the rate of intradialytic hypotension was somewhat higher among patients who started carvedilol (57.5 vs 55.2 episodes/1,000 person-treatments; adjusted IRR, 1.10, 95% CI, 1.09-1.11). A second recent study also reported rates of IDH with carvedilol within the bounds of a randomized, double-blind, placebo-controlled feasibility study (188). 72 patients entered a run-in period, 49 patients were randomised to either carvedilol or placebo but only 16 in the carvedilol group completed the study. As such, results should be treated with extreme caution and the study is obviously underpowered; the authors reported a non-significant trend to more IDH in the carvedilol group (incidence rate ratio 2.94, 95% CI, 0.70-12.28; p=0.1). It is clear therefore that more research is needed in this area. At present, prescribing decisions must be made on an individual patient basis, factoring in uncertainty about optimal blood pressure targets in HD populations, and that evidence for the use of betablockers and RAAS inhibitors for cardiovascular benefit is often extrapolated from non-dialysis populations, and weigh this against IDH risk.

Levocarnitine (LC) supplementation has been proposed to improve dyslipidemia, malnutrition and quality of life in patients on dialysis. A trial of LC is recommended in current guidelines (15, 143) as a last resort in patients who are resistant to other interventions for IDH. Recent data appear to tentatively support this position. A single-centre randomised controlled trial compared intravenous LC prior to dialysis (30 mg/kg per session) with placebo (189). The total number of IDH episodes were fewer in the

LC group (60 events complicating 9.3% of treatments versus 179 events in 33.1% of treatments; P<0.001). Zhang et al reported data from an observational study that included dialysis patients who were and were not receiving LC supplementation (190). Whilst causal associations can't be assessed in this study, lower rates of IDH and intra-dialytic symptoms were seen in those receiving LC. Paradoxically, the authors observed that IDH rates were increased in patients receiving LC with plasma free carnitine levels >300 mumol/L when compared to plasma free carnitine levels <80-199 mumol/L (P<0.05); whilst it is difficult to draw firm conclusions from this, further work to establish optimal dosing may be required.

Section 3.11 Novel interventions

Whilst not ready for clinical adoption, there have been some recent small studies describing novel approaches to preventing IDH, with early reported signals of effectiveness. These include: pneumatic compression devices designed to increase venous return from the lower limbs (191) (192); immersion therapy by which dialysis is performed in patients immersed in water up to their neck (193); and herbal acupuncture (194). Clearly, such approaches would require significant additional study and, in some cases, would have to overcome large practical barriers.

Section 3.12 Other suggested interventions

This section provides details of other interventions that were shown to benefit patients prone to IDH, but no new studies were published during the investigatory period on these approaches.

3.12.1 Dialysate Calcium

Fluctuation in the ionised calcium levels during HD can affect myocardial contractility. In studies comparing low calcium dialysate (low CD, 1.25mmol/L) to high calcium dialysate (high CD, 1.75mmol/L), drops in systolic BP and MAP were more significant in the low CD group without a difference in the peripheral vascular resistance (195). Calcium profiling (with low CD for first 2 hrs and high CD for the last 2 hrs of HD) also showed beneficial effects on BP profile and reduction of IDH (196). There is evidence suggesting the additive effect of the high CD to other interventions like cooling or midodrine in reducing IDH (197). However, the concerns about positive calcium balance and its adverse effects on vascular health inhibit the wide application of this measure.

3.12.2 Midodrine:

Midodrine acts by activation of α-adrenergic receptors on the arterial and venous systems, thus improving the BP by reducing the pooling of blood in capacitance venous vessels and direct vasoconstriction of arteries. In a meta-analysis of 10 studies including 117 patients, nadir intradialytic SBP (13.3 mmHg; 95% CI 8.6–18.0) and DBP (5.9 mmHg; 95% CI 2.7–9.1) were noted to be higher compared to the control group. The authors concluded that midodrine was effective in reducing the IDH episodes, and they also rightly acknowledged that these results should be interpreted with caution given the small sample size and quality of the studies (198). EBPG suggests using midodrine as an intervention for IDH only if all the other available treatments strategies fail (143).

Section 3.13 Conclusion

IDH remains a significant clinical problem, negatively impacting patient symptoms, dialysis adequacy and treatment time, and is strongly associated with worse patient outcomes. Evidence that emerged over the investigatory 2-year period does not support any major changes to current practice, with cooling of the dialysate and reduction of IDWG remaining cornerstones of management. It remains a priority for dialysis care that better strategies are developed for monitoring and detecting IDH, for defining optimal definitions of IDH with respect to pathophysiological consequences, and ultimately for more effective preventative strategies. Further studies to understand the mechanistic process behind the protective effects of cooling of dialysate using imaging techniques will help us to progress further to develop a novel intervention to address this problem in the future.

Chapter 4 Generic and Magnetic resonance methods

The descriptions of various methods utilised in the different clinical studies that make up this thesis are provided in this chapter.

Section 4.1 Finometer

The Finometer (Finapres Nova, FMS, Netherlands) allows continuous non-invasive monitoring of the haemodynamic parameters with the analysis of changes at the small finger (digital) artery using a finger cuff. Infrared plethysmography is used to detect the changes in the digital artery diameter. The inbuilt ultra-fast pressure servo controller in the finger cuff rapidly adjusts the cuff pressure to keep the diameter of the digital artery constant (199). Hence the pressure changes in the finger cuff represent intra-arterial pressure changes, and a pulse wave is generated. This pulse wave is utilised to calculate a full range of haemodynamic variables continuously for each heartbeat (200). These haemodynamic variables include pulse rate (HR), blood pressure (BP), stroke volume (SV), cardiac output (CO) and total peripheral resistance (TPR). The device was been fitted to the non-fistula arm at the start of the investigatory period of our study and left in place throughout the HD session as shown in Figure 4-1.

Waldron et al, assessed the inter-day reliability of Finapres in resting conditions and exercise. They reported the coefficient of variation to be smallest for MAP (CV 1.6-3.2%; Limits of agreement, LoA total error was 4.6-12 mmHg) and HR (CV 3.2-3.9%; LoA total error 6.8-11.9 b/min), which increased with exercise intensity (gradient) (201). Although there is variability in the data (more so SBP with better reliability in

MAP and DBP) obtained by this method when compared to brachial artery BP, it was shown to be accurate and precise in tracking the changes in the various parameters to physiological stimuli and hence an effective alternative to invasive techniques (202, 203). Moreover, this method has been reliably used in patients with vascular disease

Figure 4-1: Finometer (NOVA) connected to the patients on the non-fistula arm and display shows various haemodynamic parameters

and cardiac surgery patients which include significant proportion of patients with vascular disease (203, 204). These are relevant findings for HD patients as the prevalence of vascular disease is very high is this population. Similar to the previous studies in haemodialysis population (72, 205), we used this non-invasive method to examine the trends in the haemodynamic changes during dialysis in our study population.

Section 4.2 Bioimpedence

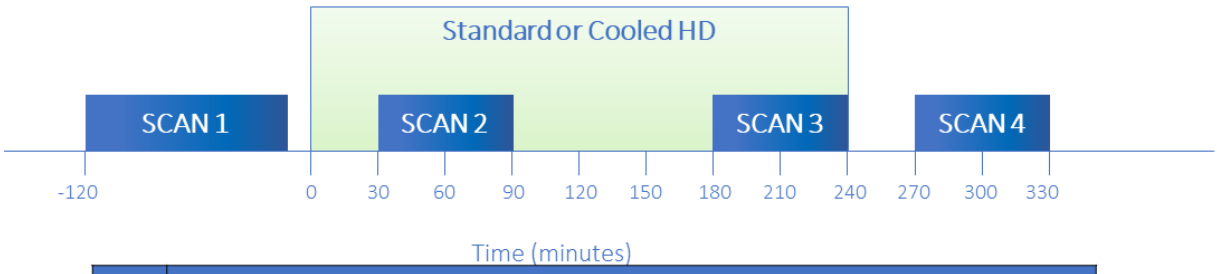
Bioimpedance (BCM) analysis provides a non-invasive approach for estimating body composition. On scheduled study sessions, BCM was performed before and after the

haemodialysis session, whilst the participant was supine position on a non-conductive surface. Leads were placed on the non-fistula hand and ipsilateral foot. The BCM device generates Bioimpedence indices (resistance, reactance and phase angle). These are calculated based on the voltage drop that occurs when a constant low voltage electrical current is applied to the tissue. This voltage drop varies based on the electrical conductivity of the tissue. These indices are used to generate the various measurements provided by the BCM monitor, including total body water, extracellular water, intracellular water and excess free fluid (overhydration status) in the body. We used the BCM monitor made by Fresenius Medical Care GmbH Germany.

Section 4.3 Magnetic resonance Methods:

4.3.1 Magnetic Resonance Imaging (MRI) Protocol

Cardiac, cerebral and renal MRI measurements were collected on a 3.0 Tesla Philips Ingenia wide-bore MR scanner (Philips Healthcare Systems, Best, Netherlands) with MultiTransmit capability, 32-channel SENSE Torso coil and 32-channel SENSE Head coil. The MRI protocol consisted of a series of non-invasive MR measurements to monitor cardiac, cerebral and renal structure and function.



	MRI scans	Parameters
Cardiac Measures	LV Short axis cine	Cardiac Index, Stroke Index, Ejection fraction, LV wall mass
	Short and Long axis LV tagging	Peak Longitudinal and circumferential strain
	LV short axis T1 native MOLLI	Myocardial T1 LV short axis
	MOLLI ASL	Myocardial Perfusion
	PC-MRI	Coronary artery flow
Brain Measures	SE- EPI ASL	Grey matter perfusion
	PC-MRI	Carotid and basilar artery blood flows
	MPRAGE	Grey and White Matter Volumes for Structural assessment
Renal Measures	SE-EPI ASL	Renal cortex perfusion
	SE-EPI IR	Renal Cortex T1
	mFFE	Renal Cortex T2*
	bTFE	Total Kidney Volume

Figure 4-2: Diagram illustrating the protocol for MRI scan collection in the MRI study day to assess structure and function of heart, brain, and kidneys.

The protocol comprised scans collected at baseline pre-dialysis (Scan 1), during dialysis (Scans 2 and 3) and post-dialysis (Scan 4) to assess the time-course of the cardiac, cerebral and renal response. The table below provides the details of the MRI measures collected.

After the run-in phase, at the baseline pre-dialysis scan (Scan 1), cardiac and renal measures were collected in a 60-minute scan session, and cerebral measures were collected in a 30-minute scan session. For subsequent scans, during dialysis (Scans 2 and 3) and following dialysis (Scan 4), data were collected in a single 60-minute scan session (Figure 4-2).

4.3.2 MRI Acquisition

4.3.2.1 Measures of Cardiac Function and Structure

Cardiac function and structure were assessed using cardiac MRI as shown in Figure 4-3. Heart rate was continuously recorded during all scan sessions using either the peripheral pulse unit (PPU) or vectorcardiogram (VCG).

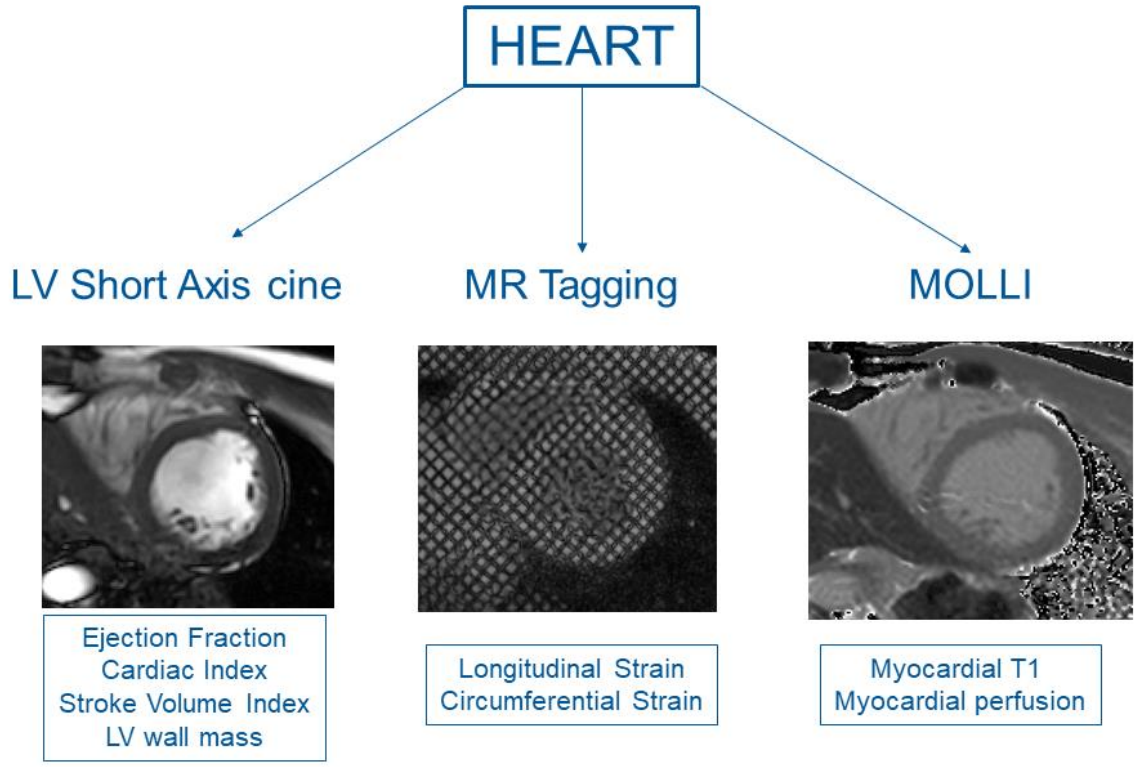


Figure 4-3: Overview of cardiac MRI measures collected

4.3.2.1.1 MRI measures of Cardiac Function

Left ventricular (LV) short-axis cine MRI data were collected to assess left ventricular function. Cardiac-gated cine images at multiple time points across the cardiac cycle were collected with a multi-slice TFE (turbo field echo) sequence (field of view (FOV) = 300 x 210 mm^2, 12 slices, 30 phases, 3 slices acquired per 15-20 s expiration breath-hold, slice thickness = 10 mm, reconstructed resolution = 1.17 x 1.17 mm, repetition time (TR)/ echo time (TE) = 3.9/1.94 ms, SENSE = 2). An example of an LV cine image is shown in Figure 4-4.

The following functional measures were calculated:

- Cardiac index (L/min/m^2) – the volume of blood pumped out of the heart in one minute, indexed to a participant's body size by dividing with body surface area (BSA).

- Stroke index (ml/m^2) – the volume of blood pumped out of the heart in one heartbeat, indexed to a participant's body size by dividing with BSA.

- Ejection fraction (%) – the percentage of blood the left ventricle pumps out in one heartbeat.

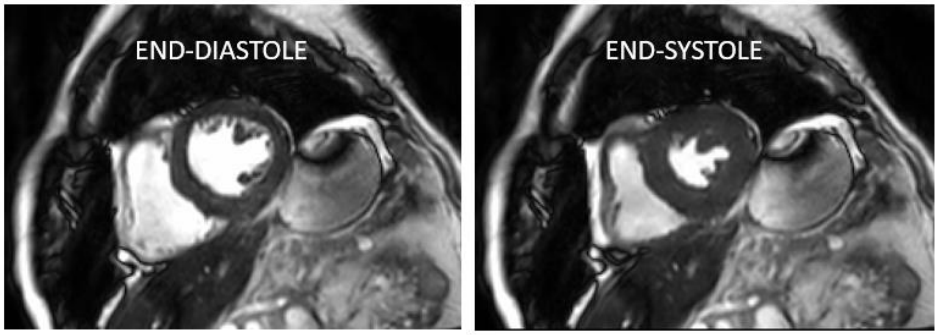


Figure 4-4: Example short axis cine image of the left ventricle at end diastole and end systole.

Myocardial wall strain was assessed using MR tagging, which provides information regarding the contractility and the deformation (twists) of cardiac muscle between diastole and systole. Tagging data were acquired through both the long and short axis of the left ventricle (Figure 4-5). Long axis data were acquired with FOV = 350 x 350 mm^2, reconstructed voxel = 0.9 x 0.9 mm^2, slice thickness = 8 mm, SENSE 2, TR/TE = 5.7/3.5 ms, flip angle 10^0, grid spacing = 7 mm, grid angle = 45^0. Short axis data were acquired with FOV = 320 x 320 mm^2, reconstructed voxel = 0.9 x 0.9 mm^2, slice thickness = 10 mm, SENSE 2, TR/TE = 5.8/3.4 ms, flip angle 10^0, TFE factor = 7, grid spacing = 7 mm, grid angle = 45^0.

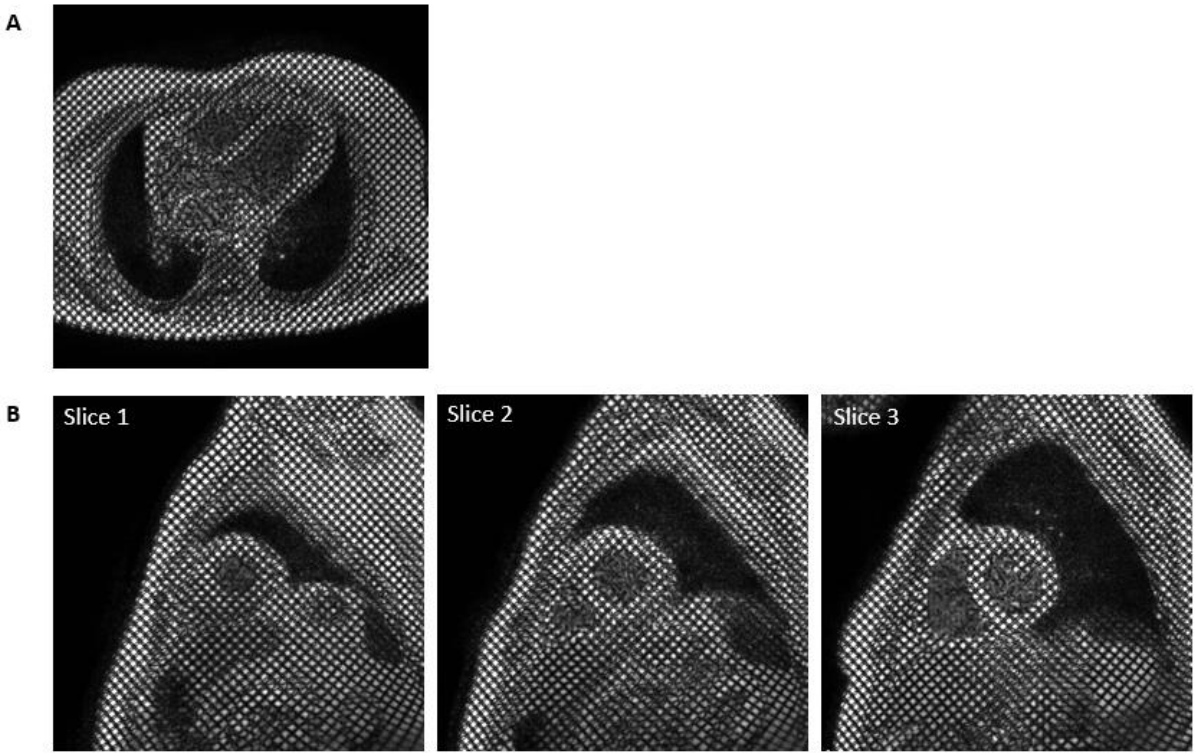


Figure 4-5: (A) Long and (B) short-axis MR tagging of the left ventricle.

Short axis tagging was acquired for three slices through the LV (base, mid, apex), which matched the T_1 mapping slices, and the mid-slice matched cardiac ASL perfusion measures. Tagging images were divided into six spatial segments, and the change in strain in each segment was assessed during dialysis.

4.3.2.1.2 MRI measures of Cardiac Structure

Structural measures of the heart comprised of Left ventricular (LV) wall mass index and the longitudinal relaxation time (T_1) of the myocardium.

Images obtained from LV short-axis cine scans described above were used for the assessment of LV mass index.

T_1 mapping data were collected using the Philips Cardiac Native 5s-(3s)-3s heart-rate triggered shortened modified Look-Locker inversion recovery (shMOLLI) scheme

(Figure 4-6). Parameters were FOV = 320 x 320 mm^2, reconstructed voxel = 0.9 x 0.9 mm^2, slice thickness = 10 mm, 3 slices, SENSE = 2.5, flip angle = 20^0, inversion delay time = 350 ms, TR/TE = 2.3/1.03 ms, 1 slice per 12 s breath-hold. Three slices through the short axis of the LV were acquired (base, mid, apex) to match the tagging with the central slice matched to the cardiac ASL.

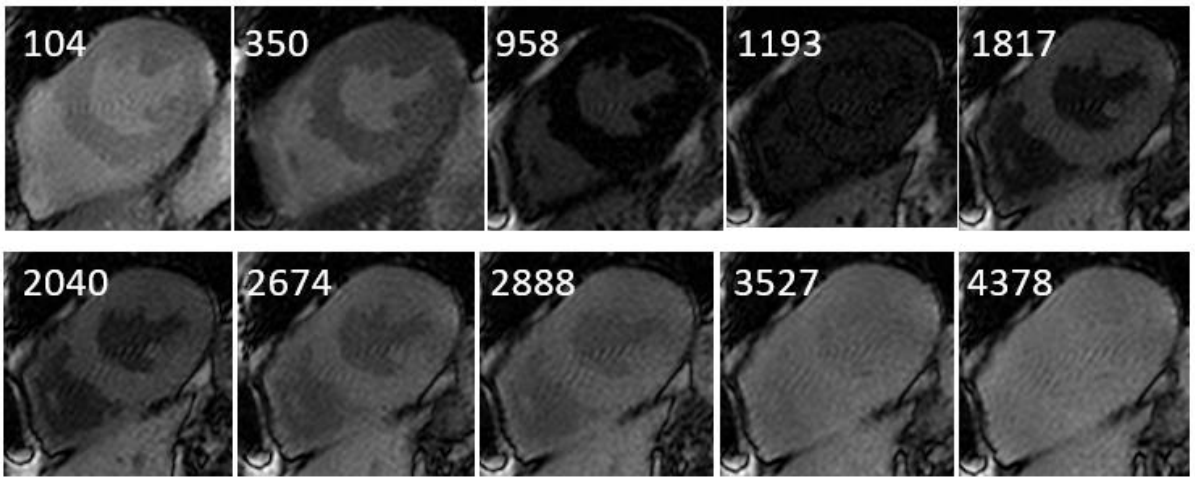


Figure 4-6: Example images of a shMOLLI T_1 (5s-(3s)-3s scheme) collected across the different inversion times (in ms). Images are shown for the mid-slice through the left ventricle.

4.3.2.1.3 MRI measures for assessment of Myocardial Perfusion and Coronary Artery Flow

Myocardial perfusion was assessed using a heart-rate triggered shMOLLI-based arterial spin labelling (ASL) technique. shMOLLI data was acquired using a 5b-(7s)-3b technique following FAIR (flow alternating inversion recovery using selective (S)/non-selective (NS) inversions) labelling, with data collected for three different inversion delay times following the second shMOLLI inversion (250, 400, 550 ms) (Figure 4-7). The FAIR labelling comprised a selective thickness of 40 mm and a non-selective thickness of 400 mm. Parameters were FOV = 320 x 320 mm^2, reconstructed voxel = 0.9 x 0.9 mm^2, slice thickness = 10 mm, SENSE = 2.5, flip angle = 20^0, TR/TE =

2.3/1.03 ms, 14 s breath-hold per individual acquisition. A single slice was acquired to match the central slice of the cardiac T_1 mapping and tagging data.

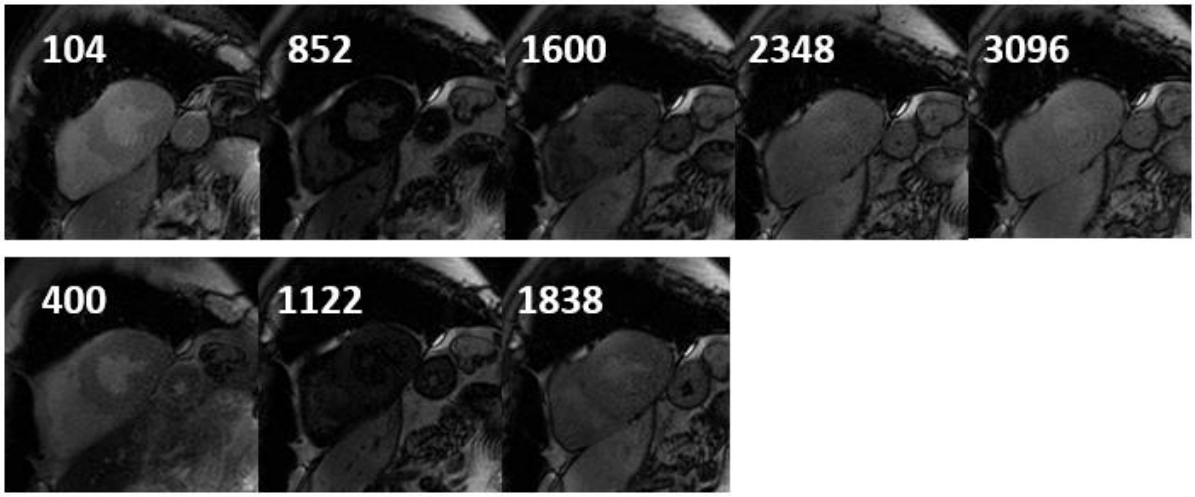


Figure 4-7: Example images of a MOLLI ASL (5b-(7s)-3b scheme) at different inversion times (in ms). This set is for non-selective inversion with a 400 ms inversion delay on the second inversion.

PC-MRI was planned to assess flow in the right coronary artery. However, due to the small size of the coronary artery, changes in coronary artery flow could not be measured reliably in all subjects (Of the first seven participants, baseline pre-dialysis coronary artery flow measurements were successful in only 1 case).

4.3.2.2 Measures of Brain Function and Structure

Brain function and structure were assessed using multi-parametric brain MRI with exemplars shown in Figure 4-8.

4.3.2.2.1 MRI measures of Brain Structure

Structural images were used to assess grey and white matter volume and cortical thickness (Figure 4-9). A high-resolution three-dimensional (3D) magnetization-prepared rapid acquisition with gradient echo (MPRAGE) T1-weighted multi-shot TFE sequence was collected with: FOV = 256 x 256 mm2, 1 mm isotropic voxel size, 162

slices, SENSE = 3.5, TFE factor = 207, shot interval 3000 ms, flip angle 8º, TR/TE = 8.0/3.7 ms, scan duration = 3 min 44 s.

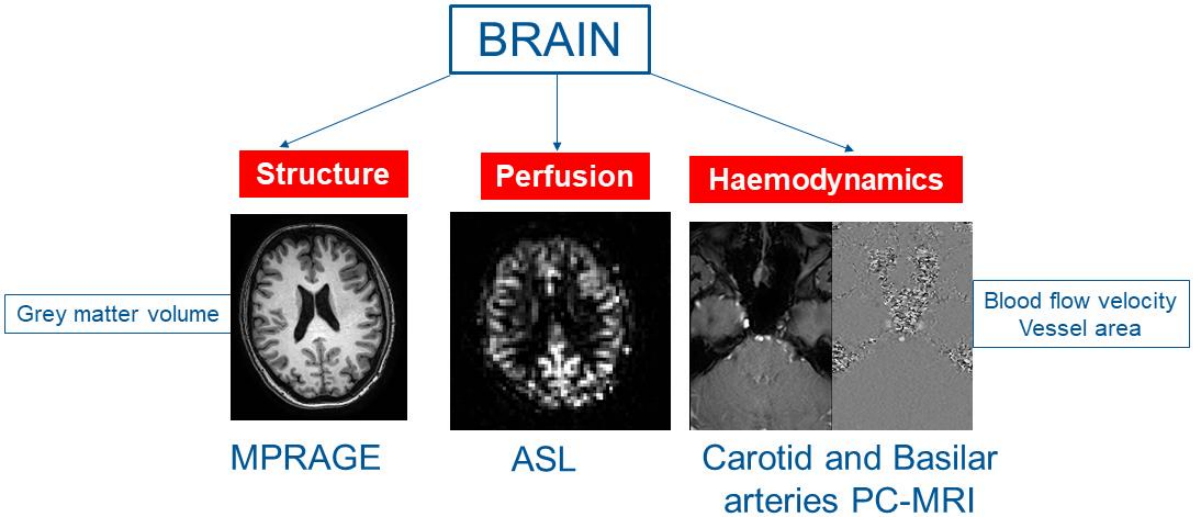


Figure 4-8: Overview of brain MRI measures collected.

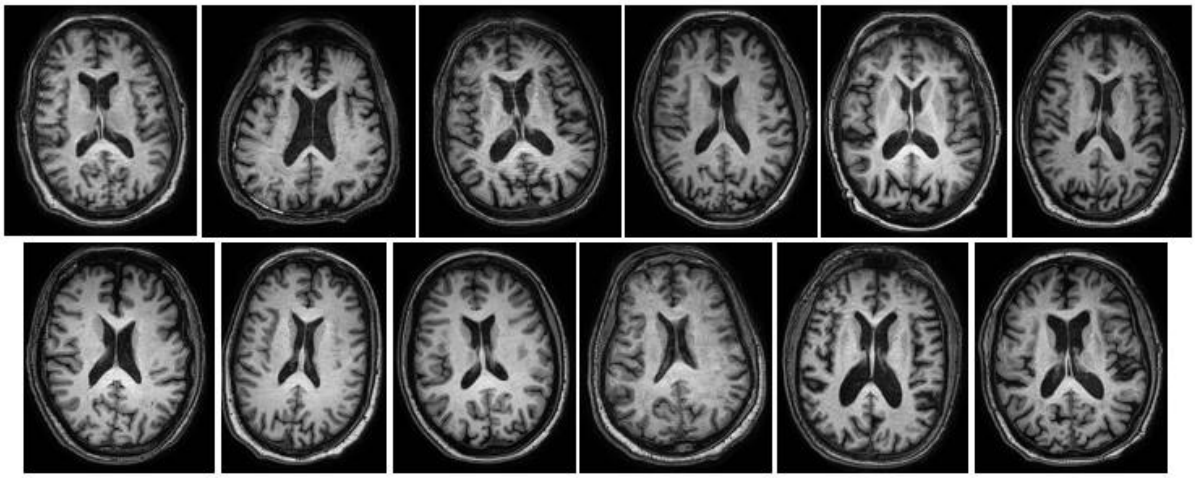


Figure 4-9: Example single slice MPRAGE brain image (of the 162 slices collected) for each participant. Images were used to assess grey and white matter volume and cortical thickness.

4.3.2.2.2 MRI measures for assessment of Brain Perfusion:

To determine grey matter perfusion, Arterial Spin Labelling (ASL) data were acquired with a FAIR labelling scheme and SE-EPI readout. Imaging parameters were FOV = 240 x 240 mm2, reconstructed voxel size = 3 x 3 mm2, slice thickness = 6 mm, 10 axial slices, SENSE = 2.3, TR/TE = 4000/16 ms, EPI factor = 29, 30 pairs of

selective/non-selective images, selective/non-selective thickness = 90/300 mm, label delay = 1500 ms, pre/post saturation, fat suppression = no, background suppression = no, vascular crushing = no, scan duration = 4 min 8 s. A Base equilibrium M0 scan with no labelling and inflow scan (20 pairs with label delay times = 300, 500, 800, 1200 ms) were also collected with the matched geometry and imaging parameters for perfusion quantification. (Figure 4-10). The T1 mapping brain scan was also matched to this geometry.

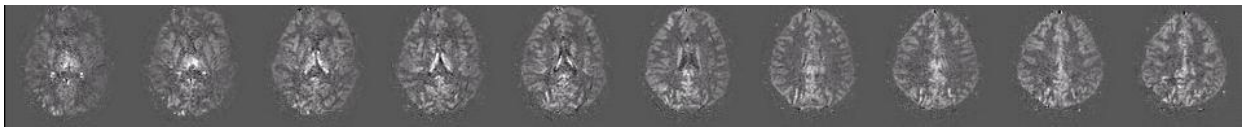


Figure 4-10: Example ASL images collected to measure grey matter perfusion.

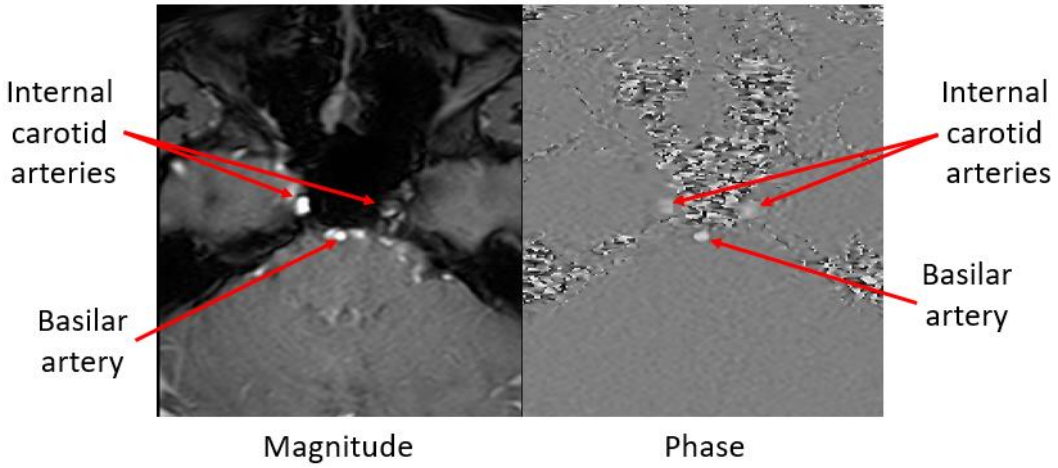


Figure 4-11: Example phase contrast measures of the internal carotid and basilar arteries, showing magnitude and phase images.

PC-MRI was acquired to determine right and left internal carotid artery flow and basilar artery flow. A single TFE slice was placed to cut perpendicular through all three vessels (Figure 4-11). Imaging parameters were FOV = 150 x 101 mm2, reconstructed voxel size = 0.6 x 0.6 x 5 mm3, SENSE = 2, flip angle = 10º, TR/TE = 9.1/5.5 ms, half scan factor = 0.625, VENC = 90 cm/s, scan duration = 39 s.

