Cardiac Ischaemic Stress in the Haemodialysis Patient

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'Science and everyday life cannot and should not be separated'

Rosalind Franklin (July 1920 – April 1958)

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ABSTRACT

Haemodialysis patients experience elevated levels of cardiovascular morbidity and mortality that has a profound effect on not only their survival and quality of life but also increases the already high social and economic cost of dialysis. It is increasingly appreciated that the circulatory stress caused by dialysis is a significant contributing factor and helps to accelerate the end organ damage this group of patients is known to experience. In particular the cumulative ischaemic insult suffered by the heart during haemodialysis sessions has been suggested as one of the principal drivers of heart failure and sudden cardiac death – the two principal causes of death in this population.

The importance of dialysis induced haemodynamic instability was reinforced as we explored the relationship between cardiac function and the measure of intra-dialytic hypotension most clearly associated with mortality (a blood pressure below 90mmHg) and found that the severity of dialysis induced cardiac injury was experienced across the whole range of dialysis induced hypotension. A nadir blood pressure below 90mmHg was strongly associated with established reduction in systolic contractile function.

We then tested two separate interventions designed to mitigate dialysis-induced injury. The first was Remote Ischaemic Preconditioning, a technique that in pre-clinical models and numerous small clinical studies

protects against the effect of the ischaemia-reperfusion injury. We found that a single application of RIPC significantly reduces dialysis induced cardiac injury for up to 28 days. The second intervention was the stepwise reduction of dialysate sodium to reduce intra-dialytic fluid accumulation and the need for aggressive ultrafiltration. We found this to be well tolerated and an effective way to reduce inter-dialytic weight gain. This intervention did not have any adverse cardiac consequences and may have resulted in a modest improvement in cardiac tolerability while still being delivered within the context of a conventional 4-hour haemodialysis treatment.

Finally, to investigate if transplantation is capable of reversing any of the factors predisposing dialysis patients to increased cardiovascular events, we chose to investigate one of the risk factors that contributes to the abnormalities of the vasculature and leave patients vulnerable to dialysis induced cardiac injury. We measured the deposition of advanced glycation end-products (via the method of skin autofluorescence) in patients who had undergone renal transplantation and compared this to existing cohorts of dialysis and chronic kidney disease patients. We found that following transplantation these markers of metabolic stress regressed to levels comparable to those seen in chronic kidney disease and much lower than seen in dialysis. This finding may suggest that the dialysis procedure itself is responsible for a great deal of metabolic stress and helps to accelerate the process by which the vasculature becomes stiff and non-compliant.

In conclusion, we tested two interventions that showed potential to reduce the cardiac stress dialysis patients are subject to. Remote ischaemic preconditioning directly reduces the severity of cardiac injury and the stepwise reduction of dialysate sodium decreases inter-dialytic fluid gains and produces a modest improvement in cardiac tolerability. We also confirmed that transplantation reverses advanced glycation end-product deposition, one of the key non-traditional risk factors for cardiovascular disease in dialysis patients, giving us further insight into the ways in which transplantation improves cardiovascular outcome.

PUBLICATIONS AND ABSTRACTS ARISING FROM THIS THESIS

Journal Articles

TISSUE ADVANCED GLYCATION END PRODUCT DEPOSITION AFTER KIDNEY TRANSPLANTATION

Crowley LE, Johnson CP, McIntyre N, Fluck RJ, McIntyre CW, Taal MW, Leung JC.

Nephron Clin Pract. 2013;124(1-2):54-9

REMOTE ISCHAEMIC CONDITIONING: THERAPEUTIC OPPORTUNITIES IN RENAL MEDICINE

Crowley LE, McIntyre CW.

Nat Rev Nephrol. 2013 Dec;9(12):739-46

Oral Presentations

TRANSPLANTATION IS ASSOCIATED WITH NORMALISED TISSUE-ADVANCED GLYCATION END PRODUCT DEPOSITION

Crowley L, Johnson, C , McIntyre N , Fluck R , McIntyre C , Taal M , Leung J

British Renal Society May 2012

REMOTE ISCHAEMIC PRECONDITIONING IN HAEMODIALYSIS: AN INITIAL RANDOMISED CONTROLLED TRIAL.

Crowley L, Odudu A, McIntyre C.

American Society of Nephrology November 2014

Poster Presentations

TRANSPLANTATION IS ASSOCIATED WITH NORMALISED TISSUE-ADVANCED GLYCATION END PRODUCT DEPOSITION

Crowley L, Johnson, C , McIntyre N , Fluck R , McIntyre C , Taal M , Leung J

American Society of Nephrology Nov 2012

STEPWISE REDUCTION OF DIALYSATE SODIUM TO REDUCE MYOCARDIAL STUNNING

Crowley LE, Hollier K, Odudu A, Dasgupta I, McIntyre CW

American Society of Nephrology November 2014

DECLARATION

Except where acknowledged, I declare that this thesis is entirely my own work and is based upon research carried out in the School of Graduate Entry Medicine and Health, University of Nottingham and three NHS research sites between November 2011 and August 2014. Ethical approval and Research and Development approvals in all centres were sought and gained by myself. This work consists of a retrospective analysis of previously obtained images, two interventional studies with wholly new collected data and a cross-sectional study of transplant patients comparing wholly new collected data with a historical database. The main funding application for the remote ischaemic preconditioning study was made by Professor Chris McIntyre and Dr. Aghogho Odudu to the British Heart Foundation. Heart Research UK provided funding for my entire training fellowship for both the sodium and remote ischaemic preconditioning studies.

Echocardiograms analysed in study one were performed by Dr. James Burton, Dr. Aghogho Odudu, Dr. Tarek Eldeheni and by myself. All patients in the interventional studies were recruited by myself with assistance from the research staff at Heart of England NHS Trust. I also collected all the data in the interventional studies, this involved travelling to all dialysis centres with the haemodynamic monitoring equipment to study patients on dialysis without changing their dialysis times. I received

appropriate training in obtaining echocardiographic images for research purposes from Professor McIntyre.

I formulated the hypothesis and designs for study one and analysed the echocardiograms with help from research fellow Steven Wong. I analysed all the echocardiograms from both interventional studies. All databases associated with the study were constructed by me. I collected the data for study 4 with the assistance of Mrs. Cathy Johnson, transplant nurse specialist. All database construction and statistical and interpretation in this study were performed by be. The statistical analysis in the thesis was performed by me in its entirety. Biochemical and haematological data were provided by the laboratories at Derby Hospitals NHS Foundation Trust or Sheffield teaching Hospitals NHS Foundation Trust. The statistical design of both interventional trials was verified by Mr. Aposotolos Fakis, an independent statistician at the Royal Derby Hospital NHS Trust.

Lisa Crowley

November 2015

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CHAPTER 1: INTRODUCTION

1.1 The Function of the Kidneys

In order for all organ systems of the body to function normally, an environment conducive to normal metabolism must be maintained. The kidneys play an essential role in the homeostatic maintenance of this environment. They are the principal means by which the body eliminates the waste products of normal metabolism, such as urea, creatinine, uric acid, metabolites of various hormones, and prevents these waste products from rising to harmful levels.

The kidneys also play an essential role in regulating the balance of water and various salts within the body. They ensure that normal cellular function be maintained by regulating the volume and electrolyte content of both intravascular and extravascular fluids. By controlling intravascular volume and sodium content and the secretion of various vasoactive substances such as renin they also play an integral part in the regulation of blood pressure to maintain organ perfusion.

Finally the kidneys also fulfill important endocrine functions. The production of red blood cells in the bone marrow is stimulated by their secretion of erythropoietin. They also release the enzymes that perform the final step in producing the active form of vitamin D, which is critical to the regulation of calcium and phosphate balance.

1.2 Chronic Kidney Disease

The definition of chronic kidney disease (CKD) is a reduction of glomerular filtration rate (GFR) to below 60 ml/min/1.73m^2 for greater than three months or other abnormalities such as structural issues or genetic traits which point to kidney disease even in the presence of a normal GFR [(1)]. CKD has been classified into five stages according to the level of reduction of GFR. Table 1 below shows the KDOQI classification of CKD.

Stage	GFR (ml/min/1.73^2)	Description
1	90	Normal kidney function but urine findings or structural abnormalities or genetic trait point to kidney disease
2	60-89	Mildly reduced kidney function, and other findings (as for stage 1) point to kidney disease
3A 3B	45-59 30-44	Moderately reduced kidney function
4	15-29	Severely reduced kidney function
5	< 15	Very severe, or **end stage** kidney disease (sometimes call **established renal failure**)

Table 1: KDOQI classification of Chronic Kidney Disease [(2)]

In clinical practice GFR is commonly estimated (eGFR) using measurements of serum creatinine. Several equations can be used, including the Modified Diet in Renal Disease formula, to estimate GFR after adjusting for age, gender

and ethnicity [3]. More recently the recommendation has been to use the CKD-EPI equation, which uses the same variables (age, gender, ethnicity) but a different relationship and mathematical model. This equation more accurately predicts risk of mortality and end-stage renal failure and is less biased when GFR is greater than 60 [4]. It is now recommended by the National Institute of Clinical Excellence as the equation that should be used for estimation of GFR [5]. Addition of the biomarker Cystatin C to this equation may increase the accuracy of its estimates further [6]. This biomarker is produced at a constant rate (by all nucleated cells) and due to its small size is freely filtered by the glomerulus and not secreted but fully reabsorbed and broken down by the renal tubules. This means the primary determinate of blood Cystatin C levels is the rate at which it is filtered at the glomerulus. Therefore, Cystatin C is in principal a better marker for the estimation of GFR as, unlike creatinine, it's levels are unaffected by age, gender, muscle mass and ethnicity.

1.3 Definition and Epidemiology of End-Stage Kidney Disease

Patients requiring renal replacement therapy (RRT) with dialysis are classified as having End Stage Renal Disease (ESRD). Generally these patients will have a Glomerular Filtration Rate (GFR) of less than 15ml/min/m^2. There is no current consensus on the optimum GFR at which to start a patient on RRT as patients can become symptomatic at different levels of kidney function. At low levels of kidney function patients may experience symptoms secondary to build up of uraemic toxins (nausea, loss of appetite, fatigue) or fluid overload

(swelling of the extremities, shortness of breath) which may require the initiation of RRT. They may also have persistently high levels of potassium, which places them at risk of cardiac arrest and would also necessitate dialysis.

According to the United Kingdom Renal Registry there were 56,940 adult patients receiving RRT as of 31st December 2010. Of these 41.6% (approximately 23,350) were receiving RRT by means of haemodialysis (HD) [7] with the rest made up of PD and renal transplantation. These figures illustrate that HD remains the dominant modality in the United Kingdom. The leading cause of mortality in ESRD patients is cardiovascular disease (CVD) which accounts for 23% of deaths [8]. It is well recognised that all patients with CKD suffer rates of cardiovascular disease that are much higher than the general population with the highest rates being present in those patients with ESRD receiving RRT. This differential in survival is proportionally greater with younger patient age [9].

1.4 Haemodialysis

Haemodialysis (HD) describes the process of removal of excess fluids, salt and waste products from a patient's blood replacing some of the loss function of the kidneys. Blood is removed from the body and pumped across one side of a semi-permeable dialysis membrane with fluid containing a fixed

concentration of major body salts (dialysate) passing in the opposite direction on the other side of the membrane. During a conventionally performed four hour session with a reasonable flow rate, the patient's entire blood volume will be removed and returned to the patient approximately 15 times. The removal of fluid, solutes or waste products occurs via diffusion, convection and adsorption (listed in descending order of relative importance)**.**

Prior to the development of dialysis, kidney failure was usually fatal (indeed the author's great-grandmother Annie died of Bright's Disease, a historical name for a nephritic syndrome often including kidney failure, in the early 1930's). HD is a life-saving treatment and the story of its development is partially the story of evolving technological and surgical development over the last one hundred years. The idea of purifying the blood from waste products dated from the early 20th century but initial publication of a clinically successful treatment was not published until 1944 [(10)].

The continuing evolution of dialysis technology and vascular access eventually allowed for the establishment of chronic HD programmes. The first such programme was established in Seattle, by Scribner and colleagues, in 1961 and they were increasingly common in the USA and Europe by the mid-sixties. In early HD coil dialysers were used and dialysis was administered once or twice per week. Management of ultrafiltration (UF) was poorly regulated. Pressure gradients between blood and dialysate compartments

had to be created through manual adjustment. Fluid removal prescription was estimated rather than being directly delivered. This led to very long dialysis sessions and unpredictable UF rates. In addition, non-physiological acetate, converted into bicarbonate in the liver, was used as a buffer. Acetate exposure led to patients experiencing many problems during treatment including hypotension and headaches. Blood leaks and bacterial contaminations were a constant concern [10]. Figure 1 illustrates the progression of dialysis technology with comparison between an early rotating drum dialyser and a modern day machine.

There have been a number of advancements in dialysis technology that allowed the shortening of dialysis times with a more comfortable experience for the patient. The most important of these have included the advent of disposable hollow fibre dialysers developed in the late sixties, the development of dry bicarbonate concentrates to be used as a buffer eliminating acetate use, and the ability to program machines to apply a pressure gradient across the membrane allowing for volumetric dialysis with prescribed and measured fluid removal.

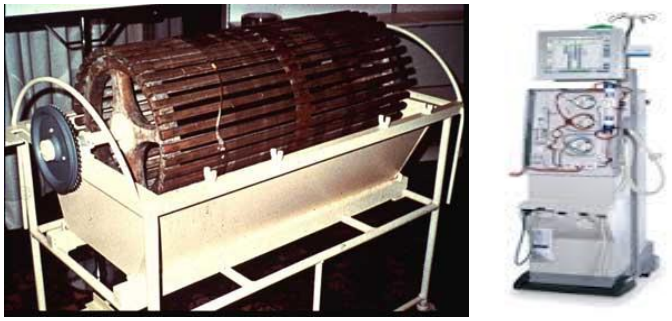

Figure 1: Original Kolff rotating drum machine for dialysis and modern dialysis machine equivalent with fully integrated production of dialysate, hydraulic and blood circuits.