4.3.2.3 Measures of Renal Structure, Function and Perfusion

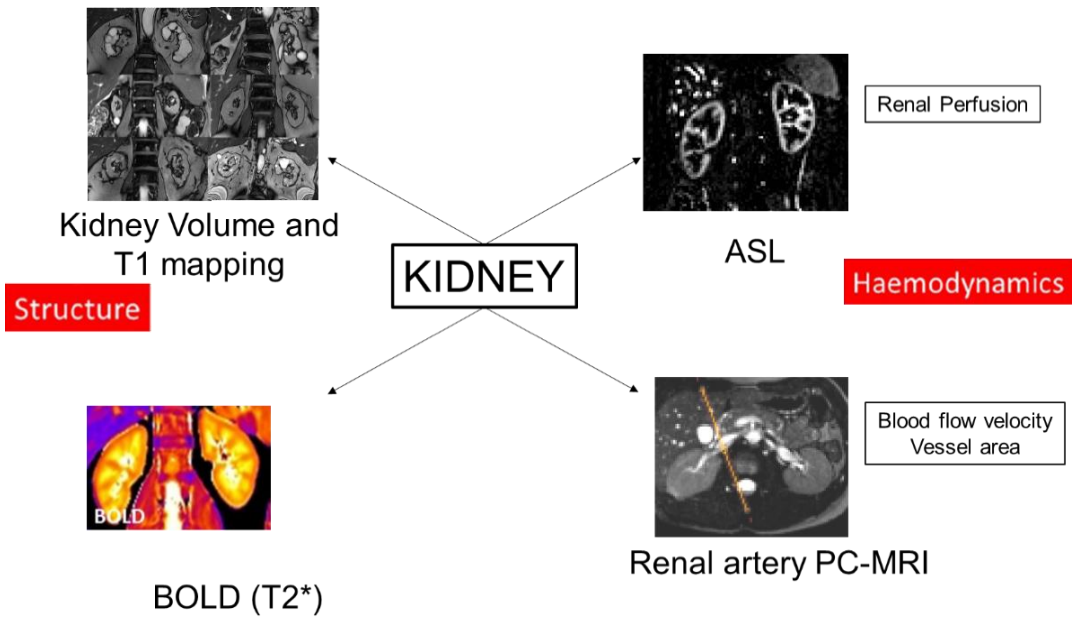


Figure 4-12: Overview of renal MRI measures collected.

Renal function and structure were assessed using multi-parametric renal MRI with exemplars shown in Figure 4-12.

4.3.2.3.1 MRI measures of Renal Structure

Total kidney volume (TKV) was computed from structural images shown in Figure 4-13. These were collected using a balanced TFE sequence with FOV = 400 x 400 m2, reconstructed voxel size = 1.6 x 1.6 mm2, slice thickness = 7 mm, 30 coronal slices, SENSE = 2, TR/TE = 3/1.5 ms, flip angle = 50º, 2 x 12 s breath holds.

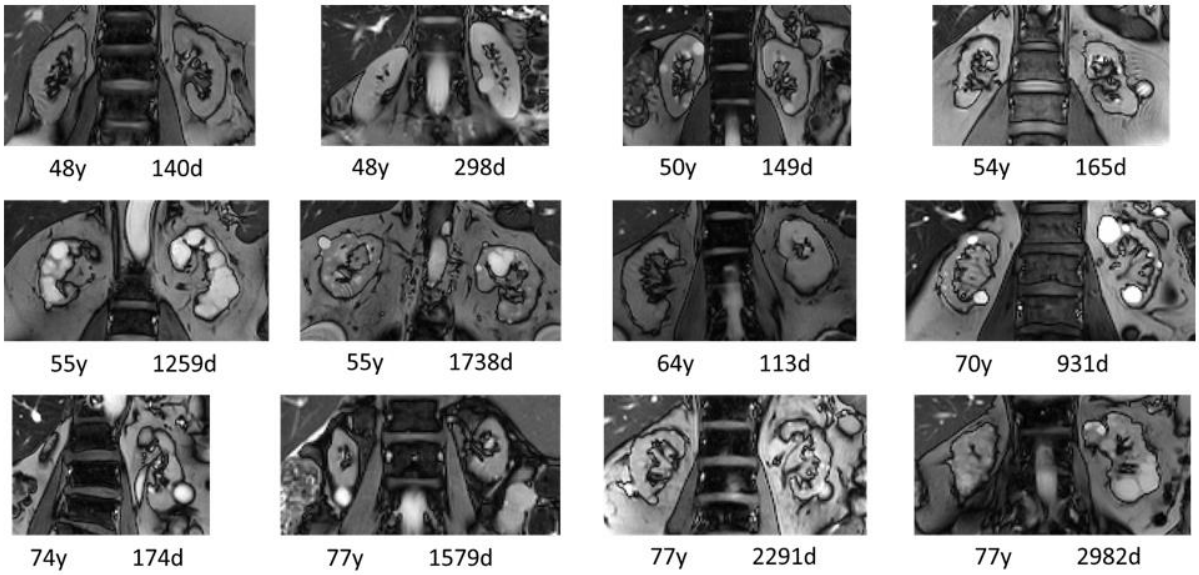


Figure 4-13: Images of all 12 subjects showing their age (in years (y)) and dialysis vintage (in days (d)) from a single slice of the coronal image used to assess total kidney volume (TKV).

Inversion recovery SE-EPI with respiratory triggering was used to assess T_1 of the renal cortex (Figure 4-14). Imaging parameters were FOV = 288x288 mm^2, resolution = 3 x 3 mm^2, slice thickness = 5 mm, 5 coronal oblique slices, SENSE = 2.3, TR/TE = 5000/27 ms, EPI factor = 27, fat suppression = SPIR, inversion times = 200, 400, 600, 800, 1000, 300, 500, 700, 900, 1100, 1200, 1300, 1500 ms, temporal slice spacing = 53 ms, scan duration 2-3 minutes.

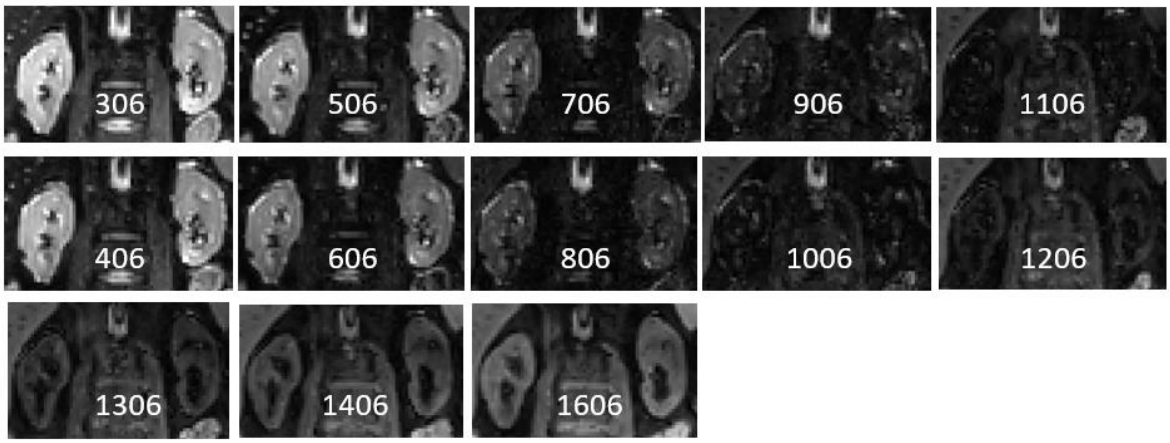


Figure 4-14: Example inversion recovery SE-EPI images of the kidney used for T1 mapping with inversion times shown in ms.

4.3.2.3.2 Functional MRI Measures of the Kidney

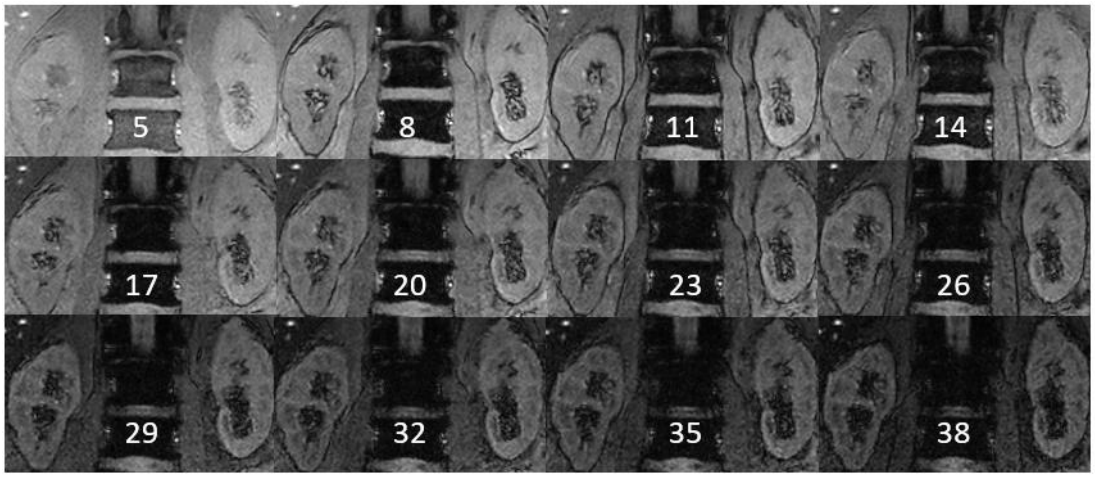


Figure 4-15: Example multiecho fast field echo (mFFE) images of the kidney with echo times shown in ms.

Multiecho fast field echo (mFFE) was used for T_2* mapping of the renal cortex (Figure 4-15). Imaging parameters were FOV = 288 x 288 mm^2, reconstructed resolution = 1 x 1 mm^2, slice thickness = 5 mm, 5 coronal oblique slices, 12 echoes, TR/TE1/ΔTE = 79/5/3 ms, flip angle = 25^0, 3 x 16 s breath hold.

We planned to acquire PC-MRI scans to determine right and left renal artery flow, however, due to the small size of the renal arteries in these participants, we were unable to assess the change in renal artery flow reliably in all subjects. Likewise, ASL MR imaging was planned to determine renal cortex perfusion. However, due to the small size of the kidneys and high numbers of cysts (Figure 4-13), analysis of renal perfusion scans could not be performed.

4.3.3 MRI Data Analysis

4.3.3.1 Measures of Cardiac Function, Structure and perfusion

4.3.3.1.1 Cardiac Function

Short axis cine images were analysed using Philips Intellispace software (Philips Medical Systems). The user identified end-diastole and end-systole phases using all slices. Epicardial and endocardial contours were drawn on the end-diastole image and propagated through all phases (Figure 4-16A). This was repeated for all slices containing the left ventricle blood pool. The basal slice was selected such that the end-diastole and end-systole images had at least fifty percent of the blood volume surrounded by the myocardium. The apical slice was defined as the last slice showing an intra-cavity blood pool. The papillary muscles were included within the blood pool. For each cardiac phase, the end-diastolic and end-systolic blood volume contours were summed across slices to provide a graph of LV blood volume against time through the cardiac cycle (Figure 4-16B). The end-diastolic volume (EDV) and end-systolic volume (ESV) were used to calculate stroke volume (SV = EDV – ESV) and cardiac output (CO = SV*heart rate). These were both indexed to a participant's body size by dividing by the body surface area (BSA) to compute stroke index and cardiac index. Ejection fraction was also calculated (EF = (EDV-ESV)/EDV).

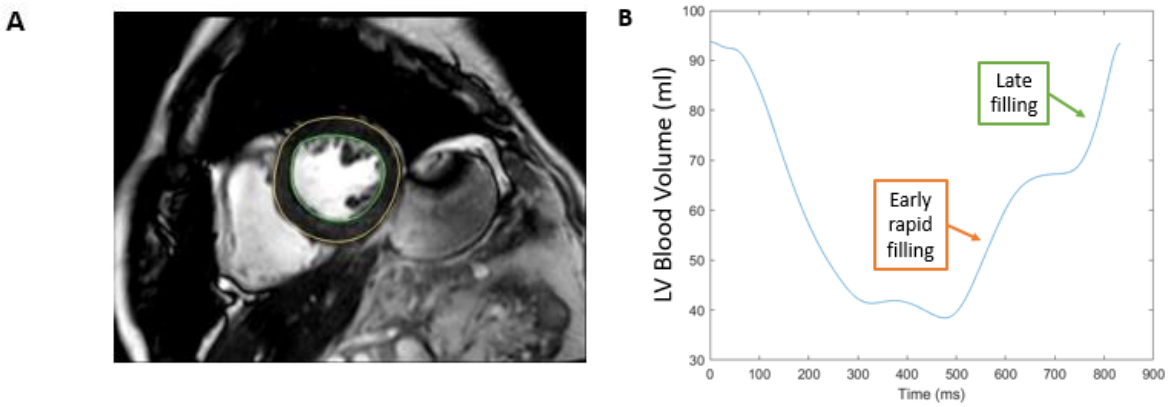


Figure 4-16: (A) Example short-axis cine image of the left ventricle with endocardial (green) and epicardial (yellow) contours shown with the left ventricle and (B) blood volume curve.

Myocardial tagging data were analysed using CIM v8.1 Tag2D (Auckland UniServices Ltd.) software. In both the long and short axis tagging, the myocardial wall was defined by placing contours around the epicardium and endocardium on the second phase and then propagating the contours to the other phases. Fiducial markers were used to define regions for regional strain analysis. In the long axis, markers were placed at the base and the apex of the left ventricle and in the short axis, one marker was placed in the center of the epicardial and endocardial contours and two markers were placed where the right ventricle free wall joins the LV (anterior – anterior septum border and inferior – inferior septum border). The tag was tracked through the cardiac phases to produce a strain graph from which the peak strain can be determined (Figure 4-17). Images with blurred tags due to poor breath breath-holding were discarded from the analysis, and only data where a peak was achieved were included.

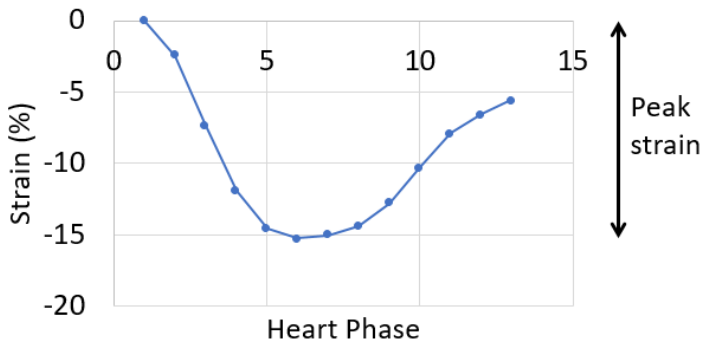


Figure 4-17: The peak strain (%) is calculated from the strain curve over the cardiac cycle.

The software separates the myocardium into six segments in both orientations for all slices (Figure 4-18). In the short axis the segments were anterior septum, anterior, anterior lateral, posterior lateral, inferior and inferior septum. Each half of the long axis was separated into the base, middle and apex (i.e., base 1 and base 2).

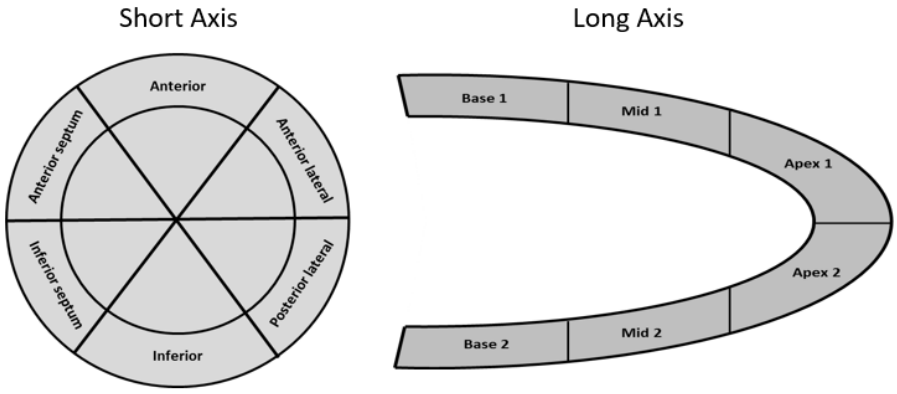


Figure 4-18: Schematics of the six segments of the short and long axes of the left ventricle.

Strain was defined as longitudinal strain in the long axis and circumferential strain in the short axis. Longitudinal strain defines how the myocardium is compressed or stretched in the direction of the long axis. Circumferential strain describes the strain of the short axis as the myocardial wall is contracting inwards.

4.3.3.1.2 Cardiac Structure

LV wall mass was calculated from the short axis cine data using Philips Intellispace (Philips Medical Systems). The epicardial and endocardial contours were used together to calculate the wall mass, excluding the papillary muscles. The wall mass was adjusted for BSA to give LV wall mass index.

Analysis of MOLLI T1 data (Figure 4-19) was performed using in-house (SPMIC) Matlab (The MathWorks, Inc) software. Images were first assessed for motion. Any data points with motion were discarded and then fit voxel-by-voxel to a Look-Locker inversion recovery to calculate a T1 map:

$$Signal = abs\left(A - B.exp\left(-\frac{TI}{T1^*}\right)\right) \quad \text{where} \quad T1 \approx T1^*\left(\frac{B}{A} - 1\right)$$

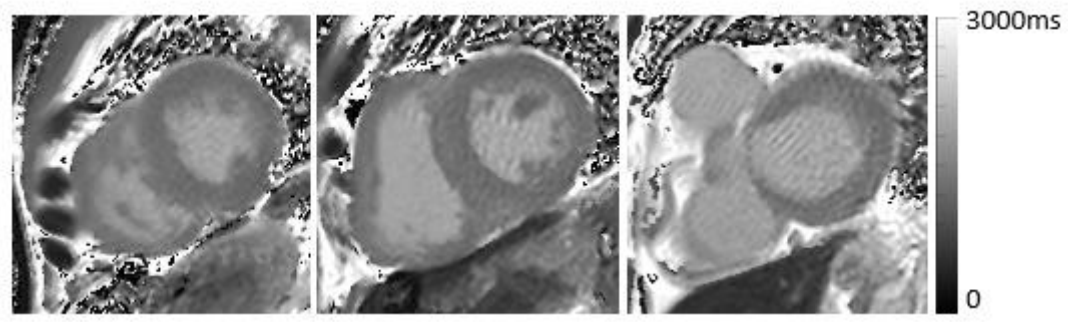


Figure 4-19: Example myocardial T1 map. Three slices through the short axis of the left ventricle were acquired.

The goodness of fit was computed for each voxel and voxels with poor fits were removed from ROI analysis. A ROI was manually drawn to cover the myocardium using MIPAV v.8.0.2 software to obtain a median myocardial T1 value.

4.3.3.1.3 Myocardial Perfusion

Myocardial perfusion data acquired using MOLLI based ASL were analysed using in-house (SPMIC) Matlab (The MathWorks, Inc) code. Images were assessed for motion,

translated linearly if possible or discarded if the slice was misaligned due to incorrect breath-hold position. Non-selective and selective data were each fit to a Look-Locker inversion recovery on a voxel-by-voxel basis to obtain selective and non-selective T1 maps. An ROI in the myocardium was drawn manually to obtain the median selective and non-selective T1 values (Figure 4-20). In addition, an ROI was drawn in the LV blood pool of the non-selective T1 map to estimate the median T1 of blood (Figure 4-21). These T1 values were then used in the Belle model to calculate myocardial perfusion:

$$Perfusion = \frac{\lambda}{T1_{blood}}\left(\frac{T1_{non-selective}}{T1_{selective}} - 1\right)$$

where λ is the tissue-blood partition coefficient = 0.92 ml/g for the heart.

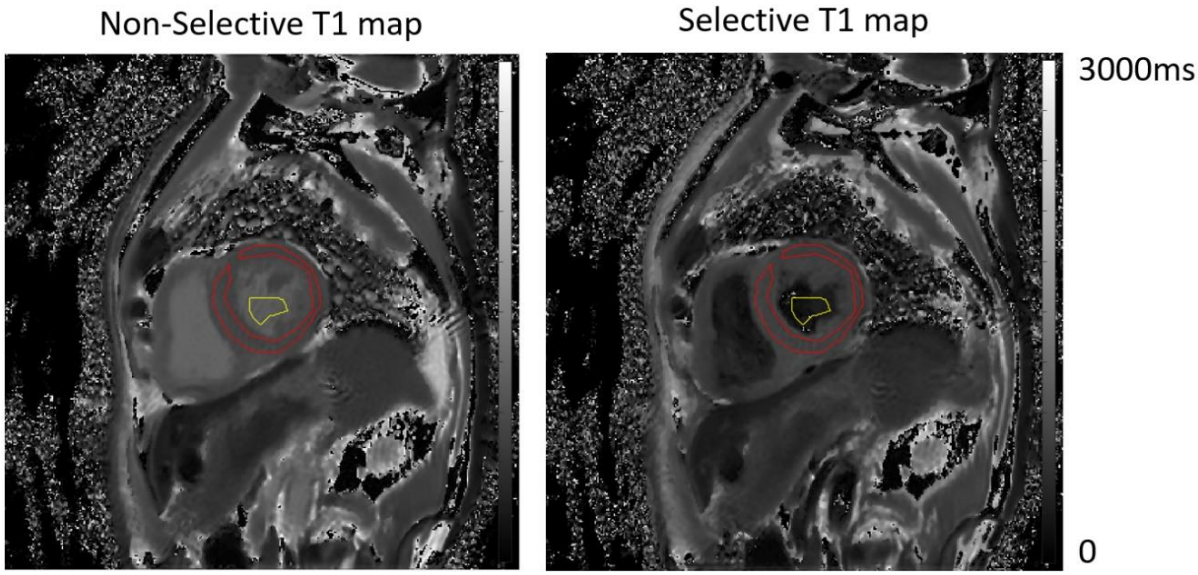


Figure 4-20: Example non-selective and selective T1 maps with ROIs of the myocardium and the left ventricular blood pool.

4.3.3.2 Measures of Brain structure, function and perfusion

4.3.3.2.1 Assessment of Brain Morphology

Structural MPRAGE images were pre-processed using SPM12 [SPM12; Wellcome Department of Cognitive Neurology, University of London] and the associated toolbox CAT12 running on MATLAB [The MathWorks, Natick, MA] with default parameters given by the CAT12 toolbox. The T1-weighted MPRAGE data were segmented into grey matter volume (GMV), white matter volume (WMV), and cerebral spinal fluid (CSF) tissue classes and then transferred into the Montreal Neurological Institute (MNI) stereotactic coordinate space and normalized using a high- dimensional DARTEL normalization (Figure 4-21). The images were then smoothed with a Gaussian smoothing kernel of 12 mm (FWHM) to allow parametric comparisons across subjects. The final smoothed, modulated, and normalized images had a 1.5 mm isotropic voxel size.

Voxel-based morphometry (VBM) statistical analysis was performed using a General Linear Model (GLM) design including the total intracranial volume (TIV) as a regressor of no-interest to remove the effect of variation in brain size across participants from the data. Possible edge effects between tissue types were avoided by excluding all voxels with GM values of less than 0.1 (absolute threshold masking). The statistical threshold for assessing voxel-wise differences was set at $P<0.001$, uncorrected, cluster size > 5. A multiple regression model was used to investigate the potential associations between GMV, WMV, and CSF with age and dialysis duration with results threshold at $P<0.001$, uncorrected, cluster size > 5. Two sample T-test was used to

explore the voxel-wise GMV and WMV differences between HD and HC groups, with TIV and age (to remove the effects of these parameters) as covariates of no interest.

Region of interest analysis of VBM: Brain regions that showed a significant correlation in VBM statistical analysis were independently selected for correlation analysis using the Region of Interest (ROI) extraction tool in MaRSBar in the SPM toolbox. Brain volumes extracted from different regions were correlated with variables of interest in SPSS (IBM SPSS Statistics 26).

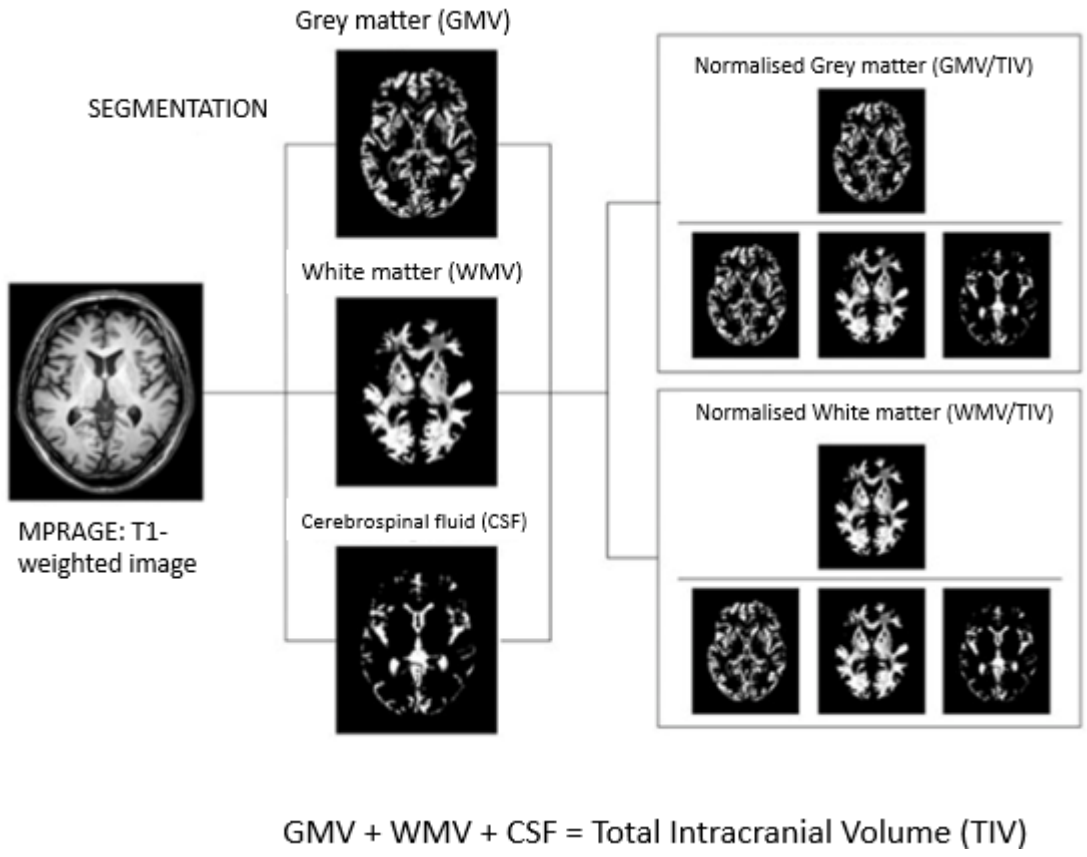


Figure 4-21: Illustration of the pipeline to segment the MPRAGE T_1-weighted image into grey matter volume (GM, white matter volume (WM), and cerebral spinal fluid (CSF) tissue classes.

To assess brain T1 mapping measures, the inversion recovery data were fit on a voxel-by-voxel basis to a standard inversion recovery equation to generate T1 and M0 maps using in-house (SPMIC) Matlab (The MathWorks, Inc) code. Partial volume (PV) maps

of grey matter, white matter, and cerebral spinal fluid (CSF) were created from the T1 maps (skull removed) using the FAST segmentation algorithm (Figure 4-22). Partial volume maps indicate how much of each voxel is comprised of each tissue type and is given as a fraction of 1.

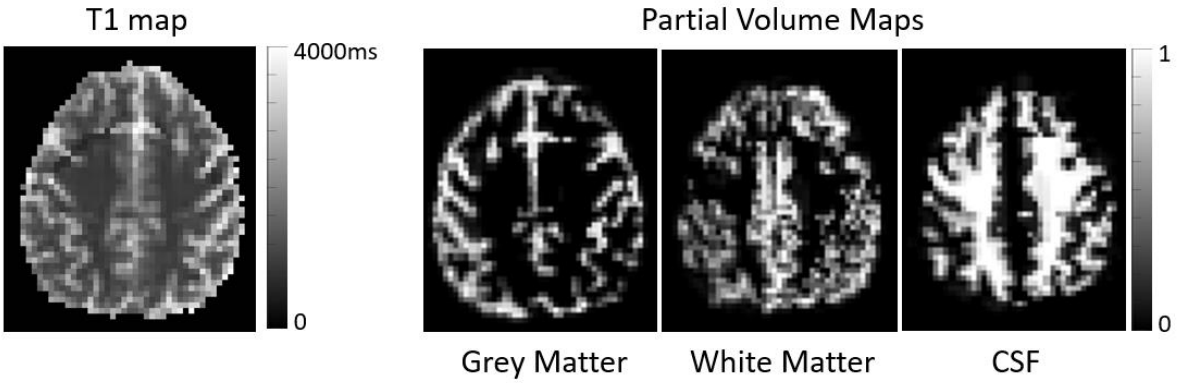


Figure 4-22: Example T1 map and partial volume (PV) maps showing the proportion of grey matter, white matter and CSF in each voxel.

The PV maps were moved into MNI space to create common masks across all visits and scans for each of the subjects (these common masks are also used to assess grey matter perfusion). The common masks were transformed back into native space to interrogate the T1 maps. Two thresholds were used (0.5, 0.75) to create two masks each for grey matter, white matter and CSF from the PV common masks. For each mask and tissue type, a histogram was plotted, and the mode and full width at half maximum (FWHM) were calculated. In addition, the number of voxels of each tissue type was counted to provide an indicator of percentage contributions within the section of brain acquired for this scan (60 cm), which can be compared with the VBM of T1w measurements of GMV, WMV and CSF volume.

4.3.3.2.2 Cerebral Perfusion and Blood Flow

To analyse the perfusion data, all the selective and non-selective images (ASL and inflow) were realigned to the base M0 scan using MCFLIRT. Perfusion weighted difference maps were calculated for each selective/non-selective pair and then averaged to create a single perfusion-weighted (ΔM) map for each inversion time. The ΔM maps and base M0 were used to calculate perfusion maps using BASIL (Figure 4-23). For each subject, the grey matter common mask (obtained previously from the T1 maps) was then applied to the perfusion map at each time point with two thresholds (0.5, 0.75 as for the T1) and the median grey matter perfusion calculated at each threshold.

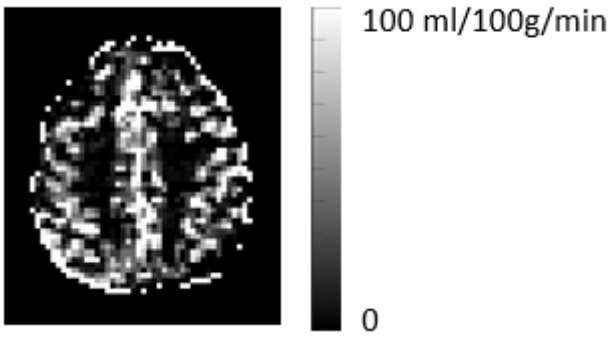


Figure 4-23: Example perfusion map measured using arterial spin labelling.

Philips Viewforum Qflow software (Philips Medical Systems) was used to analyse each vessel within the PC-MRI slice through the internal carotids and basilar arteries. A region of interest (ROI) was drawn around the vessel wall and propagated through all phases across the cardiac cycle. From this, blood velocity, area and flux waveforms over the cardiac cycle were derived. The mean across the cardiac cycle of each parameter was calculated to obtain mean vessel cross-sectional area (mm2), mean blood velocity (cm/s), and mean flux of blood flow (ml/s) over the cardiac cycle.

4.3.3.3 Renal Structure and function

MIPAV v.8.0.2 software was used to calculate TKV. Regions of interest were manually drawn on each slice on each kidney and summed across all slices and kidneys to obtain the total kidney volume (ml). This was corrected for patient body size by dividing by BSA.

Inversion recovery data were initially inspected for motion and discarded if necessary. The remaining data were fit on a voxel-by-voxel basis to a standard inversion recovery equation to generate a T1 map using in house Matlab (The MathWorks, Inc) code (Figure 4-24). The median T1 across both kidneys was calculated by drawing ROIs on the renal cortex using MIPAV v.8.0.2 software.

T2* data were fit voxelwise using a weighted echo time fit to form T2* maps from the log of the exponential signal decay using in house Matlab (The MathWorks, Inc) code (Figure 4-24). The median T2* across both kidneys was calculated by drawing ROIs on the renal cortex using MIPAV v.8.0.2 software.

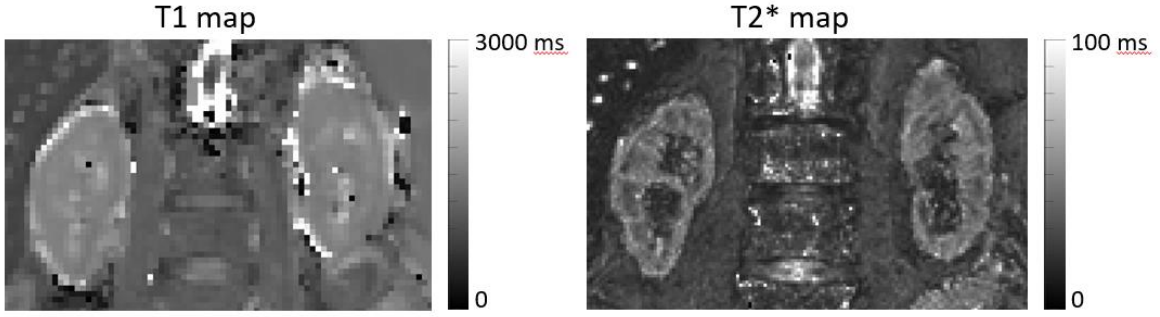


Figure 4-24: Example T1 and T2* maps of the kidneys. Renal cortex T1 was measured using an inversion recovery technique and renal cortex T2* was measured using a multi echo technique.

4.3.4 Reproducibility of MRI data

Reproducibility indicates the ability to obtain same results from a test/ experiment even when it is conducted by the different team in different setting. Repeatability is the ability to obtain the consistent results by the same team in the same setting. These characteristics of myocardial T1, T2 and T2* relaxation time depends on multiple factors like patient's characteristics, MRI hardware, acquisition protocol, post processing protocol and manual vs automatic segmentation. Review by Ogier et al suggests that parametric mapping is currently reproducible at the level of the individual MR scanner, but direct comparison cannot be made different the mapping techniques or different set ups (206).

It is important to note that our studies were not designed to assess the reproducibility or repeatability of the MRI measures. Limited studies explored the reproducibility of MRI measures in haemodialysis patients, but the results seem to be promising. Graham et al reported good inter-study and inter-observer variability of native T1 mapping with coefficient of variation of 0.7% and 0.3% respectively (207) and are independent of the volume status of the patient (body weight and left ventricular end diastolic volume were used as markers for volume status). In another study, excellent interstudy reproducibility was demonstrated for ascending aortic distensibility using MRI measures (208).

Section 4.4 Set-up of dialysis for intra-dialytic MRI and validation of extended dialysis blood lines for use with BTM device

The set-up for the provision of dialysis at the MRI centre is illustrated in Figure 4-25. We used extension lines to standard blood lines on both arterial and venous sides to cover the distance between the participant (inside the scan room) and the dialysis machine (located outside the scan room). The total length of blood lines was 2m each. Participants spent the duration of dialysis during this study session on an MRI-safe trolley for ease of transfer if required. As one of the chapters in this thesis used a Blood Temperature Monitor (BTM) biofeedback device in this setting, it was important to determine whether the performance of the BTM was affected by the extended length of the blood lines.

4.4.1 Study set up at Magnetic resonance imaging centre

Figure 4-25: Research setting of the Magnetic Resonance Imaging area.

Left side is the participant and research nurse in the scan room; long extension lines are visible on the floor in the scan room; on the right side (outside the scan room), is the dialysis machine

4.4.2 : Calculation of thermal energy flow in the Extracorporeal circuit

During dialysis treatment, thermal exchange in the extracorporeal circuit raises the patient body temperature during standard dialysis (37ºC) on average ~0.5 to 0.7ºC (76, 209). This thermal exchange leads to so-called heat stress, which causes vasodilatation. However, vasoconstriction is the normal physiological response to the intravascular volume depletion secondary to ultrafiltration during dialysis. The net result of these two counter-productive mechanisms, to a certain extent, determines the haemodynamic changes during dialysis and its effects on intradialytic blood pressure.