Current conventional practice is for patients with an estimated GFR of less than 15ml/ml/m^2 to be considered for RRT in the form of renal transplantation, peritoneal dialysis (PD) or HD. The number of patients fit enough to undergo organ transplantation and the number of available donor organs limits access to the first option. PD is a form of home therapy that involves the patient performing at least four dialysis 'exchanges' per day via a tube inserted and indwelling in their peritoneal cavity. It is an effective method of providing RRT (despite fairly low efficiency) but it has a number of challenges for the patient including the ability to successfully perform exchanges, and adequate storage space in the home. PD also becomes less effective in terms of solute clearance over time due to sclerosis of the peritoneum caused by longstanding exposure to the dialysis fluid. Failure of UF is usually a consequence of peritoneal neovascularization, increasingly high transporter status and the loss of the osmotic gradient. While PD is a very good option for some people (and in many parts of the world is the most frequently used form of RRT) the most common form in the UK remains HD.

1.5 Principles of Haemodialysis

HD principally utilises diffusion across a semi-permeable membrane to exert its effect. Small solutes such as creatinine and urea and ions such as potassium diffuse out down a concentration gradient. The counter current flow principle, with blood flowing in the opposite direction to the dialysate, is utilised to ensure the concentration gradient remains maximal. Diffusion occurs across the concentration gradient between the dialysate and the blood. Conversely, the dialysate has a higher concentration of bicarbonate allowing diffusion in the opposite direction to correct the acidosis associated with chronic kidney disease.

The composition of the various solutes therefore plays an important role in haemodialysis allowing for the control of various important salts via dialysis treatment. Table 2 below shows the ranges of various solutes found in the typical dialysate. These can be adjusted according to the individual patients metabolic profile. Control of plasma tonicity is of paramount importance in order to maintain cellular integrity and this is largely influenced by plasma sodium content. The management of sodium via dialysis will be discussed in more detail later in this thesis.

Ions	Concentrations
Na^+	132-145 mEq/L
K^+	0-4 mEq/L
Cl^-	103-110 mEq/L

HCO_3	0-40 mEq/L
Ca^{2+}	0-3.5 mEq/L
Mg^{2+}	0.5-1 mEq/L
Glucose	0-200 mg/dL

Table 2: Typical composition of dialysate solution to be used with intermittent haemodialysis

Ultrafiltration (UF) also occurs by the generation of a negative pressure within the dialysate compartment. This causes a pressure gradient along which free water can move. The amount of fluid removal required is based on two key concepts 1) an estimate of a dialysis patients 'dry' weight, prior to each session the patient is weighed and the fluid removal prescribed according to how far over the target weight the patient is, and 2) the accumulation of fluid between dialysis sessions. Inter-dialytic weight gain (IDWG) depends upon factors such as residual renal function and fluid and salt, and the subsequent ingestion of free water (to remain isotonic).

While the development and availability of HD (at least in the developed world) remains a tremendous medical achievement it is still a rather crude and imperfect treatment. We have replaced the continuous function of the kidneys with an intermittent treatment replicating a continuous GFR of 10-15 (CKD stage 5). Removal of waste products via dialysis is a life supporting measure but cannot match the efficacy of the native kidneys. Nephrologists can alter various parameters (the dialysis prescription) according to each patient to try and improve the efficiency of the procedure. For example dialysate potassium

concentration can be modulated to optimize potassium removal. The efficiency of waste product removal can be altered by measures such as increasing blood flow rates and extending dialysis time as well as dialyser characteristic choice. All of the changes the nephrologist may make have implications in terms of tolerability and acceptability to the patient. The physiological needs of the patient need to be carefully balanced with the effect the treatment has upon them.

Conventional HD typically involves 3-5 hours per session three times per week administered in a dialysis centre. The conventional length of a dialysis session is largely a result of the increasing number of people receiving dialysis leading to an 'industrialisation' of the process and the resulting need for operational and cost expediency. Measurement of dialysis efficacy, with regard to small solute clearance in order to determine optimal dose is a somewhat controversial subject but at present a number of measures are used to determine whether the dose of dialysis is 'adequate', the most common of which is Kt/V where K=dialyzer clearance of urea, t=dialysis time and v=volume of distribution of urea**.** Adequate dialysis has been defined from previous pivotal randomized controlled trials with low dialysis doses (as measured by low removal of blood urea nitrogen) resulting in a Kt/V of less than 1.2 associated with an increased morbidity and mortality [(11)]. These trials were conducted before the further evolution of dialysis technology and more recent randomized trials have not shown a benefit to increasing Kt/V. The

clearance of urea alone may not be a good surrogate marker for clearance of the many other potentially toxic metabolic waste products [12].

1.6 Cardiovascular Disease in Haemodialysis Patients

Patients on regular HD suffer from a very large treatment burden. They require the formation of special vascular access to facilitate connection to the dialysis machine, are more vulnerable to infection and are prone to extremely high rates of cardiac failure and sudden death. The annual mortality rates of dialysis patients are shown in figure 2 and are estimated to be up to 30 times that found in the aged matched general population on average with rates being much higher than that in younger people on dialysis [13].

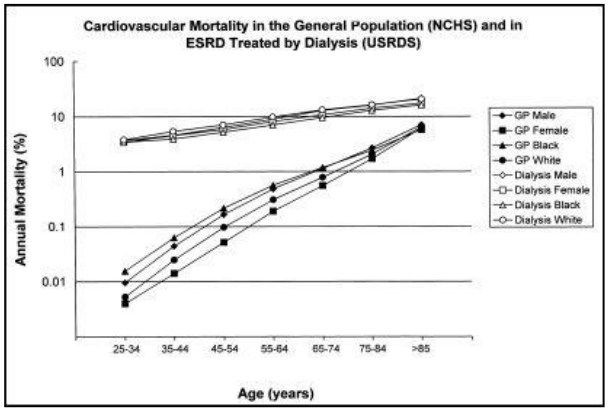

Figure 2: Graph showing the cardiovascular mortality rates by age and ethnicity for both dialysis patients and healthy controls [13]

The most recent mortality data from the United States confirms that CVD continues to be the most common cause of death in both incident (under 180 days exposure to dialysis – data shown in figure 3) and prevalent (greater than

180 days exposure) patients. The largest proportion of this is due to arrhythmia or sudden cardiac death with the next most common being congestive heart failure [14].

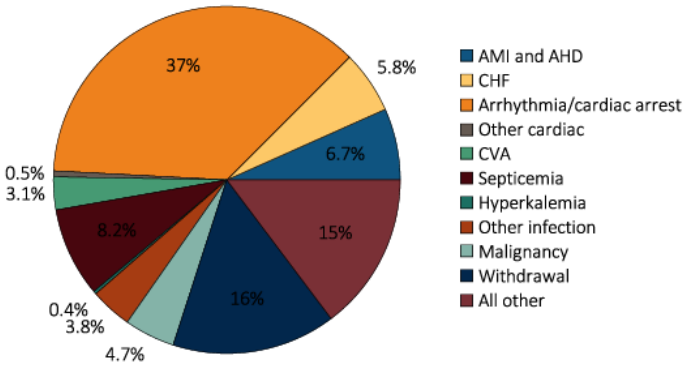

Figure 3: Causes of death in incident haemodialysis patients in the United States (2014) [14]

1.7 Abnormalities of the Vasculature in Haemodialysis Patients

1.7.1 Classical vs. Non-Classical Risk Factors

In the general population, cardiovascular mortality is well recognised to be driven by a set of risk factors that include hypertension, smoking and raised cholesterol. These risk factors might be considered 'traditional' due to their long recognized importance in the general population. The underlying pathophysiology principally consists of thrombotic occlusive coronary artery disease leading to acute myocardial infarction. All of these 'traditional' risk factors may be present to a greater or lesser extent in the end-stage renal disease population, however they do not fully explain the disproportionate

mortality rates, particularly those in younger patients with shorter medical histories. This discrepancy suggests an alternative pathophysiological explanation for these disproportionate mortality rates.

In the general population the prevention of cardiovascular disease is tightly focused on mitigating the 'traditional' set of risk factors via lifestyle interventions and medications and has met with some success. However trials attempting to assess their efficacy in haemodialysis patients have reported more disappointing results. For example, the role of lipid lowering therapy is disputed with some trials showing no benefit in terms of reducing cardiovascular events in dialysis [15]. Further large trials in patients with all stages of chronic kidney disease suggested a reduction in cardiovascular events overall but no effect on mortality in dialysis patients [16].

ESRD is the endpoint of CKD and rates of cardiovascular disease rise throughout the stages of CKD [17]. As an individual with CKD progresses towards ESRD, there is an accumulation of risk factors unique to the CKD patient and it has been hypothesized that these drive a change in pathophysiology. The large vessel occlusive disease that predominates in early CKD becomes less of a factor. Instead, patients with later stage CKD demonstrate a tendency towards microvascular disease with an increased propensity for demand ischaemia [18]. Rather than acute myocardial infarctions the most common cause of death in these patients is sudden cardiac death

followed by heart failure. It can be hypothesised that the accumulation of risk factors that drives this change in clinical presentation is happening throughout the course of a patients 'CKD life' e.g. patients developing progressive cardiac fibrosis, abnormalities of rhythm, and with reducing contractile function, congestive cardiac failure. The most extreme manifestation of this disease spectrum is seen in patients in end stage renal failure who have progressed to the stage where they require RRT. These patients have accumulated additional vascular risk through all the stages of CKD and as a result their vascular disease has reached the point where they are exquisitely vulnerable to ischaemic challenge.

There are a number of individual microvascular risk factors that drive cardiovascular risk in haemodialysis patients and potentially explain this vulnerability to cardiovascular stress. These factors are a result of the milieu associated with chronic kidney disease and their confluence is relatively unique to CKD.

1.7.2 Endothelial Dysfunction

Endothelial dysfunction provides an important pathological link between cardiac and renal disease. The endothelial layer has a pivotal role in vascular function. The functions of endothelial cells include control of vasomotor tone via release of factors such as nitrous oxide and the mediation of coagulation and platelet adhesion. In conditions that classically lead to an increased risk

of cardiovascular disease (smoking, diabetes, hyperlipidaemia etc.) the endothelium undergoes structural and functional change that leads to a loss of these protective capabilities [19]. In endothelial dysfunction there is an imbalance between the vasodilating and vasoconstricting capacities of the endothelial layer particularly an increase in angiotensin II and a decrease in nitrous oxide [20]. Endothelial dysfunction is also involved in the development of large vessel atheromatous disease, but plays an important part in the pathophysiology of cardiovascular disease without it.

The physiological effects of CKD have the potential to further exacerbate endothelial dysfunction. Amongst these are the chronic inflammatory state present in CKD and ESRD, the disturbance of Vitamin D metabolism and increased oxidative stress [21]. At present there are no effective therapeutic strategies to counteract endothelial dysfunction in CKD. Limited study of various medications, including ACE inhibitors has been undertaken. A meta-analysis of these trials has suggested that ACE inhibitors provide limited improvement of endothelial dysfunction (as measured by brachial flow-mediated vasodilatation) when compared to other anti-hypertensives [22].

1.7.3 Vasomotor Regulation

By the time a patient has progressed to ESRD they have undergone multiple insults that have compromised the functioning of their autonomic nervous system. The combination of the underlying disease processes (e.g. diabetes

mellitus), medication such as beta-blockers and peripheral vasodilators and the consequences of the dialysis therapy itself all contribute to deteriorating vasomotor regulation.

In the event of circulatory stress the role of the autonomic nervous system is to maintain end-organ perfusion in the face of variable blood pressure through alterations in vascular resistance (contributing to autoregulation). A combination of impaired sensing and response to these changes (baroreflex sensitivity) [23], defective autonomic control, and impaired microcirculation [24] all combine to disrupt autoregulation. The failure to compensate in the event of circulatory stress exposes vulnerable vascular beds to the possibility of ischaemia during the haemodynamic challenge of dialysis.

1.7.4 Vascular Calcification and Arterial Stiffness

Calcification of the vascular system is common in patients with CKD. It is particularly severe and progresses most rapidly in those with significant renal impairment. In this group medial arterial calcification is characteristic rather than intimal (relating to atheroma) [25]. The pathophysiological effects of this change are not fully understood. Vascular calcification (amongst other factors) contributes to increased arterial stiffness, which is an independent predictor of all-cause mortality in HD patients. Arterial stiffness can be determined via measurement of pulse wave velocity. A positive correlation between lower limb pulse wave velocity and superficial femoral artery calcification score in

CKD patients (CKD 4-5D) [26] has been demonstrated, and this and other studies provide evidence of the connection between a calcified vasculature and arterial stiffness.

The process that links this increased arterial stiffness to cardiovascular events is not well established. HD patients have been reported as having a markedly reduced coronary flow reserve (CFR). CFR is the maximum increase in blood flow through the coronary arteries above the normal resting volume. When the myocardial demand for oxygen increases, the coronary arteries dilate to reduce resistance and increase blood flow through the vessels. A reduced CFR suggests an inability to do this in response to increased myocardial oxygen demand and an inability for the microcirculation to adapt to conditions of increased cardiac work. An increase in arterial stiffness may be, at least in part, responsible. In HD patients there is a widening of central pulse pressure resulting in an increase in systolic BP peak and an increasing myocardial oxygen demand. This is matched with a lack of reinforcement of diastolic blood pressure and a reduction in coronary artery filling pressure in diastole. Both factors combine to predispose to demand myocardial ischaemia [25].

1.7.5 Metabolic Stress

The process of atherosclerosis is accelerated by both inflammation and oxidative stress. It has been suggested that the presence of these two factors in advanced CKD and HD patients is the ‘missing link’ that explains the disproportionate incidence of cardiovascular disease in this group.

One measure of cumulative metabolic stress is the accumulation of Advanced Glycation End Products (AGEs). These compounds form as a result of biochemical reactions and are able to bind to cell surface receptors, structural proteins and to type IV collagen leading to stiffening of cardiovascular tissues. AGE deposition can be measured in one of two ways, either via direct measurement from blood tests where enzyme-linked immunosorbant assays of serum can be used [27] or AGE deposition can be determined non-invasively via skin autofluorescence (SAF) using a skin autofluorescence reader. SAF levels have been found to correlate to specific skin AGE levels detected in skin biopsy samples [28]. Measurement of tissue AGE is likely to be more accurate than measurement in blood as long-lived proteins may accumulate in the tissues when chronic complications develop [29].