The following formula helps to calculate thermal energy flow in the extracorporeal circuit (210)

$$E = C\ P * (Tven - Tart) * (Qb - UFR)$$

- Product of C (specific caloric capacity of the blood) and P (haematic density) equals 3.81J/ml and depends on the haematocrit.
- $Tven$ is the temperature in the venous line
- $Tart$ is temperature in the arterial line
- Qb is the blood flow.
- UFR is ultrafiltration rate

4.4.3 Blood Temperature Monitor

Blood temperature monitor (BTM) in the Fresenius 5008 dialysis machine enables measurement of Tart and T ven continuously throughout the dialysis session. Short segments of the venous and arterial blood lines are inserted into the temperature sensors which enables these measurements. Using this closed loop biofeedback system, body temperature can be controlled by adjusting the dialysate temperature to compensate for increase or decrease in the body temperature during dialysis with the help of BTM (211). The machine can be programmed to deliver isothermic ($\Delta T=0$) or hyperthermic (in cases of hypothermia) or hypothermic (in cases of fever) dialysis, in other terms thermo-controlled dialysis.

The measured Tart and Tven by BTM depend on various factors as outlined below:

- Actual blood flow
- Assumed ambient temperature
- Thermal conductivity of the blood tubing system
- Distance between patient and sensor
- Total blood recirculation

4.4.4 Aims and Objectives

Due to the factors mentioned in the previous section, it is plausible that the thermal conductivity of the lines and the distance between the patient-sensors are affected by the long extension bloodlines that we have used in the study set up as described

above, thus affecting the measurement of Tart and Tven. This needed to be studied prior to conducting a trial comparing standard and thermocontrolled dialysis.

The aim was to evaluate the effects of the long extension bloodlines used during the dialysis sessions (to adapt the layout to the MRI scanner room) during multiorgan randomised cross-over study on the thermoregulation of patients. To achieve this, the following comparisons were made:

Test 1: Compare the effects of standard lines (SL) vs. longlines (LL) on change of body temperature at a set dialysate temperature (ΔTT, calculated by the difference in the post-dialysis tympanic temperature and pre-dialysis tympanic temperature) and BTM measured change of temperature (Δ BTM).

Test 2: Compare the effects of standard lines (SL) vs longlines (LL) on ΔTT and Δ BTM during thermo-controlled dialysis sessions using BTM.

Test 3: Compare the effects of insulated long lines (ILL) vs non-insulated longlines (NILL) on ΔTT and Δ BTM.

4.4.5 Methods

We dialysed participants using Fresenius 5008 machines using their routine dialysis prescriptions for 4 hours, either using standard lines or long lines. We collected data regarding:

- Dialysate temperature
- Tubing length (Standard or longlines)
- Tympanic temperature of the patient- pre and post dialysis

- Arterial and venous temperature from the BTM- pre and post
- Change of temperature from the BTM

Data were analysed using Graph Pad Prism 8. Bland-Altman plots were used to evaluate the agreement among the different methods of measuring the change in the temperatures using different lines. Mann Whitney U test was used to assess the statistical significance difference between the two methods and a p value of <0.05 was considered significant.

4.4.6 Results

There was no difference in the change of tympanic temperature, and the BTM measured change of body temperature in test 1. These results are summarised in Table 4-1 &Table 4-2. This rules out increased thermal conductivity when long lines were used.

Table 4-1: Comparison of standard vs long lines at dialysate temperature of 37°C

Dialysate temperature 37°C	**Standard line n=11**	**Long line n=9**	**p value**
∆ TT	0 (0.2)	0.2 (0.45)	0.46
∆ BTM	0.3 (0.3)	0.3 (0.25)	0.66

Table 4-2: Comparison of standard vs long lines at dialysate temperature of 35.5°C

Dialysate temperature 35.5°C	**Standard line n=8**	**Long line n=5**	**p value**
∆ TT	0.05 (0.65)	0 (0.4)	0.73
∆ BTM	-0.15 (0.6)	-0.1 (0.3)	0.83

In test 2, we compared the effects of long lines on body temperature monitor (BTM) performance to deliver thermo-controlled dialysis sessions. As demonstrated in Figure 4-26, there was no significant difference in ΔTT or Δ BTM when dialysis was performed using BTM with a pre-set target to achieve a change in temperature of -0.5ºC/hour.

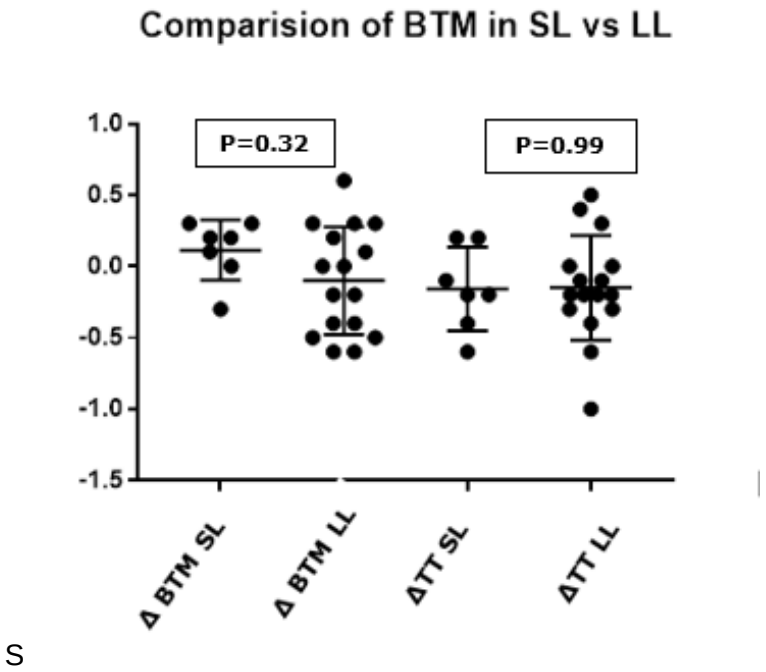


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Figure 4-26: Comparison of change of temperature measured by BTM (Δ BTM) and change of tympanic temperature (Δ TT) using standard lines (SL) vs long lines (LL) during thermo-controlled dialysis sessions using BTM functionality.

Finally, in test 3, we examined if insulation was required to address the potential difference in the thermal conductivity of long lines. There was no statistical significance between insulated (ILL) and non-insulated longlines (NILL), as demonstrated in Figure 4-27.

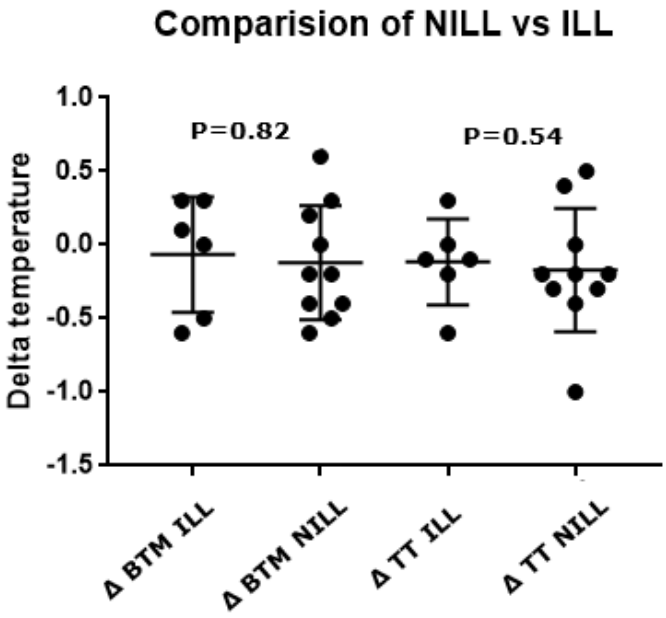


Figure 4-27: Comparison of change of temperature measured by BTM (Δ BTM) and change of tympanic temperature (Δ TT) using insulated long lines (ILL) vs non-insulated long lines (NILL) during thermo-controlled dialysis sessions using BTM functionality.

4.4.7 Conclusion and Limitations

We demonstrated that long lines with or without insulation did not affect the performance of the BTM device. Hence, long extension lines without insulation were suitable for use in our clinical study without affecting the performance of the BTM to deliver the prescribed thermo-controlled treatment.

However, there were limitations to the methodology that we adopted. We have standardised the equipment utilised for dialysis in the comparison groups in the above tests. But the comparisons made were not paired assessments, i.e., the participants were not the same in the comparison groups. Thus, participant-related factors like the ultrafiltration volume might be affecting the results of the tests. This approach was used to avoid alternation in the dialysis prescription of the participants.

Chapter 5 An analysis of the frequency of continuous blood pressure variation and haemodynamic responses during HD

Section 5.1 Abstract

5.1.1 Background

During dialysis, higher beat-to-beat blood pressure (BP) variation, measured by extrema point (EP) frequency analysis of continuous BP monitoring, is associated with elevated cardiac damage markers and white matter ischaemic changes in the brain thus suggesting its relevance to end-organ perfusion. Therefore, we utilised EP analysis to study intra-dialytic BP variation to improve the individualised description of the haemodynamic response to haemodialysis (HD).

5.1.2 Methods

We recruited 50 participants receiving in-centre HD and performed continuous non-invasive haemodynamic monitoring during dialysis. EP MAP frequencies were extracted. Participants were divided into those with a greater proportion of low frequency (LF) (n=21) and high frequency (HF) EP values (n=22). Clinical and haemodynamic data were compared between groups.

5.1.3 Results

Median EP MAP frequencies of mid-week HD sessions were 0.54 Hz (IQR 0.18) and correlated with dialysis vintage (r=0.32, p=0.039), NT pro-BNP levels (r=0.32,

p=0.038), and average real variability (ARV) of systolic BP (r=0.33, P=0.029), ARV diastolic BP (r=0.46, p=0.002) and ARV MAP (r=0.57, P<0.001).

In LF group, MAP positively correlated with Cardiac Power Index (CPI) in each hour of dialysis, but not with total peripheral resistance index (TPRI). In contrast, in HF group, MAP correlated with TPRI in each hour of dialysis but only with CPI in first hour.

5.1.4 Conclusions

EP frequency analysis of continuous BP monitoring during dialysis allows assessment of BP variation and categorisation of individuals into low or high frequency groups, which were characterised by different haemodynamic responses to dialysis. This information highlights the complex haemodynamic responses during HD and this improved understanding may assist in better individualisation of dialysis therapy.

Section 5.2 Introduction

Intradialytic hypotension is a commonly encountered problem during haemodialysis (HD), with a reported incidence of 10-40% (139, 140, 212). It is associated with ischaemic end-organ damage (75, 115) and mortality (141, 142). However, arbitrary blood pressure (BP) thresholds do not reliably predict end-organ ischaemia (213), and asymptomatic IDH may result in reduced organ perfusion (214). Furthermore, subclinical myocardial ischaemia has also been demonstrated during continuous renal replacement therapies with apparently stable haemodynamics and low ultrafiltration rates (215). These observations imply that factors other than the absolute drop-in BP play a key role in inducing ischaemic organ damage, one of which may be the degree and frequency at which BP varies.

Higher variation in systolic BP (SBP) has been linked to cardiovascular events, cerebrovascular events and increased mortality in the general population (216, 217) and in those with CKD (218, 219). In HD populations, higher variability in pre-dialysis SBP is associated with a 15% increase in the risk of mortality (220, 221). Likewise, greater interdialytic SBP variability assessed using average real variability (derived from ambulatory BP monitoring during the 44-hour interdialytic period) is independently associated with cardiovascular mortality (222). In addition, the HD population face unique haemodynamic stresses related to dialysis treatment and are often subject to acute BP variations during dialysis. Many studies reporting intradialytic BP changes relied on intermittent, infrequent (every 15-60 minutes) BP readings from an arm cuff, which do not provide detailed resolution of more rapid BP variations that may occur during HD (223). Detailed study of intradialytic beat-to-beat BP variation using continuous monitoring is lacking. In patients with Transient Ischaemic Attacks or

non-disabling stroke, higher beat to beat BP variability is a better predictor of recurrent strokes and cardiovascular events than day-to-day variations (216).

Extrema point (EP) frequency analysis, a method of measuring BP variation, utilises peaks and troughs of a continuously recorded BP waveform to calculate the frequency of variation (224). In a previous study, the EP frequencies of MAP during HD were reported to rise during HD and reached a peak during the 3rd quarter of the HD session, at which time there was a drop in the absolute BP. Higher EP MAP frequencies during HD were associated with higher cardiac troponin levels and greater ischaemic changes in the brain white matter detected by magnetic resonance imaging. This suggests that higher EP frequencies, representing greater beat-to-beat BP variation, may adversely affect organ perfusion (115, 225). Importantly, in a randomised trial of standard versus cooled dialysate HD where the latter prevented subclinical brain white matter ischaemic injury, EP frequencies increased during standard HD but did not change significantly during cooled dialysis, indicating that EP frequency could be a potential modifiable target for interventions (115). Therefore, we aimed to prospectively study EP frequencies during dialysis to identify the factors associated with higher values and describe how EP frequencies relate to changes in central haemodynamics during dialysis. To do so, we propose a new method of categorising patients based on their intra-dialytic EP frequency data.

Section 5.3 Methods

5.3.1 Patients and Data Collection

We performed a prospective observational study at University Hospitals of Derby and Burton NHS Foundation Trust, the United Kingdom, between January 2018 to August 2019.

This study was conducted ethically in accordance with the World Medical Association Declaration of Helsinki and was approved by the West Midlands Ethics Committee (IRAS number: 217655). Informed written consent was obtained from all participants.

Participants were aged ≥18 years and had been receiving HD for more than 3 months. Baseline characteristics, details of the dialysis prescription, medication history and laboratory parameters were collected. HD was performed thrice weekly using Gambro Artis machines with participants' usual dialysis prescription. Net ultrafiltration was based on the individual's prescribed dry weight and anticoagulation was provided as per the participant's standard prescription.

5.3.2 Continuous blood pressure monitoring

Continuous non-invasive monitoring of blood pressure and haemodynamics was performed using pulse wave analysis (Finapres NOVA, FMS, Netherlands) for the entirety of three consecutive dialysis treatments. This method has been described in detail in Chapter-4 and has been validated in HD populations previously (72, 76). This device was fitted to the non-fistula arm (in participants with arteriovenous fistula as HD access) at the start of the investigatory HD session and left in place throughout.

5.3.3 Signal processing and identification of extrema points

The haemodynamic data generated by the Finapres were analysed by first identifying the frequency and amplitude of local extrema points (maxima and minima; EP) for MAP as previously described (224), summarised in Figure 5-1. A modified Short-time Fourier Transform method was then applied as a moving asynchronous filter to extract the sinusoidal frequency and phase content of time-varying MAP signals (226). These spectra were then decomposed into constituent frequency events using the Freedman-Diaconis rule (227), and plotted as histograms for each individual patient (example shown in Figure 5-2).

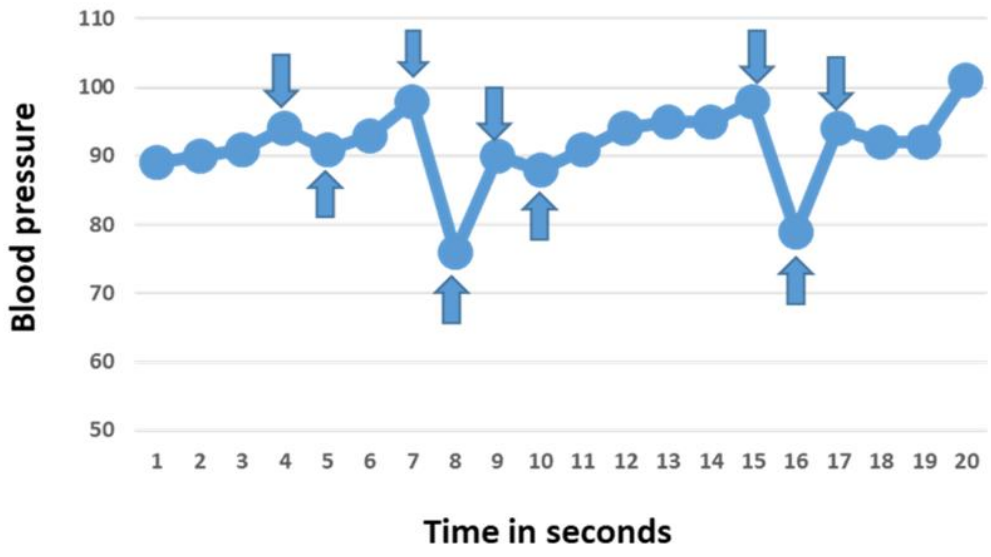


Figure 5-1: Illustration of identification of extrema points (minima and maxima identified by arrows) on a 20 second trace of MAP measurements.

Once identified, frequency is calculated using the following formula:

$$f = {}^{1}/_{time\ difference\ between\ 2\ consecutive\ extrema\ points}$$

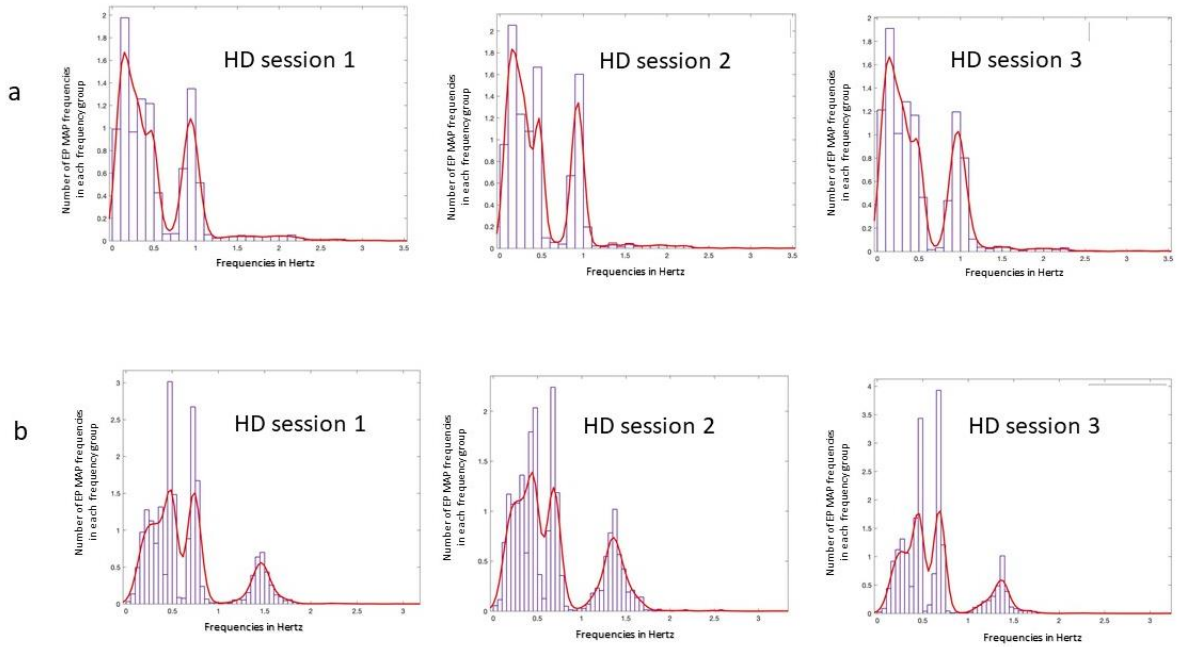


Figure 5-2: Histograms of EP MAP frequencies of 2 participants (a, b) across three consecutive monitored HD sessions (4 hours duration).

These demonstrate bimodal distribution of EP MAP frequencies and are similar across the 3 sessions in each participant. X-axis represents frequency values; Y-axis represents number of frequencies.

5.3.4 Categorisation of participants based on EP MAP frequencies

As higher EP frequencies have been shown previously to associate with ischaemic brain injury, we hypothesised that patients could be characterised using the ratio of high to low EP frequency values during dialysis. To calculate ratios of high to low EP frequency values, we plotted histograms of EP frequencies for each individual (Figure 5-3) from processed data from a mid-week HD session (48-hour interdialytic gap) and defined:

- HFC (high frequency changes) as EP frequencies occurring within the same frequency range as the mean intradialytic heart rate ± two standard deviations, representing beat-to-beat variation in BP.

- LFC (low frequency changes) were defined as those occurring in the frequency range of three or more cardiac cycles, corresponding to those with a frequency range below one third of mean heart rate + two standard deviations.

Based on the median HFC/LFC ratio the study population was divided into two groups: Low frequency (LF) group with HFC/LFC ratio ≤0.5 and High frequency (HF) group with HFC/LFC ratio >0.5. Haemodynamic trends and clinical variables were then compared between these groups.

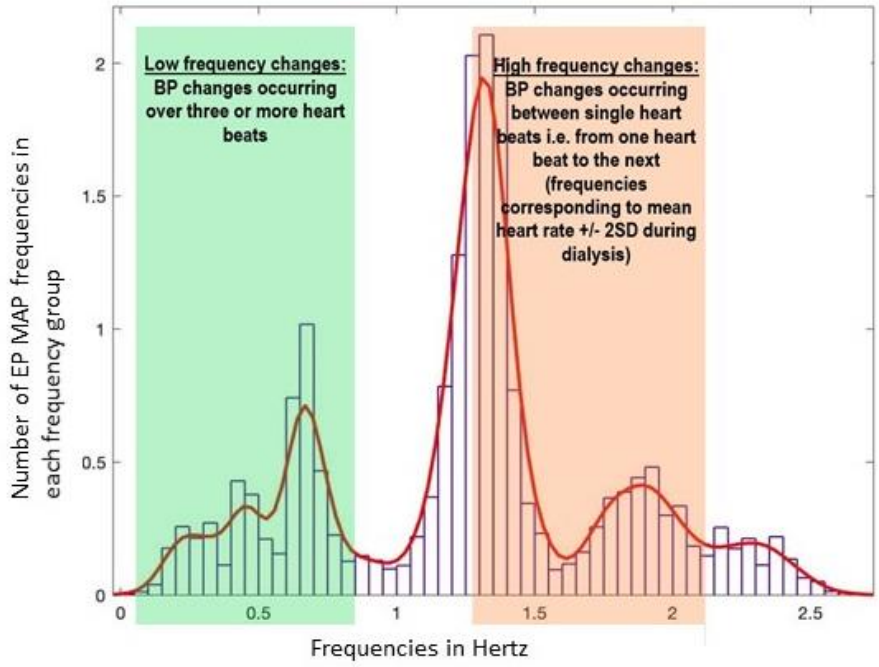


Figure 5-3: Schematic diagram demonstrating the categorisation of the EP MAP frequencies into low frequency changes (LFC) and high frequency changes (HFC) used to calculate the HFC/LFC ratio.

X-axis represents frequency values, Y-axis density of frequencies.

5.3.5 Haemodynamic data processing and definitions

In addition to the haemodynamic data generated by the Finapres, we calculated

- Cardiac power index (CPI=*MAP x CI x 0.0022 w/m², normal range = 0.45 to 0.85 w/m²)* which has been shown to be independently associated with adverse outcomes in cardiogenic shock (228) and has been utilised to categorise patients with differing intradialytic haemodynamic responses to fluid removal (229)].

- Average real variability ($ARV = \frac{1}{N-1}\sum_{K=1}^{N-1} x\ |BP_{K+1} - BP_K|$; *where K ranges from 1 to N-1; N is the number of intradialytic BP readings (230)*. While EP analysis evaluates the frequency of BP change, ARV describes the magnitude of BP variation. Higher ARV (24-hour BP monitoring) has been shown to be associated with increased all-cause mortality, cardiovascular mortality and non-fatal strokes (222, 231, 232) but has not been used to describe continuous BP recordings during dialysis.

- Baroreflex sensitivity (BRS) as a measure of cardiovascular autonomic integrity was derived as a regression of pulse interval against systolic blood pressure. Using Matlab (R2011a, MathWorks®, MA, US) inter-beat intervals and corresponding systolic blood pressures were computed from Finapres data. The geometric mean for the whole dialysis session was then used to assess BRS during HD for each individual.

Haemodynamic measures during the intradialytic period were averaged over 10-minute blocks to study the trends in BP and other haemodynamic measures during HD (apart from BRS).

Traditional definitions of IDH are difficult to apply to continuous BP data, hence we assessed IDH by recording:

1. The proportion of the SBP readings below 90mmHg from the total number of intra-dialytic BP measurements,
2. The proportion of the SBP readings is 20mmHg below the pre-dialysis brachial SBP from the total number of intra-dialytic BP measurements.

5.3.6 Statistical analysis

Continuous variables are expressed as mean ± SD for normally distributed variables, median and interquartile range for non-parametric data. Categorical variables were expressed as percentages. Spearman's correlation was used where the data were non-parametric. Friedman's nonparametric analysis of variance was used to test variations between time points and the Kruskal Wallis test was used to test variance between groups. Matlab (R2018a) was used for extraction of EP frequencies. Statistical analysis was performed using IBM SPSS (Version 24). A $p< 0.05$ was considered significant.

Section 5.4 Results

A total of 50 participants was recruited, from which 43 participants completed at least one monitored mid-week HD session (48-hour interdialytic gap) (Figure 5-4).

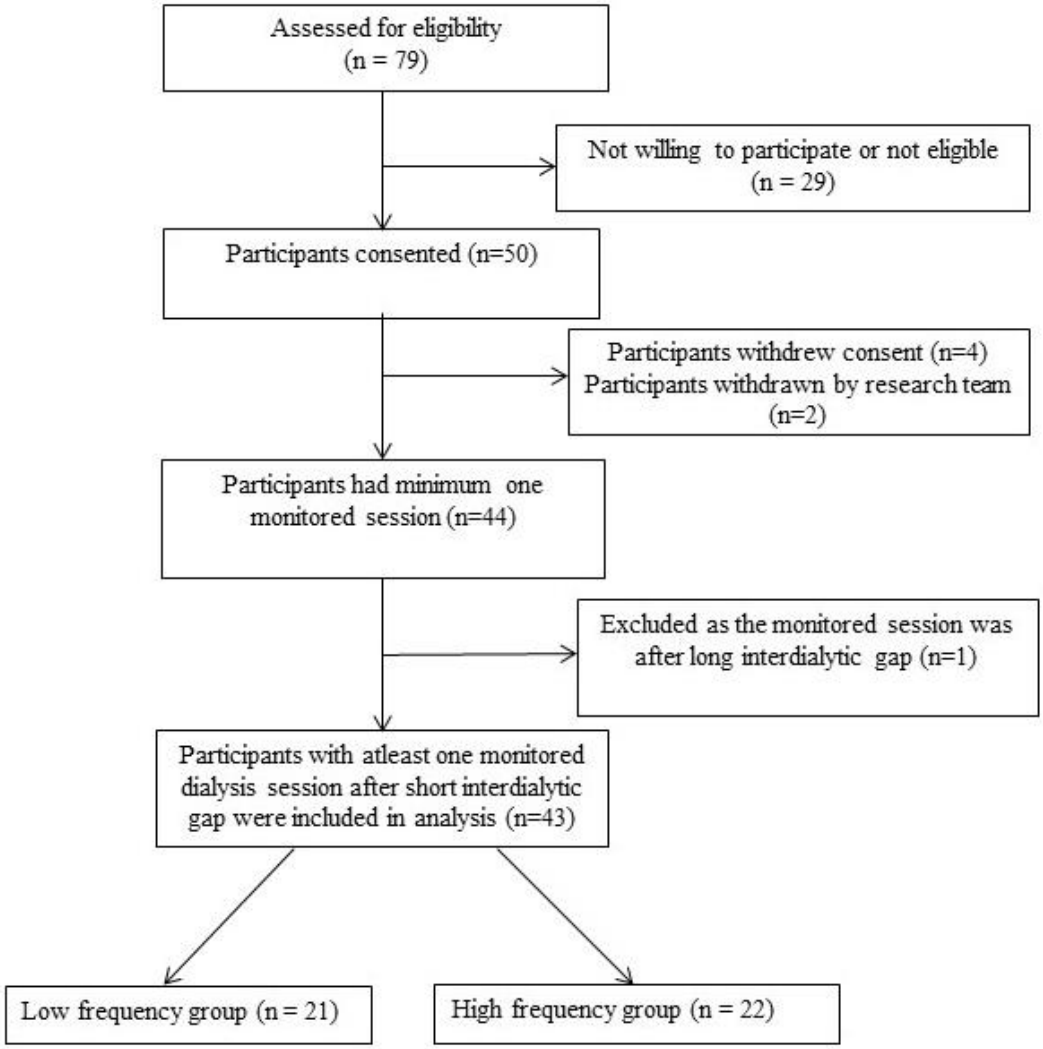


Figure 5-4: Consort diagram illustrating participant flow through the study

Characteristics of the study population are presented in Table 5-1a. Mean age was 61.5±16.6 yrs, 26 (60.5%) were male and 19 (44.2%) had diabetes. Median time since dialysis initiation was 24 months (IQR 75), and arteriovenous fistula was the predominant vascular access (83.7%). Median Charlson comorbidity index (CCI) was 4 (IQR 2).

Haemodynamic parameters for the study population are described in Table 5-1b. Intradialytic trends of BP and other haemodynamics are illustrated in Figure 5-5**.** On average an initial brief rise was followed by gradual decline in SBP, MAP, DBP, Cardiac index and CPI. TPRI increased during dialysis.

Table 5-1: Characteristics of the Participants and intradialytic haemodynamic findings

(a) Demographics of the study population (N=43 included)	
Age (years)	61.5 ± 15.63
Gender (% male)	26 (60.5%)
Ethnicity (%) White	37 (86%)
Diabetes	19 (44.2%)
Charlson Comorbidity Score	4 (2)
Weight	71 kg (31.9)
Body Mass Index	26.7 kg/m^2 (9.8)
Mean Interdialytic weight gain over 4 weeks prior to recruitment in Kilograms	1.51 ± 0.68
Number of participants with Intradialytic hypotension episodes in 4 weeks prior to recruitment	4 (9.3%)
Number of participants on Anti-hypertensives • Angiotensin Converting enzyme Inhibitors • Beta-Blockers • Calcium Channel blockers	25 (58.9%) 9 (20.9%) 13 (30.2%) 12 (27.9%)
Number of participants receiving Dialysable anti-hypertensive therapy	3 (7%)
Dialysis vintage in months	24 (75)
Access type • Arteriovenous fistula • Arteriovenous graft	 36 (83.7%) 4 (9.3%)

• TNL	3 (7%)
Access flow (Qa) ml/min	754 (554)
(b) Intradialytic Haemodynamics of study population	
Systolic Blood Pressure in mmHG	140 (25)
Diastolic Blood Pressure in mmHG	75 (18)
Mean Arterial Blood Pressure in mmHG	99 (18)
Cardiac Power Index in w/m^2	0.67 (0.32)
Cardiac Index in L/min/m^2	3.44 ± 1.03
Stroke Volume Index in ml/m^2	46.36 ± 14.67
Total Peripheral Resistance Index in dynes·sec/cm^5/m^2	0.39 (0.28)

Median and interquartile range (IQR) are reported for non-parametric data, mean ± standard deviation (SD) for normally distributed data; numbers (percentages) are reported for categorical data.

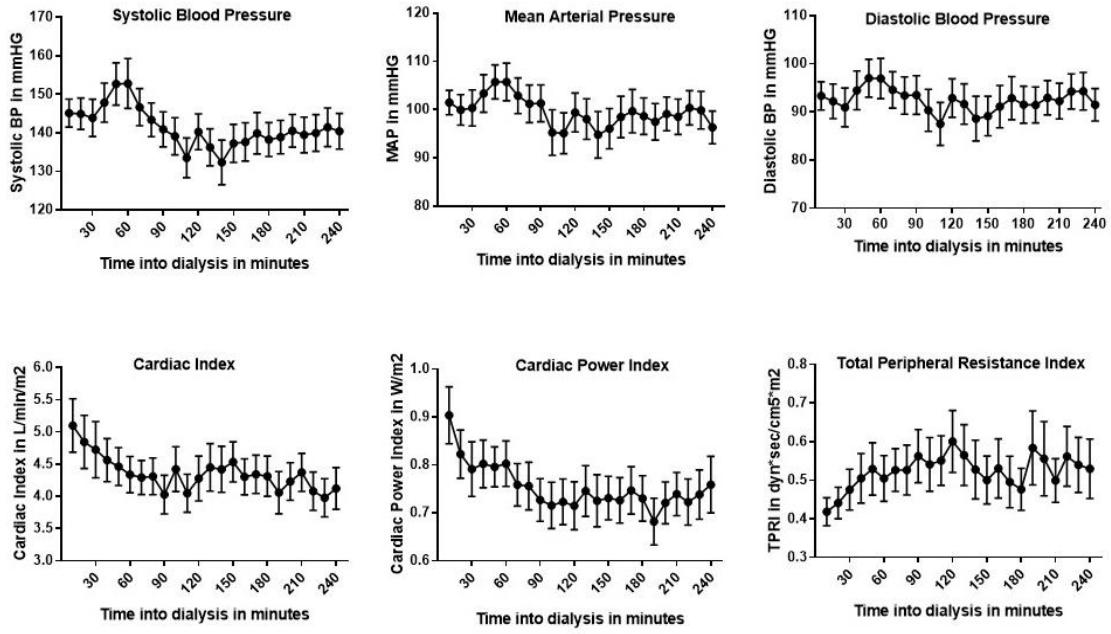


Figure 5-5: Intradialytic population trends in Systolic BP (SBP), Diastolic BP (DBP), Mean arterial pressure (MAP), Cardiac Index, Cardiac Power Index (CPI) and total peripheral resistance index (TPRI).

The total monitored dialysis duration (4 hours) was divided into 10minutes blocks, and the average of each block is represented as a data point. An initial brief rise was followed by gradual decline in SBP, MAP, DBP, CI and CPI but TPRI increased as the dialysis progressed.

The median proportion of recorded BP measures per participant that were <90mmHg was 0.79% (IQR 3.03%). The median proportion of SBP readings 20mmHg below the pre-dialysis brachial SBP was 9% (IQR 27.5%).

5.4.1 EP MAP frequencies during dialysis and association with clinical variables

The median of EP MAP frequencies was 0.54 (IQR 0.18) Hz across all participants. While BP declined during HD, EP MAP frequencies showed a tendency to increase with peak values in the third hour (Figure 5-6), although this did not reach statistical significance (p=0.671).

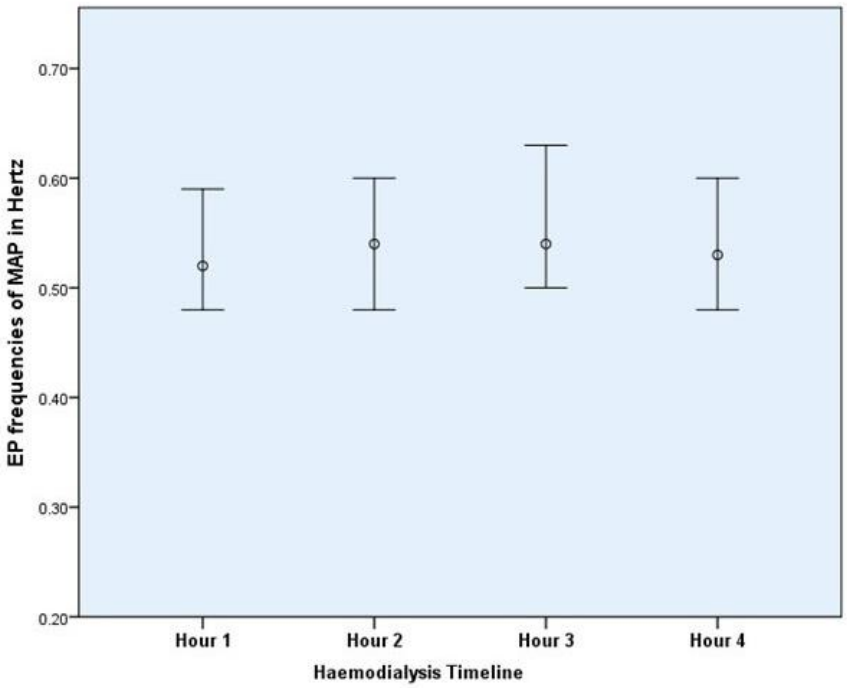


Figure 5-6: A graphical representation of population trend of EP MAP frequencies during 4 hours of Haemodialysis (HD).