Elevated SAF levels have been demonstrated in CKD and transplant patients, and grossly elevated levels in dialysis patients providing an illustration of the spectrum of metabolic stress in CKD [30, 31]. AGEs are strongly implicated in the arterial wall disturbances of CVD through inducing alterations in endothelial function [32, 33]. The degree of tissue AGE deposition has also been shown to correlate well with the development of microvascular and macrovascular complications in diabetes [34-36]. Assessment of tissue AGE correlates well to cardiovascular outcomes in HD patients [37]. AGE deposition is considered to be relatively ‘fixed’ in the general population but patients

undergoing renal transplantation show lower levels of AGE deposition than dialysis patients suggesting mobilization following transplant *(see chapter 4).*

1.7.6 Systemic Inflammation

Inflammation appears to be strongly associated with worsening survival and negative (non-fatal) cardiovascular outcomes in patients with cardiac and renal dysfunction [38]. Dialysis patients have a number of indicators that point to a systemically inflamed state. Some of these are the commonly used markers of an inflammatory state such as an elevated CRP or a reduced albumin while some are less recognized but potentially more sensitive markers such as elevated serum free light chain (SFLC) levels [39]. In studies that have assayed both monoclonal proteins (including monoclonal FLC) and polyclonal FLC in patients with advanced CKD and high CV comorbidity, independent associations have been found between high SFLC levels and markers of cardiac injury [40].

Elevated polyclonal free light chains are a dynamic marker of the adaptive immune system. The clearance of the molecules is far more rapid than that of immunoglobulins at 21 days so high levels represent elevated immune activity and inflammation. Elevated polyclonal FLC levels (but still within the normal range) have been found to be a strong independent predictor of mortality in patients with CKD stage 3 displacing all the other more conventional risk factors [41].

Inflammation is considered to be a general factor in the promotion of abnormal remodeling of the ventricle and the development of myocardial fibrosis. Other pro-inflammatory factors are thought to have a more direct effect on the heart. Endotoxin is initially released via the breakdown of bacterial cell walls either as a result of autolysis or the activation of host defence mechanisms. It enters the bloodstream via translocation across the intestinal barrier as well as from dialysis water and contamination from vascular access routes[42]. Higher endotoxin levels have been associated with cellulose membranes that are less biocompatible than polysulfone coated dialyzers, which have a more potent ability to adsorb endotoxin[43]. There is also in-vitro and in-vivo evidence that high-flux polysulfone coated dialysers prevent back-diffusion of endotoxin and so potentially attenuate the inflammatory process [44] as exposure to endotoxin results in the release of pro-inflammatory cytokines [45]. Endotoxin also been implicated in other pathophysiological responses, including endothelial dysfunction and impaired autoregulation [46, 47], that are of importance in the abnormal vasculature of patients with CKD. As discussed, impaired auto regulation is of particularly critical importance as without mechanisms to respond to drops in blood pressure organ perfusion becomes extremely dependent on the maintenance of blood pressure at just the time it begins to drop.

As can be seen in figure 4, endotoxin levels increase progressively through each of the stages of CKD. In patients on dialysis (both HD and PD) the numbers jump significantly and are five times higher than in patients with CKD 5 [43].

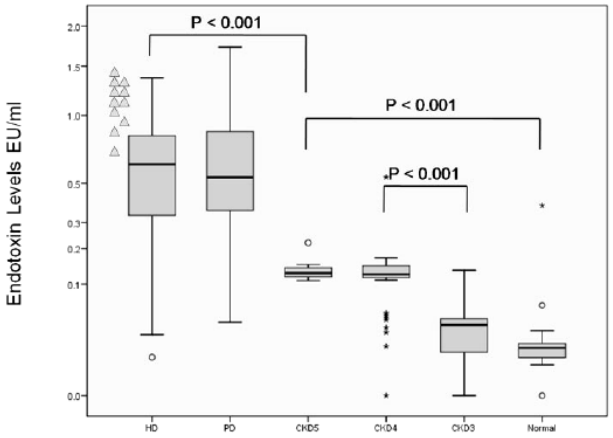

Figure 4: Endotoxin Levels in CKD, Dialysis and healthy controls [43]

1.7.7 Summary

Patients with advanced kidney failure require RRT that can be given in the form of HD. While this is a life-saving therapy patients with kidney disease still suffer from very high rates of cardiovascular mortality. A series of structural and functional abnormalities act to 'prime' the microvasculature of the haemodialysis patient leaving it vulnerable to ischaemic insult.

1.8 Cardiac Structural Abnormalities in Haemodialysis Patients

Our appreciation of how cardiac abnormalities develop in dialysis patients has been dependent on developments in cardiac imaging. This has evolved from earlier 2D echocardiography based studies to more advanced ultrasound based techniques and cardiac magnetic resonance imaging (CMR). The development of these techniques has led to a more nuanced understanding of the complexities of coordinated cardiac contraction/relaxation, segmental function and to more refined measures of global ventricular function.

1.8.1 Abnormalities of Coronary Circulation

Patients on dialysis may suffer from 'traditional' occlusive thrombotic coronary artery disease but this is relatively less common in comparison to the incidence of sudden cardiac death and heart failure [48]. Instead, the predominant changes in the coronary circulation may be microvascular and impede the patients ability to respond to circulatory stress. The large vessel and microcirculatory changes described above result in reduced coronary fractional flow reserve (FFR). The reduction of FFR may be in part due to left ventricular hypertrophy (LVH) and in part due to increased arterial stiffness. LVH alone reduces FFR and is associated with a myocyte-capillary mismatch while increased arterial stiffness has an adverse effect on myocardial perfusion and reduces ischaemic [49].

1.8.2 Abnormalities of Strain

Cardiac assessment by echocardiography is non-invasive, inexpensive to perform, and generates detailed information about gross cardiac anatomy, objective quantification of LV mass and the geometry of LVH, along with measures of function during systole and diastole. Previously dynamic (i.e. intra-dialytic) echocardiographic assessment of systolic dysfunction was conducted by measuring fractional shortening, which is defined as the fraction of any diastolic dimension which is lost in systole [(50)]. Increasingly two-dimensional speckle tracking images are being used to assed global and segmental contractility. This technique measures strain rather than fractional shortening.

Strain is a dimensionless measure of deformation, or the relative displacement of two particles caused by the application of a force. Myocardial strains can be positive (lengthening or expansion) or negative (shortening or compression). Strain as it applies to the myocardium was first described in 1973 [(51)]. Strain rate can also be measured and is the temporal derivative of strain and is a measure of the rate of deformation, with units of 1/s.

The heart deforms in a complex way that cannot be described in just one direction. This complexity stems from how the myocardial fibres are orientated as shown in figure 5. It is commonly measured in the longitudinal, circumferential and radial vectors. In dialysis patients there is some evidence

that longitudinal strain is the superior method of detecting subtle myocardial dysfunction and cardiovascular outcome [52, 53] in patients with preserved left ventricular ejection fraction (LVEF) [54, 55].

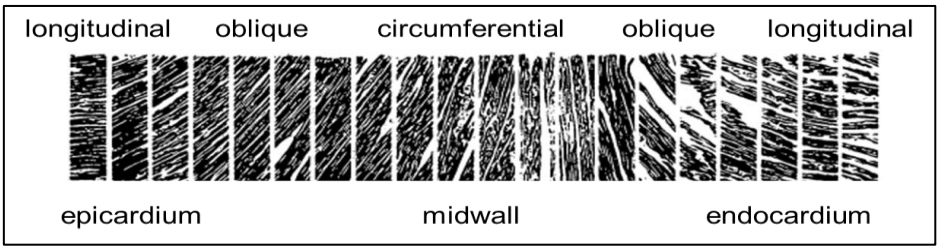

Figure 5: Myocardial fibre orientation of the left ventricle from the epicardial to endocardial layer [56]

Longitudinal myocardial strain is the ratio of change between longitudinal length during systole, and the original length. The value of measuring strain compared to displacement is in being able to distinguish active from passive displacement. An ischaemic basal segment may still show normal motion as it is tethered to healthy tissue but will not show deformation. It has been proven that the human eye is not capable of appreciating the subtle changes seen in deformation [57], thus strain and strain rate changes may be more sensitive markers of myocardial function [58, 59]. Global longitudinal strain (GLS) is now accepted as a sensitive and reproducible method for assessing cardiac function and is predictive of a poor prognosis in patients with a preserved LVEF [60].

A more detailed description of how strain analysis is achieved with the use of 2D speckle tracking analysis of echocardiograms has been supplied in the methods section of this thesis (page 92). There is work looking specifically at strain in haemodialysis patients with preserved left ventricular ejection fraction. The study used a cutoff of a GLS less than 15% as a 'low' GLS. Those in the 'lower' GLS group had a higher mortality. This group also had a higher rate of cardiovascular death. Overall, a GLS below the normal range was a strong independent predictor of all-cause mortality [55].

1.8.3 Left Ventricular Abnormalities

Various static and dynamic cardiac parameters that can be measured by echocardiography can be used to predict cardiovascular mortality in dialysis patients. These parameters include left ventricular mass index (LVMI), left ventricular ejection fraction (LVEF), left atrial volume index (LAV) and the development of regional wall motion abnormalities (RWMA's) secondary to dialysis induced cardiac injury.

Approximately three quarters of dialysis patients meet the criteria for left ventricular hypertrophy on echo and one-third have evidence of left ventricular dilatation. High left ventricular cavity volume and mass index at the time of dialysis initiation were independently associated with death after two years [61] and the risk of developing heart failure can be stratified according to the pattern of left ventricular organization [62] as seen in figure 6.

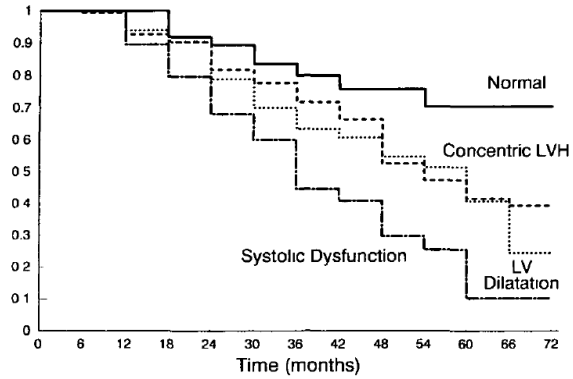

Figure 6: Time in months after starting dialysis to first episode of heart failure stratified according to left ventricular geometry [(62)]

LVH is considered to be an adaptive response to certain conditions that allows for the preservation of a normal LVEF in the presence of abnormal pressure or volume load. Multiple contributory risk factors for it's development have been proposed including volume and pressure overload, increased vascular stiffness providing ventriculo-aortic uncoupling, the presence of an arterio-venous fistula, hyperphosphatemia and neurohormonal activation [(63)]. Recent experimental work has further complicated this picture with the implication of endogenous digitalis glycosides [(64)] and fibroblast growth factor 23 (FGF 23) [(65)]. Both are found in higher concentrations in patients undergoing dialysis and both independently associated with increased left ventricular mass and eccentric left ventricular hypertrophy. Of particular note, sodium is now appreciated to induce adrenal gland secretion of endogenous digitalis glycosides which in vitro studies have shown directly induces myocyte

hypertrophy. This potentially provides a novel mechanism linking high dietary salt intake and impaired sodium excretion to hypertension and LVH in dialysis patients [66].

In patients exhibiting LVH, an elevated left atrial volume index (LAVI) has been shown to be a strong independent predictor of death. In the same study neither ejection fraction nor LVMI were significantly associated with mortality [67]. This study was performed in patients being assessed for renal transplantation and may not be the most representative sample of dialysis patients due to selection bias of patients with less overt cardiovascular co-morbidity. However, these findings are in line with previous studies [68] suggesting that in terms of the prognostic value of various echocardiographic markers, LAVI is superior to measures of left ventricular structure. Enlarged left atrial volumes are considered to be useful markers of left ventricular diastolic dysfunction [50] as it is less sensitive to acute changes in preload than markers related to mitral flow Doppler indices.

CMR to characterise abnormalities in cardiac mass is the current gold standard for assessing cardiac structure and function at rest although it must be noted that in ESRD patients the use of gadolinium contrast enhancement is not possible due to the increased risk of developing nephrogenic systemic fibrosis as a serious complication [69]. Using tagged CMR to provide additional regional functional information and characterize the hearts of dialysis patients,

our study group identified some measure of reduced contractile function in nearly 81% of all patients within 6 months of starting HD [70]. Incident HD patients show significantly reduced circumferential strain and left ventricular ejection fraction in comparison to age-matched controls. Strain continued to be significantly reduced even in those with preserved LVEF. It was significantly correlated to LVEF, troponin-T, measures of cardiac hypertrophy and arterial stiffness.

These results are in line with previous studies of prevalent (dialysis for greater than 90 days) HD patients published without strain data. A study using gadolinium-enhanced CMR scanning as part of renal transplant assessment showed a prevalence of 72% for LVH, 11.2% for LV dilatation and 8.2% for reduced LVEF using cut-offs derived from published reference range studies [71]. This compares to 37%, 28% and 35% respectively in our groups study (of a whole cohort, not just those patients being considered for renal transplantation). Another study (which also did not measure strain) compared 44 prevalent patients with 11 age-matched controls and showed findings similar to the study in incident patients detailed above, demonstrating lower LVEF and greater LV mass in HD patients but with significantly different absolute values [72]. Direct comparisons between these studies are difficult as there is a difference in the study populations, different cut-offs being used and different decisions with regard to manual contouring of the LV to determine the mass and volumes when analyzing images. These factors may account

for the difference in results. Overall there appears to be a high incidence of structural abnormalities seen in dialysis patients undergoing CMR.

1.8.4 Diastolic Dysfunction

So far we have only considered the abnormalities of systolic function that are described above and associated with a poor prognosis. However it is also true that abnormalities of diastolic function are strongly associated with adverse outcomes. These abnormalities are characteristically present in the setting of advanced CKD [73].

The importance of diastolic dysfunction can be appreciated by a closer consideration of ventricular mechanics. Ventricular filling during diastole is of critical importance to efficient cardiac function and is determined by the relationship between left ventricular filling pressures and the physical properties of the ventricle. Ventricular compliance, the ability of the ventricle to relax and 'torsional recoil' (essentially the 'untwisting' of the ventricle during diastole, the systolic contraction motion contains a torsional element) are all physical properties that have an impact on filling pressure. The release of potential energy and the untwisting during diastole serve to create a 'suction' effect that promotes ventricular filling [74].