This graph demonstrates the comparison of EP frequencies of mean arterial blood pressure 1st, 2nd, 3rd and 4th hours of dialysis for the study population. Each circle represents the median EP MAP frequencies of the cohort for every hour with 95% confidence intervals as error bars. There is trend towards slow rise to the third hour and drop in the fourth hour (p=0.671).

Dialysis vintage (r=0.32, p=0.039), NT-pro BNP levels (r=0.32, p=0.038), average real variability (ARV) of SBP (r=0.33, p=0.029), ARV of DBP (r=0.46, p=0.002) and ARV of MAP (r=0.57, p≤0.0001) were correlated with higher intradialytic median EP MAP frequencies. Variables that were not associated with median EP frequency included age (r=0.2, p=0.2), CCI (r=0.07, p=0.659), diabetic status (z=-1.48, p=0.139), prescription of beta-blockers (z=-1.84, p=0.278), ultrafiltration volumes (r=0.1, p=0.542), baroreflex sensitivity (r= -0.27, p=0.08) and the blood volume change during HD (r=-0.27, p=0.096).

5.4.2 HFC/LFC ratio and association with clinical variables

Median intradialytic HFC/LFC ratio was 0.517 (IQR 0.42) for the study population. There was no significant difference in the median intradialytic HFC/LFC ratios of consecutively monitored HD sessions of each participant on repeated measures non-parametric ANOVA (p=0.697), indicating intra-individual repeatability of this measure.

The demographics, clinical and biochemical variables across the groups defined by MAP frequency patterns are shown in Table 5-2. There were no differences in age, proportion with diabetes, CCI or dialysis vintage between the groups.

In the HF group, there was a higher proportion of participants on anti-hypertensive therapy including beta-blockers (HF group 45.5% vs LF group 14.3%, p=0.026). This group also had higher NT-pro BNP levels (HF group 6285.5 [IQR 20217] vs LF group 1949 [3941], p=0.045). ARV of BP during HD was higher in HF group (Table 5-2). However, there was no difference in BRS between the groups (HF group 8.23 [IQR 3.54] vs LF group 9.55 [IQR 6.29]) (p=0.903).

Table 5-2: Comparison of low frequency (LF) versus high frequency (HF) group

Clinical Variable	LF group (n=21)	HF group (n=22)	P-value
Age	58.2±17	64.9 ±13.8	0.164
Sex (females)	47.6%	31.8%	0.289
Charlson Comorbidity Score	3 (4)	4 (2)	0.292
Current Smokers/ex-smokers	10 (50%)	9 (42.9%)	0.68
Diabetes	38.1%	50%	0.432
Dialysis Vintage in months	24 (56)	26.5 (80)	0.846
Participants on Antihypertensive therapy	7 (33%)	18 (18.8%)	**0.02**
Participants on Dialysable Antihypertensives	0	3 (16.7%)	0.25
Participants on B-blockers	14.3%	45.5%	**0.026**
Participants on ACEi	9.5%	31.8%	0.072
Pre-dialysis-Troponin t (ng/L)	54 (52)	74 (64)	0.15
Pre-dialysis NT Pro BNP (ng/L)	1949 (3941)	6285.5 (20217)	**0.045**
Initial Brachial Systolic BP (mmHG)	130 (32)	154.5 (23.5)	**0.018**
Mean Systolic BP (SBP in mmHg)	132.45 (19.52)	140.19 (37.3)	0.264
Mean Diastolic BP (DBP in mmHG)	81.51 (19.44)	72.47 (19.24)	**0.037**
Mean Mean Arterial Pressure (MAP in mmHg)	99.01 (25.24)	98.46 (15.46)	0.409
Stroke Volume Index (ml/m2)	42.35±13.79	50.18±14.76	0.08
Cardiac Index(L/min/m2)	3.356 ±1.13	3.523±0.952	0.602
Cardiac Power Index (w/m2)	0.65 (0.39)	0.68 (0.30)	0.771
Total Peripheral Resistance Index (dynes·sec/cm5/m2)	0.425 (0.29)	0.372 (0.34)	0.395
Baroreceptor Sensitivity (millisec/mmHG)	9.55 (6.29)	8.23 (3.54)	0.903
RR-interval (milliseconds)	845.57±132.23	944.90±177.91	**0.045**

Heart rate (beats/min)	73.11 ±13.73	66.46±17.9	0.181
ARV SBP (mmHG)	4.08 (3.26)	5.65 (4.56)	**0.004**
ARV MAP (mmHG)	2.496 (1.05)	3.394 (3.31)	**0.004**
ARV DBP (mmHG)	2.361 (1.61)	3.4042 (2.99)	**0.008**
Net UF (Ultrafiltration) in litres	1.58±0.959	1.56±1.07	0.955
Difference between prescribed and achieved UF	0.4 (0.35)	0.4 (0.53)	0.51
Kt/V	1.257 ± 0.245	1.263 ± 0.271	0.942
Participants on Dialysate temperature of <37°C	15 (71.4%)	13 (59.1%)	0.396
Median Frequency of MAP in Hertz (Hz)	0.4866 (0.19)	0.6129 (0.36)	**0.002**
Max percentage drop in SBP during HD compared to initial brachial SBP	-18.8 % (19.7)	-25.4% (27.43)	0.913
Proportion of 20mmHg drop in SBP (%)	6.81 (15.17)	14.55 (32.77)	0.145
Proportion of <90mmHg drop in SBP (%)	0.736 (3.6)	0.914 (2.35)	0.884

HF group- participants with higher proportion of high frequency extrema point (EP) MAP changes, LF group- participants with higher proportion of low frequency EP MAP changes. ARV is average real variability which is calculated using the formula as described in methods section for Systolic, diastolic and mean arterial BP measurements and provides magnitude of variation. Median and interquartile range (IQR) are reported for non-parametric data, Mean ± standard deviation (SD) for normally distributed data, numbers (percentages) are reported for categorical data. P values < 0.05 are highlighted in bold.

We studied the changes in the HFC/LFC ratio during dialysis. To so do we calculated the average HFC/LFC ratios for each hour of dialysis (as the monitoring was performed over 4 hours of dialysis for each patient, we had 4 values represented the average HFC/LFC ratio for each hour of dialysis) and compared them. In the entire study population, average hourly HFC/LFC ratios did not demonstrate any specific direction of change (Figure 5-7). However, there was a decline in HFC/LFC ratio in the LF group during HD (reaching nadir in 3rd hour of HD) and no change in HF group (Figure 5-8). The HFC/LFC ratios differed significantly between the groups during every hour of HD (Figure 5-8).

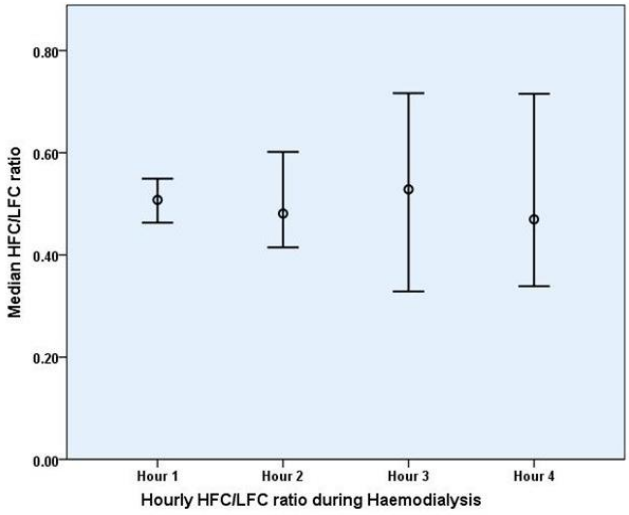


Figure 5-7: A graphical representation of population trend of HFC/LFC ratio during 4 hours of Haemodialysis (HD).

This graph demonstrates the comparison of HFC/LFC (High and low frequency changes ratio) ratio of 1st, 2nd, 3rd and 4th hours of dialysis for the entire study population. Each circle represents the median HFC/LFC ratio of the cohort for every hour with 95% confidence intervals as error bars. There is no significant direction of change in HFC/LFC as dialysis progressed.

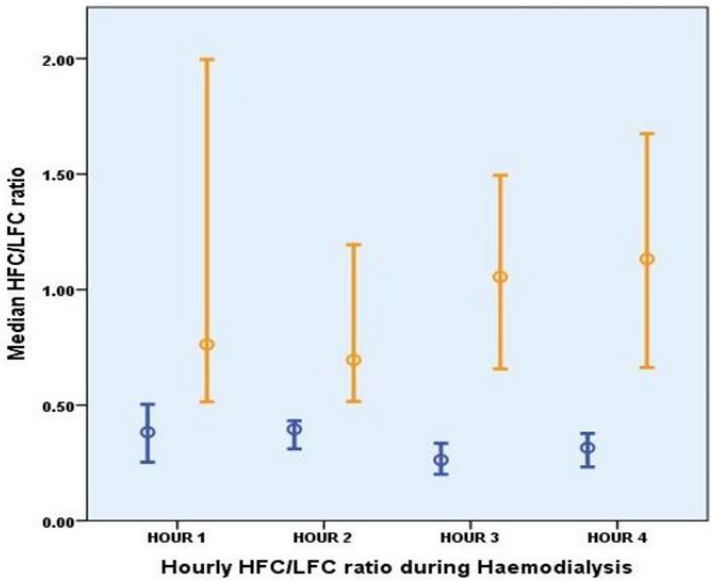


Figure 5-8: A graphical representation of HFC/LFC ratio patterns in the low frequency (LF) group (represented in blue) and high frequency (HF) group (represented in orange) during haemodialysis.

This graph demonstrates the comparison of HFC/LFC (High and low frequency changes ratio) ratio of 1st, 2nd, 3rd and 4th hours of dialysis for the Low frequency and high frequency groups of

the study. Medians are represented with the circles for each hour of HD and the 95% confidence intervals as error bars. Friedman's test was used to compare between various time points and was significant in LF group (p value: 0.036) and not significant in HF group (p value: 0.532). Kruskal Wallis test was used to compare both groups at each time point and was significant across all 4 hours with p values of <0.001.

5.4.3 Intradialytic haemodynamic responses in LF and HF groups

We examined the associations between intradialytic haemodynamic variables within the groups to assess if haemodynamic responses to dialysis differed depending on HFC/LFC ratio. To do so we calculated the mean intradialytic haemodynamics for the entire HD session (4 hours) and also means for each hour of HD.

In the LF group, mean intradialytic MAP correlated with mean intradialytic CPI (r=0.64, p=0.002) but not with mean intradialytic TPRI (Figure 5-9). The opposite was observed in HF group, with correlation of mean intradialytic MAP with mean intradialytic TPRI (r=0.66, p=0.001) but not with mean intradialytic CPI (Figure 5-9).

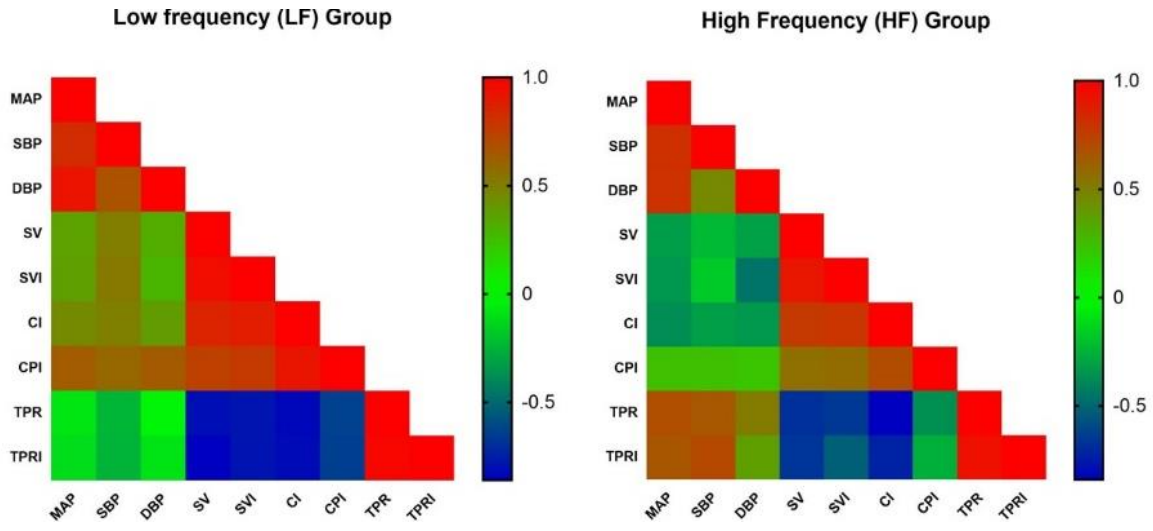


Figure 5-9: Correlation matrix for haemodynamic variables for entire haemodialysis session in the low frequency (LF) and high frequency (HF) groups.

The colours are representative of the values of rho for each correlation. In LF group, MAP positively correlates with CPI (r=0.64, p=0.002) however in HF group, MAP positively correlates with TPR (r=0.69, p<0.0001) and TPRI (r=0.66, p=0.001). *MAP- Mean arterial pressure, SBP- Systolic blood pressure, DBP- Diastolic blood pressure, SV-Stroke Volume, SVI- Stroke Volume Index, CI-cardiac index, CPI- Cardiac Power Index, TPR- Total peripheral resistance, TPRI- Total peripheral resistance index.

Comparisons between the hourly means of haemodynamics demonstrate two findings. Firstly, the hourly means of MAP, CPI and TPRI were not different between LF and HF groups (Table 5-3). Secondly, the association between various haemodynamic variables changed differentially in the two groups. In the LF group, MAP was positively correlated with CPI in each hour of dialysis, but not with TPRI (Table 5-4**,** Figure 5-10a and 5-10b). In contrast in the HF group, MAP correlated with CPI in the first hour of dialysis only; but MAP did correlate with TPRI in each hour of dialysis (Table 5-4**,** Figure 5-10a and 5-10b).

Table 5-3: Comparisons of hourly haemodynamic data between Low frequency (LF) and High frequency (HF) groups

	Groups	**Hour 1**	**Hour 2**	**Hour 3**	**Hour 4**	**p value** (across the timeline)
MAP in mmHG	LF group	101.71 (18.22)	97.89 (19.46)	92.61 (22.06)	98.43 (19.96)	0.180
	HF group	98.34 (22.9)	94.35 (23.71)	95.43 (25.1)	93.59 (25.47)	0.760
	p value	0.285	0.451	0.99	0.811	
CPI in w/m2	LF group	0.763 (0.49)	0.657 (0.35)	0.751 (0.4)	0.623 (0.29)	0.026
	HF group	0.784 (0.37)	0.705 (0.25)	0.633 (0.41)	0.644 (0.35)	0.09
	p value	0.528	0.662	0.95	0.772	
TPRI in dynes·sec/cm5/m2	LF group	0.4164 (0.33)	0.4402 (0.34)	0.3971 (0.34)	0.4472 (0.34)	0.491
	HF group	0.3503 (0.31)	0.3838 (0.35)	0.4210 (0.42)	0.3937 (0.32)	0.029
	p value	0.181	0.437	0.678	0.473	

Heart Rate in beats per minute	LF group	77.06 (20.57)	77.86 (20.44)	82.35 (16.41)	82.03 (20.18)	0.468
	HF group	66.21 (17.95)	66.32 (15.63)	68.53 (15.55)	67.43 (22.6)	0.564
	p value	0.029	0.049	0.016	0.013	

HF group- participants with higher proportion of high frequency extrema point (EP) MAP changes, LF group- participants with higher proportion of low frequency EP MAP changes. MAP: Mean arterial pressure; CPI: Cardiac Power Index[2]; TPRI: Total peripheral resistance index. Median and interquartile range (IQR) are reported for non-parametric data.

Table 5-4: Correlations between Mean Arterial Pressure vs Cardiac Power Index and Mean Arterial Pressure vs Total Peripheral Resistance Index

	Groups	**Hour 1**	**Hour 2**	**Hour 3**	**Hour 4**
MAP vs CPI	LF group	0.50 (0.021)	0.43 (0.053)	0.59 (0.005)	0.74 (<0.0001)
	HF group	0.53 (0.012)	0.33 (0.135)	0.27 (0.246)	0.17 (0.471)
MAP vs TPRI	LF group	0.09 (0.687)	0.20 (0.382)	-0.04 (0.871)	-0.32 (0.153)
	HF group	0.63 (0.002)	0.60 (0.003)	0.59 (0.005)	0.53 (0.014)

MAP: Mean arterial pressure, CPI: Cardiac Power Index, TPRI: Total peripheral resistance index. HF group- participants with higher proportion of high frequency extrema point (EP) MAP changes; LF group- participants with higher proportion of low frequency EP MAP changes. Correlation coefficient (r) values are provided with p-values (in brackets)

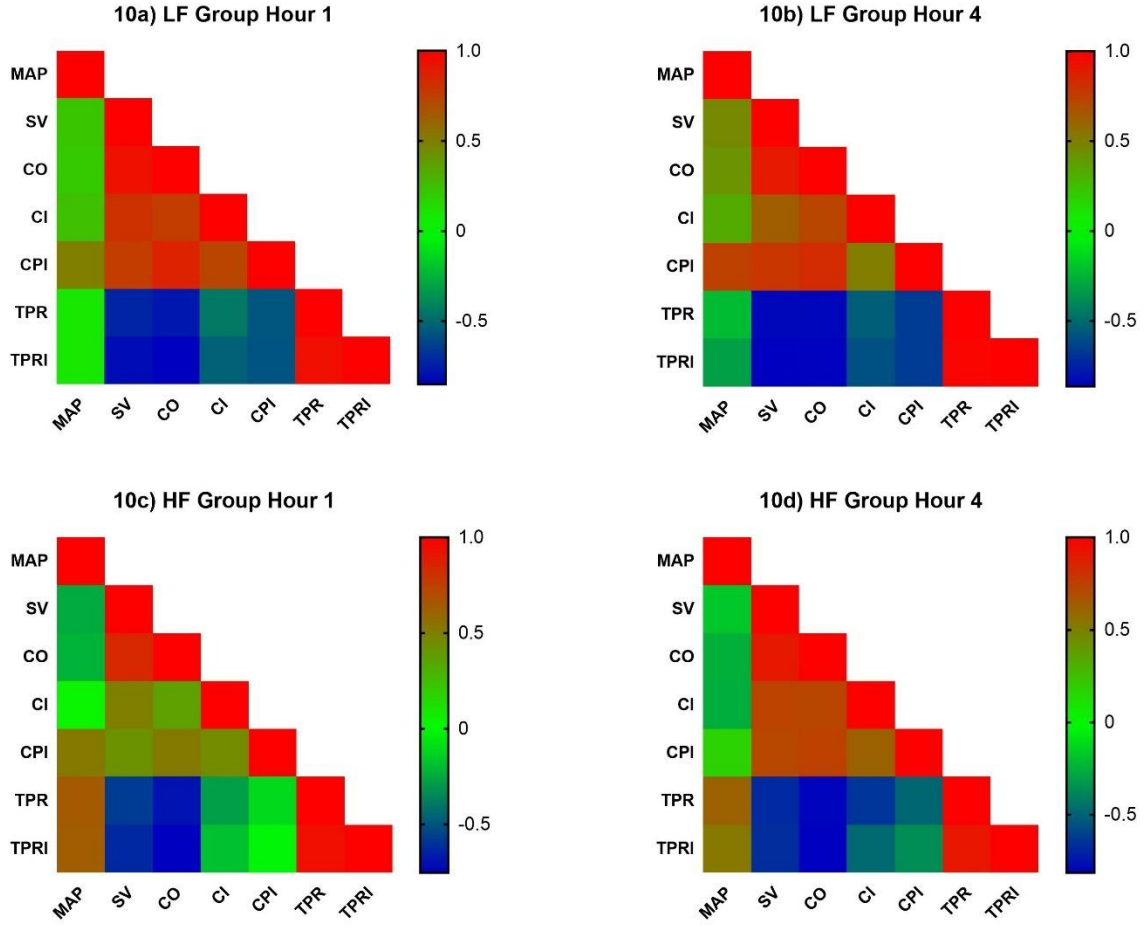


Figure 5-10: Correlations between average intradialytic variables in hour 1 and hour 4 of dialysis in the low frequency (LF) group (a, b) and high frequency group (c, d).

The colours are representative of the values of rho for each correlation. In the LF group, MAP was positively correlated with CPI in each hour of dialysis, but not with TPRI (Table 8-4). In contrast in the HF group, MAP correlated with CPI in the first hour of dialysis only; MAP then correlated with TPRI in each subsequent hour of dialysis (Table 8-4). *MAP- Mean arterial pressure, SBP- Systolic blood pressure, DBP- Diastolic blood pressure, SV- Stroke Volume, CI- cardiac index, CPI- Cardiac Power Index, TPR- Total peripheral resistance, TPRI- Total peripheral resistance index. *HF group- participants with higher proportion of high frequency extrema point (EP) MAP changes, LF group- participants with higher proportion of low frequency EP MAP changes.

We performed a sensitivity analysis using intradialytic SBP instead of intradialytic MAP. Correlations between intradialytic SBP, CPI and TPRI were similar to that of intradialytic MAP. In the LF group there was a strong positive correlation of SBP with

CPI (r=0.59, P=0.005) but not TPRI, and in the HF group SBP correlated with TPRI (r=0.71, P=<0.001) but not CPI (Figure 5-9).

5.4.4 Intradialytic Hypotension

The average proportion of BP measurements with SBP <90mmHg in the LF group was 0.74% (IQR 3.6%) vs 0.91% (IQR 2.35%) in HF group (p=0.145). The proportion of measured BP values 20mmHG below initial pre-dialysis SBP was 6.81% (IQR 15.17) and 14.56% (IQR 32.77%) in LF and HF groups respectively (p=0.884).

Section 5.5 Discussion

We have utilised Extrema Points frequency analysis to analyse continuous BP measurements during HD and have further developed the method by proposing a ratio of high to low EP frequency changes to categorise dialysis patients based on the proportion of BP variation that occurs on a beat-to-beat basis versus variation which happens more slowly over several cardiac cycles. We have demonstrated that lower versus higher HFC/LFC ratios were associated with differing haemodynamic responses and diverging trends of HFC/LFC ratios during the course of HD treatments.

BP variability has been described using several different methods and has been linked to adverse outcomes, including in HD populations. Shafi et al demonstrated that each standard deviation increase in BP variability was associated with increased risk of all-cause mortality (HR 1.18; 95% CI 1.13-1.22), cardiovascular mortality (HR 1.18; 95% CI 1.12-1.24) and first cardiovascular event (HR 1.11; 95% CI 1.07-1.15) (220). Similarly, Wang et al reported that every 1% increase in the coefficient of variation of pre-dialysis BP was associated with increased cardiovascular (HR 1.71; 95% CI 1.01-2.90) and all-cause mortality (HR 1.80; 95% CI 1.11-2.92) (233). In a study involving 103 HD patients with interdialytic ABPM (ambulatory BP monitoring) and average real variability (ARV) assessment, Feng et al reported that higher ARV was independently associated with higher cardiovascular mortality after adjustment for demographics and clinical factors (HR: 1.143; 95% CI 1.022-1.279) (222). However, a comparable study by Sarafidis et al involving 227 HD patients, after adjusting for other clinical and demographic factors, reported no significant association of higher ARV of interdialytic SBP with composite endpoint of all-cause mortality, non-fatal MI or non-fatal stroke (232)]. To our knowledge there are a limited number of studies examining intradialytic

BP variability. Flythe et al (234) studied intradialytic BP using absolute SBP spline curves (i.e. curve fitting as opposed to assuming a linear change in SBP) and demonstrated that greater ultrafiltration volume (UFV), older age and shorter dialysis vintage were associated with increased SBP variability. They also reported high SBP variability (more than the observed median in their study population) was associated with greater risk of all-cause mortality with HR 1.26, 95% CI 1.08-1.47, when compared to the patients with lower SBP variability (less than observed median) (223).

All the above-described methods assess the magnitude of BP variability. However, our method of EP analysis approaches BP variability by assessing *frequency* of BP change. Previous studies involving EP MAP frequencies have shown that higher frequency of BP variation is associated with ischaemic changes in the brain and interventions (cooled dialysate) that reduce frequency of BP variation are protective against this (115). This suggests that frequency of BP variation is an important determinant of organ perfusion. The lack of correlation between UFV and EP MAP frequencies in our analysis indicates that EP frequency analysis may be less dependent on external factors that may affect magnitude of BP change and may be more reflective of the physiological reserve of the individual. This is supported by insignificant intra-individual variability of median EP frequencies between the three monitored HD sessions. In addition, we demonstrated a strong correlation between EP MAP frequencies (frequency of variability) and ARV (magnitude of variation) of BP, suggesting greater frequency of variation is associated with greater magnitude of variation. Thus, interventions that reduce EP frequencies may help to address the magnitude of variability and we can speculate as to whether this will in turn lead to clinical benefit.

We have confirmed the intradialytic trends of EP MAP frequencies (reaching peak during the third hour of HD) and their associations with higher cardiac biomarkers (positive correlation with NT-pro BNP levels) as described in previous studies (225). Although there is ongoing debate about the appropriate cuff off values for BNP/NT-pro BNP in HD population, published literature supports their prognostic value at least at population level. A meta-analysis by Cheng et al (235) involving 27 studies with 8666 patients with ESRD reported that elevated BNP/NT-pro BNP were significantly associated with increased all-cause mortality [OR: 3.85 (95% CI: 3.11 to 4.75)], cardiovascular mortality [OR: 4.05 (95% CI: 2.53 to 6.84)] and cardiovascular events [OR: 7.02 (95% CI, 2.21 to 22.33)]. Thus, we may conclude that patients with cardiac disease (known or subclinical) tend to have higher frequency of BP variability.

We also demonstrate for the first time that there are distinct patterns in the hemodynamic responses to HD in those with low vs high HFC/LFC ratio. In the LF group, MAP appeared to be more dependent on cardiac function (stronger associations with CPI), without significant dependency on TPRI. This suggests that these patients have sufficient cardiac reserve to maintain BP during dialysis without the need for maximal vasoconstriction. In contrast, in the HF group, MAP was more dependent on TPRI. As the equation for CPI includes MAP values, it was therefore surprising to observe a loss of corelation of MAP and CPI in the HF group as dialysis progressed, and this may suggest a reduced cardiac reserve. As a result, participants in the HF group appeared to become dependent on TPRI for maintenance of BP. Comparable findings were demonstrated in a study of 54 HD patients by Levin et al. The authors described three different profiles of haemodynamic response during HD sessions with intradialytic hypotension episodes: (i) a reduction in CPI with little change in TPRI; (ii) a reduction in TPRI with little change in CPI; and (iii) reduction of

both CPI + TPRI (236)]. Demonstration of these different patterns of haemodynamic response to dialysis in our study suggests that EP frequency analysis may allow better assessment of an individual's physiological behaviour. This may ultimately lead to more individualised approaches to prevent or manage IDH.

Although we have demonstrated novel interesting relationships and differences in the intradialytic haemodynamic behaviours of the individuals, there are some limitations of our study. Firstly, the categorisation of the participants in our study was based on 4 hours of intradialytic haemodynamic data that is not feasible in the standard clinical setting. Given that HFC/LFC ratios adopted diverging patterns as dialysis progressed (figure 9), there is a potential to categorise based on a shorter period of haemodynamic monitoring during HD and this should be evaluated in future. Secondly, we included patients with a low incidence of IDH and were therefore unable to adequately assess possible associations between BP frequency patterns and IDH. Further studies that include more hypotension-prone patients are required to explore associations with IDH and other clinical outcomes. Finally, our study also highlights an important technological gap in the haemodynamic monitoring adopted for HD patients. Intermittent BP measures, the current standard of practice, are not sufficient to allow EP analysis, whilst the currently available non-invasive continuous BP monitoring methods are not practical for HD patients outside of a research setting. Methods to achieve less burdensome continuous intradialytic BP monitoring are in development (237).

In conclusion, EP frequency analysis of continuous BP monitoring during dialysis allows assessment of BP variation, and individuals can be categorised into having patterns of low or high frequency variation. This may provide information on patients'

physiological responses to haemodynamic stress during HD, which seems to separate into two categories. BP variation and other HD treatment-related risk factors might have long-term implications on end-organ perfusion. Along with the characterisation of the haemodynamic responses, understanding the extent of this end-organ damage in this population might allow us to under the magnitude of the clinical problem and persuade us to develop individualised treatment strategies to mitigate dialysis-induced ischaemic injury.

Chapter 6 Comparison of structural brain changes in haemodialysis population to healthy controls

Section 6.1 Abstract

6.1.1 Introduction

Ischaemic end-organ damage during haemodialysis (HD) is a significant problem and leads to functional/structural deterioration in the long term. We compared the structural morphology of the brain in a prevalent HD group compared to age-matched healthy controls using magnetic resonance imaging (MRI).

6.1.2 Methods

Structural 1mm isotropic T1-weighted magnetization-prepared rapid gradient echo (MPRAGE) MR images were acquired on a 3T Phillips Ingenia scanner in prevalent HD patients (n = 10) and age/gender-matched healthy controls (HCs) (n = 10). HD patients were scanned 1 hour prior to their scheduled dialysis session. All participants completed the Montreal cognitive assessment (MoCA) and trail making tests (TMT) A & B on the day of their MRI scan. Voxel-based morphometry analysis was performed using Statistical Parametric Mapping software (SPM12). Images were registered to the Montreal Neurological Institute (MNI) template and segmented into grey matter volume (GMV), white matter volume (WMV), and cerebrospinal fluid (CSF) (whole-brain volumetrics). A general linear model of HD patients and HCs, adjusted for total intracranial volume (TIV) and age, was interrogated to assess differences between the groups using a voxel-wise two-sample t-test ($p<0.001$, uncorrected). Multiple

regression was used to evaluate the effect of age and dialysis vintage (DV) in the HD group ($p<0.001$, uncorrected).

6.1.3 Results

Median age of the HD group (HDs) was 55 (22) vs. 59.6 (18) years in the HCs ($p=0.791$). In the HD group, DV was 19.8 months (IQR 55.8).

The GMV/TIV ratio was lower in the HDs (HD group: 0.384 ± 0.039 vs HC group: 0.421 ± 0.032; $p= 0.026$), as was the WMV/TIV ratio (HD group: 0.346 ± 0.032 vs HC group: 0.372 ± 0.012; $p= 0.031$), whilst the CSF/TIV ratio was higher (HD group: 0.270 ± 0.057 vs HC group: 0.206 ± 0.036; $p= 0.007$). Correlation of these parameters with age demonstrated an accelerated ageing pattern, more so in WMV/TIV ratio.

Voxel-wise analysis substantiated the findings of the lower GMV and WMV, which were widespread affecting several regions of interest (ROI) in the HDs. Furthermore, regional GMV and WMV correlated negatively with age and DV.

6.1.4 Conclusion

There were significant alternations in the structural brain morphology in HD patients compared to HCs. The greater decline in the whole-brain volumetric and the voxel-based morphometric results in the HD group supports an accelerated ageing phenomenon in this group. Further studies linking these morphological findings with diffusion tensor imaging and perfusion studies will improve our understanding of the pathogenesis, enabling us to develop interventions to reduce the burden of the changes.

Section 6.2 Introduction

Prevalence of cognitive impairment is reported to range from 6.6-87% in the haemodialysis (HD) population depending on the type of cognitive assessments used (95, 96, 238). Initiation of HD has been associated with a rapid fall in cognitive and functional status. The presence of cognitive impairment in this population has been linked to increased dependency, hospitalisation rates (239), mortality (239-242), and dialysis withdrawal (241). Despite these issues, cognitive impairment remains under-diagnosed in this population group. In large retrospective cohort studies, the prevalence of dementia in end-stage kidney failure (ESKF) patients was reported to be as low as 3%-4% (240, 241).

Various structural changes have been reported in the HD population, including cerebral atrophy (101, 102), cerebral microbleeds (CMBs) (103), and silent infarcts (243). High prevalence of traditional risk factors in this population like hypertension, vascular disease, atrial fibrillation, diabetes, and other ESKF-related factors like inflammation, oxidative stress, uraemia, and anaemia contribute to the neurological sequelae. Moreover, the prevalence of adverse neurological consequences were reported to be higher in ESKF patients receiving HD compared to those managed conservatively (244) and with peritoneal dialysis (PD) (245), which raises the possibility of the role of HD-related risk factors. HD-induced circulatory stress may contribute to recurrent temporary ischaemic insults to the brain. This phenomenon is supported by a one-year follow-up study by Eldehni et al. These authors demonstrated less cortical white matter changes in patients who were randomised to cool dialysis to reduce intradialytic hypotension (115). In a study by Findlay et al., a decline in the cerebral blood flow during HD was shown to correlate with intradialytic cognitive

decline and was associated with cognitive dysfunction plus worsening white matter changes in the brain at one-year follow-up (114). Furthermore, studies demonstrating an acute reduction in cognition during a dialysis session (246) and independent association of dialysis vintage with cognitive decline (247, 248) support adverse implications of HD on neurological function.

A better understanding of the prevalent structural changes in this population group will enable us to understand the magnitude of the problem and the potential consequences. Hence, the aims of this chapter are 1) To explore the differences in the grey matter volume (GMV), white matter volume (WMV), and cerebrospinal fluid volumes (CSF) between HD group and healthy controls (HC) using whole-brain volumetric and voxel-based morphometric analysis. 2) To correlate these changes with cognitive scores and dialysis vintage.

Section 6.3 Methods

6.3.1 Basic protocol and participant recruitment

Stable and chronic HD patients who had been on dialysis for more than 90 days were recruited into this study. HD patients who had an active infection or malignant disease, were unstable on dialysis in the four weeks prior to recruitment, had significant heart failure (NYHA class IV), unwilling to provide informed written consent, or had any contraindication for MRI scan were excluded. Structural MR images of the brain were acquired pre-dialysis for all the participants as a part of our randomised controlled study described in the next chapter.

HCs were recruited from an existing participant database in the renal research unit and the general public advertised through fliers in the Renal Department and University. Volunteers who met the study inclusion criteria were sent an invitation letter and a patient information sheet. HCs were age and gender-matched with the HD patients. Potential healthy volunteers who were pregnant, breastfeeding, or had significant comorbidities (heart disease, kidney disease, neurodegenerative diseases, diseases that might affect brain morphometry including multiple sclerosis, epilepsy, stroke, diabetes, Parkinson's disease; depression, subjective memory impairment) were excluded from the study. All HD participants and HCs gave written informed consent before recruitment.