Assessing diastolic dysfunction is a more challenging prospect in HD patients. Many echocardiographic measurements are load-dependent and so will alter

during the inter-dialytic period and in the course of a dialysis session. Classically, diastolic function can be assessed using Doppler measurements of early mitral flow velocity (E) which decreases in the face of raised intraventricular pressure, resulting in increased reliance on atrial filling (A) and a reduced E/A ratio. However, volume expanded dialysis patients have an increased pre-load and this can elevate the mitral flow velocity resulting in an underestimate of diastolic dysfunction. On the other hand, falls in circulating volume due to ultrafiltration result in reductions of cardiac chamber size and stroke volume by the end of dialysis. Declines in filling pressure of this degree will exaggerate reductions of E and E/A.

Despite some of the inherent difficulties of measuring diastolic dysfunction in this population there is now some evidence that it is a better predictor of cardiovascular events than the more traditional echocardiographic parameters [73, 75]. These studies use the ratio of early transmitral blood flow to early mitral annular velocity (known as E/E') as measured by tissue Doppler imaging which has been proposed as an index of filling pressure [76] and therefore of diastolic dysfunction. In these studies, an elevated E/E' was found to be predictive of cardiovascular events.

1.8.5 Circulating Biomarkers of Cardiac Distress

B-type natriuretic peptide (BNP) and N-terminal Pro-B-type natriuretic peptide (NTpro-BNP) are predominantly released by the ventricles in response to

myocardial stretch or pressure and volume overload and are used as markers in the diagnosis and monitoring of heart failure [77]. In HD patients, levels of natriuretic peptide have been shown to correlate with the degree of LVH, left-ventricular end-diastolic wall stress and the extent of coronary artery disease [78, 79]. Baseline levels of NTpro-BNP reflect the individual patients likelihood to develop dialysis-induced cardiac injury at any time during the next 12 months [80].

Troponins are structural proteins found in skeletal and cardiac muscle. Certain troponin subtypes, most notably I and T are sensitive and specific markers biochemical markers of myocardial damage [81].They are also, as measured by fully automated standard assays, currently superior to all other clinically available biomarkers for the diagnosis of acute myocardial ischaemia. It is now well recognized that high circulating levels of cardiac troponin are found in HD patients even in the absence of acute coronary syndromes [82-84] and thus the usefulness of Troponins as a marker or acute myocardial cell necrosis in dialysis patients is more limited.

Elevated levels of circulating cardiac troponin are indicative of a more general level of ‘ventricular distress’ however the precise reason for this remains controversial in the literature. It does not appear that coronary artery disease alone triggers the release of troponin T in dialysis patients. Studies in clinically indicated coronary angiography found elevated Troponin levels in in up to half

of dialysis patients who did not have overt coronary artery disease [85]. Breidthardt et al found that pre-dialysis troponin T was strongly associated with the presence and severity of dialysis-induced cardiac injury in a sample of 70 prevalent dialysis patients [86].

1.9 Haemodialysis Induced Cardiac Injury

1.9.1 Occurrence of Dialysis Induced Cardiac Injury

As discussed, the condition of the dialysis patient's heart makes them prone to demand ischaemia. This is due to the low FFR, which results from a multitude of factors, which include, but are not limited to, 'traditional' coronary artery disease. There is now strong evidence that the dialysis procedure itself is responsible for a significant degree of circulatory stress[87] as a result of the complex interplay of haemodynamic changes caused by the dialysis procedure and changes in the microvasculature that are described above. The interaction between the two causes perfusion anomalies that accelerate the process of end organ damage. Most of the initial work describing this process has been performed in the heart [88].

In patients suffering from coronary artery disease without kidney disease, a phenomenon known as myocardial stunning has been well demonstrated. It occurs in patients subject to transient myocardial ischaemia leading to left ventricular dysfunction, persisting for several hours following the restoration of

normal perfusion and eventually followed by functional recovery. Repetitive episodes of stunning may be cumulative and result, in the long term, in hibernation and irreversible contractile dysfunction [89]. It has long been suspected that the HD process could itself induce myocardial ischaemia. Previous studies have shown 'silent' ischaemic changes on ECG, elevated cardiac troponins and up to 30% of HD treatments complicated by significant intra-dialytic hypotension [48].

A number of proof-of-concept studies have been conducted in order to investigate whether HD induces circulatory stress and consequently, myocardial ischaemia. It has been demonstrated in studies using $H_2{}^{15}O$ positron emission tomography that HD is characterised by a 30% reduction in regional myocardial blood flow. This reduction and how it relates to time on dialysis is seen in Figure 7.

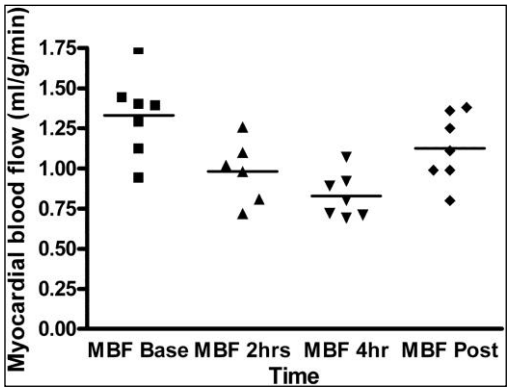

Figure 7: The effect of dialysis on global myocardial blood flow as measured by positron emission tomography[90]

This decreased myocardial blood flow localises to the same segments identified as regional wall motion abnormalities (RWMA) by simultaneous 2D echocardiography and occurs in patients who have angiographically normal coronary arteries [90]. These RWMA's can therefore be used as a more practical surrogate measurement of myocardial ischaemia during dialysis.

Further study has supported the contention that this phenomenon is primarily perfusion based. Segmental strain analysis of echocardiograms taken pre dialysis and at peak dialysis stress has identified anatomical 'clustering' of affected segments. The most commonly affected segments were the apical-inferior, mid-anterior, basal-anterior and basal-lateral segments. This clustering demonstrates a higher degree of vulnerability to demand ischaemia occurring in specific areas [91].

Finally, to add to the evidence that occlusive coronary artery disease is not solely responsible for dialysis induced cardiac injury, studies conducted in paediatric dialysis patients demonstrated the development of regional wall motion abnormalities during dialysis with some degree of compensatory hyperkinesis in those segments that were unaffected[92].

1.9.2 Determinants and Long-term Effects of Dialysis Induced Cardiac Injury

A natural history study of 70 prevalent HD patients demonstrated that new RWMA's developed in 45 patients at peak stress compared to their pre-dialysis echocardiography. In these participants there was 30% mortality and a 13% reduction in mean left ventricular ejection fraction at 12 months [93, 94]. Further analysis of the survivors of the stunning subgroup showed persistence in RWMA's at 12 months. Multivariable analysis showed that age, UF volume, intra-dialytic hypotension and serum troponin-T concentration were all significant determinants of these stress-RWMA's, displacing all other variables including diabetes and pre-existing ischaemic heart disease. Of these variables, the most significant was ultrafiltration volume where a UF volume of 1.5L was associated with an 11 fold increase in the likelihood of developing new RWMA's [93].

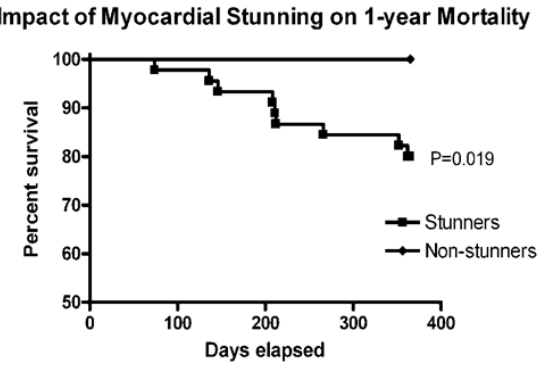

Figure 8: One-year mortality in patients who stun versus non-stunners

The finding that myocardial stunning is a predictor of mortality has been replicated in work carried out by other groups and then taken further. It appears that not only is the presence of stunning a determinant of death but the severity also. Work by Assa et al demonstrated that the higher the number

of wall motion abnormalities developed during dialysis, the worse the long-term survival [95] (see figure 9) up to 700 days. This finding has significant implications as it suggests that even a small reduction in affected segments has the potential to confer an overall survival benefit.

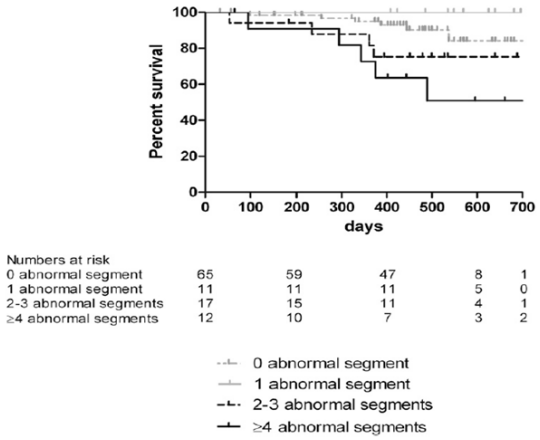

Figure 9: Survival in dialysis patients according to number of regional wall motion abnormalities that develop during dialysis

Aside from the implications for mortality, the haemodynamic tolerability of dialysis has an impact on quality of life. The recovery time post dialysis is affected by dialysis induced cardiac injury. The greater the wall motion abnormality score the more likely the patient is to report severe post-dialysis fatigue [96] (see figure 10). Post dialysis fatigue is an important and debilitating symptom that directly affects quality of life.

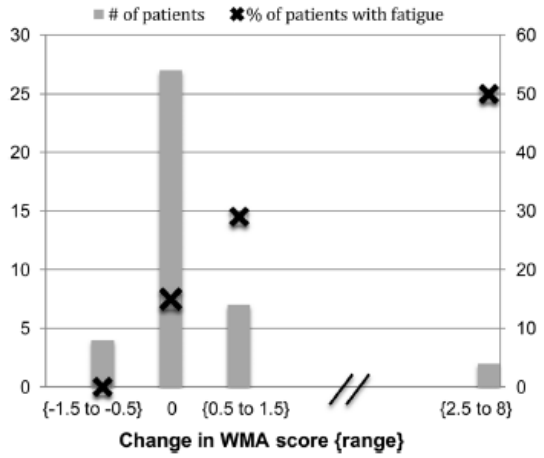

Figure 10: Relationship between wall motion abnormality score and post dialysis fatigue[96]

1.9.3 Cycles of Injury

The occurrence of dialysis induced cardiac injury causes the patient to enter into a vicious cycle of self-propagating cardiac injury. The development of this injury results in the fixed reduction of contractile function leading to an inability to maintain blood pressure during dialysis and further aggravating the cardiac insult [94]. Intra-dialytic hypotension (IDH) is a significant and common complication of HD occurring in 20-30% of treatment sessions [97]. The causes of IDH appear to be multifactorial with UF rate, sodium removal, and levels of bacterial endotoxin all implicated [43, 98]. Previous studies have concluded that IDH is a determinant factor for myocardial stunning and is associated with increased mortality [93, 95, 99].

The most recent work in this field looked retrospectively at outcomes associated with a number of definitions of IDH that have been used in the literature. The only definition clearly associated with mortality was an intra-

dialytic nadir systolic blood pressure of less than 90mmHg. Furthermore, as seen clearly in figure 11, there was a 'dose-response' relationship with higher mortality seen with a greater number of dialysis sessions where a nadir BP of 90 systolic was reached. Even definitions that considered the symptoms the patient reported were not as clearly associated with mortality as a low nadir BP [100]. This raises the question of whether this means there is a 'safe' blood pressure in HD and whether a systolic of 90mmHg constitutes a threshold above which dialysis is tolerable. However, we can also hypothesize that these are the patients that have developed the most significant fixed cardiac dysfunction and are therefore the most vulnerable to a poor cardiovascular outcome as an extension of the already well described negative effects of reduced systolic function [101].

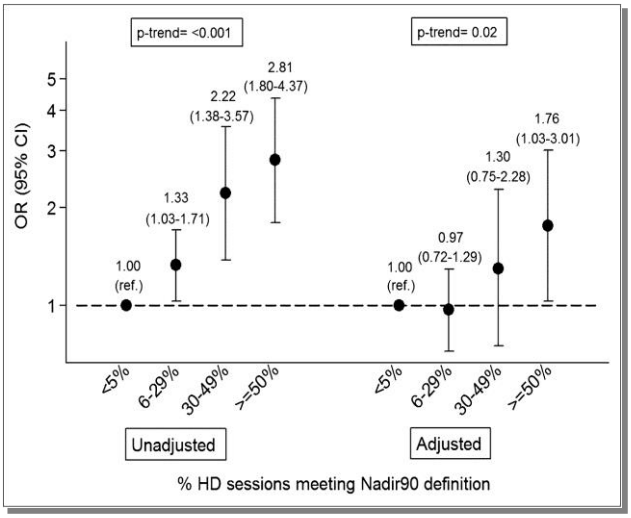

Figure 11: Relationship between IDH and mortality odds ratio increasing with number of sessions complicated by falling to a nadir BP of 90 systolic [100]

These cycles of injury and their wider effects are not confined to the heart. For example, in HD patients, the severity of endotoxaemia was associated with biochemical and echocardiographic evidence of cardiac injury [43, 102]. As the gut is a vascular bed subject to the same changes in microvasculature as the rest of the body, we can hypothesize that it also suffers an ischaemic insult during dialysis resulting in the translocation of endotoxin into the circulation and the pro-inflammatory consequences of this endotoxinaemia that are discussed above.

Although there is no formal work looking at it, we can also speculate that dialysis induced cardiac injury has an effect on residual renal function, which can be rapidly lost once a patient has commenced haemodialysis. The loss of residual function results in increased IDWG and therefore increasing requirements for fluid removal, which is a determinant of cardiac injury.

1.9.4 Summary

The multiple structural and functional abnormalities of the heart and vasculature present in HD patients leave them vulnerable to injury under the haemodynamic stress of dialysis. This injury appears to be at least partly determined by factors such as UF volumes and blood pressure and has implications for long-term cardiac function, mortality and quality of life

1.10 Abrogating Dialysis Induced Cardiac Injury

The most effective way to abrogate dialysis induced cardiac injury would be to reduce the changes to the vasculature that leave patients vulnerable to the haemodynamic stresses of HD. However, as detailed above, the multi-factorial causes of this change remain difficult to treat with the current available therapeutic options. As a result the focus has switched to the dialysis procedure itself and the alterations that can make it more tolerable.