6.3.2 Ethical approval

Approvals were granted by the National Research Ethics Service [NRES] Committee East Midlands [REC reference 17/EM/0235]. Nottingham University Research Ethics Committee approved HC Study in February 2019.

6.3.3 Cognitive assessments and blood tests

Montreal Cognitive Assessment (MoCA), trail making test A (TMT-A) and B (TMT-B) were used to assess cognition before the scheduled MRI scans.

HD group had pre-dialysis blood test to check full blood count, sodium, potassium, urea, creatinine. HC group had the same blood tests, and in addition, they had urine dipstick analysis to rule out significant haemato-proteinuria, i.e., to rule out significant renal disease.

6.3.4 MR Image acquisition and analysis

Acquisition of the Structural MR images with T1-weighted magnetization-prepared rapid gradient echo [MPRAGE] sequence, the processing of this data, and the statistical analysis were described in detail in the MR methods section in Chapter 4.

6.3.5 Statistical analysis

Continuous variables are expressed as mean ± SD for normally distributed variables, median and interquartile range for non-parametric data. Pearson's or Spearman's correlation were used based on the distribution of the data. Likewise, based on the distribution, two-sample T-test or Mann-Whitney U tests were used to compare the groups. Statistical analysis was performed using IBM SPSS (Version 24). A $p< 0.05$ was considered significant. Statistical analysis of the MRI data was performed using Matlab program as described in Chapter 4.

Section 6.4 Results

6.4.1 Recruitment

The consort diagram (Figure 6-1) below shows the number of consented, recruited and included participants in the brain structural analysis.

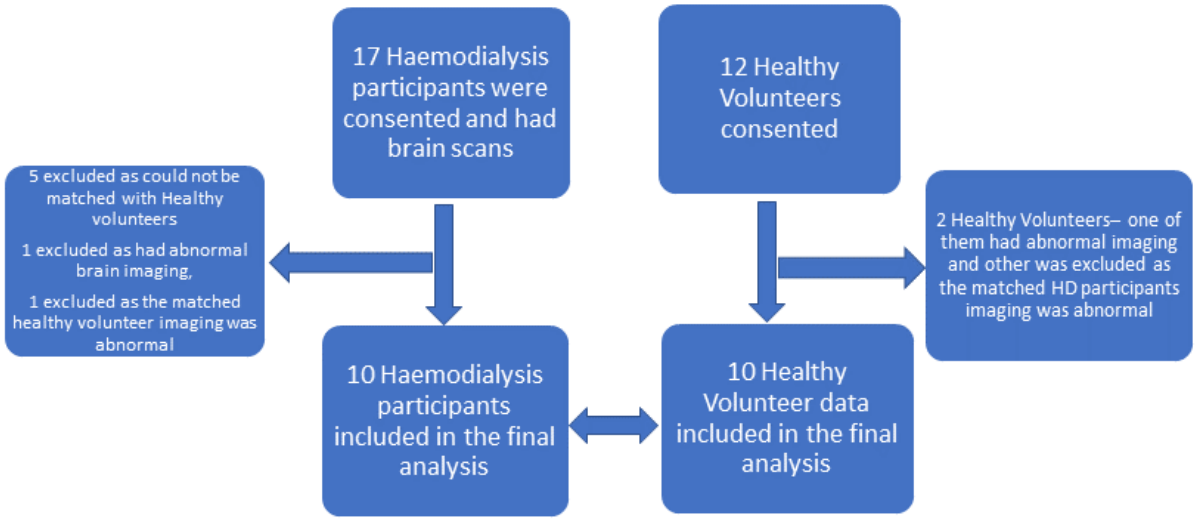


Figure 6-1: Consort diagram illustrating the number of participants recruited, scanned and included in the final analysis

6.4.2 Subject demographics

10 HD patients were matched 1: 1 with HCs on age and gender for the structural assessment of the brain. Demographics and the clinical parameters for the HD and HC groups are outlined in Table 6-1 and cognitive scores in Table 6-2. Dialysis vintage for the HD group was 19.82 (55.8) months.

Table 6-1: Demographics and clinical parameters

Parameters	Haemodialysis (HD) group	Healthy Control (HC) group	P value
Age (years)	55(22)	59.6(18)	0.791
Total Body Water (Litres)	41.2(7.8)	45.95(9.4)	0.131
Extracellular Water (Litres)	19.75(3.6)	20.6(5.7)	0.406
Intracellular Water (Litres)	21.9(4.4)	24.75(4.5)	0.096
Excess free body fluid (Litres)	1.25(1.4)	0.2(2.1)	0.307
Haemoglobin (grams/litre)	114(18.5)	145(15.5)	0.001
White Cell Count (x 10^9/L)	7.195(3.14)	6.35(0.68)	0.07
Platelets (x 10^9/L)	267.5(106.75)	214.5(52.25)	0.427
Mean Corpuscular Volume fl*	92.25(4.83)	87.75(6.35)	0.034
Haematocrit	0.35(0.06)	0.416(0.05)	0.002
Sodium (mmol/L)	141(2)	142(4.25)	0.53
Potassium (mmol/L)	5.05(0.78)	4.05(0.78)	0.001
Urea (mmol/L)	17.5(8.25)	4.85(1.9)	0.000
Creatinine (µmol/L)	715(264)	83.5(17.25)	0.000

Mean ± SD or Median (interquartile range) are provided. fl=femtolitres.

Table 6-2: Cognitive scores in HD and HC groups

	HD group (n=9)	HC group (n=10)	P value
MoCA	27.56 ± 1.01	28.7 ± 0.95	0.021
TMT-A	27.34 ± 8.7	23.19 ± 9.92	0.349
TMT-B	60.78 ± 24.42	49.4 ± 16.33	0.244

Mean ± SD are provided for Montreal Cognitive assessment (MoCA), trail making A test score (TMT-A) and trail making B test score (TMT-B) with p values for independent 2 sample t-tests.

6.4.3 Whole-brain volumetry between HD patients and HCs

Table 6-3 provides the whole brain volumetry data for HD and HC groups. GMV and WMV were significantly lower in the HD group compared to the HC group.

Table 6-4 provides the whole brain volumetry of HD and HC groups normalised to Total intracranial volume (TIV). Normalised GMV (GMV/TIV ratio) and WMV (WMV/TIV ratio) were lower in the HD group, and normalised CSF volume (CSF/TIV ratio) was higher in the HD group.

Table 6-3: Whole-brain Volumetrics in HD and HC groups

Whole brain morphometric parameter	**HD [n=10]**	**HC [n=10]**	**Significance**
Total intracranial volume (TIV) cm^3	1404 ± 142	1466 ± 141	0.34
Grey matter volume (GMV) cm^3	532 ± 42	613 ± 51	0.01
White matter volume (WMV) cm^3	486 ± 64	545 ± 56	0.042
Cerebral spinal fluid (CSF) cm^3	383 ± 104	304 ± 73	0.066
Cortical Thickness (CT) in mm	2.66 (0.48)	2.53 (0.34)	0.089

Values presented as Mean ±SD or Median (IQR)

Table 6-4: Whole-Brain Volumetrics normalised to Total Intracranial Volume for HD and HC groups

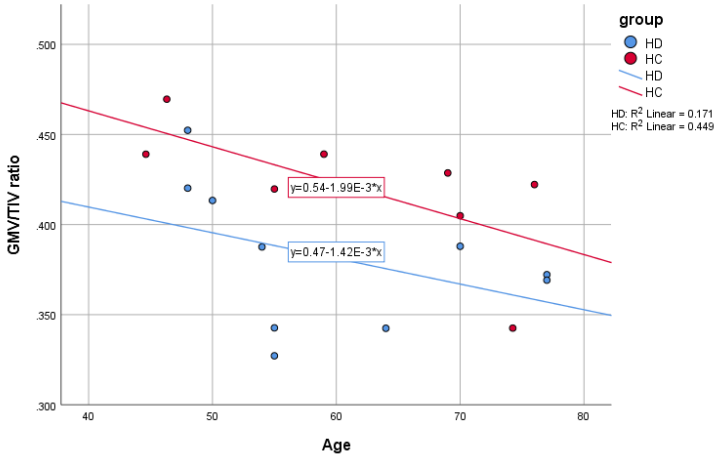
Whole-brain morphometric parameter adjusted to brain size	**HD group [n=10]**	**HC group [n=10]**	**Significance**
GMV/TIV ratio	0.382 ± 0.039	0.421 ± 0.032	0.026
WMV/ TIV ratio	0.346 ± 0.032	0.372 ± 0.012	0.031
CSF/TIV ratio	0.270 ± 0.057	0.206 ± 0.036	0.007

Values presented as mean ± SD.

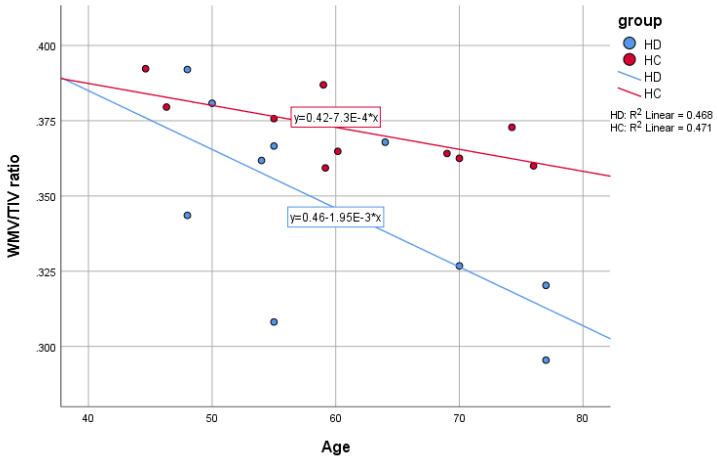
6.4.3.1 Correlation of Whole-brain volumetry data of HD patients and HCs with age

As demonstrated in the below Figure 6-2a-d, age correlated negatively with GMV/TIV, WMV/TIV ratios, and Cortical thickness (CT), but positively with CSF/TIV ratio in both HD and HC groups. However, this negative correlation appears to be more pronounced in HD, especially in WMV/TIV ratio (Figure 6-2b). The corresponding correlation and p values are provided in Table 6-5.

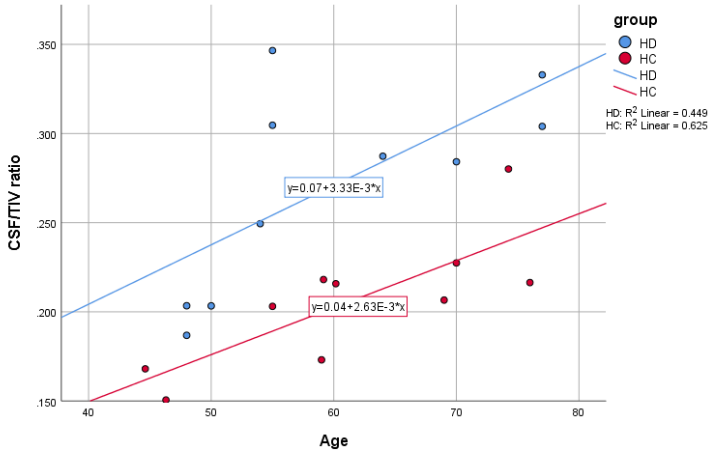
2a)



2b)



2c)



2d)

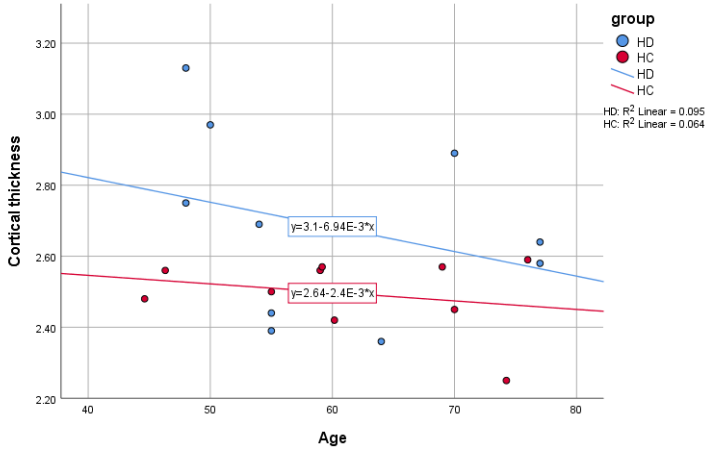


Figure 6-2: Correlations of Whole-brain volumetry data with Age in HD (blue fit line and dots representing individual values) and HC (red fit line and dots representing individual values) groups.

The X-axis represents Age in years in all the graphs. Y-axis represents Grey matter volume (GMV)/ Total intracranial volume (TIV) ratio in Figure 3a, White matter volume (WMV)/TIV ratio in Figure 3b, Cerebrospinal fluid volume (CSF)/TIV ratio in Figure 3c, and Cortical thickness (millimetres) in Figure 3d respectively.

Table 6-5: Correlation of Whole-Brain Volumetrics with Age in HD and HC groups

	HD group [n=10]		HC group [n=10]	
	r-value	**p-value**	**r-value**	**p-value**
GMV/TIV ratio	-0.414	0.234	-0.670	0.034
WMV/ TIV ratio	-0.684	0.029	-0.686	0.029
CSF/TIV ratio	0.670	0.034	0.790	0.006
Cortical thickness	-0.308	0.386	-0.252	0.482

6.4.3.2 Correlation of Whole-brain volumetry data in HD group with Dialysis Vintage

DV correlated negatively with WMV/TIV ratio (r= -0.841, p= 0.002) and positively with CSF/TIV ratio (r= 0.766, p= 0.008). There was no significant correlation of DV with GMV/TIV (r= -0.438, p= 0.205) and cortical thickness (r= -0.362, p= 0.304).

6.4.3.3 Correlation of Cognitive scores with Age and Dialysis Vintage

Age correlated positively with TMT-B score in HCs (r= 0.853, p= 0.002) and with TMT-A score in HD group (r= 0.733, p= 0.025).

We also noted positive correlation of DV with TMT-A (r= 0.706, p= 0.034) and TMT-B (r= 0.683, p= 0.042) but not with MoCA (r= -.167, p= 0.668).

6.4.3.4 Correlation of Whole-brain volumetry data of HD and HC groups with Cognitive scores

We assessed correlations between the MoCA, TMT-A, TMT-B scores versus GMV/TIV, WMV/TIV, CSF/TIV, and cortical thickness. These are provided in Table 6-6.

In HC group, there was a significant negative correlation between GMV/TIV with TMT-A (r= -.0742, p=0.014) and TMT-B (r=-0.721, p=0.019); WMV/TIV with TMT-B (r=-0.732, p=0.016). There was also positive correlation between CSF/TIV ratio with TMT-A (r=0.694, p=0.026) and TMT-B (r= 0.840, p=0.002) in HC group.

In HD group, CSF/TIV ratio positively correlated with TMT-A (r=0.741, p=0.022) and there was a trend towards significance in negative correlation of WMV/TIV with TMT-A (r= -0.656, p=0.055).

Table 6-6: Correlation of Whole Brain Morphometrics with Cognitive scores in HD and HC groups

		MoCA		TMT-A		TMT-B	
Group		**r value**	**p value**	**r value**	**p value**	**r value**	**p value**
Haemodialysis (HD)	GMV/TIV ratio	-0.178	0.646	-0.594	0.092	-0.222	0.566
	WMV/TIV ratio	0.173	0.656	-0.656	0.055	-0.322	0.398
	CSF/TIV ratio	0.021	0.958	0.741	**0.022**	0.319	0.403
	Cortical thickness	-0.121	0.756	-0.396	0.292	-0.078	0.842
Healthy Controls (HC)	GMV/TIV ratio	0.405	0.246	-0.742	**0.014**	-0.721	**0.019**
	WMV/TIV ratio	-0.590	0.072	-0.166	0.646	-0.732	**0.016**
	CSF/TIV ratio	-0.170	0.640	0.694	0.026	0.840	**0.002**
	Cortical thickness	0.526	0.119	-0.626	0.053	-0.331	0.350

The above table provides correlation and p values of the cognitive test (Montreal Cognitive score, MoCA; Trail making A, TMT-A; Trail making B, TMT-B) versus whole-brain volumetrics (GMV/TIV, WMV/TIV, CSF/TIV, and Cortical thickness) for HD and HC groups.

6.4.4 Voxel-Based Morphometric Analysis of HD and HC groups

6.4.4.1 Assessing Grey Matter Volume changes in the HD group

6.4.4.1.1 Comparison of Grey Matter Volume changes between HD and HC groups

Figure 6-3 shows the result of the VBM analysis to assess regional GMV differences between the HD patients and HCs, with the demonstration of a region of GMV which was reduced in HD patients compared to HCs for illustration (one area for illustration purpose; right middle frontal gyrus). Analysis was performed using age and TIV as covariates-of-no-interest in the statistical model. Compared to the HC group, regional GMV in the HD patients was lower in several regions (ROI's), as outlined in Table 6-7. No brain regions showed significantly greater GMV in HD patients compared with HCs.

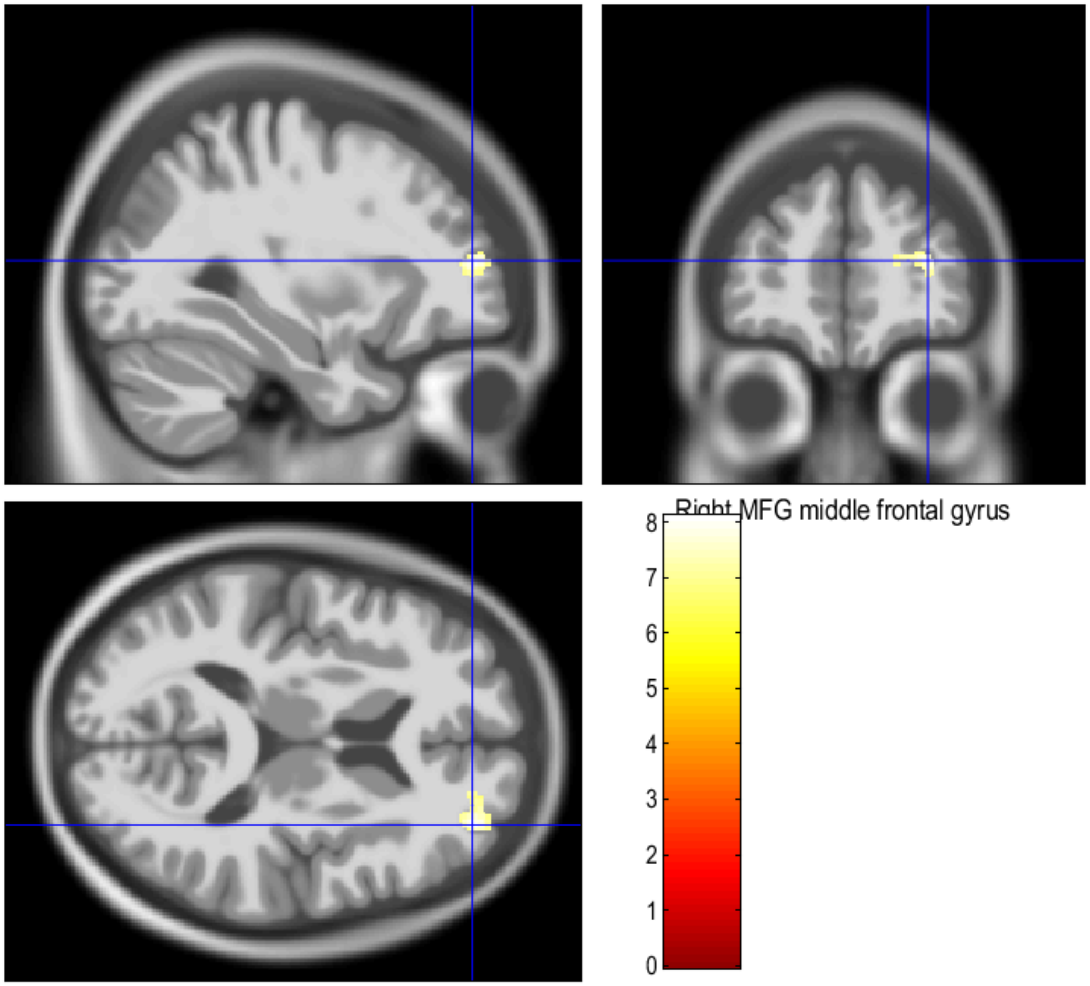


Figure 6-3: Regional GMV was lower in HD patients compared to HCs.

The SPM display of a T1-weighted normalised anatomical image (P< 0.001, uncorrected). The colour bar represents the range of the T value. Data of HD <HC is shown using age and TIV as covariates of no interest. Table 6 provides those areas (ROI) which showed GMV loss in HD patients compared with HCs when using age and TIV as covariates of no interest.

Table 6-7: Results of GMV regions in HD group that were lower when compared to HC group

Anatomical regions	T value (voxel level)	MNI coordinates			Cluster size (k)
		x	y	z	

Left Angular Gyrus	6.070544	-34.5	-66	30	905
Left Precuneus	5.445656	-16.5	-55.5	37.5	1159
Left Fusiform Gyrus	5.401755	-36	-36	-22.5	2068
Left Middle Frontal Gyrus	5.175507	-33	54	-6	501
Left Lingual Gyrus	5.107005	-21	-46.5	-3	228
Left Occipital Fusiform Gyrus	4.992358	-34.5	-66	-9	566
Left Supplementary Motor Cortex	4.890671	-10.5	-12	49.5	432
Left Superior Parietal Lobule	4.803156	-28.5	-39	45	145
Right Precentral Gyrus	4.740642	33	-12	49.5	114
Left Ventral DC/Thalamus	4.689363	-21	-16.5	-6	293
Right Posterior Cingulate Gyrus	4.620747	10.5	-33	37.5	584
Right Precuneus	4.547338	18	-54	37.5	74
Right Ventral DC/Thalamus	4.496895	18	-10.5	-7.5	77
Right Angular Gyrus	4.252011	43.5	-58.5	46.5	130
Right Middle Frontal Gyrus	3.955204	33	15	48	74
Left Putamen	3.949248	-22.5	18	-10.5	13
Left Posterior Insula	3.853592	-39	-19.5	-1.5	57
Left Middle Temporal Gyrus	3.821904	-58.5	-18	-13.5	14
Left Superior Frontal Gyrus	3.798218	-10.5	46.5	34.5	5

All the regions of Grey matter with lower volumes in HD patients compared to HC using age and TIV as covariates of no interest (p <0.001, uncorrected) are outlined above. Montreal Neurological Institute (MNI) coordinates provide the location of the cluster in the brain. T value donates the statistical value of the two-sample t-test by contrasting HD patients to HC's.

6.4.4.1.2 Effect of ageing on Regional Grey Matter Volume in HD and comparison to HC group

The effect of ageing was assessed on regional GMV in HD patients and compared with the HC group. Grey matter ROI's negatively associated with age in the HD group are outlined in Table 6-8. A more substantial effect of ageing was observed in the HD patients than HC group in the affected ROIs as demonstrated in Figure 6-4.

No brain regions in the HD or HC group showed a higher GMV with increasing age.

Table 6-8: GMV ROI's in the HD group which correlated negatively with Age

Anatomical regions	T value (voxel level)	MNI coordinates			Cluster size (k)
		x	y	z	
Right Precuneus	8.241986	16.5	-58.5	25.5	204
Right Inferior Occipital Gyrus	7.593189	40.5	-78	-6	39
Left Middle Frontal Gyrus	6.956195	-33	28.5	22.5	19
Left Post Central Gyrus	5.53362	-52.5	-18	24	47
Left Fusiform Gyrus	5.251925	-42	-42	-24	10
Right Supramarginal Gyrus	5.109307	45	-43.5	33	17

The above table outlines all the brain regions demonstrating a negative correlation of GMV (Grey Matter Volume) with age, P<0.001, uncorrected. Montreal Neurological Institute (MNI) coordinates provide the location of the cluster in the brain. Cluster size provides the number of voxels in each cluster.

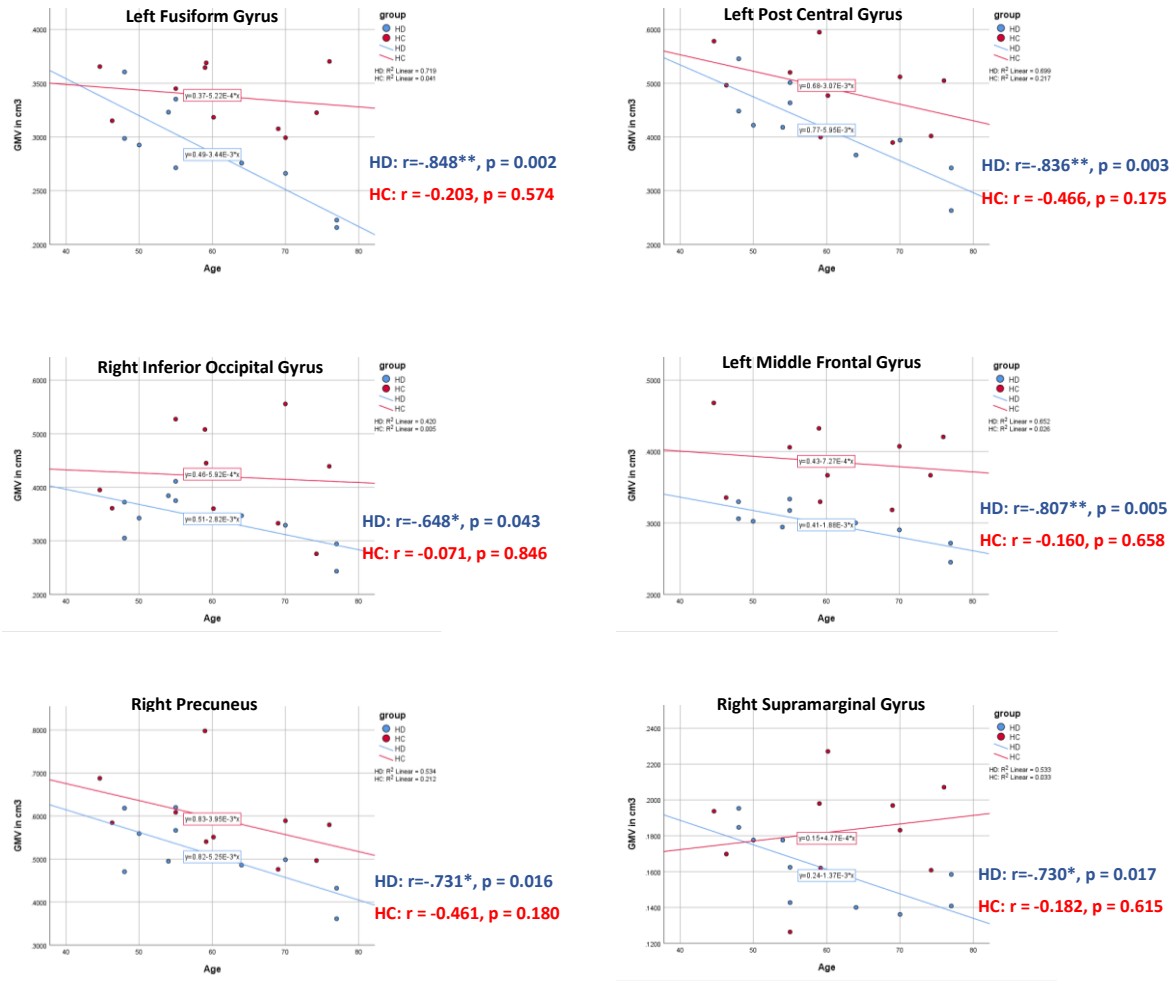


Figure 6-4: Correlation plots of regional GMV with age in HD and HC groups.

HD patients exhibited a more substantial effect of ageing (as shown by the correlation coefficient r values and p values) compared with HC group.

6.4.4.1.3 Effect of Dialysis Vintage on regional Grey Matter Volume in HD group

Several regions of GMV negatively correlated with DV on VBM analysis, adjusted for age and TIV. As outlined in Table 6-9, they include

1) Left- calcarine cortex, supplementary motor cortex, occipital fusiform gyrus, precentral gyrus, cuneus, middle frontal gyrus.

2) Right- middle frontal gyrus, middle frontal gyrus.

There were no regions of GMV that positively correlated with DV.

Table 6-9: GMV regions in HD group that negatively correlated with Dialysis Vintage

Anatomical regions	Uncorrected p	T value	MNI coordinates			Cluster size (K)
	(Peak level)	(Voxel level)	x	y	z	
Left Calcarine Cortex	0.0000	17.3875	-9	-81	9	63
Left Supplementary Cortex	0.0000	10.5930	0	-16.5	51	676
Left Occipital Fusiform Gyrus	0.0002	7.2506	-21	-90	-18	155
Right Middle Frontal Gyrus	0.0002	7.1872	30	3	60	91
Left Precentral Gyrus	0.0002	6.9830	-51	3	25.5	117
Right Middle Frontal Gyrus	0.0003	6.6063	40.5	10.5	57	31
Left Calcarine Cortex	0.0005	6.0102	-13.5	-84	-1.5	8
Left Cuneus	0.0006	5.6856	-3	-88.5	22.5	25
Left Middle Frontal gyrus	0.0006	5.6658	-33	21	36	22

All the regions of Grey matter volume (GMV) which correlated negatively with Dialysis Vintage in HD patients using age and TIV as covariates of no interest (p <0.001, uncorrected) are outlined in the above table. Montreal Neurological Institute (MNI) coordinates provides the location of the cluster in the brain. Cluster size provides information about the number of voxels in each cluster.

6.4.4.2 Assessing white matter volume changes in HD

6.4.4.2.1 Comparison of white matter volume between HD and HC groups

VBM analysis was performed to assess WMV differences between the HD patients and HCs, adjusted to age and TIV. As demonstrated in Figure 6-5, several regions of white matter were lower than HCs. Table 6-10 provides the details of the affected areas.

No brain regions showed significantly greater WMV in HD patients compared with the HC group.

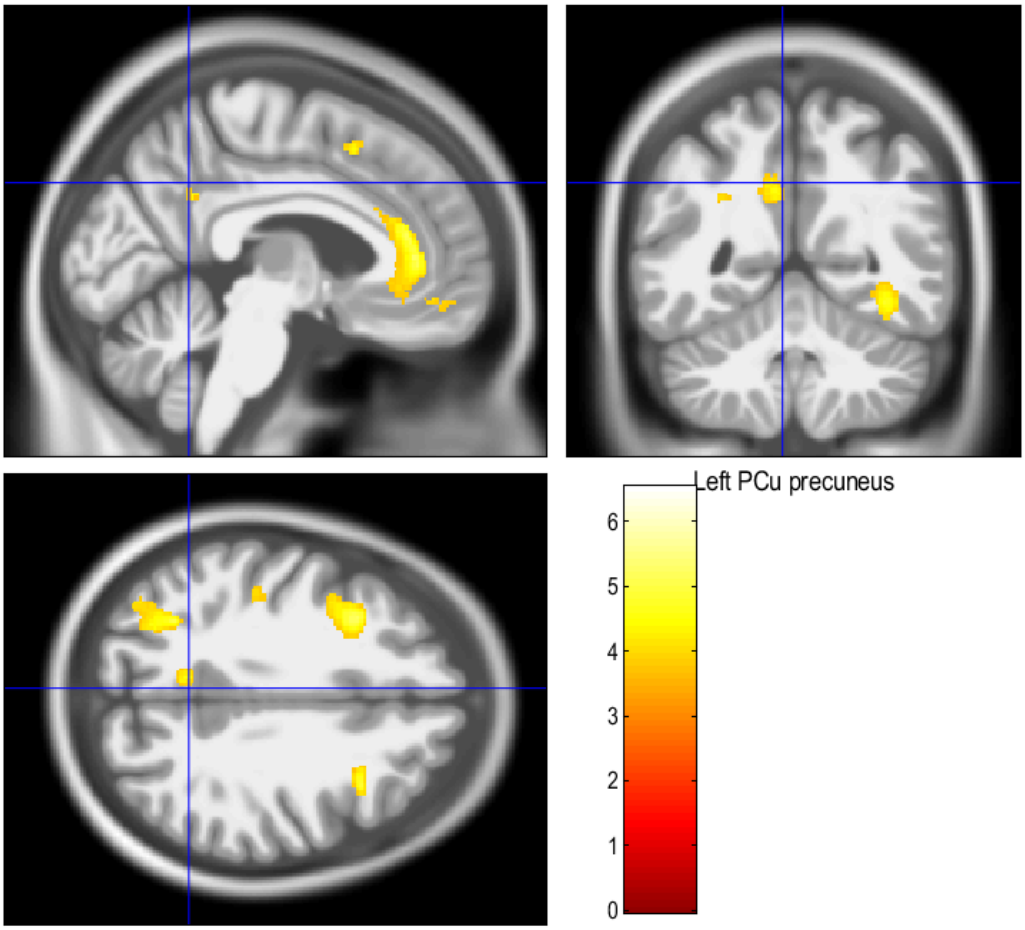


Figure 6-5: Illustrates the result of VBM analysis to assess WMV differences between HD patients and HC group.

Image displays regions (highlighting Left precuneus) where WMV in HD was less than HC, using age and TIV as covariates of no interest. Table 9 provides the regions that showed WMV loss in HD patients compared with HC group when using age and TIV as covariates of no interest.

Table 6-10: Comparison of WMV between HD and HC groups: Regions of the brain with less WMV in HD groups compared to HCs

Anatomical regions	T value	MNI coordinates			Cluster size (k)
	(voxel level)	x	y	Z	
Left Angular Gyrus	6.508	-31.5	-57	31.5	961
Left anterior cingulate gyrus	5.538	-1.5	36	15	9164
Right middle temporal gyrus	5.502	51	-34.5	-3	1088

Left middle temporal gyrus	5.373	-33	15	39	678
Left middle temporal gyrus	4.852	-34.5	31.5	28.5	174
Left middle temporal gyrus	4.825	-54	-33	-13.5	119
Left precuneus	4.821	-7.5	-51	33	169
Right cerebral white matter	4.734	34.5	16.5	37.5	108
Left supplementary motor cortex	4.518	-3	12	51	60
Left postcentral gyrus	4.353	-43.5	-27	40.5	69
Right middle temporal gyrus	4.259	52.5	-3	-19.5	95
Right caudate	3.973	12	9	10.5	56
Right supplementary motor cortex	3.826	6	10.5	43.5	8

All the regions of White matter volume (WMV) which were lower in HD patients when compared to HC using age and TIV as covariates of no interest (p <0.001, uncorrected) are outlined above. Montreal Neurological Institute (MNI) coordinates provides the location of the cluster in the brain. T value donates the statistical value of the two-sample t-test by contrasting HD patients to HC's.

6.4.4.2.2 Effect of Age on White matter volume in HD patients and comparison to HC group

The effect of ageing was assessed on WMV in HD patients and compared with HCs using VBM analysis with TIV as covariate of no interest. Regions of white matter (ROI's) that correlated negatively with age in the HD group are summarised in

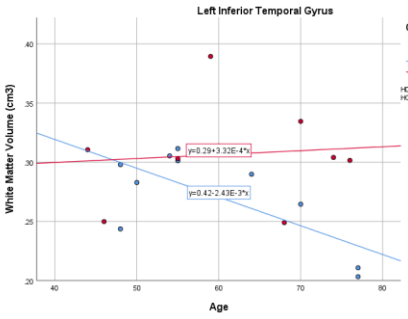
Table 6-11. In the affected ROI's, a more substantial effect of ageing was observed in the HD group than in the HC groups, as demonstrated in Figure 6-6.