Alterations to standard dialysis prescriptions include more frequent dialysis, nocturnal dialysis and dialysis where fluid removal is regulated by biofeedback mechanisms built into the machine. While some of these changes have shown some promise on a small scale there remains no large-scale evidence that any improve long term cardiovascular outcome. It is likely that no one single change to the procedure will improve outcomes alone and instead a rethinking of several aspects of the dialysis prescription and procedure is necessary.

1.10.1 Cooled Dialysate

Standard dialysis sessions set the temperature of the dialysate fluid at 37°C Systematic review of available evidence confirms that cooling the temperature of the dialysate below this is associated with an improvement in haemodynamic stability [(103)]. This has generally been thought to be related to improved systemic vascular resistance and patients dialyzing at 35°C do demonstrate an increase in total peripheral resistance and a decrease in heart rate, stroke volume and therefore cardiac output [(104)]. They also show

increased baroreflex variability with less variation in systolic blood pressure suggesting that cooling increases the ability of the autonomic nervous system to respond to haemodynamic challenge [105].

Pre-clinical work in animals has suggested that a moderate therapeutic hypothermia has the potential to reduce the size of an acute myocardial infarction [106] after experimental coronary artery ligation. The mechanism for this is not solely due to changes in systemic vascular resistance or indeed reduction in myocardial energy requirements. This experimental work suggests that a mild to moderate hypothermia can induce the activation of a number of signaling kinases that can mitigate the effect of the ischaemia-reperfusion injury. It has not been possible to successfully translate this into the clinical arena, in part because the timing of cooling appears to be important and in part because of limitations relating to the various techniques used to generate the cooled state. In the experimental setting, initiating hypothermia after reperfusion begins can have the effect of increasing the size of the infarction. It is possible that a predictable model of ischaemia is required in order to fully utilize the effects of mild hypothermia.

Dialysis represents a predictable model of ischaemia with clearly defined windows for potential intervention and studies have been carried out to determine whether cooling of the dialysate can provide organ protection. Temperatures of 0.5 and 1°C were tested in a preliminary trial of 11 patients

as it was found that the low dialysate temperatures used in the previous studies were uncomfortable for some patients. Patients were also dialysed at temperatures identical to their tympanic temperature. Isothermic dialysis was associated with a significant reduction in dialysis induced cardiac injury compared to dialyzing at 37°C and 0.5°C below isothermic an even greater reduction still (there was no increased effect at 1°C below isothermic) [107].

Following this, a larger trial of 73 incident HD patients (exposed to dialysis for less than 90 days) was conducted. Patients were randomized to two groups dialyzing at 37°C and 0.5°C below body temperature. They underwent baseline cardiac and brain magnetic resonance imaging and then were scanned again at 12 months. The study showed that patients dialyzing at cooler temperatures had complete protection from changes in white matter microstructure at 12 months. They also had a relative preservation of systolic and diastolic strain and regression of left ventricular dilatation and mass [108, 109]. Individualized cooling represents a promising method of limiting the effect of dialysis induced organ injury and is now undergoing large scale testing in a cluster randomized study with a composite of all-cause mortality and cardiovascular events as the primary endpoint.

1.10.2 More Frequent Haemodialysis

One of the key modifiable factors associated with dialysis induced cardiac injury is UF volume and the intra-dialytic drop in systolic blood pressure [93]. A more frequent HD schedule than conventional thrice weekly would be

associated with lower UF volumes and potentially lower falls in systolic blood pressure. Small studies have shown that more frequent dialysis regimes, such as short daily HD (more than of equal to five times per week) and nocturnal HD (more than or equal to five times per week), are associated with lower levels of dialysis-induced cardiac injury as compared to conventional HD [(110)]. Larger trials of frequent nocturnal HD have reported improvements in blood pressure control [(111)]. However they also found there to be no difference in markers of cardiac structure and function on MRI [(112)]. In patients followed up on a longer term basis there appeared to be a higher mortality [(113)], although it should be noted that there was a surprisingly low mortality rate in patients randomized to conventional haemodialysis and that these studies were not designed or powered to study the impact of daily dialysis on mortality. There may also have been significant carry over effects as the majority of the patients had been established on dialysis for many years.

The reason for this disappointing result is not entirely clear (although it may be at least partially the result of the difficulties of designing and recruiting to trials in this patient group). However, regardless of results the take up of frequent nocturnal HD would likely be limited somewhat by the acceptability to the patient and the ability to perform the procedure at home. Frequent HD alone does not hold the answer to reducing cardiovascular mortality.

1.10.3 Biofeedback Dialysis

A number of modern HD machines from different manufacturers are now equipped with biofeedback technology. These devices monitor blood pressure or infer plasma refilling from blood volume monitoring. Inbuilt software is then able to respond to physiological changes and then automatically adjust factors such as the dialysate conductivity and the UF rate in order to obtain a better balance between fluid removal and plasma volume. Most studies of biofeedback dialysis are small but a meta-analysis suggests that use of this system improved the tolerability of dialysis by reducing episodes of IDH although in the same meta-analysis there was no evidence of this having any long-term effect on outcomes [114]. The quality of evidence reviewed in this study was considered to be low.

One small study suggested that biofeedback dialysis was associated with a lower incidence of regional wall motion abnormalities compared to standard [115]. While it seems clear that appropriately applied biofeedback dialysis has the potential to improve haemodynamic tolerability, the long term effects of this approach and the potential cost-benefit ratio is unknown. Further investigation would be needed.

1.10.4 Haemodiafiltration

Haemodiafiltration (HDF) is an alternative form of dialysis, which increases the rate of UF to the point where volume replacement is required to maximize convection and maintain cardiovascular stability. As this results in more fluid

removal than required then fluid must be replaced using a substitute fluid infused directly into the blood line. Theoretically this allows for better clearance of larger uraemic toxins that are normally inefficiently removed by standard dialysis and have been considered to play a pathogenic role in uraemia (the 'middle molecule' hypothesis). Many of these molecules are newly detected and have been shown to effect endothelial, leucocyte and thrombocyte function making them potential culprits in cardiovascular damage [116].

The most recent evidence suggests that high-dose intermittent HDF is associated with an improvement in cardiovascular mortality [117, 118]. The mechanism by which HDF improves cardiovascular mortality is unknown with investigators hypothesizing that it may be due to the improved clearance of 'middle molecules'. There are some barriers to widespread uptake of HDF. There has been almost no uptake in the United States as re-infused fluid is considered a drug by the Federal Drug Administration and thus would be required to go through the process of FDA approval. This has not yet occurred, the substitution fluid could be administered with bags but this would be very expensive.

1.10.5 Summary

A patient with ESRD characteristically has a stiff, non-compliant vasculature due to a number of pathophysiological changes that occur during the course of chronic kidney disease. This leaves them vulnerable to the haemodynamic

challenge presented during a conventional HD session and results in ischaemic cardiac injury the cumulative effect of which drives cardiovascular mortality. Modifications in the dialysis procedure can help to abrogate this injury.

1.11 Remote Ischaemic Preconditioning

1.11.1 Background

In order to reduce the haemodynamic stress of dialysis there has to be a change in either the supply or demand of oxygen to the myocardium. Previously, the evidence for a number of proposed methods of reducing dialysis induced cardiac injury has been considered. This thesis will now consider in detail the feasibility of two further novel methods of potentially improving the cardiovascular tolerability of HD. The first method uses the activation of a variety of innate mechanisms already present in the human body to protect against injury caused by reperfusion of an organ following an insult and by doing so potentially improve perfusion to the target organ (thus increasing supply). The second method will use an alteration in the dialysis prescription to effect a change in IDWG and thus reduce UF requirements during dialysis (reducing demand).

Much of the damage that can happen to a tissue following the restriction of blood supply occurs after the restoration of circulation. During the ischaemic period the tissue is deprived of oxygen and nutrients. Following the return of

circulation there is an inflammatory response caused by the generation of reactive oxygen species. The inflammatory response promotes cell death and fibrosis.

In the experimental setting it has been repeatedly observed that the application of a transient, non-lethal episode of ischaemia prior to a larger insult results in an endogenous cardioprotective effect. This effect 'conditions' the heart to tolerate the larger insult and limit the extent of the ischaemia-reperfusion injury. Animal experiments have shown that myocardial infarct size can be dramatically reduced by directly subjecting the myocardium to one or more episodes of non-lethal myocardial ischaemia prior to the coronary artery occlusion [119]. This has emerged as a potentially powerful new tool in a variety of clinical settings and has been tested experimentally in a number of different organs including the kidney, liver and brain [120].

1.11.2 Timeline of Development

Murry et al conducted laboratory work in 1986 in which they subjected the myocardium of dogs to 4, 5-minute direct occlusions of a coronary artery alternating with 5 minutes of reperfusion. Following this sequence the circumflex coronary artery was occluded for a total of three hours. The pre-conditioned group was compared to a non-preconditioned control group. The size of the myocardial infarction (MI) was reduced by as much as 75% in the pre-conditioned group [119]. This effect was named 'ischaemic preconditioning'

(IPC). The same response has been reproduced remotely from the heart by occlusion of other vessels such as the mesenteric and renal arteries [121]. This effect has now been replicated in numerous laboratory based experiments.

This large body of experimental evidence also suggests that the brief episodes of ischaemia and reperfusion that constitute the IPC stimulus lead to two distinct windows of cardioprotection. The first window of protection (classical IPC) manifests immediately and wanes after 2-3 hours. A second window of protection develops 12-24 hours later and seems to disappear after 2-3 days [122].

In 1997 the critical observation was made that briefly restricting blood flow to the lower limb and then pacing the gastrocnemius muscle reduced MI size in rabbits [123]. This offered one of the first indications that the preconditioning effect could be achieved by administering the stimulus to the lower limb and thus paved the way towards using a more practical method of delivery than direct occlusion of a major blood vessel. The final barrier to translation into the clinical arena was removed when the use of a non-invasive RIPC stimulus in humans was developed in the simple form of a blood pressure cuff applied to the upper limb and inflated to 200mmHg [124]. The provision of an amenable delivery method has allowed RIPC to be tested in a number of clinical trials which have looked not only at whether preconditioning can protect from

ischaemia, but also if application of the stimulus during or after an ischaemic insult can be protective (peri and post conditioning).

1.11.3 Proposed Underlying Mechanisms

Since the initial observations of remote ischaemic preconditioning, extensive effort has been made to determine the underlying mechanisms. It appears that both pre and post conditioning share a common final signaling pathway, involving the generation of mitochondrial reactive oxygen species that then mediate the cardioprotective effect through a number of pathways [125]. These pathways involve the recruitment of a number of G-protein coupled receptors to ligands such as adenosine and opioids which in turn leads to the activation of signaling protein kinases and the opening of mitochondrial ATP-sensitive potassium channels (K_{ATP}) which inhibit the formation of the mitochondrial permeability transition pore (MPTP), a key step in preventing apoptosis [126]. The MPTP opens during initial stages of reperfusion by uncoupling oxidative phosphorylation and promoting mitochondrial swelling and preventing MPTP opening promotes myocyte survival [127]. The protective effect of the stimulus is abolished by administration of the K_{ATP} inhibitor glibenclamide [128]. These processes are direct actions at a cellular level in the myocardium and represent then final pathway for all forms of ‘conditioning’. With regard to remote conditioning (as opposed to direct intermittent pre-conditioning`) how these pathways are ultimately activated is the main research question.

Much is now known about the cellular effects of IPC. In remote pre and post conditioning focus has been on trying to determine the 'messenger' conveying the preconditioning signal to the target organ. An overview of the proposed messengers is provided in figure 12. Neural, humoral and combined neurohumoral mechanisms have all been proposed. In the study by Gho et al involving occlusion of the anterior mesenteric artery and left renal arteries it was noted that the remote effect was abolished by nerve ganglion blockade, something that did not occur with direct coronary artery occlusion [121].

The MI size can be reduced in rabbits pretreated with plasma and dialysate from a donor animal that had received RIPC, suggesting a messenger circulating in the blood mediates the remote conditioning response. Moreover, cellular necrosis induced by ischaemia and reperfusion of *in vitro* cardiomyocytes treated with the RIPC dialysate from either rabbits or humans was substantially reduced compared with controls [129].

Reactive oxygen species, generated during the preconditioning stimulus, might trigger a series of mechanisms that provide the second window of protection, an effect that is partially mediated through nitric-oxide (NO) release or bradykinins [130]. In mice, a 30-minute ischaemic insult to the kidneys increased the expression of both inducible and endothelial NO synthetase (NOS), and the expression of heat shock protein 25. Treatment with NO synthesis inhibitors increased the kidney's susceptibility to ischaemia as did a

reduction of inducible NOS. This effect seems to be crucial in the later phase of IPC as a partial protection was achieved with a preconditioning regime that did not alter NOS expression.(131) NO reduces inflammatory responses, which are important contributors to ischaemic reperfusion injury.(131)

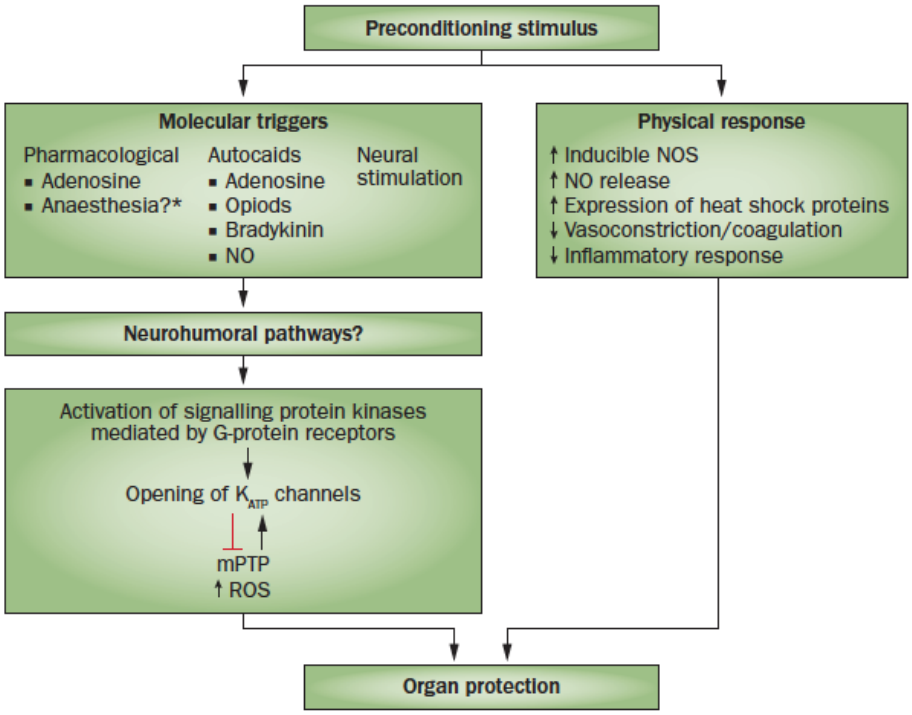

Figure 12: Overview of proposed signaling pathways underlying the cardioprotective phenomenon of 'conditioning' (120)

The factors mediating remote conditioning might also be conveyed via a humoral or neuro-humoral path. Blocking nerve ganglions with hexamethonium can prevent remote preconditioning using the anterior mesenteric artery and left renal arteries, an effect that did not occur after direct

coronary artery occlusion [121]. Moreover, renal RIPC increased afferent nerve discharge, whereas renal nerve ablation abolished this effect [132]. Taken together, these findings indicate that substances released from the preconditioned organ or tissue stimulate local afferent nerve pathways and in turn activate efferent nerve pathways that terminate in the myocardium. The dependence of remote conditioning on intact neural pathways would explain why its effects seem to be attenuated in patients with diabetes mellitus and neuropathy [133, 134].

However, no absolute consensus exists regarding a neuro-humoral pathway and some work suggests that a purely humoral messenger conveys the conditioning signal. For example, pigs who receive a donor heart were still able to respond to a preconditioning stimulus and reduce infarct size despite the heart being denervated [135] supporting a hypothesis that signals from the remote organ act via the bloodstream to activate the cardioprotective pathways [136]. Adenosine, opioids, bradykinins and endocannibinoids have all been suggested as potential humoral messengers.[137]

1.11.4 Clinical Translation of Ischaemic Conditioning

Translating the experimental findings of RIPC into the clinical arena has proven to be challenging. There have been a number of small clinical trials that have attempted to investigate whether laboratory findings can be replicated on a larger scale. The focus has largely been on scenarios involving

a predictable ischaemic insult such as patients undergoing planned cardiac surgery or elective percutaneous coronary intervention. In more acute medical settings such as MI or cerebrovascular accident remote ischaemic *post*conditioning has been used with mixed results.

The first demonstration of a potential clinical translation of Remote Ischaemic Preconditioning in humans was made during work by MacAllister et al. Using a non-invasive protocol in human volunteers where a blood pressure cuff was applied to the upper arm, they demonstrated a positive effect on endothelial function using flow mediated dilatation as a surrogate marker of vascular effect [124]. The protocol involved inflation of the cuff to a pressure of 200mmHg for five minutes and then deflation for five minutes, repeated a further three times. This has formed the basis for clinical trials in RIPC since with some variations in the number of cycles used.

The translation of RIC from laboratory to clinical setting has focused on situations with predictable ischaemic injury, for example, during cardiac surgery, percutaneous coronary intervention and aortic aneurysm repair. Early trials of preconditioning in cardiac surgery showed some promise [138] but more recent trials in the setting of coronary artery bypass grafting have proven to be disappointing [139, 140]. These studies have largely relied on biomarker measurement to determine reduction in tissue injury[138, 141]. No detailed analysis of cardiac function using imaging based methods has been

undertaken in these trials. The most recent large trials in cardiac surgery looked specifically at clinical outcomes and did not show any benefit of using RIPC prior to elective cardiac surgery [142, 143].

There have been relevant smaller cardiac trials that have shown positive results in the setting of primary PCI. For example Botker et al investigated RIPC administered before cardiac angioplasty (but during an evolving ST elevation MI) and demonstrated that patients who received RIPC had a higher myocardial salvage index at 30 days than those who did not [144]. This result was similar to one demonstrated by Crimi et al in a similar scenario (prior to primary PCI) [145]. A similar more recent study by White et al, again looking at remote ischaemic conditioning administered prior to angioplasty following a ST-elevation MI showed a reduction in MI size of 27% in the RIC group as compared to the control group. The RIC group also had a lower high sensitivity troponin T level and reduced myocardial oedema seen on T2-weighted MRI images [146].

There are a number of possible explanations for the mixed performance of RIPC in clinical trials and the current inability to reliably translate the pre-clinical work into clinically meaningful outcomes. There may be issues with patient selection and the clinical scenarios being used. While the small and medium sized trials in primary PCI that are detailed above have been promising other clinical situations appear less so. Cardiac surgery for example

may involve the selection of older patients with multiple co-morbidities and more limited physiological ability to respond to an RIPC stimulus. There is also evidence that the use of inhalation anaesthetic gas and certain medications may attenuate the effect of preconditioning [147]. Some trials have attempted to use more standardized intravenous anaesthetic protocols to account for this but have still not managed to yield positive results [142]. In addition, there is no clear information available on the optimum number of cuff inflations to use in each scenario and the ideal timing at which to administer the RIPC stimulus. Formal dose-response studies and investigations into the precise window for administration of RIPC are potential future targets for study but would need to be undertaken in patients with predictable episodes of ischaemia.

Several studies suggest that application of postconditioning can have beneficial effects on infarct size and oedema following an acute ST-elevation myocardial infarction. For example, patients who had undergone percutaneous coronary intervention and postconditioning had a smaller infarct size and reduced myocardial oedema, on T2 weighted MRI images.[148] At least one study has suggested that postconditioned patients had a better ejection fraction three years after an infarct.[149] These studies are relatively small, and other similar trials have suggested contradictory results [139, 150].

There has also been much interest in broadening the application of pre-conditioning away from simply being a cardio-protective measure and towards

exploring the possibilities in other organ systems and clinical scenarios. There have been some interesting results in clinical trials looking at the use of RIPC to protect against contrast-induced nephropathy [151]. Other suggested potential sites of action are skin during surgery [152], the liver in transplantation [153] and prior to carotid endarterectomy [154] although no extensive clinical studies have been conducted in any of these areas as yet.

1.11.5 Preconditioning and Kidney Disease

The high-energy demand and intricate microvascular network of the kidney make it especially vulnerable to the effects of the ischaemia and reperfusion injury. While, a number of animal studies have demonstrated a protective effect on renal function following RIPC, a number of small clinical trials have shown conflicting results.

A systematic review and meta-analysis of 58 animal studies suggests that ischaemic preconditioning reduces serum creatinine levels, blood urea nitrogen and histological damage compared with untreated controls. In these studies no difference between local and remote conditioning was observed and the efficacy of preconditioning was greatest during the second window of protection. However, the available evidence was not sufficient to determine what may be the optimal window of protection [155].

Secondary analysis of two randomized controlled trials has suggested that RIPC is associated with decreased AKI incidence in patients without diabetes mellitus and who undergoing elective cardiac surgery [156]. A meta-analysis of randomized controlled trials investigating AKI in patients undergoing complex cardiac or vascular surgery identified 10 trials with a total of 924 patients. The combined results of these trials suggest that patients who received RIPC had significantly lower incidence of AKI using a fixed-effects model (although not when using a random effects model). However, the meta-analysis did not indicate any difference in levels of renal biomarkers, incidence of renal replacement therapy or mortality between those patients receiving vascular interventions with or without RIPC. The authors concluded there was not enough evidence to suggest RIPC provides renal protection [157].

Other trials investigating different clinical scenarios resulting in AKI have yielded more promising results. In a trial of 50 patients undergoing elective cardiac angiography, RIPC before contrast use protected against contrast-induced nephropathy (in this instance defined as a rise in serum creatinine >25% or 0.5 mg/dl above baseline 48 hours after contrast exposure) [158].

There has been some interest in the use of RIPC to improve outcomes following renal transplantation. In one study transplant recipients received 4 cycles of RIPC following induction of anaesthesia. The primary endpoint of the study was estimated GFR at 1 and 3 months. The trial did not show any effect

on the primary endpoint [159]. A further trial used a 2x2 factorial design to investigate the timing of administration of RIPC in living donor kidney transplantation. Both the donor and recipient received 2 RIPC treatments either immediately or 24 hours before surgery (or a combination of both). The trial again used eGFR as an endpoint, this time at 12 months post transplantation. Early RIPC seemed to result in a weak effect on the primary outcome at 12 months which the authors considered clinically important [160]. There remains uncertainty over optimal timing of RIPC administration in relation to anaesthesia and also the comparative benefits of conditioning the donor, the recipient or both.

How the complex pathophysiological milieu in patients with CKD may affect the efficacy of RIPC is unclear. Some data suggest that the benefits of preconditioning might be limited in older patients [161] and those with diabetes mellitus [162] both of whom would be well represented among those with CKD. However, animal studies suggest that ischaemic conditioning might still benefit the heart in chronic uraemia. In rodent MI models, all conditioning strategies efficaciously reduce infarct size in the uraemic heart,[163, 164] a finding replicated in animals that have undergone a sustained period of uraemia[164].

The overall failure of RIPC to translate into definitive positive clinical outcomes has caused a great deal of discussion amongst researchers in this area with many feeling that further work needs to be done in elucidating the underlying

mechanisms [165]. There are likely to be many underlying reasons for these failures. Firstly, relatively little attention has been given to the dose-response characteristics of preconditioning, i.e. whether more frequent preconditioning, application on multiple limbs etc. intensifies the effect. There has also been little exploration of whether the effect can be sustained over a longer period of time or if the effect diminishes.

Secondly, the end-points used in many studies are surrogate endpoints of organ function or damage such as eGFR or troponin t release. While there have been a handful of studies that have utilized imaging to more directly visualize organ damage, many of the larger studies have used these surrogate endpoints and thus are relying on them to detect a clinically meaningful effect.

Finally, the clinical scenarios in which RIPC has primarily been tested are difficult to show an effect in. Acute scenarios such a MI or stroke presents problems in terms of the timing of RIPC application and whether it may be administered when reperfusion is already occurring and thus complicating it's ability to have a clinically meaningful effect. In scenarios such as cardiac surgery the complicated nature combined with co-morbidities and the multiple insults the patient faces may simply represent too complex a clinical scenario for RIPC to have an effect. The best opportunity for RIPC lies in situations where a predictable model of ischaemic insult is present.

1.11.6 Potential Application of RIPC in Haemodialysis

Given that HD represents a model of a predictable ischaemic insult it would seem a logical target for modification with pre-conditioning. However there has previously only been one study in this area which found that one dose of RIPC caused a reduction in post-dialysis Troponin T levels which lasted up to 28 days [166]. This pilot study, like many others, used biomarkers as a surrogate marker of the interventions effects. It did not look in detail at the intra-dialytic cardiac effects.

There are potential pitfalls in applying RIPC to a HD population. Most notably that there is evidence that the effect of preconditioning may be at least attenuated or perhaps negated entirely in patients who are diabetic [167] or elderly [133]. Given that these two groups represent a sizable proportion of the HD population this could limit potential clinical application.

While the 'dose' of RIPC that has been used in previous clinical trials is four 5-minute inflations to 200mmHg there has never been a formal study to assess dose-response to different degrees of cuff inflation. The number of inflation-deflation cycles used in RIPC differs in the literature. In one of the first translational studies in humans, Loukogeorgakis et al. used three 5-minute cycles to demonstrate protection against endothelial ischaemia-reperfusion injury [124]. This protocol has been used in subsequent cardiac trials with mixed results [139, 141]. More recently a study of ninety-four patients undergoing ad-

hoc PCI suggested a one-cycle protocol of RIPC applied to the upper arm reduced Troponin I release [168]. Further trials, which have been suggestive of a benefit in the setting of acute MI, have used a four-cycle protocol [169, 170]. The dose, and need to repeat the application, of RIPC clearly need to be refined in this particular target patient group.

1.11.7 Summary

Since the initial observation of RIPC extensive work has been carried out to determine the underlying mechanism. Development of more practical methods of delivering the stimulus has allowed for translation into the clinical arena. The predictable ischaemic insult of dialysis provides an ideal model for further study.

1.12 Sodium and Haemodialysis

1.12.1 Salt and Water Physiology

Sodium is the main cation present in extracellular fluid where it is found at a concentration of 136-145mEq/L. It is the main determinant of plasma and intracellular tonicity (the pressure gradient between two solutions separated by a semi-permeable membrane). Tonicity needs to be conserved by the body in order to maintain an isotonic balance between the intracellular and extracellular fluid and thus preserve cellular integrity.

Almost 100% of dietary sodium is absorbed in the small intestine. Following glomerular filtration most of the filtrated sodium is reabsorbed in the renal tubule. Two-thirds of this reabsorption occurs in the proximal tubule where it passes along an electrochemical gradient. Approximately 25% occurs in the thick ascending limb of the Loop of Henle via the Sodium-Potassium co-transporter and approximately 5% occurs in the distal convoluted tubule, again via a co-transporter.

The collecting duct system of the kidney accounts for roughly 5% of the body's reabsorption of sodium, which in this part of the kidney is regulated by Aldosterone via the expression of sodium channels on the apical membrane. Aldosterone also increases the number of sodium-potassium pumps. Along with anti-diuretic hormone (vasopressin) the actions of Aldosterone allow the principal cells of the collecting duct to regulate the quantity of water that is reabsorbed by the kidneys [171].

1.12.2 Dietary Sodium and Cardiovascular Disease

The minimum physiological requirement for sodium intake is 500 milligrams per day. The principle source is sodium chloride in the diet, which accounts for 95% of sodium intake. The majority of the world's population will consume between 3 and 6g/day of sodium[172]. It is well recognized that the salt intake of an individual contributes to their systolic blood pressure and this has led to

guidelines recommending limiting dietary intake of sodium to less than 1.5g/day [173]. Whether strategies to limit salt intake can improve cardiovascular outcomes remains unclear from current evidence [174].