No brain regions in the HD or HC group showed a greater WMV with increasing age.

Table 6-11: The effect of Age on WMV in HD group

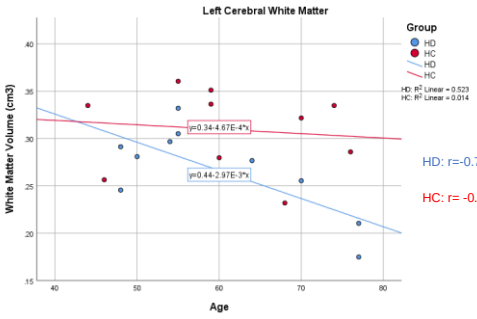
Anatomical regions	T value	MNI coordinates			Cluster size (k)
	(Voxel level)	x	y	z	
Left inferior temporal gyrus	6.340365	-40.5	-12	-28.5	108
Right fusiform gyrus	5.605501	40.5	-13.5	-27	46
Left inferior temporal gyrus	5.390348	-48	-28.5	-24	20
Right fusiform gyrus	8.173069	40.5	-36	-18	272
Right superior temporal gyrus	5.347282	52.5	-6	-16.5	17
Left middle temporal gyrus	5.555498	-57	-31.5	-13.5	5
Left superior temporal gyrus	5.551746	-55.5	-6	-13.5	5
Right medial frontal cortex	4.926502	6	52.5	-12	10
Right caudate	5.003953	12	24	-4.5	8
Right anterior cingulate gyrus	4.962833	13.5	39	3	8
Right middle frontal gyrus	6.926549	37.5	4.5	40.5	99
Right precuneus	5.089996	6	-57	37.5	17
Left superior frontal gyrus	6.818759	-9	33	48	160
Left middle frontal gyrus	6.91388	-24	13.5	51	27

The above table outlines the brain regions demonstrating a negative correlation of WMV with age, P<0.001, uncorrected. Montreal Neurological Institute (MNI) coordinates provide the location of the cluster in the brain. Cluster size provides the number of voxels in each cluster.



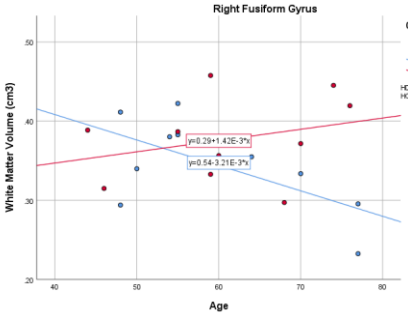
HD: r=-0.703; p=0.023

HC: r= 0.091; p=0.802



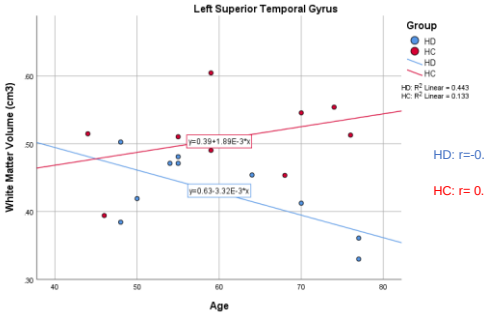
HD: r=-0.724; p=0.018

HC: r= -0.119; p=0.744



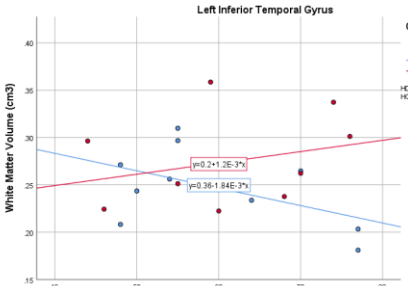
HD: r=-0.623; p=0.054

HC: r= 0.291; p=0.415



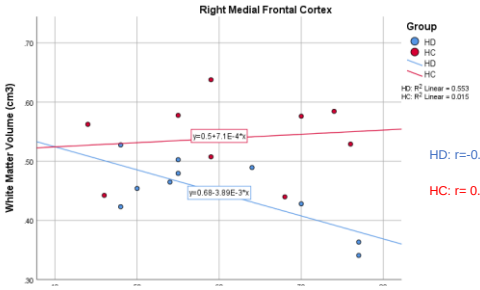
HD: r=-0.666; p=0.036

HC: r= 0.365; p=0.300



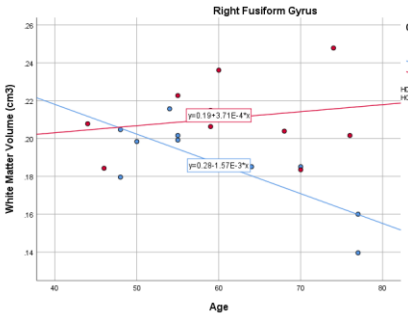
HD: r=-0.507; p=0.134

HC: r= 0.279; p=0.435



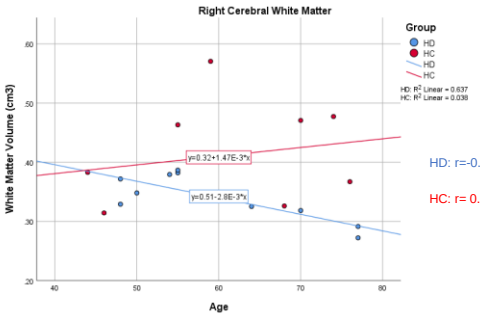
HD: r=-0.744; p=0.014

HC: r= 0.124; p=0.733



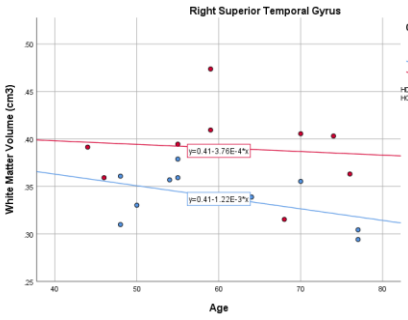
HD: r=-0.786; p=0.007

HC: r= 0.199; p=0.582



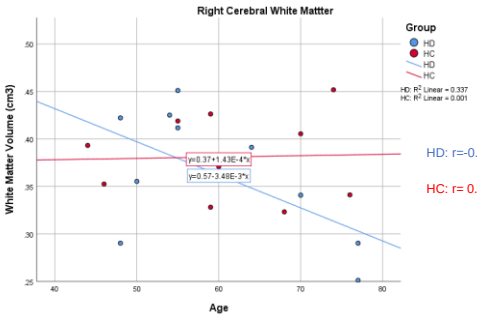
HD: r=-0.798; p=0.006

HC: r= 0.196; p=0.588



HD: r=-0.490; p=0.150

HC: r= -0.101; p=0.782



HD: r=-0.581; p=0.078

HC: r= 0.035; p=0.923

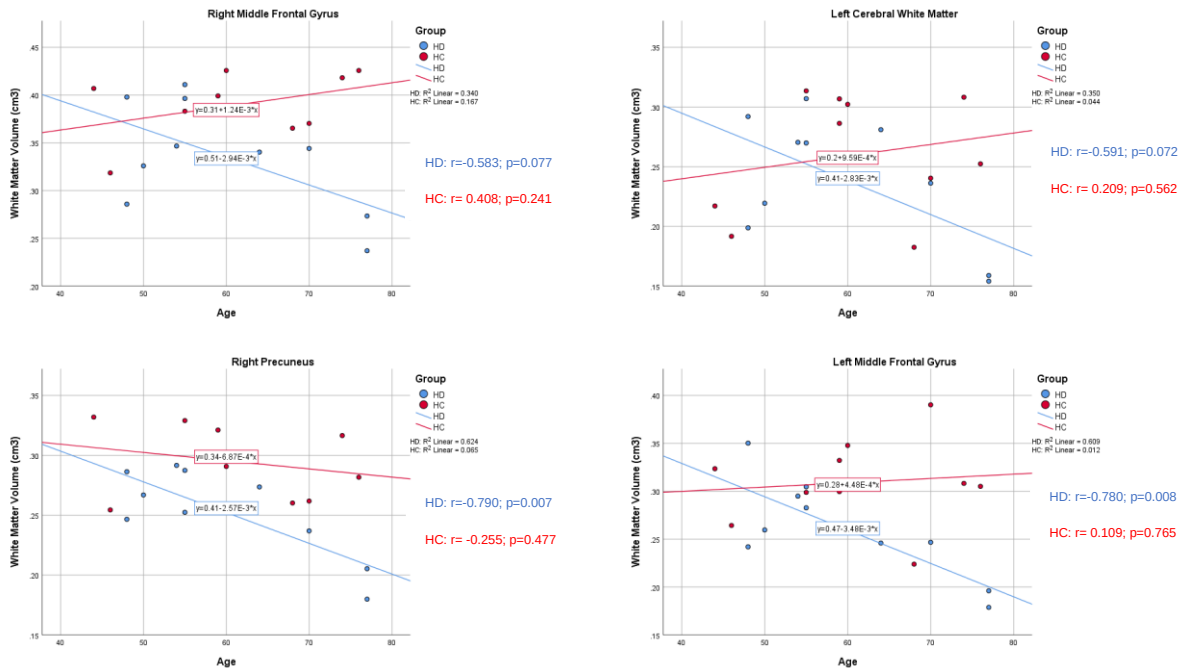


Figure 6-6: Plots of correlation between regional WMV and age in HD and HC groups.

HD patients exhibited a stronger effect of ageing (as shown by the correlation coefficient r values and p values) compared with HCs.

6.4.4.2.3 Effect of Dialysis Vintage on White Matter Volume in HD group

The effect of DV on regional WMV using VBM analysis and was adjusted for age and TIV. Several ROI's of the white matter were negatively correlated with DV, as outlined in Table 6-12.

Only one cluster (right inferior temporal gyrus; cluster size, K= 7) of WM correlated positively with DV.

Table 6-12: Th effect of Dialysis Vintage on WMV in HD group

Anatomical regions	uncorrected p (peak level)	T value (voxel level)	MNI coordinates			Cluster size (K)
			x	y	z	
Left cuneus	0.0000	13.4956	-15	-78	18	1461
Left Superior Frontal Gyrus Medial Segment	0.0000	12.4410	-7.5	45	15	492
Left Posterior Insula	0.0000	10.3707	-31.5	-25.5	12	1299
Left Middle Frontal Gyrus	0.0000	9.3686	-33	4.5	33	659
Left Middle Frontal Gyrus	0.0001	9.0755	-36	28.5	40.5	134
Right Angular Gyrus	0.0001	7.9880	37.5	-58.5	49.5	13
Right Superior Frontal Gyrus	0.0001	7.5225	22.5	6	49.5	100
Right Superior Temporal Gyrus	0.0002	7.2524	45	-10.5	-16.5	277
Left Middle Temporal Gyrus	0.0002	6.9708	-39	-60	7.5	79
Left Middle Frontal Gyrus	0.0003	6.5952	-42	6	46.5	6
Left Supramarginal Gyrus	0.0006	5.7242	-30	-34.5	34.5	196
Left Caudate	0.0008	5.4793	-21	-9	22.5	13
Right Anterior Cingulate Gyrus	0.0009	5.3529	12	30	19.5	6

The above table outlines the brain regions demonstrating a negative correlation of WMV with dialysis vintage, P<0.001, uncorrected. Montreal Neurological Institute (MNI) coordinates provide the location of the cluster in the brain. Cluster size provides the number of voxels in each cluster.

Section 6.5 Discussion

This study demonstrated significant alternations in the structural brain morphology in HD patients compared to HCs. After adjustment for variation in brain size, global (Whole Brain Volumetrics) GMV and WMV were much lower, and CSF volume was much higher in the HD group compared to HCs. The correlation curve of the WMV/TIV with age was suggestive of an accelerated ageing phenomenon in the HD group than HCs. This decline correlated negatively with DV. VBM analysis demonstrated a reduction in the GM and WM volumes in several regions (ROIs) compared to HCs. The correlation of GM and WM volumes extracted from adversely affected ROIs with age further substantiates the accelerated ageing phenomenon in the HD group.

The demonstrated global reduction in the GMV and WMV in HD patients accompanied by an increase in CSF volume in our study suggests the presence of significant cerebral atrophy. These findings are consistent with the previously reported results. Savazzi et al. reported the presence of cerebral atrophy in 46.6% of HD patients, evenly distributed across the brain in half of the patients and predominant in the frontal region in the other half, assessed by computed tomography scanning (249). Higher sulcal prominence and ventricular volumes were shown in the HD population compared to healthy volunteers and the peritoneal dialysis population (102, 245, 250).

In our study, on comparison with HC group, there was a consistent reduction in the GMV/TIV, WMV/TIV, cortical thickness and increase in CSF/TIV ratios in HD group in all the age ranges (even the younger HD patients, the volumes are much lower than the HCs). Moreover, the degree of WMV loss appears to increase as HD patients get older. GMV and WMV are known to be lower in healthy older adults than in younger healthy persons (251, 252). A review of 56 longitudinal MRI studies involving 2211

HCs suggests an estimated loss of annual 0.2% global whole-brain volume starting at the age of 35 years, which gradually increases to 0.5% per year by 60 years and the rate of decline remains steady at this level thereafter (253). The so-called 'normal expected age-related loss' seems to be more pronounced in the HD population as they get older. Prohovnik et al. demonstrated 8% less brain matter in HD patients than in HCs, despite the latter group being 17 years younger; this atrophy was attributed to approximately 10-20 years of chronological age in HCs (250).

Our VBM analysis findings further supported the observed changes in the whole-brain volumetrics. In HD patients, several GM and WM ROI's had lower volumes when compared to the HC group after adjustment for variation in TIV and age. In addition, several regional GM and WM volumes demonstrated a significant negative correlation with age in the HD group. Ledig et al. showed similar findings in patients with Alzheimer's disease (AD) (254). They performed a longitudinal follow-up of structural brain changes in HCs and AD patients with MRI scans at baseline, 1 and 2 years. They demonstrated a greater % loss of regional brain volumes in several areas in the AD group compared to HCs, suggesting an association of accelerated loss with cognitive impairment. The positive association of CSF/TIV ratio and a trend towards negative correlation WMV/TIV ratio with TMT-A scores support the association of accelerated brain atrophy with cognitive impairment in our study. The trend of association of whole-brain morphometric results with TMT-A of our study is consistent with those reported by Pi et al., in some ways, where they demonstrated association of the lower white matter volume (HR: 1.80; CI 1.22-2.64), increased ventricular volume (HR: 1.67; CI 1.09-2.57), and hippocampal atrophy (HR: 2.49; CI 1.07-5.77) with global cognitive impairment in a group of 30 HD and 30 non-dialysis CKD patients (245). Chiu et al. demonstrated the presence of reduced attention, information

processing speed, and executive function in 56 dialysis patients without known dementia. They also reported a reduction in the total brain volume, subcortical volume, and white matter integrity in this group compared to the non-dialysis participant cohort; however, they have not assessed the correlation between cognitive impairment and structural changes (255). We acknowledge that there was no consistent association of the cognitive assessments with the whole-brain morphometrics in our study. We have to note that the MoCA scores for HD patients in our study were within the normal range (although lower than the HC's) (256). TMT scores vary significantly depending on the age and education levels of the individuals (257), so this makes it difficult to compare the scores of our participant group with the general population; however, they were not different from the scores of HC's in our study. So, the presence of relatively normal cognition based on the assessments we used, accompanied by a small study group involving a wide age range of participants (48 to 77 years), might have influenced the results of our study.

We demonstrated a negative correlation of dialysis vintage with WMV/TIV and vice versa with CSF/TIV ratios. DV was also negatively associated with higher executive function (as demonstrated by a positive correlation with TMT-A and TMT-B score). In VBM analysis, the two largest clusters of the GMV which negatively correlated with DV in our study are the left supplementary motor cortex (responsible for complex motor sequencing (258)) and left occipital fusiform gyrus (responsible for higher visual processing, facial recognition(259)). Likewise, the two largest clusters of WMV which negatively correlated with DV are the left cuneus (responsible for visual processing (260)) and left posterior insula (responsible for somatosensory, limbic/emotional, and cognitive integration(261)). Several other regions of the brain, both in GM and WMV, negatively correlated to DV. In the literature, there is heterogeneity in the reports of

regions associated with the poor performance of the trail making test (262), so it is challenging to correlate the VBM analysis results with poor performance in our study. Despite this, it is plausible that these changes affect the overall functional performance of HD patients and make us consider the potential role of dialysis in the pathogenesis of end-organ injury.

There are several possible explanations for extensive structural alterations in HD patients. Firstly, a reduction in the regional cerebral blood flow (CBF) reported in the literature might be contributory. Jiang et al. assessed cerebral blood flow using Arterial Spin Labelling technique in patients with ESKF receiving PD (n=32), HD (n=33) and those not receiving dialysis therapy (n=32). The timing of scanning in HD patients was not specified but was not during the dialysis session in this study. A reduction in the regional CBF in bilateral frontal and insular lobes, anterior cingulate gyrus, right precuneus, and left occipital lobe in the HD group was demonstrated compared to the non-dialysis patient group (263). These findings support some of our results. For example, our study identified the left anterior cingulate gyrus as one of the largest ROIs with reduced WMV. The anterior cingulate gyrus is responsible for complex cognitive functions like decision-making and emotional behaviour like social interactions and empathy (32). These findings may explain the previously reported impairment of decision-making capacity in HD patients (264). Secondly, factors like oxidative stress and activation of the renin-angiotensin system (265), inflammation (266), high prevalence of intracranial calcifications (267), and anaemia (263) may also be contributory. Although the role of such factors supports the negative association with dialysis vintage with structural changes, they do not explain the higher prevalence of structural alterations in HD when compared to PD and non-dialysis CKD groups. Thirdly, recurrent ischaemic stress caused by HD treatment might explain such

differences (268). Reduction in the mean flow velocity in the middle cerebral artery using doppler-based study (269) and global reduction in the CBF demonstrated using PET-CT study (214) support this hypothesis.

The following limitations in the current study should be considered. Our cross-sectional study helped us understand the extent of the structural alterations in the brain, but we were unable to adjust for other comorbidities given the small sample size and should be taken into consideration in the interpretation of our results. We acknowledge that comparing the extent of structural changes in the brain with aged matched healthier controls although provided insights into the severity of adverse changes, it did not help to understand the pathogenesis of these adverse effects. Some of the consequences that we demonstrated could be due to high prevalence of comorbidities like vascular disease, diabetes and hypertension in HD population and may not be representation a manifestation of HD related processes. Study involving comparison with group of patients who experience similar comorbidities to the haemodialysis population (like peritoneal dialysis or pre-dialysis CKD groups) might have provided better insight into the adverse effects of the haemodialysis treatment. We need longitudinal studies to provide additional insights to understand the clinical significance of such adverse structural changes. In addition, studies utilising more thorough and specific cognitive assessments to detect subtle cognitive alterations may help evaluate the functional consequences of these morphological changes. Finally, a multiparametric assessment of the brain linking the structural brain changes to other changes like perfusion might enhance our understanding of the pathogenesis, enabling us to develop interventions to reduce the extent of the changes.

In conclusion, there were significant alternations in the structural brain morphology in HD patients compared to HCs. The whole-brain volumetric and the voxel-based morphometric analysis results suggest an accelerated ageing phenomenon in HD patients. The negative correlation of these adverse findings with dialysis vintage emphasises the potential contribution of HD- specific processes in the pathogenesis of these changes. This study highlights the need for future work focusing on the interventions to reduce the adverse impact of HD-specific processes on end-organs.

Chapter 7 A randomised cross-over trial using intradialytic magnetic resonance imaging to compare the effects of standard versus thermocontrolled haemodialysis on structure, function and blood flow of heart, brain and kidneys

Section 7.1 Abstract

7.1.1 Background

Ischemic end-organ damage during haemodialysis (HD) is a significant problem that may be ameliorated by intradialytic cooling. We performed a randomised trial to compare standard HD (SHD) and thermocontrolled HD (TCHD), using multiparametric magnetic resonance imaging (MRI) to simultaneously assess the structural, functional and blood flow changes in heart, brain and kidneys.

7.1.2 Methods

12 prevalent HD patients were randomly allocated to receive 4 hours either SHD (dialysate temperature 37ºC) or TCHD (programmed cooling using Body Temperature Monitor, BTM device). The rest of the HD prescription and other operating conditions remained constant. Participants were exposed to initial modality for two weeks before undergoing serial multiparametric MRI (Phillips 3T Ingenia) of the heart, brain and kidneys at pre, during (30min and 180min) and post-dialysis (4 time points).

Participants then crossed over to the other modality, and the study protocol was repeated.

7.1.3 Results

Median age of participants was 55 yrs (IQR 27) and three had diabetes. There was a reduction in blood temperature (measured by BTM) with TCHD (-0.1 ± 0.3^0C) and a rise with SHD (+0.3 ± 0.2^0C, p=0.022), although no difference in change in tympanic temperature was observed between groups (SHD arm +0.2 ± 0.3^0C vs TCHD arm +0.1 ± 0.5^0C, p=0.7).

Pre-dialysis myocardial T1 and left ventricular wall mass were lower after two weeks of TCHD as compared to SHD (1255ms (IQR 41) vs 1293 ms (IQR 100), p=0.021; 66.5 ± 22.2 g/m2 vs 72.6 ± 23.7 g/m2, p=0.004). There was a significant reduction in the cardiac index, reaching a nadir at 180mins, with a fall of 26 ± 8% and 28 ± 6% in SHD and TCHD arms, respectively, and partial recovery post-dialysis. During dialysis, blood flow velocities fell in the left carotid and basilar arteries, reaching a nadir in the fourth hour. Changes in cerebral blood flow and cardiac function were not different between TCHD and SHD arms.

There were no differences in total kidney volume (indexed to body surface area), cortical T1, and cortical T2* between SHD and TCHD groups, although all three measures declined significantly during dialysis.

7.1.4 Conclusions

HD adversely affects cardiac function, carotid and basilar artery blood flow, along with a reduction in the total kidney volume. Cooling the dialysate using a biofeedback

module did not result in significant differences in intradialytic MRI measures as compared to dialysis at 37^0C but did result in reductions in pre-dialysis myocardial T1 and LVMI that may reflect less dialysis-induced cardiac injury over the preceding two weeks.

Section 7.2 Introduction

The burden of organ dysfunction is high in HD populations, despite advances in therapy. The prevalence of heart failure is as high as 43% (270), and cognitive dysfunction affects between 6.6% to 87% of patients depending on the type of assessments and the cut-offs used (95, 96, 238). Cognitive deficits are clustered in the domains of attention, orientation and higher executive functioning, which are essential for functional status (271). These comorbidities are associated with high hospitalisations rates, poor quality of life and increased mortality (272). Several haemodialysis treatment-related factors are now recognised to play a role in the aetiology of end-organ injury. Repeated intradialytic cardiac stunning (a reversible, subclinical ischaemia-driven myocardial dysfunction) during HD has been shown to cause fixed reductions in myocardial contractility, left ventricular systolic dysfunction and increased mortality (66, 273). Similar recurrent ischaemic insults occur in the brain, in particular in the cortical white matter, leading to neurological sequelae (268). Studies using transcranial doppler have demonstrated reductions in the blood flow velocity of the middle cerebral artery (274) and a reduction in global cerebral blood flow by 10±15% was reported in a study using intra-dialytic PET CT (214). The latter study also described associations of global intradialytic cerebral blood flow reduction with higher tympanic temperature and greater ultrafiltration volumes.

A variety of dialysis-based interventions may reduce haemodynamic instability including cooling the dialysate, which is recommended by The European Best Practice Guidelines for prevention of IDH (143). Previous studies have revealed that cool dialysate helps to reduce dialysis-induced regional left ventricular wall motion abnormalities in comparison to standard dialysis (275). In a randomised controlled

study comparing cool vs standard dialysis, less cerebral white matter changes assessed using magnetic resonance imaging (MRI) were noted after 1 year of follow up in participants who received cool dialysis when compared to standard dialysis at 37ºC (115). However, symptoms like shivering and discomfort are experienced more frequently with empirical reductions in dialysate temperature when compared to standard HD (150). In response, biofeedback systems have been developed that automatically adjust dialysate temperature in response to blood temperature. These can be programmed in different ways to achieve isothermic dialysis (maintaining core body temperature by creating a negative thermal balance) (153) or deliver a prescribed rate of programmed cooling. Whilst these approaches have been shown to reduce the incidence of IDH, their effect on changes in organ perfusion during dialysis have not been previously studied.

The mechanisms by which cooling the dialysate offers protection are not fully characterised. One explanation is the preservation of the normal physiological vasoconstrictor response to hypovolemia caused by ultrafiltration by avoiding thermal energy accumulation. This is supported by data that suggest that total peripheral resistance increases more significantly during cool dialysis compared to standard HD (148), although this fits less well with changes in myocardial blood flow that have been reported as early as 30min into dialysis (179). Evidence suggests that dialysate cooling increased plasma norepinephrine levels (276) and improved left ventricular contractility leading to more stable haemodynamics has also been reported based on echocardiographic assessment (149). Effects on venoconstriction are also possible.

Therefore, we aimed to study the intradialytic effects of dialysate cooling using multi-organ quantitative MRI assessments of cardiac, brain and native kidney structure,

function and blood flow during dialysis. Specifically, we designed a randomised controlled trial to compare standard dialysis with cooled dialysis, using programmed cooling delivered by a biofeedback device in the intervention arm to ensure its precise delivery.

Section 7.3 Methods

7.3.1 Study Design and participants

This was a prospective, randomised, open-label, blinded endpoint, crossover study in prevalent HD participants. The aim of the trial was to compare the intra-dialytic effects of standard dialysis against dialysis with programmed cooling of the dialysate, using magnetic resonance imaging (MRI) to assess changes in the heart, brain and kidneys (ClinicalTrials.gov Identifier: NCT03280901). Participants were recruited from a single site (University Hospitals of Derby and Burton NHS Foundation Trust, UK) between March 2018 and May 2019. The study was reviewed by an Independent Ethics Committee (East Midlands Research Ethics Committee), and all the participants provided written informed consent.

Participants aged between 18 to 80 years who had kidney failure and had been receiving treatment with HD for more than three months were eligible. All patients had a functioning arteriovenous fistula as dialysis access. Exclusion criteria were hypotension during HD in the four weeks prior to recruitment, bilateral nephrectomy, New York Heart Association Stage IV heart failure, active infection or malignancy, contraindications to MRI, pregnancy, or medical conditions/physical frailty that precluded participation.

7.3.2 Randomisation and blinding

Participants were randomised to one of two study arms: standard HD (SHD) with a fixed dialysate temperature of 37^0C; or thermocontrolled HD (TCHD) in which a commercially available biofeedback system (Fresenius Body Temperature Monitor, BTM) was programmed to deliver a change in blood temperature of -0.5^0C/hour during

dialysis. Online software (Sealed Envelope) was used for randomisation and was performed in blocks of varying sizes to achieve equal numbers in each arm. After randomisation, participants underwent two weeks (six sessions) of the initial dialysis modality before crossing over and receiving two weeks of the alternative dialysis modality. During each arm, a study session was performed on the day of the final dialysis treatment (6th and 12th dialysis sessions) that included serial MRI scans before, during and after dialysis. The study design is summarised in Figure 7-1. Due to the nature of the intervention, it was not possible to blind the clinical team to the study, but members of the study team who were responsible for acquiring and analysing the MRI data were blinded to treatment allocation.

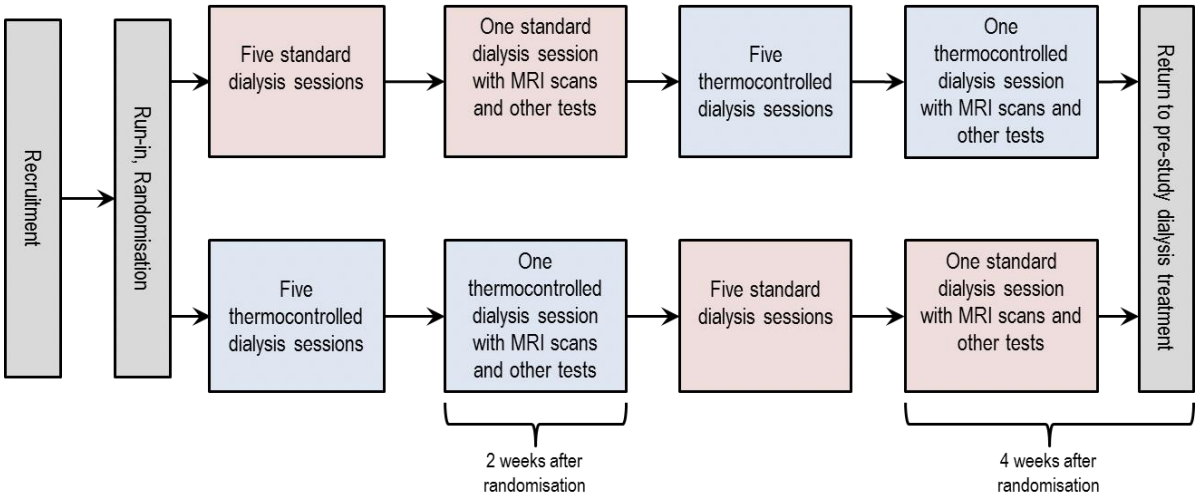


Figure 7-1: Study treatment schedule

7.3.3 Study procedures and schedules

All dialysis sessions were conducted by a research nurse using a Fresenius 5008 machine™. Dialysate flow was 500mls/min with a minimum blood pump speed of 300mls/min. Anticoagulation was achieved using the standard anticoagulation prescription of the participants and the composition of dialysate was sodium

137mmol/L, potassium 2.0mmol/L, calcium 1.25mmol/L, magnesium 0.5mmol/L, glucose 1.0g/L.

Study sessions on the days of the 6th and 12th dialysis treatments were performed at the Sir Peter Mansfield Imaging Centre (University of Nottingham), where the scanner facilities have been adapted to enable MRI scanning during dialysis as illustrated in the methods chapter. Participants underwent serial MRI scanning at the following time points: Pre-dialysis (scan 1); 30 mins into dialysis (scan 1); 180 minutes into dialysis (scan 3); and 30 mins after dialysis session (scan 4). MRI was performed using a 3 Tesla Philips Ingenia wide bore Scanner (Philips Medical Systems, Best, Netherlands). The dialysis machine was situated immediately outside of the scan room, with a specially positioned wave guard to minimise the distance between dialysis machine and scanner table. The blood lines ran through the wave guard and connected to non-ferrous 15G silicon dialysis needles that were used to cannulate the AV fistula. The total length of the blood lines was 2m. In between MRI scans, the participants were transferred to an MR-compatible bed inside the scan room for comfort.

Blood pressure was recorded every 15mins during dialysis using the integrated blood pressure cuff, and IDH was defined as a drop in systolic BP to <90 mmHg or a drop in systolic BP of >20mmHg from baseline. A number of clinical measurements were taken before and after dialysis. These comprised of body weight, cognitive function tests (30 point Montreal Cognitive Assessment (MOCA) and Trail Making Tests Part A (TMT-A) and B (TMT-B), bioimpedance measurements of total body, intracellular and extracellular water (Fresenius BMC machine), tympanic temperature, and laboratory tests (haematology and biochemistry panels, NT-pro-BNP, troponin-T).

Ultrafiltration volume and relative blood volume change were also recorded at the end of dialysis treatments. Routine clinical data and demographic details were collected from the medical records at the start of the study.

7.3.4 MRI measures

Acquisition of the MRI measures and the processing/ analysis of these measures were outlined in chapter 4 (Section 4.3).

7.3.5 Outcomes

A primary outcome measure was chosen for each of the three organs assessed as follows: cardiac index measured with cardiac MRI, cerebral perfusion measured with ASL-MRI (Arterial Spin Labelling MRI), and renal perfusion measured with ASL-MRI.

Pre-specified secondary MRI outcome measures included left ventricular strain, myocardial T1, cerebral blood flow, kidney volume, kidney cortical T1 and kidney BOLD-MRI (T2*).

Safety endpoints were also collected to determine the tolerability of MRI during dialysis, symptoms during TCHD, and frequency of adverse events.

7.3.6 Statistical Analysis

Continuous variables were expressed as mean ± SD, mean ± SEM or median (IQR) depending on the distribution of the data. Categorical variables were expressed as percentages. Repeated measures ANOVA was used to compare the outcome measures between the two dialysis modalities across the four time points at which MRI scans were performed. Based on the distribution of the data, appropriate paired sample tests were used to compare the parameters between the two arms. Statistical

analysis was performed using IBM SPSS (Version 24) and Graphpad PRISM 9. A $p \leq 0.05$ was considered significant.

7.3.7 Sample size

Using data from the previous study of our team, of cardiac MRI during dialysis, which measured cardiac index pre-and during standard dialysis, observing corresponding values of 3.6±0.2 L/min/m2 (pre-dialysis) versus 2.6±0.2 L/min/m2 at 230minutes (168), 9 patients per group would be required to detect a difference between groups of 0.25 L/min/m2 (250ml/min/m2) with 90% power, significance at the 5% level. This would represent a clinically meaningful difference between treatment arms, and to ensure the minimum sample size was achieved, we planned to recruit 12 patients.

Section 7.4 Results

7.4.1 Enrolment and Participant Characteristics

A total of 17 participants were recruited to the study (Figure 7-2). One withdrew consent prior to commencing, a further four were withdrawn before completion of the first study session and one participant received a kidney transplant during the second arm of the study. Therefore, eleven participants completed both arms of the study and were included in analyses comparing SHD and TCHD.

Demographics and clinical characteristics of the 11 patients are shown in Table 7-1. There were no differences between SHD and TCHD in the majority of clinical or laboratory variables measured before and after dialysis, as shown in Table 7-2. The change in core body temperature was significantly greater with TCHD (-0.1 ± 0.3^0C) when compared to SHD (+0.3 ± 0.2^0C, p=0.022), although this was not matched by a difference in change in tympanic temperature between groups (SHD arm +0.2 ± 0.3^0C vs TCHD arm +0.1 ± 0.5^0C, p=0.7). There were no differences in blood pressure, ultrafiltration volume, body weight, bioimpedance measurements or relative blood volume change between the study arms.