Our understanding of how the body handles sodium is imperfect and evolving. Chronic exposure to hypernatraemic states may result in sodium deposition in a number of different body compartments including bone, skin and skeletal muscle [175, 176]. Dialysis patients in many centres are advised to restrict their salt intake as a means to controlling their blood pressure and IDWG with the suggestion that this approach will lead to reduced prescription of antihypertensive drugs and may limit LVH, better preserve LV function and reduce IDH [177].

1.12.3 Sodium in Dialysis

The concentration of sodium in dialysate has changed as the efficiency of dialysis delivery and especially fluid removal has changed. In the early days of dialysis sodium concentrations in the dialysate were low as this was prior to the development of ultrafiltration technology [178]. Low dialysate sodium concentrations (of approximately 126mEq/L) provided the principal means of volume control via diffusive sodium loss from the patient to the dialysate. In combination with a low salt diet this provided excellent blood pressure control without the need for pharmaceutical therapy. There was a lag effect before

blood pressure control was achieved, adequate control of BP took four to six weeks suggesting that the effect of sodium was not entirely due to extra-cellular water and increased volume [179].

The development of automated UF meant that low sodium concentrations were no longer desirable as low sodium and convective fluid loss in combination would lead to a rapid drop in serum osmolality and a net fluid shift out of the vascular space, osmolality was also reduced by rapid falls in urea due to dialyser efficiency. Maintenance of high plasma tonicity is necessary to maintain plasma refill during rapid UF. As high efficacy dialysers and shorter hours became standard patients would suffer symptoms including profound hypotension, headache, nausea and cramps due to more dramatic osmolar shifts. In order to counteract this, dialysis facilities began to shift to higher sodium concentrations of between 140mEq/L and 145 mEq/L [180]. The use of a consistently hypertonic dialysis sodium concentration minimized the symptoms of ‘disequilibrium’ (the specific state of cerebral disequilibrium is quite rare) and importantly maintained consistent blood pressure. Current standard practice in dialysis units is to dialyse all patients against a fixed sodium concentration, usually of 140mEq/L.

There is however concern regarding the effects of using hypernatraemic dialysate. A sodium concentration of 140mEq/L is likely to be higher than the serum sodium of a significant percentage of dialysis patients thereby setting

up the possibility of a sodium gradient between the dialysate and the patient and effecting a net transfer of sodium into the patient. Many patients will leave the dialysis unit with a relative hypernatraemia leading to increased IDWG, thirst and difficulties in controlling blood pressure [178].

1.12.4 Implications of Osmolar Set Point

Data in subjects with normal renal function suggest that individuals have a specific osmolality value above which thirst is generated and fluid is ingested. The osmolar 'set-point' varies amongst individuals. Gotch et al. studied pre dialysis plasma sodium concentrations over a protracted period. Plasma sodium concentration varied from 132 to 144 mmol/l between patients, but varied by less than 2 mmol/l within individual patients [181]. Published data suggests that this set point is present in haemodialysis patients also and dialyzing a patient at a concentration above their serum sodium is associated with increased inter-dialytic weight gain and hypertension [182, 183]. The implication is that current dialysate sodium concentrations are sufficient to cause a relative hypernatraemia post-dialysis resulting in the stimulation of thirst and requiring them to drink enough free water to drive their osmolality back to the 'set-point'. While some feel that the significance of the osmolar set-point is overstated [184] it is has been suggested that a more individualized sodium prescription may be beneficial.

1.12.5 Diffusive Sodium Loss in Dialysis

Removal of sodium during dialysis is the sum of diffusive and convective losses. Adequate dialysis should ideally be isonatric allowing for complete removal of the inter-dialytic sodium gain and avoiding intra dialytic sodium loading [185]. It is estimated that more than 80% of sodium removal is convective and perhaps only 15% to 20% is diffusive [186].

The proportion of sodium that is free in plasma water and not associated with proteins or other ions is available for diffusive loss. Movement is determined by the gradient between the concentrations of non-complexed, electrochemically active ions from plasma to dialysate. The Gibbs-Donnan effect refers to a phenomenon describing the actions of charged particles near a semi-permeable membrane that can result in an uneven distribution of charge across the membrane. In this case it is caused by positively charged plasma coating the dialysis membrane and directly impeding movement of positively charged ions, thus reducing diffusive sodium loss by approximately 3-5% [187]. This effect allows for the movement of sodium and water to become uncoupled during HD [182]. Uncoupled movement of sodium and water makes it possible to ‘load’ sodium during dialysis (even if the dialysate sodium concentration is lower than the serum sodium concentration). Furthermore, as discussed above there is evidence of sodium storage in other tissues due to chronic exposure to hypernatraemic states, this has the potential to further decouple sodium and water movement [188].

The effect of this is likely to be negligible over the course of a single dialysis session, however it would seem that a potential reservoir of sodium ions may be present to buffer sodium transportation over a longer period of time and this would presumably have some influence over IDWG and blood pressure control.

It should be noted that measurement of plasma sodium is imperfect and further complicates efforts to study the movement of sodium during dialysis. Laboratories typically use flame spectrophotometry or indirect potentiometry with diluted samples to measure total sodium concentration, which is a practical method for handling multiple samples but is typically 4–6% lower than the electrochemical activity of ionised sodium in plasma water measured directly with an ion-selective electrode [(189)]. The challenge in quantifying sodium is that we can only measure the concentration in plasma free water and the assays are limited in terms of measuring sodium itself.

While previous attempts to study sodium mass balance during dialysis have been hampered by a lack of available tools and an imperfect understanding of sodium movement, more recent attempts have been made to use online conductivity monitoring to study sodium loss. This technology is built into many modern dialysis machines and allows the monitoring of plasma conductivity, which is the positive electrical activity of a substance. The main plasma cation (positively charged ion) is sodium, which, therefore accounts for nearly all of

the estimated conductivity of plasma. By placing conductivity cells at the dialyser inlet and outlet and measuring every 2 minutes it becomes easy to determine 'effective plasma conductivity' using in-built software [190].

This technology remains fairly specialized and not necessarily available on all dialysis machines in day-to-day use. A few studies have attempted to use this information to gain more information on ionic mass balance (IMB) during dialysis. IMB is an estimate of sodium transferred during treatment derived from measuring changes in dialysate conductivity during treatment. One study looked at the IMB at two different dialysate sodium concentrations (140mmol/L and 144mmol/L). The authors found that diffusive sodium transfer into the patient was greatest when the difference between dialysate sodium and pre-dialysis serum sodium was greater than 5mmol/L. These patients also underwent concurrent blood volume monitoring and this did not significantly change during HD. The study also found that patients with low pre-dialysis serum sodium had enhanced sodium removal (again without a significant change in blood volume) at these levels of dialysate sodium [191].

A prospective study of 24 stable chronic HD patients over four weeks who were dialyzing at a fixed sodium concentration of 140mmol/L showed a mean IMB of 338mmol which indicated a net sodium removal across the cohort. However, as seen in the graph in figure 13, there was a wide inter-patient variability in this cohort with mean IMB estimates between 96 and 515mmol.

In this small study only three patients demonstrated evidence of a positive dialysate to plasma concentration gradient. The wide variability in this study (which encompassed 228 treatments) did suggest a degree of individualization of sodium prescriptions would be desirable [192]. This variability differs from the study mentioned above but can perhaps be explained by a smaller sample size and different dialysis conditions in the earlier study (different treatment times and frequencies). Of note, there was very little *intra-patient* variability in this study, which is further evidence for the presence of an osmolar set-point.

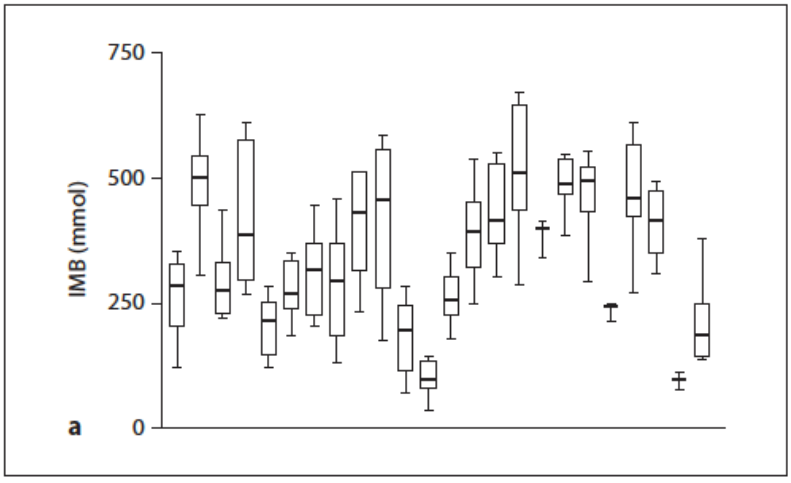

Figure 13: Inter- and Intra-patient variability in ionic mass balance over the course of a dialysis session. Each box plot equates to an individual patient [192]

From the rather limited evidence available it is reasonable to believe that fixed dialysate sodium prescriptions may lead to a wide range of sodium removal with potential consequences for blood pressure and IDWG.

1.12.6 Sodium Profiling

While increasing dialysate sodium concentrations helped to maintain more consistent blood pressures through treatments the continued persistence of intra-dialytic complications has led to use of sodium profiling to alleviate symptoms of cramps, dizziness and nausea. This involves a programmed means of varying dialysate sodium concentrations during dialysis using built-in software. The most common programmes begin with hypertonic sodium concentrations at the start of dialysis with the intention of offsetting the decrease in plasma osmolality caused by the removal of urea and other solutes. This is greatest at the beginning of dialysis where the gradient between plasma and dialysate is highest and thus the decrease in plasma osmolality is the greatest in the early stages of dialysis treatment. After starting at a high level the dialysate sodium concentration is then decreased during the treatment with the aim of ending with a serum sodium in physiological range and minimizing transfer of sodium to the patient and the attendant complications of thirst and weight gain that this brings [193].

Like most dialysis interventions it has proven difficult to subject sodium profiling to large studies but the evidence from the small studies so far conducted is that sodium profiling does appear to have benefits relating to reduction of *intra*-dialytic symptoms [194]. However, there remains an issue of patients experiencing more *inter*-dialytic symptoms with increased weight gain, fatigue and thirst reported [195] likely due to profiles resulting in a positive increase in sodium balance [196].

1.12.7 Isonatric Dialysis

To deliver an isonatric dialysis session dialysate sodium concentration should approximately match patient's serum sodium, and all sodium gained in the inter-dialytic period must be removed by a combination of convection and a degree of additive diffusion [197]. Again, developments in dialysis technology had raised the possibility that dialysis sessions could be modeled to be approximately isonatric. The use of continuous on-line conductivity monitoring allows measuring of the conductivity (and therefore sodium concentration) of the dialysis ultrafiltrate. If used concurrently with haemodiafiltration the reinfusion of fluid can be adjusted aiming to preserve plasma conductivity at approximately normal levels. This system allows for a biofeedback loop with the machine programmed to reach a prescribed serum sodium concentration [198]. however on the latest dialysis platforms manufactured by the same company (Artis) this system is no longer being offered.

These systems aim for the benefits of sodium profiling mentioned above without the excess transfer of sodium to the patient, which complicates it. They have been evaluated in a small number of studies with intra-dialytic sodium removal being predicted using mathematical models that require pre-dialysis plasma sodium. Recent studies have incorporated a sensor to detect pre-dialysis sodium and further refine sodium removal. The studies are small in scope and suggest that this method could indeed reduce intra-dialytic

episodes of IDH without the same effects on IDWG [199, 200]. However, as yet there are no larger scale studies assessing this effect and uptake of these complex methods would likely be limited in the near future.

1.12.8 Relationship between Ultrafiltration Volume and Outcome

Concern regarding high UF volumes stems from the decreased survival seen in patients requiring greater fluid removal as illustrated in figure 14. In one of the more widely quoted studies higher weight gains between 2 subsequent dialysis sessions were associated with higher all cause mortality. The hazard ratios of CVD for weight gain >1.0 kg and >4.0 kg (compared with 1.5 to 2.0 kg as the reference) were 0.67(0.58 to 0.76) and 1.25 (1.12 to 1.39), respectively [201].

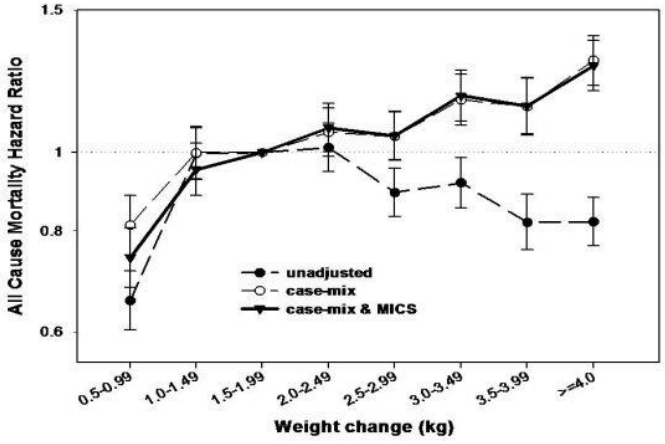

Figure 14: Relationship between all-cause mortality and inter-dialytic weight gain [201]

This association holds true when considering relative UF volume rather than absolute UF volume. This means ‘indexing’ inter-dialytic weight gain to the

patient dry weight (or their post-dialysis weight) and expressing it as a percentage gain. This turns out to be a more consistent predictor of outcome with a greater than 3.5% gain from post-dialysis weight independently associated with all-cause and cardiovascular mortality amongst other indicators [202]. Finally, the failure to consistently dialyse someone to dry weight is also associated with a greater mortality. Patients who were above their target weight in above 30% of treatments exhibit greater all-cause mortality and post-dialysis weights more than 2kg above (and, it should be noted, below) are associated with a worse outcome [203].