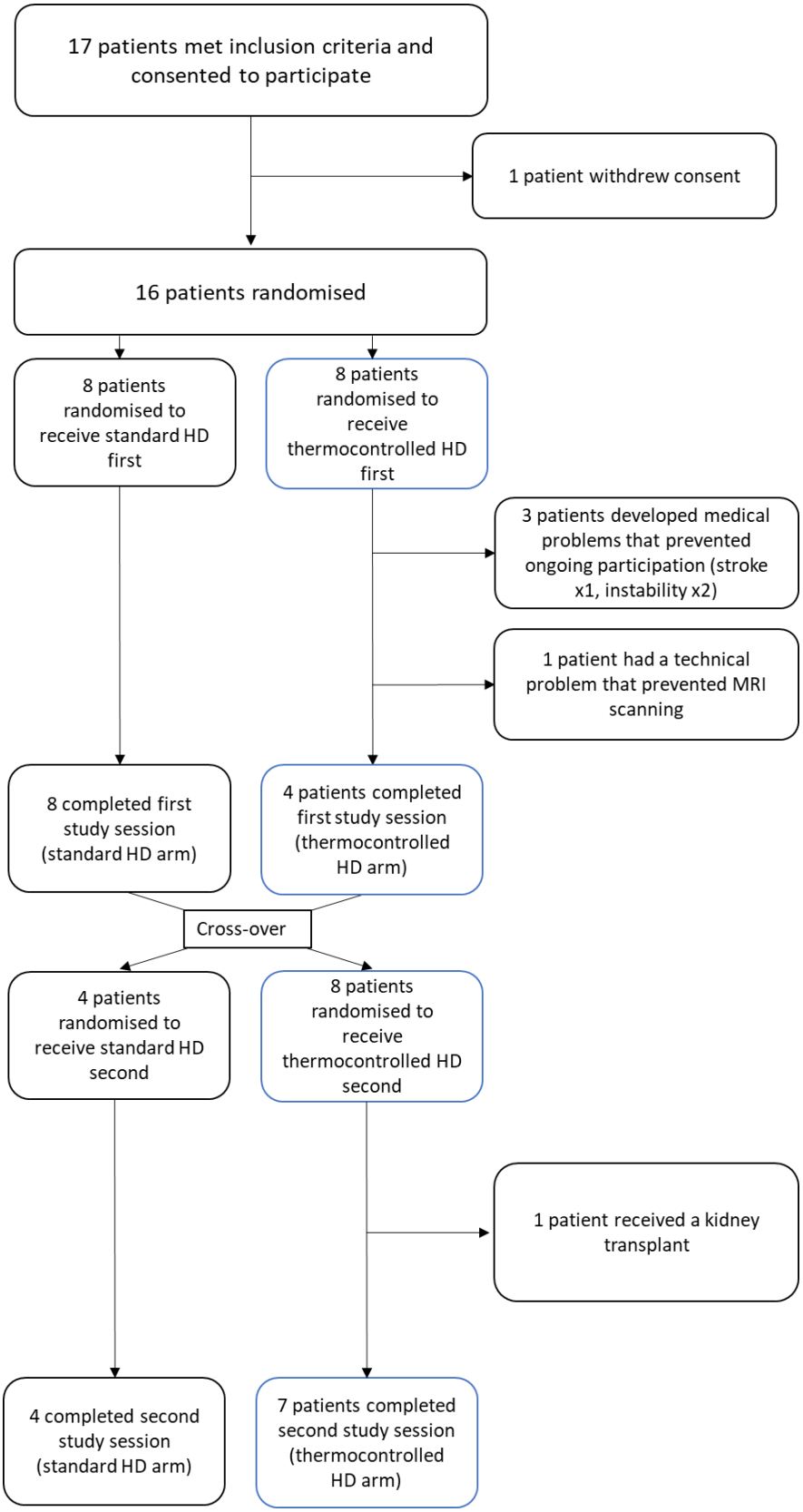


Figure 7-2: Flow of Participants in the study

Table 7-1: Demographics and Clinical Characteristics of the 11 participants who completed both arms

Age (years)	55 (IQR 27, range 48-77)
Male	9 (81.8%)
Ethnicity:	
White	8 (72.7%)
Black	1 (9.1%)
Asian	2 (18.2%)
Aetiology of kidney disease:	
Diabetes	3 (27.3%)
Glomerulonephritis	2 (18.2%)
Hypertension/ischaemic nephropathy	2 (18.2%)
Chronic pyelonephritis	2 (18.2%)
Interstitial nephritis	1 (9.1%)
Unknown	1 (9.1%)
Time in months since End Stage Kidney Failure	9.6 (IQR 51, range 4-96)
Co-morbid conditions:	
Ischaemic heart disease	2 (18.2%)
Cerebrovascular disease	1 (9.1%)
Hypertension	10 (90.9%)
Ischaemic/neuropathic ulcers	1 (9.1%)
Previous fracture	2 (18.2%)
Taking blood pressure lowering medication	6 (54.5%)
Smoking status:	
Current	1 (9.1%)
Ex	5 (45.5%)
Never	5 (45.5%)
Vascular access:	

Arterio-venous fistula (AVF) AVF blood flow by ionic dialysance method (ml/min)	11 (100%) 719 (IQR 318, range 572-1570)
Vascular access by location: Left arm Right arm	 9 (81.8%) 2 (18.2%)
Residual renal function: Urine output >500ml/24hrs Creatinine clearance (ml/min)	 4 (36.4%) 0.42 (IQR 8, range 0-9)
Anthropomorphic measurements: Euvolaemic body weight (kg) Height (m) BMI (kg/m2)	 80.3 ± 15.8 1.71 ± 0.08 27.4 ± 4.43

Mean ± Standard deviation or Median (Interquartile range) are presented depending on the distribution of data

Table 7-2: Clinical and laboratory variables pre and post dialysis

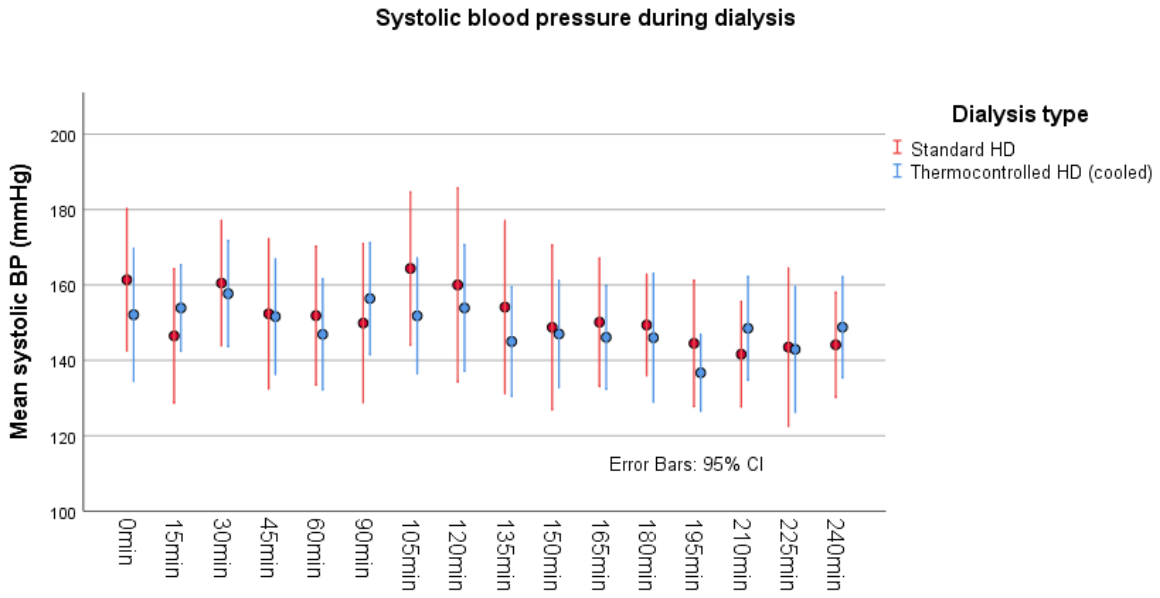
	Pre-dialysis			**Post-dialysis**		
	SHD	**TCHD**	**p value**	**SHD**	**TCHD**	**p value**
Body weight (kg)	83.0 ± 16	82.4 ± 16	0.99	81.1 ± 16	80.3 ± 16	0.96
Systolic blood pressure (mmHg)	154 ± 20	150 ± 21	0.96	157 ± 16	148 ± 24	0.14
Diastolic blood pressure (mmHg)	80 ± 16	80 ± 15	0.77	80 ± 16	80 ± 15	0.25
Pulse rate (bpm)	74 ± 10	74 ± 13	0.28	77 ± 13	78 ± 6	0.07
Anticoagulation Heparin Argatroban	 10 (91%) 1 (9%)	 10 (91%) 1 (9%)	 1.0 1.0			
Blood pump speed (ml/min)	300 ± 31	293 ± 21	0.81			

Target Ultrafiltration volume (UFV in ml)	2181 ± 753	2345 ± 782	0.95			
Tympanic temperature (^{0}C)	36.4 ± 0.4	36.4 ± 0.4	0.74	36.6 ± 0.4	36.4 ± 0.1	0.26
Change in tympanic temp (^{0}C)				0.2 ± 0.3	0.1 ± 0.5	0.70
Change in body temp (^{0}C) from BTM				0.3 ± 0.2	-0.1 ± 0.3	0.022
Online CDn monitor Kt/V				1.23 ± 0.2	1.20 ± 0.2	0.74
Online CDn monitor plasma Sodium (Na+)				137 ± 1	137 ± 1	0.2
Achieved UFV (ml)				2182 ± 753	2304 ± 744	0.31
Relative blood volume at the end of dialysis (%)				91.9 ± 4.69	89.02 ± 4.05	0.59
Bioimpedance Measurements (in litres)						
Total Body Water	40.4 ± 4.8	40.5 ± 5.0	0.93	38.0 ± 4.6	37.5 ± 5.2	0.7
Extracellular Water	19.4 ± 2.3	19.4 ± 2.3	0.77	17.8 ± 2.1	17.3 ± 2.2	0.95
Intracellular Water	21.1 ± 3.1	21.1 ± 3.4	0.90	20.2 ± 3.4	20.2 ± 3.7	0.90
Over-hydration	1.64 ± 1.6	1.70 ± 1.8	0.61	0.68 ± 2.0	0.13 ± 2.1	0.79
Laboratory measurements						
Haemoglobin (g/l)	116 ± 8	116 ± 6	0.49	119 ± 9	123 ± 9	0.84
Haematocrit	0.36 ± 0.03	0.35 ± 0.01	0.26	0.36 ± 0.03	0.37 ± 0.03	0.92
Sodium (mmol/l)	140 ± 2	140 ± 2	0.81	138 ± 2	149 ± 1	0.29
Potassium (mmol/l)	5.7 ± 1	5.9 ± 1	0.54	3.9 ± 0.5	3.9 ± 0.5	0.55
Urea (mmol/l)	21.6 ± 6	22.7 ± 7	0.36	5.9 ± 2	6.8 ± 3	0.19
Creatinine (µmol/l)	750 ± 281	735 ± 277	0.87	298 ± 124	304 ± 127	0.89
Calcium (mmol/l)	2.28 ± 0.12	2.28 ± 0.20	0.41	2.29 ± 0.06	2.29 ± 0.09	0.30
Phosphate (mmol/l)	1.6 ± 0.6	1.5 ± 0.4	0.49	0.8 ± 0.2	0.8 ± 0.2	0.98
Bicarbonate(mmol/l)	22 ± 2	22 ± 2	0.65	27 ± 2	27 ± 1	0.75
Glucose (mmol/l)	6.0 ± 4.5	5.7 ± 2.6	0.29	7.6 ± 1.9	7.2 ± 1.8	0.89

Albumin (g/l)	34 ± 3	33 ± 3	0.82	35 ± 4	37 ± 4	0.49
Brain Natrieutic Peptide (ng/l)	2320 (IQR 18543)	2308 (IQR 11395)	0.45	1801 (IQR 1991)	1371 (IQR 2808)	0.61
Troponin T (mg/ml)	58 (IQR 56)	58 (IQR 68)	0.78	50 (IQR 53)	53 (IQR 59)	0.90

Mean ± Standard deviation or Median (Interquartile range) are presented depending on the distribution of data

Blood pressure (BP) measurements during SHD and TCHD are shown in Figure 7-3. A two-way repeated ANOVA (dialysis modality and time) showed that both systolic (SBP) and diastolic BP (DBP) fell significantly over the course of the four-hour treatment (in both analyses, p<0.001 for interaction effect of time) but that there was no significance difference between SHD and TCHD arms (interaction effect of modality p=0.80 for SBP and p=0.61 for DBP). There were 5 episodes of IDH (SBP <90mmHG) in 3 participants in TCHD arm and 16 episodes in 4 participants in SHD arm during the entire study period (p=0.066).



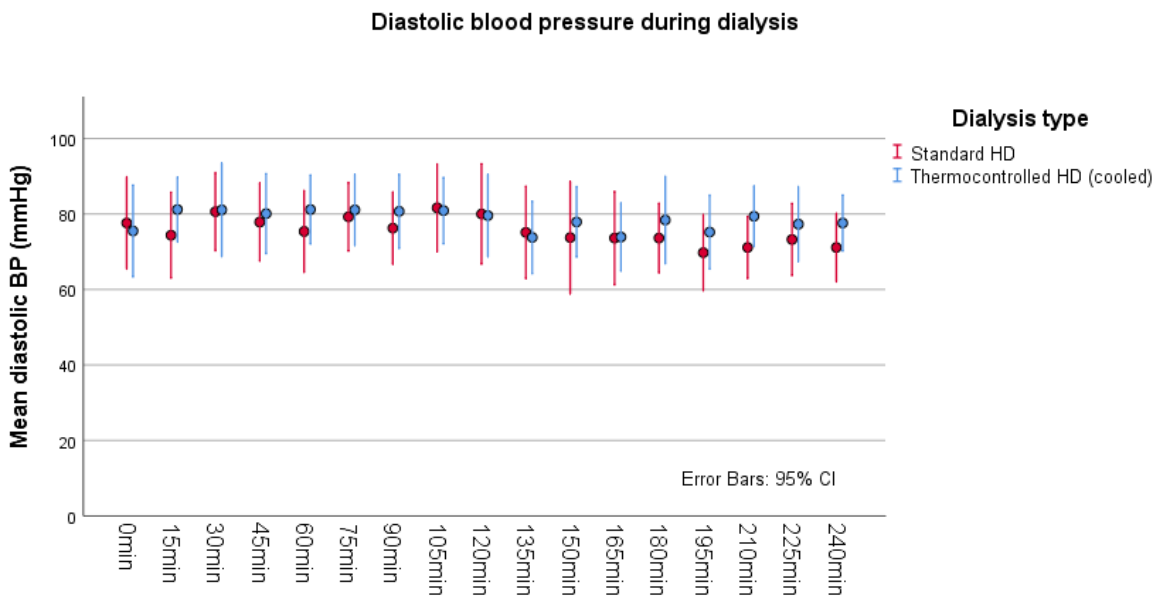


Figure 7-3: Trends in the Systolic and Diastolic blood pressure (in mmHG) during dialysis in Standard (represented in red) and Thermocontrolled (represented in blue) arms.

Mean ± Standard error of mean are provided in 15-minute intervals.

7.4.2 Outcomes

7.4.2.1 Comparison of Cardiac MRI changes between SHD and TCHD arms

The baseline cardiac index was 2.77 ± 0.6 l/m^2 in the SHD group, and 2.81 ± 0.47 l/m^2 in the TCHD group (p=0.863). The rest of the parameters are provided in Table 7-3. There was a significant reduction in the cardiac index during dialysis in both arms, but there were no differences between SHD and TCHD. The biggest reduction in the cardiac index was seen at Scan 3, with 26 ± 8% and 28 ± 6% reductions in SHD and TCHD groups, respectively, with partial recovery observed at Scan 4 (post-dialysis). These data are summarised in Figure 7-4. A similar pattern was observed for stroke volume index.

Table 7-3: Baseline (pre-dialysis) Cardiac MRI parameters for Standard and thermocontrolled HD

Baseline Cardiac Parameters	Standard HD	Thermocontrolled HD	P-value
Cardiac index (L/m2)	2.77 ± 0.60	2.81 ± 0.47	0.989
Stroke index (mL/m2)	39.37 ± 8.59	39.59 ± 8.25	0.225
Ejection fraction (%)	61.88 ± 8.24	63.37 ± 10.53	0.566
Peak longitudinal strain (%)	-13.7 ± 2.8	-12.9 ± 3.6	0.020
Peak circumferential strain apical slice (%)	-20.83 ± 5.41	-21.69 ± 3.61	0.727
Peak circumferential strain mid slice (%)	-17.86 ± 4.36	-18.48 ± 2.39	0.118
Peak circumferential strain basal slice (%)	-14.9 (6.12)	-13.4 (1.9)	0.753
LV wall mass index (g/m^2)	72.6 ± 23.7	66.5 ± 22.2	0.004
Myocardium T1 (ms)	1293 (100)	1266 (41)	0.021
Myocardium perfusion (ml/g)	3.44 (3.17)	2.15 (2.01)	0.241

Mean ± Standard deviation or Median (Interquartile range) are presented depending on the distribution of data

Myocardial T1 and LVMI were lower pre-dialysis (scan 1) in TCHD arm. Median myocardial T_1 was 1266ms (IQR 41) in TCHD as compared to 1293 (IQR 100) in the SHD group, p=0.021. Corresponding values for LVMI were 72.6 ± 23.7 g/m^2 (SHD group) and 66.5 ± 22.2 g/m^2 (TCHD group), p=0.004. As expected, neither of these variables changed during dialysis in either arm.

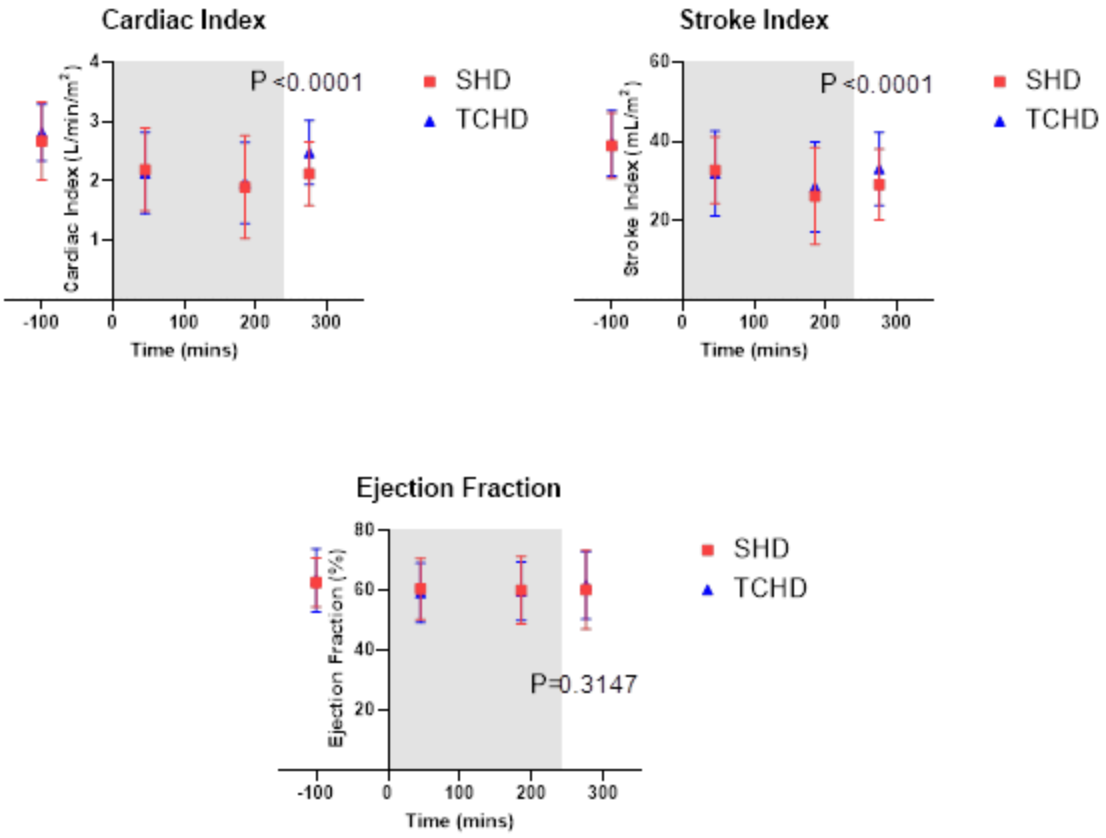


Figure 7-4: Left ventricular function measured using the short axis cine to assess cardiac index (L/min/m2), stroke index (ml/m2) and ejection fraction (%) during Standard (SHD) and thermocontrolled (TCHD) haemodialysis.

The grey box indicates the period of dialysis.

Changes in myocardial strain during dialysis are shown in Figure 7-5. During dialysis, there was a reduction in whole wall strain in both SHD and TCHD arms (strain values became less negative, indicating a reduction in myocardial contractility). This was seen in global longitudinal strain and circumferential strain in apical and mid-ventricular slices. There was no significant difference in strain values at the intra- and post-dialytic timepoints between SHD and TCHD, although peak longitudinal whole wall strain was significantly more negative before dialysis in the SHD arm (-13.7 ± 2.8) as compared with the TCHD arm (TCHD -12.9 ± 3.6%, p=0.02). There were no differences in the pre-dialysis circumferential strain values between study arms.

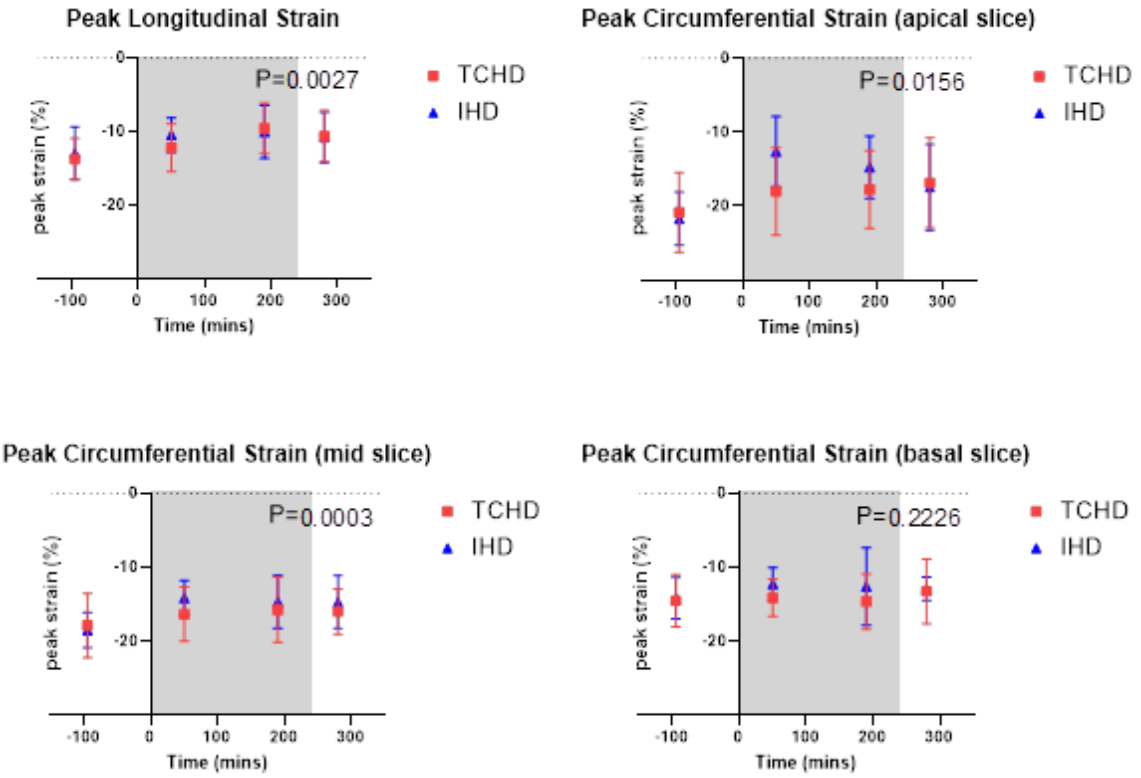


Figure 7-5: Peak longitudinal and circumferential strain (in three different slices) measured using cardiac tagging during Standard HD (SHD) and Thermocontrolled HD (TCHD).

The grey box indicates the period of dialysis. P-values for the interaction of time factor are presented in the graphs and were significant. P-values for the modality factor were not significant for any demonstrated parameters.

Myocardial perfusion changes during dialysis are shown in Figure 7-6. Perfusion values were lower during dialysis in both arms, but this did not reach significance (interaction effect for time, p=0.099), and there was no difference between the two arms (interaction effect for modality, p=0.686).

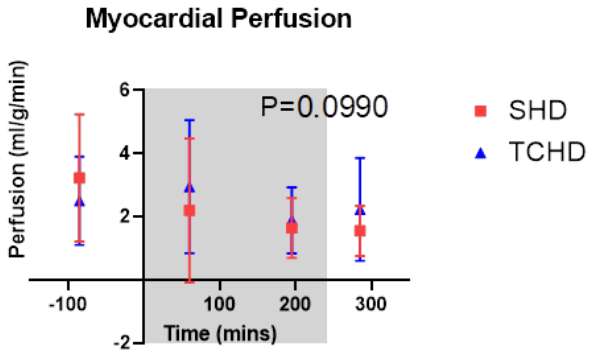


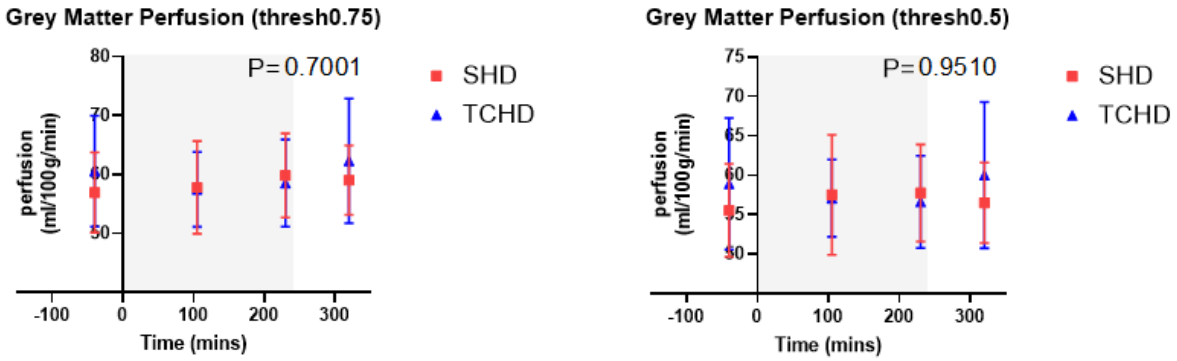
Figure 7-6: Myocardial perfusion during SHD and TCHD.

Grey box indicates period of dialysis.

7.4.2.2 Comparison of Cerebral MRI changes between SHD and TCHD arms

Grey matter perfusion (measured with ASL-MRI, mask threshold 0.75) was similar before dialysis in SHD (56.9 ± 6.8ml/100g/min) and TCHD (60.6 ± 9.4ml/100g/min) groups, p=0.12. As shown in Error! Reference source not found., there was no change in grey matter perfusion during dialysis and no difference between SHD and TCHD. Similar results were seen when analyses were performed with a mask threshold of 0.5.

A)



B)

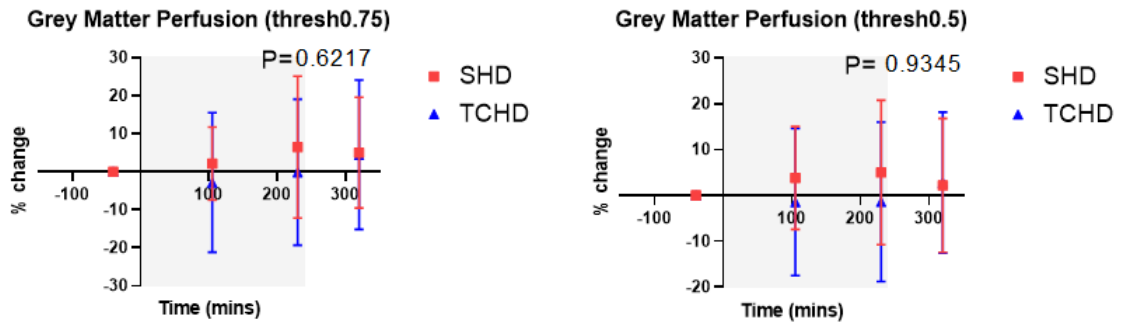


Figure 7-7: A) Grey matter perfusion during dialysis for thresholds of 0.75 and 0.5. B) Percentage change in grey matter perfusion during dialysis for thresholds of 0.75 and 0.5.

The grey box indicates the period of dialysis.

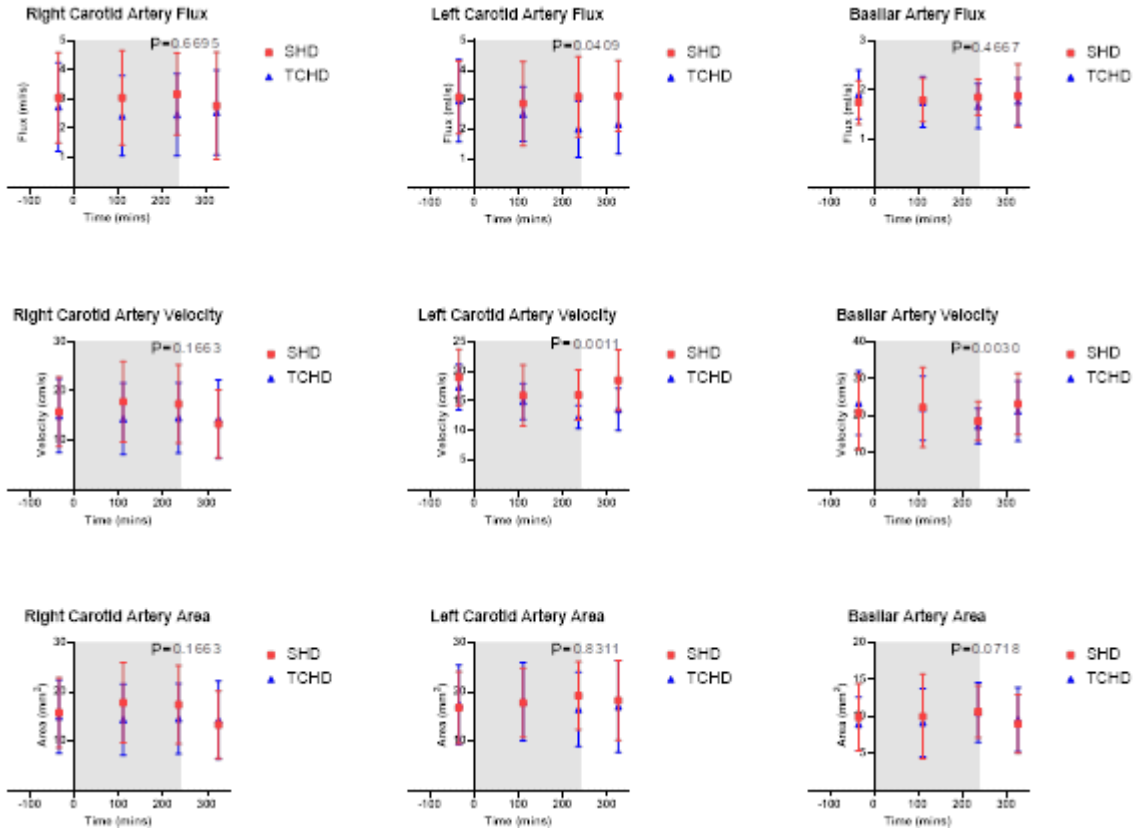


Figure 7-8: Carotid arteries and basilar artery blood flow during dialysis.

Grey box indicates period of dialysis.

In contrast, cerebral artery blood flow changes did occur during dialysis, although again these were not different between SHD and TCHD arms, as shown in Figure 7-8.

There were significant reductions in left carotid artery and basilar artery velocity during dialysis, and a reduction in left carotid artery flux.

7.4.2.3 Comparison of renal MRI measures between SHD and TCHD arms

Due to the degree of anatomical abnormalities in the kidneys of participants, it was not possible to obtain reliable measurements of renal perfusion with ASL-MRI. Data were collected on total kidney volume (indexed to body surface area), cortical T1 and cortical T2*, which are shown in Figure 7-9. There were no differences between any of these measures between SHD and TCHD groups, although all three measures declined significantly during dialysis. The largest reductions were seen at scan 3 (180mins during HD), and whilst partial recovery was seen in cortical T1 and cortical T2* values post-dialysis, this was not seen for TKV.

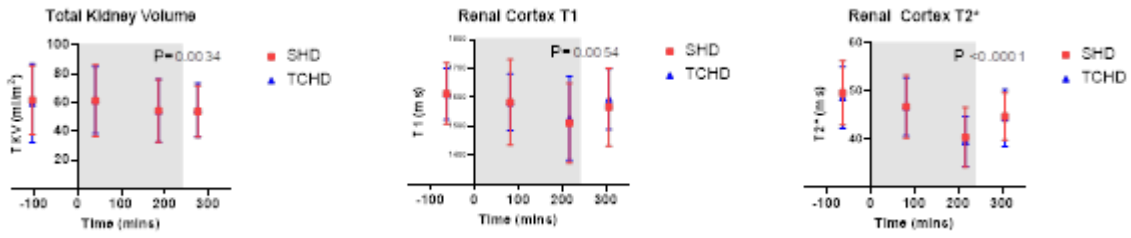


Figure 7-9: Change in renal MRI parameters during dialysis. There was a significant effect of time on all three measures with P values indicating the effect of time. There was no difference between treatments.

Grey box indicates period of dialysis.

Section 7.5 Discussion

This study demonstrates the effects of dialysis on multiple organs simultaneously using intradialytic MRI. We observed a significant reduction in cardiac function, carotid and basilar artery blood flow and total kidney volume during dialysis. Furthermore, we demonstrated significant differences in the pre-dialysis structural measures of the heart with lower myocardial T1 and left ventricular mass index (LVMI) in the TCHD arm. However, there were no differences between standard (SHD) and thermocontrolled (TCHD) haemodialysis in any of the observed intradialytic changes.

Cooling the dialysate helps restore the expected physiological vasoconstrictor response to hypovolemic-stress related to ultrafiltration (277) and improves intradialytic haemodynamic stability by preventing gain in thermal energy. In a meta-analysis, which included 26 randomised trials, Mustafa et al. concluded that cool dialysis reduced the risk of IDH (Relative risk of 0.32, 95% CI: 0.18-0.56) and was associated with higher MAP by 12 mmHg (95% CI: 8 to 16 mmHg) when compared with standard dialysis (151). Moreover, evidence suggests that cool dialysis offers end-organ protection. Previous studies reported a reduction in dialysis-induced myocardial stunning during cool dialysis (76, 278). In a one-year follow-up study, Odudu et al. reported preservation of left ventricular (LV) systolic and diastolic strain parameters along with a reduction in the LV mass with dialysate cooling (152). Comparable protective effects on brain cortical white matter were reported by Eldehni et al. with less ischaemic changes (as detected by magnetic resonance imaging) with cool dialysis at the end of one year follow up (115). Furthermore, some evidence from observational studies suggests improved survival with cool dialysis although this remains to be tested in randomised controlled trials (279, 280).

The intradialytic cardiac MRI findings of our study are consistent with previously reported results (168) with a significant reduction in cardiac and stroke volume index during dialysis along with a reduction in the myocardial contractility, as assessed by global longitudinal and circumferential strain. In tandem, a trend towards a fall in myocardial perfusion is consistent with studies from our group and others that have previously described significant falls in perfusion during dialysis (168). Additional insights are provided from multi-organ imaging that allows these changes in cardiac function to be viewed in parallel with the effects of dialysis on other organs. A fall in cardiac output is likely to be a factor in the observed reduction in carotid and basilar artery blood flow, and these findings are consistent with previously demonstrated fall in middle cerebral artery flow velocity using transcranial doppler (114, 269). It was interesting that differential changes were observed between right and left carotid arteries. Lateralisation of changes in the extracranial vascular haemodynamics to the side of HD access along with a negative correlation with access flow has been reported previously by Chung et al (281). A similar phenomenon may have led to the observed differences in the right and left carotid artery changes in our study, as 82% of the participants had vascular access on the left side in our study. Despite changes in carotid and basilar artery blood flow, we did not observe a change in grey matter perfusion as assessed by ASL, which is in contrast to a previous study that used PET-CT to demonstrate a fall in cerebral perfusion in elderly dialysis patients during HD (214). One possible explanation for this inconsistency is the difference in the age of the patients in the two studies, with the average age of participants in our study approximately 20 years younger. Acquisition of renal measures was challenging due to the extent of anatomical abnormalities in participants that included atrophy, acquired cystic disease and ADPKD. Despite this, we demonstrated a significant reduction in

the TKV, T1 and T2* during HD. These observed changes are likely to reflect a reduction in the water content of the kidneys and thus may indirectly represent an alteration in renal blood flow during HD, or alternatively, the effects of ultrafiltration per se. These findings support previous suggestions that loss of RRF in HD patients could be related to renal ischaemia driven by ultrafiltration (282) and are in keeping with a computed tomography based study demonstrating an intra-dialytic drop in renal perfusion (129). In summary, we have confirmed the negative effects of dialysis on myocardial contractility and cardiac output and show synchronous changes in carotid and basilar artery blood flow and indirect evidence of similar processes affecting the kidney, which together describe the acute multi-organ effects of dialysis.

Previous studies have shown that a fixed reduction in dialysate temperature reduces the development of left ventricular wall motion abnormalities. However, we did not observe significant differences in any of the intradialytic MRI measures between the two arms in our study. On the MRI study days, only modest separation in blood temperature was achieved, and this might have limited the extent of physiological responses. Despite this, there were differences in pre-dialysis structural measures in the heart (lower myocardial T1 and LV mass index with TCHD), which could reflect the cardio-protective effects of dialysate cooling during the two-week run-in phase, during which there was a trend towards fewer recorded episodes of IDH with TCHD. Myocardial T1 is sensitive to changes such as tissue oedema, collagen deposition, or fibrosis. In large animal models, hypothermia protects against ischaemia-induced myocardial injury that is characterised by cellular processes that would be consistent with an increase in myocardial T1 such as cell stress, apoptosis and inflammation. Reductions in myocardial T1 in response to dialysis have also reported which would be consistent with higher T1 values being reflective of myocardial oedema (283). Thus,

it is plausible that if cool dialysis was protective against subclinical dialysis-induced ischaemia during the two-week run-in period prior to the MRI study session, such mechanisms might underlie the lower pre-dialysis myocardial T1 and LVMI values in the TCHD group.

Strengths of our study include its novelty with simultaneous structural and functional assessments of different organs during dialysis using multiparametric MRI, and its efficient randomised cross-over design to allow comparisons of an intervention in pragmatically sized cohorts without differences between participants in the study arms (as each participant acts as their own control). Weaknesses of our study include exclusion of unstable HD participants (who perhaps would have benefitted more from cool dialysis) and a relatively small effect of the programmed cooling that may have potentially influenced our results with a bias towards the null hypothesis. We investigated important effects of dialysis on organ function and perfusion but did not report effects on the longer-term organ dysfunction.

In conclusion, this study provides novel insights into the structural and haemodynamic effects of dialysis on the heart, brain and kidneys and how these may interact. Cooling the dialysate using a biofeedback module did not result in significant differences in intradialytic MRI measures as compared to dialysis at 37^0C but did result in reductions in pre-dialysis myocardial T1 and LVMI that may reflect less dialysis-induced cardiac injury over the preceding two weeks.

Chapter 8 Conclusions

Intradialytic haemodynamic instability plays a role in the pathophysiology of the multiorgan morbidity of haemodialysis patients. The aim of this thesis was to characterise the haemodynamic impacts of dialysis and its effects on end-organs.

In our observational study, beat to beat BP variation during HD using extrema point frequency analysis and its impact on intradialytic haemodynamic responses were explored. In the general population, higher BP variation was associated with adverse outcomes. Our study demonstrated comparable findings in HD patients, with a positive association of higher frequency of BP variation with elevated cardiac markers. Furthermore, a higher frequency of BP variation did not correlate with ultrafiltration volumes, suggesting that the frequency of the variation in BP was independent of the fluid shifts during dialysis. This finding potentially makes it a more ideal biomarker than the absolute drop in BP in dialysis patients for future work. Moreover, we noted distinct and differing haemodynamic responses during HD in the two groups categorised based on the frequency of BP variation. The group with a lower frequency of variation seemed to be more dependent on cardiac function. In contrast, the group with higher variation was dependent on total peripheral resistance, suggesting the possibility of reduced cardiac reserve in this group. This advanced characterisation of the haemodynamic responses during HD might help develop more targeted and individualised therapies to overcome the adverse effects of HD.

Evidence is well established suggesting the adverse impact of the process of HD on the cardiac structure and function, and there is growing research to understand the similar effects on the brain. We demonstrated extensive structural alterations in the

brain of dialysis patients, and some of these changes correlated negatively with cognitive function, suggesting functional significance. Regional volumes of grey and white matter in several areas were much lower in HD patients than HCs. Furthermore, these changes were more pronounced with increasing age in the HD group than HCs. The correlation of these adverse changes with dialysis vintage suggests the possible role of dialysis-induced haemodynamic stress in pathogenesis. The previous studies support this phenomenon by demonstrating a higher prevalence of cognitive impairment in HD than PD patients and studies showing reduced cerebral perfusion during HD. We do acknowledge that a better comparator would have been peritoneal dialysis patients with similar levels of comorbidities and might have provided better insight into unique haemodialysis induced stress.

From the above studies, it is evident that the process of HD might have a significant impact on intradialytic haemodynamics and likely contributes to end-organ injury. Hence, we explored the role of cool dialysis as an intervention to mitigate the risks of dialysis-induced haemodynamic stress. Dialysate cooling has been previously demonstrated to reduce intradialytic myocardial stunning and hypotension (associated with higher mortality in the HD group). In our randomised controlled study, we compared the effects of cool vs. standard dialysis on the structure, perfusion and function of multiple organs simultaneously (heart, brain and kidneys). We demonstrated a significant intradialytic reduction in cardiac function, carotid and basilar artery blood flow and total kidney volume. There were no differences in these observed changes in both arms. Characteristics of study population could be one of the possible explanations for the lack of differences in the primary end point. We have excluded the patients who had significant intradialytic events. This was done to avoid compromise in patient safety as our study setup was such that MRI scans were

performed away from acute hospital setting. This may have excluded the patients that may have benefitted the most from our intervention (cool dialysis) and these factors need to be considered when designing a study. We used BTM device available on the dialysis machine to deliver our intervention of cooling the dialysate as it allows gradual reduction in the dialysate temperature (ranging from 0ºC/ hour to -0.5ºC/ hour), but we could not achieve significant separation in the post dialysis body temperatures between the study arms and we have observed heterogeneity in the effect size to cooling in the study participants. Moreover, although previous meta-analysis supports that all types are cool dialysis have a significant impact on improving intradialytic haemodynamic stability, fixed cooling appear to more beneficial in reducing the difference between pre and post dialysis MAP (150). Perhaps, a study design aiming to reduce the body temperature to pre-agreed levels based on the pre-dialysis body temperature may have more appropriate to test our hypothesis but understandably one can expect practical challenges to achieve this.

This study identified a significantly lower baseline myocardial T1 and LVMI in the cool HD arm. These changes might reflect the protective effect of cool HD during the run-in period and may represent underlying early molecular changes secondary to cooling in the myocardium. Exploring these changes further might help understand the long-term protection offered by cool HD.

In conclusion, the results presented in this thesis demonstrated novel findings in the haemodynamic responses to dialysis, the impact of dialysis on end-organs, and highlighted the potential mechanisms underlying the long-term protective effects of the cool dialysis. These need further exploration to understand the modifiability of these responses, clinical significance, and the long-term consequences.

Section 8.1 Future plans

This thesis provided novel insights into the potential mechanisms of HD-induced haemodynamic stress. However, some of the results are themselves hypothesis-generating.

Although we have demonstrated significant structural alternations in the brain of dialysis patients and extrapolated that these may reflect the impact of the dialysis treatment process because of their correlation with dialysis vintage, we were unable to explain the mechanisms for such a high prevalence of these adverse changes in the HD group or the associated clinical significance. Cardiac studies clearly demonstrate the role of HD induced haemodynamic stress in the pathogenesis of cardiac dysfunction, but it is not well established in other organ systems. Our study generated lots of data about the simultaneous changes in multiple organs during dialysis and exploring these data further might provide additional insights. For example, mapping the intradialytic regional cerebral perfusion changes in the brain regions where volume loss was noted will enable us to understand if HD- induced subclinical ischaemia occurs in the brain and explore how these changes relate to intradialytic changes in the cardiac function.

Our observational study involving non-invasive monitoring during dialysis highlighted a significant technological gap in the haemodynamic monitoring of dialysis patients. The current monitoring system is intermittent, minimalistic and not adequate to pre-emptively identify patients at risk of developing haemodynamic instability. Although the monitoring approach we used in our study was non-invasive, it is not practical to adopt in everyday practice. We are currently collaborating with a team of engineers from the University of Derby and working towards developing sensors that can be

connected to the arterial and venous dialysis lines to enable continuous monitoring of the patients during dialysis. This work has attracted funded from Kidney Research UK and is currently in the preliminary stages, but we are excited about our progress in generating pressure waveforms from these lines to derive arterial blood pressure. Hopefully, these can be developed further and can be calibrated to the brachial BP waveforms to generate information regarding continuous BP changes.

It was exciting to learn about the different haemodynamic behaviours of the patients based on the categories of the BP variation. Further work is required 1) to categorise patients from a shorter monitoring session, 2) to explore if this haemodynamic behaviour is similar in the follow-up dialysis sessions (long term) and 3) to understand its modifiability using dialysis-based interventions to enable us to understand the clinical significance of our findings. In this thesis, only the baseline results of this study were presented. Further analysis of the follow-up data is planned and will help answer some of the above questions.

Chapter 9 List of Abbreviations

ABPM	Ambulatory Blood Pressure Monitoring
ACE	Angiotensin Converting Enzyme
ARB	Angiotensin Receptor Blocker
ARV	Average Real Variability
ASL	Arterial Spin labelling
AVF	Arterio-Venous Fistula
BCM	Bioimpedance
BMI	Body Mass Index
BNP	Brain Natriuretic Peptide
BOLD	Blood oxygenation level dependent
BP	Blood Pressure
BRS	Baroreceptor Sensitivity
BSA	Body Surface Area
BTM	Blood Temperature Monitor
CBF	Cerebral Blood Flow
CCB	Calcium Channel Blocker
CCI	Charlson Comorbidity Index
CI	Confidence Interval
CKD	Chronic Kidney Disease
CMB	Cerebral Microbleeds
CO	Cardiac Output
CPI	Cardiac Power Index
CSF	Cerebrospinal Fluid
CT	Computed Tomography
CVD	Cardiovascular Disease
CWMC	Cerebral White Matter Changes
DBP	Diastolic Blood Pressure
DV	Dialysis Vintage
EBPG	European Best Practice Guidelines
ECG	Electrocardiogram
ECHO	Echocardiogram
EDV	End Diastolic Volume
EF	Ejection Fraction
EP	Extrema Points
ESKF	End Stage Kidney Failure
ESV	End Systolic Volume
FA	Fractional Anisotropy
FAIR	Flow Alternating Inversion Recovery
FOV	Field of View
GFR	Glomerular Filtration Rate
GIT	Gastrointestinal Tract
GLM	General Linear Model
GM	Grey Matter

GMV	Grey Matter Volume
GP	General Population
HC	Healthy Control
HD	Haemodialysis
HDF	Hemodiafiltration
HF	High Frequency
HFC	High Frequency changes
HR	Hazard Ratio
IDH	Intradialytic Hypotension
IDWG	Intradialytic Weight Gain
ILL	Insulated Longlines
IQR	Inter Quartile Range
IRAS	Integrated Research Application System
KDOQI	Kidney Disease Outcomes Quality Initiative
LC	Levocarnitine
LF	Low Frequency
LFC	Low Frequency Changes
LL	Longlines
LV	Left Ventricle
LVH	Left Ventricular Hypertrophy
LVMI	Left Ventricular Mass Index
MAP	Mean Arterial Pressure
MoCA	Montreal Cognitive Assessment
mFFE	Multi-echo fast field echo
MI	Myocardial Infarction
MNI	Montreal Neurological Institute
MOLLI	Modified Look-Locker Inversion Recovery
MPRAGE	Magnetization Prepared - RApid Gradient Echo
MRI	Magnetic Resonance Imaging
NILL	Non- insulated longlines
NRES	National Research Ethics Service
NSAIDS	Non-Steroidal Anti- Inflammatory Drugs
NT proBNP	N-Terminal Pro Brain Natriuretic Peptide
NYHA	New York Heart Association
OL- HDF	Online Hemodiafiltration
OR	Odds ratio
PC-MRI	Phase Contrast MRI
PD	Peritoneal Dialysis
PET	Positron Emission Tomography
PPU	Peripheral Pulse Unit
RAAS	Renin Angiotensin Aldosterone System
RBV	Relative Blood Volume
RCT	Randomised Controlled Trial
ROI	Region of Interest
RR	Relative Risk

RRT	Renal Replacement Therapy
RRF	Residual Renal Function
RWMA	Regional Wall Motion Abnormality
SBP	Systolic Blood Pressure
SD	Standard Deviation
SE-EPI	Spin Echo-Echo Planar Imaging
SEM	Standard Error of Mean
SHD	Standard Haemodialysis
SL	Standard Lines
SMI	Silent Myocardial Ischaemia
SPM	Statistical Parametric Mapping
SPMIC	Sir Peter Mansfield Imaging Centre
SPSS	Statistical Package for the Social Sciences
SV	Stroke Volume
SVI	Stroke Volume Index
TCHD	Thermo-controlled Haemodialysis
TE	Echo Time
TFE	Turbo Field Echo
TIV	Total Intracranial Volume
TKV	Total Kidney Volume
TMT	Trail Making Test
TPR	Total Peripheral Resistance
TPRI	Total Peripheral Resistance Index
TR	Repetition Time
TT	Tympanic Temperature
UF	Ultrafiltration
UFR	Ultrafiltration Rate
UFV	Ultrafiltration Volume
UK	United Kingdom
US	United States
USRDS	United States Renal Data System
VBM	Voxel Based Morphometrics
VCG	Vectorcardiogram
WM	White Matter
WMV	White Matter Volume

Chapter 10 References

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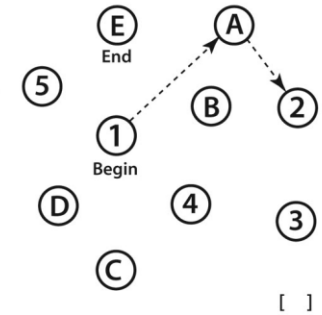
Chapter 11 Appendices

Section 11.1 Montreal Cognitive Assessment (MoCA)

MONTREAL COGNITIVE ASSESSMENT (MOCA)
Version 7.1 Original Version

NAME :
Education : Date of birth :
Sex : DATE :

VISUOSPATIAL / EXECUTIVE — POINTS



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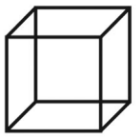


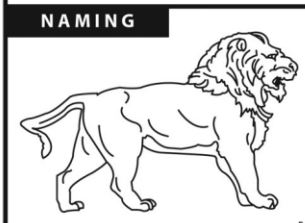
[ ] [ ]

Draw CLOCK (Ten past eleven)
(3 points)

[ ] Contour [ ] Numbers [ ] Hands

__/5

NAMING



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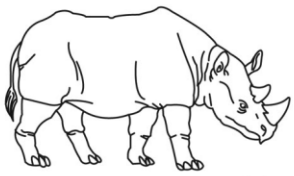

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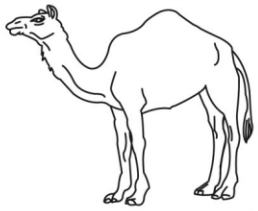

[ ]

__/3

MEMORY Read list of words, subject must repeat them. Do 2 trials, even if 1st trial is successful. Do a recall after 5 minutes.

	FACE	VELVET	CHURCH	DAISY	RED
1st trial					
2nd trial					

No points

ATTENTION Read list of digits (1 digit/ sec.).
Subject has to repeat them in the forward order [ ] 2 1 8 5 4
Subject has to repeat them in the backward order [ ] 7 4 2
__/2

Read list of letters. The subject must tap with his hand at each letter A. No points if ≥ 2 errors
[ ] F B A C M N A A J K L B A F A K D E A A A J A M O F A A B
__/1

Serial 7 subtraction starting at 100 [ ] 93 [ ] 86 [ ] 79 [ ] 72 [ ] 65
4 or 5 correct subtractions: **3 pts**, 2 or 3 correct: **2 pts**, 1 correct: **1 pt**, 0 correct: **0 pt**
__/3

LANGUAGE Repeat : I only know that John is the one to help today. [ ]
The cat always hid under the couch when dogs were in the room. [ ]
__/2

Fluency / Name maximum number of words in one minute that begin with the letter F [ ] _____ (N ≥ 11 words)
__/1

ABSTRACTION Similarity between e.g. banana - orange = fruit [ ] train – bicycle [ ] watch - ruler
__/2

DELAYED RECALL

	Has to recall words WITH NO CUE	FACE [ ]	VELVET [ ]	CHURCH [ ]	DAISY [ ]	RED [ ]	Points for UNCUED recall only
Optional	Category cue						
	Multiple choice cue						

__/5

ORIENTATION [ ] Date [ ] Month [ ] Year [ ] Day [ ] Place [ ] City
__/6

© Z.Nasreddine MD www.mocatest.org Normal ≥ 26 / 30

TOTAL __/30
Add 1 point if ≤ 12 yr edu

Administered by: _____

Section 11.2 Trail Making Tests A and B

Trail Making Test (TMT) Parts A & B

Instructions:

Both parts of the Trail Making Test consist of 25 circles distributed over a sheet of paper. In Part A, the circles are numbered 1 – 25, and the patient should draw lines to connect the numbers in ascending order. In Part B, the circles include both numbers (1 – 13) and letters (A – L); as in Part A, the patient draws lines to connect the circles in an ascending pattern, but with the added task of alternating between the numbers and letters (i.e., 1-A-2-B-3-C, etc.). The patient should be instructed to connect the circles as quickly as possible, without lifting the pen or pencil from the paper. Time the patient as he or she connects the "trail." If the patient makes an error, point it out immediately and allow the patient to correct it. Errors affect the patient's score only in that the correction of errors is included in the completion time for the task. It is unnecessary to continue the test if the patient has not completed both parts after five minutes have elapsed.

Step 1:	Give the patient a copy of the Trail Making Test Part A worksheet and a pen or pencil.
Step 2:	Demonstrate the test to the patient using the sample sheet (Trail Making Part A – *SAMPLE*).
Step 3:	Time the patient as he or she follows the “trail” made by the numbers on the test.
Step 4:	Record the time.
Step 5:	Repeat the procedure for Trail Making Test Part B.

Scoring:

Results for both TMT A and B are reported as the number of seconds required to complete the task; therefore, higher scores reveal greater impairment.

	Average	Deficient	Rule of Thumb
Trail A	29 seconds	> 78 seconds	Most in 90 seconds
Trail B	75 seconds	> 273 seconds	Most in 3 minutes

Sources:

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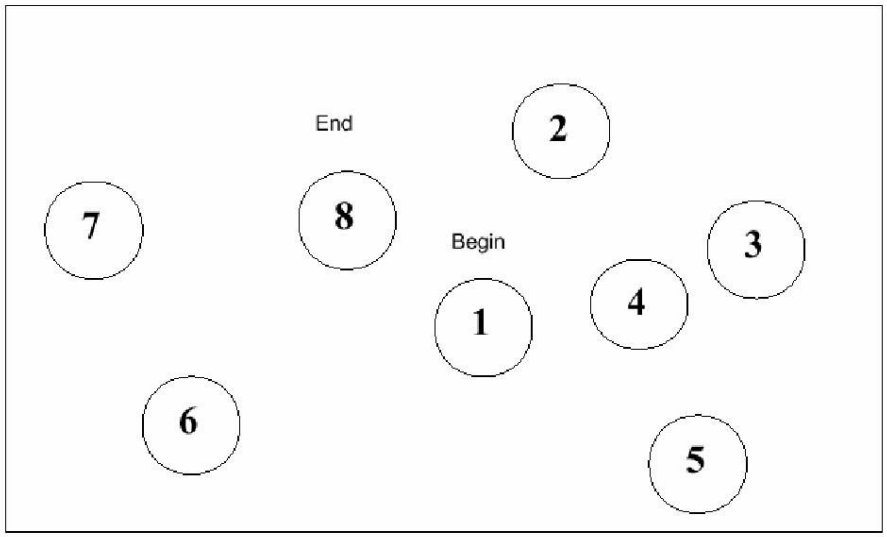
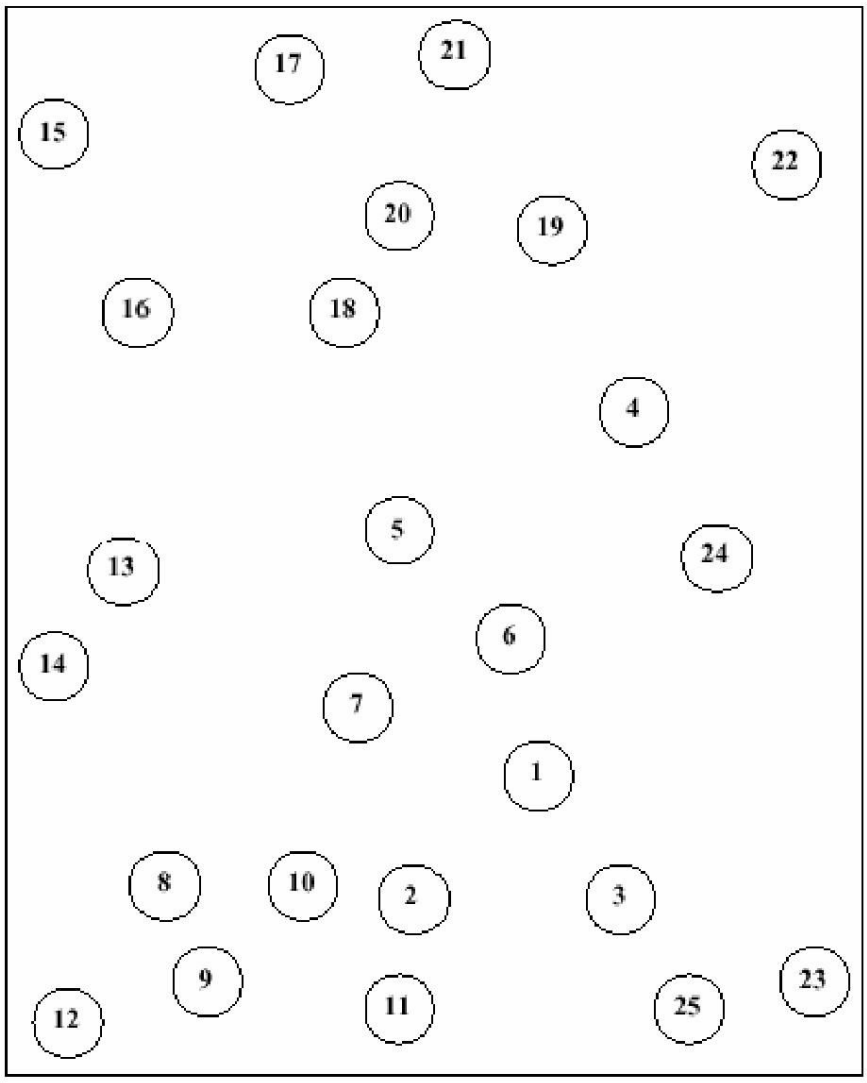
Trail Making Test Part A – *SAMPLE*

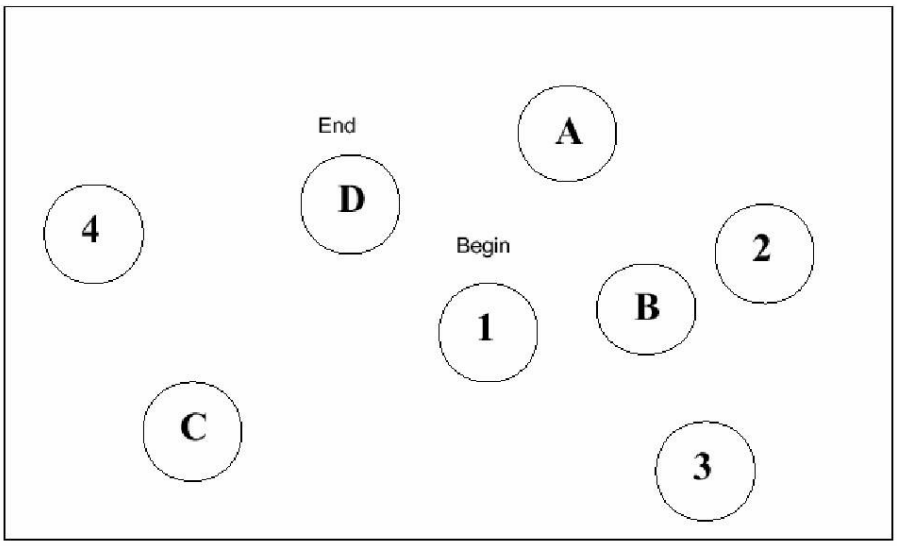


Trail Making Test Part A

Patient's Name: _____ Date: _____

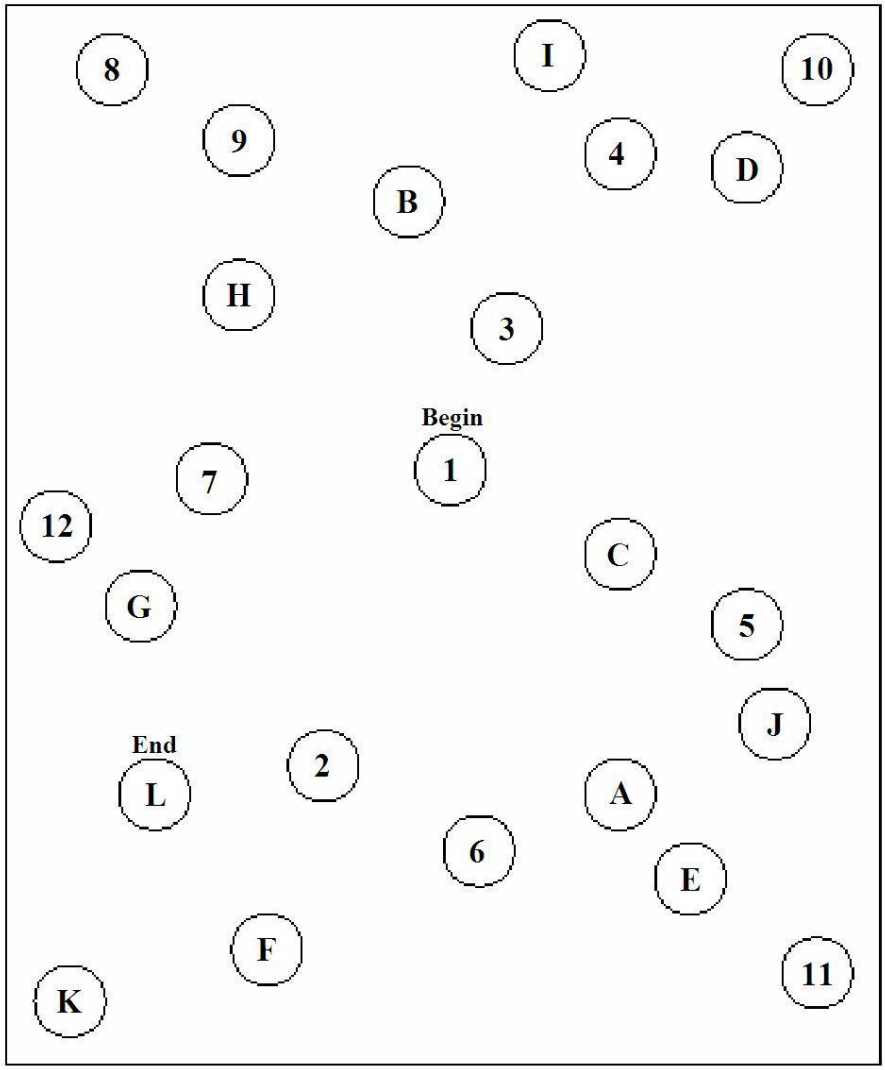


Trail Making Test Part B – *SAMPLE*



Trail Making Test Part B

Patient's Name: _____ Date: _____



Section 11.3 Magnetic Resonance Imaging Safety Questionnaire

The University of Nottingham

Sir Peter Mansfield Magnetic Resonance Centre

MR Volunteer Safety Screening Questionnaire:

NAME	Date of Scan	Date of Birth
ADDRESS	Volunteer Number	
	Ethics Code	
Phone number	Weight	Height if applicable

MR scanning uses strong magnetic fields. For your own safety and the safety of others it is **very important** that you do not go into the magnet halls with any metal in or on your body or clothing. Please **answer** the following questions **carefully** and ask if anything is not clear. All information is held in the strictest confidence.

1. Do you have any implants in your body? e.g. replacement joints, drug pumps Y/N
2. Do you have aneurysm clips (clips put around blood vessels during surgery)? Y/N
3. Do you have a pacemaker or artificial heart valve? *(These stop working near MR Scanners)* Y/N
4. Have you ever had any surgery? Please give brief details over. Y/N
   (We do not need to know about uncomplicated caesarian delivery, vasectomy or termination of pregnancy)
5. Do you have any foreign bodies in your body (e.g. shrapnel)? Y/N
6. Have you ever worked in a machine tool shop without eye protection? Y/N
7. Do you wear a hearing aid or cochlear implant? Y/N
8. Could you be pregnant? (Pregnancy tests are available in the female toilets) Y/N
9. Have you ever suffered from tinnitus? Y/N
10. Do you wear dentures, a dental plate or a brace? Y/N
11. Are you susceptible to claustrophobia? Y/N
12. Do you suffer from blackouts, epilepsy or fits? Y/N
13. Do you have any tattoos? Where? Y/N
14. Do you have any body piercing jewellery that cannot be removed? Y/N
15. Do you have any skin patches (trans-dermal patches)? Y/N
16. Do you have a coil in place (IUD) for contraception? Do you know what type? Y/N
17. Do you have any condition that may affect your ability to control your temperature (e.g. Do you have a fever, cardiovascular disease, hypertension, diabetes or cerebrovascular disease? Are you taking diuretics, beta-blockers, calcium blockers, amphetamines, muscle relaxants or sedatives?) Y/N
18. Will you remove all metal including coins, body-piercing jewellery, false-teeth, hearing aids etc before entering the magnet hall.? *(lockers available by the changing rooms)* Y/N
19. Is there anything else you think we should know? Y/N

I have read and understood all the questions **Signature:**	**Date:**
Verified by: **SPMMRC Staff Signature:**	**Date:**

MRI Exercise Dev: MRI Questionnaire, version1.0, Date: 28/04/2014

Section 11.4 Research setting of the Magnetic Resonance Imaging area

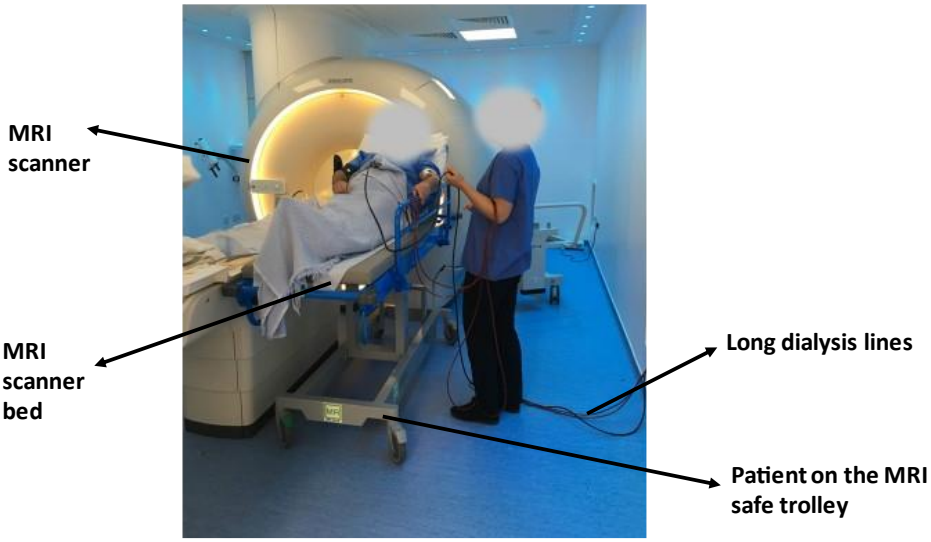
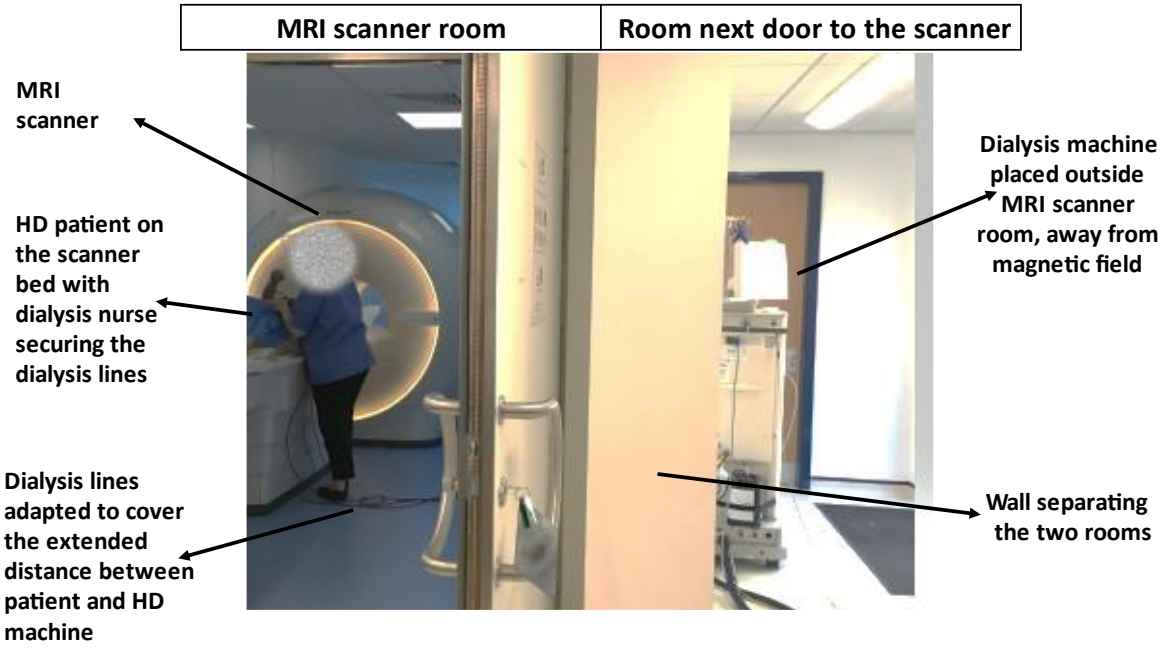


Image of the Magnetic Resonance Imaging (MRI) scanner room with participant on the MRI safe trolley and research nurse supervising him. Long lines are visible on the floor, extending from patient to the wall where they pass through the line guard to the space



Left side is the participant and research nurse in the scan room; long extension lines are visible on the floor in the scan room; on the right side (outside the scan room), is the dialysis machine