The management of fluid removal and volume overload in patients continues to be a considerable challenge for dialysis physicians and has led to detailed consideration of the ideal methods of measuring volume in a patient and the causes of high IDWG. Choosing the optimum dry weight is challenging and determining when a patient is truly fluid overloaded can be difficult clinically. Many physicians feel that paying more attention to a precise determination of optimum dry weight should be a priority [204]. Given the consistent association of poor volume control with morbidity and mortality the chief medical officers of the largest dialysis providers in the United States have recently issued guidance to units prioritizing volume control as a key component of adequate dialysis. Amongst their recommendations were the following 1) Extracellular fluid status should be a component of sufficient dialysis, such that approaching normalization of extracellular fluid volume should be a primary goal of dialysis

care. 2) Fluid removal should be gradual and dialysis treatment duration should not routinely be less than 4 hours without a justification based on individual patient factors 3) Intra-dialytic sodium loading should be avoided by incorporating dialysate sodium concentrations set routinely in the range of 134-138 mEq/L, avoidance of routine use of sodium modeling, and avoidance of hypertonic saline solution and 4) Dietary counseling should emphasize sodium avoidance [205].

1.12.9 Rationale Behind Reduction of Dialysate Sodium

As shown in a previous chapter, the most significant determinant of the development of RWMA is UF volume, therefore the reduction of UF volume and rate could potentially lead to a reduction in dialysis-induced injury. The concentration of sodium in the dialysate fluid has been proposed as a contributing factor to high UF volume due to the development of a positive sodium gradient between dialysate and blood. This concern has led to a re-evaluation of the dialysate sodium prescription and a number of small-scale studies looking at the effect of a reduction of sodium concentration on IDWG.

To define whether a reduction in dialysate sodium would be helpful, it is necessary to understand the relative contribution of salt to inter-dialytic fluid gain. An interesting study conducted in 17 HD patients sought to answer this question by examining serum sodium in the inter-dialytic period and calculating isotonic weight gain (due to sodium intake) and net pure water gain using balance formulae (figure 15).

The authors estimated that in non-diabetic subjects almost all of the inter-dialytic fluid gain was isotonic in nature and none due to free water ingestion. In diabetic subjects the picture was a little different with roughly half of fluid gains due to free water ingestion and half due to isotonic fluid gain. The difference can probably be explained by hyperglycaemia in diabetic patients driving greater ingestion of free water than in non-diabetic subjects [206].

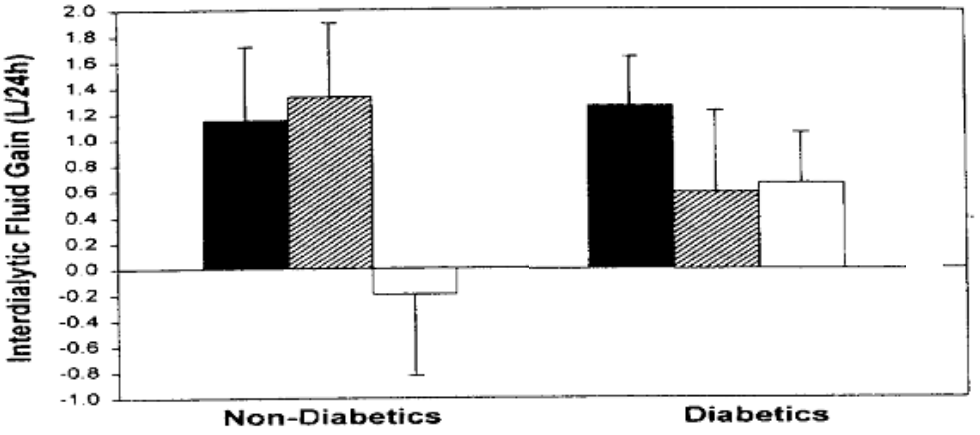

Figure 3. Interdialytic weight (fluid) gain in nondiabetic and diabetic patients. The solid bars indicate the total fluid gain (difference between predialysis and preceding post dialysis weights). The shaded bars indicate isotonic fluid gain (from formula [5]). The open bars indicate pure water gain (from formula [6]).

Figure 15: Contribution of isotonic fluid gains in non-diabetic and diabetic patients according to the balance principal illustrating that in non-diabetic patients the majority of inter-dialytic fluid gain is isotonic [206]

An audit conducted by Davenport in 469 maintenance dialysis patients demonstrated that centres which primarily used a concentration of 140mEq/L had higher inter-dialytic weight gains and more difficult blood pressure control (higher rate of patients receiving prescriptions for anti-hypertensive

medication) [185]. An observational study conducted in facility that changed practice from a dialysate sodium level of 141mEq/L to 138mEq/L reported that the change was safe and well tolerated while improving blood pressure control. It did not have a similar effect in IDWG, which the authors ascribed to not accompanying the change with reinforcement of dietary salt restriction [184].

The safe reduction of dialysate sodium has been replicated in further published work. A small study of 16 patients, the result of which are summarized in figure 16, looked at the gradual stepwise reduction of dialysate conductivity (analogous to reducing dialysate sodium) compared to a group continuing to dialyse at standard conductivity. The study reported no episodes of 'disequilibrium' and a significant reduction in inter-dialytic weight gain [186].

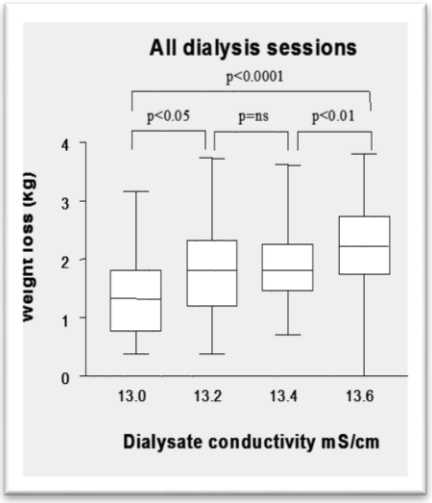

Figure 16: Achieved dialysate conductivity versus change in inter-dialytic weight gain in patients undergoing gradual stepwise reduction of dialysate conductivity[186]

This result has been consistently replicated in other small studies some of which have chosen a similar stepwise reduction over a period of weeks in order to find a level that is tolerable for an individual patient [207] and others which have taken the approach of assigning groups a pre-determined sodium concentration [208]. In both cases the reduction was well tolerated with no increase in reported muscle cramps and a decreased IDWG once the patient had dialysed at the lower sodium for a period of time.

It would therefore seem that short-term reduction in sodium can be easily tolerated and will likely have effects on IDWG and potentially on blood pressure control. There is however, a lack of long-term studies to show the potential positive effects. Concern remains that in the long-term, dialyzing at lower sodium may be harmful. This has been driven in part by a large retrospective, observational study taken from the Dialysis Outcomes and Practice Patterns (DOPPS) study, which found that patients dialyzed at facilities using a lower dialysate sodium concentration also had a higher risk of hospitalization and all-cause mortality [209]. The authors hypothesized that lower dialysate sodium leads to greater haemodynamic instability and thus increases the incidence of dialysis-induced cardiac injury leading over time to higher mortality rates from cardiovascular disease.

The authors have followed up this study with further work examining associations with lower dialysate sodium. In a similar observational study

looking at factors influencing post dialysis recovery time they found low dialysate sodium to be one (of many) factors associated with a prolonged patients reported recovery time [210]. They also found that a higher serum sodium level was associated with lower adjusted all-cause mortality. Mortality analyses restricted to the serum sodium tertile with the highest mortality (serum sodium <137 mEq/L) showed lower mortality risk in patients with dialysate sodium prescriptions >140 mEq/L suggesting that the low sodium was equalized by the dialysate and this 'protected' patients from cardiovascular instability during dialysis [211].

When interpreting the results of these studies we should bear in mind that these are large, retrospective observational studies that are only looking at units with a standard 'low' sodium prescription and not units that are individualizing their sodium prescription on a patient by patient basis. In none of these studies can causality be established nor do the studies take account of the potential effect on thirst and weight gain or the dietary strategies that each unit may employ. However it is important to take these observations into account and consider that in a certain subset of 'frail' HD patients, the consequences of reducing dialysate sodium may involve increasing cardiovascular instability on dialysis [212].

1.12.10 Summary

Sodium is an important constituent of the diet and vital for determining plasma tonicity. Dialysate sodium concentrations have risen as dialysis technology has evolved with implications for control of blood pressure and IDWG. As IDWG is heavily implicated in increased morbidity and mortality in dialysis patients there has been interest in reducing dialysate sodium concentrations as a strategy for tacking IDWG. However, low dialysate sodium concentrations may contribute to cardiovascular instability on dialysis, particularly in physiologically frail patients. Thus the optimum strategy for the prescription of dialysate sodium remains unknown and further study is required.

1.13 Role of Transplantation in Reduction of Cardiovascular Risk

The clearest way of reducing cardiovascular risk in the ESRD population is via renal transplantation. While still carrying a higher cardiovascular risk than the general population, figure 17 shows that renal transplant patients still show a much lower risk in comparison to dialysis patients [(9)].

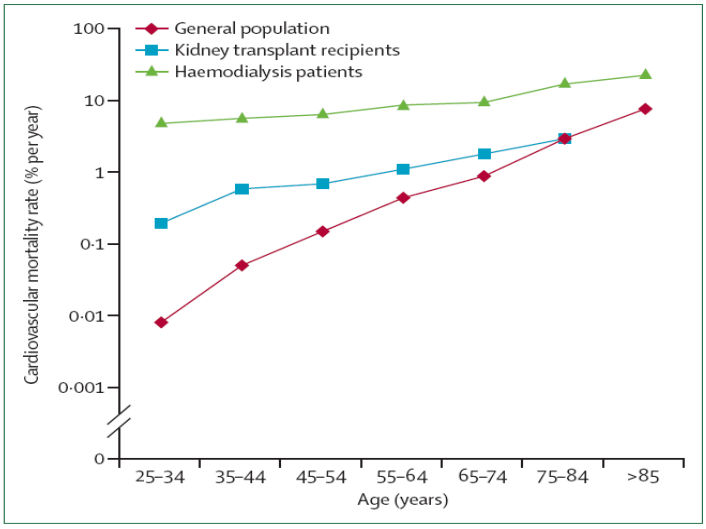

Figure 1: Cardiovascular mortality rate by age group

Figure 17: Cardiovascular mortality rate by age group in the general population as compared to haemodialysis patients and transplant recipients [213]

This improvement in cardiovascular mortality raises a number of questions. Presumably the restoration of normal (or near normal) physiological function due to reversal of the uraemic state plays a role in this improvement, as does the removal of the recurrent cardiac injury associated with dialysis. Balanced against this is the issue of immunosuppressive medications, which as well as increasing the risk of infection and certain malignancies also increases the risk of cardiovascular disease via their impact on weight gain, lipids, blood pressure and association with NODAT (new onset of diabetes after transplant) [214].

It also raises the question of how 'plastic' the changes to the vasculature and the heart that occur during the spectrum of CKD are and to what extent they

may regress following transplant. Surprisingly little is know regarding the performance of the heart and changes in the vasculature post transplantation. There are a number of studies that suggest that not only does transplantation stop deterioration in cardiac function but also that there is significant improvement in LVEF. The largest of these studies by Walli et al examined 138 patients with CKD and a LVEF less than 40% who were referred for either a kidney, or kidney-pancreas transplantation. All patients underwent assessment for reversible ischaemia prior to transplantation, approximately 57% were considered to be in stage IV heart failure (according to the New York Heart Association classification of heart failure). The investigators found that mean LVEF increased from 31.6% to 47.2% at 6 months and to 52.2% at 12 months (both changes were highly statistically significant) following transplant. The LVEF improved in 69% of patients to greater than 50% and 16% increased to greater than 40% but less than 50%. Notably, the investigators also found an inverse relationship between the duration of dialysis and the normalization of left ventricular ejection fraction. Those patients improving to a LVEF greater than 50% had significantly fewer days on dialysis than those who improved to only 40-50%. Those with an LVEF improving to greater than 50% were also found to have a significant improvement in functional status [215].

The effect of renal transplantation on the progression of vascular calcification has been studied. While it would seem reasonable that restoration of the

phosphate-calcium-vitamin D axis by transplantation would reduce the propensity of the patient to vascular calcification this has not been clearly shown. The largest study looking at the effect of transplantation on VC used computer tomography scanning to assess coronary artery calcification scores in 150 renal transplant patients. The average follow up time was almost 3 years. The baseline prevalence of coronary artery calcification in this study was approximately 35% and by the end of the follow up period this had increased to 64.6%. The investigators concluded that renal transplantation neither halts nor reverses coronary artery calcification [(216)]. A number of smaller studies have shown similar results with progression occurring in as low as 12% [(217)] and as high as 25% [(218)]. Only one study shows evidence of regression in 14% of their cohort [(219)].

The weight of evidence clearly suggests that transplantation may slow the progress of vascular calcification (particularly in comparison to haemodialysis which accelerates it) but it does not halt it and regression is a rare event. One or more of these studies have shown that factors such as baseline coronary artery calcification score, lipid concentration, bisphosphonate use and blood pressure control are independent determinants of progression.

In terms of the other ‘non-classical’ risk factors discussed above, recent studies have attempted to assess the progress of arterial stiffness post

transplantation, which again gives information regarding the plasticity of changes to the vasculature. Two recent studies have looked at arterial stiffness in the year after transplant, one used aortic pulse wave velocity (PWV) [220] and the other measured brachial-ankle pulse wave velocity (baPWV) [221]. In the first study mean aortic PWV was 9.25m/s at baseline versus 8.97m/s at 12 months with the change being non-significant. In the second study baPWV was significantly decreased at 12 months with 86.9% of the cohort showing no progression of arterial stiffness. These studies at least indicate that there is no progression in arterial stiffness in the 12 months post transplantation, however they do not provide any indication of long-term trends.

Finally, measurement of tissue AGE deposition is possible indirectly via determination of skin autofluorescence (SAF), a method that utilises the correlation between collagen linked fluorescence and AGE content observed in skin biopsies. A simple non-invasive, test that makes use of a specialized auto-fluorescence reader and gives a reading in arbitrary units. SAF has been validated against AGE deposition in skin biopsies and thus provides a useful marker of the degree of metabolic stress a patient has accumulated.

As discussed above, SAF is independently predictive of cardiovascular mortality in a variety of clinical scenarios including dialysis (peritoneal and haemodialysis) where patients have extremely high readings that are comparable to and often greater than patients suffering from diabetes with

multiple complications [37, 222]. There is little study regarding the trend in SAF following transplantation. Skin-AF has been measured in order to assess its potential impact on graft loss where it seems to be an independent predictor of transplant failure [223]. A study of 285 transplant recipients where SAF levels were measured and compared to 231 normal controls showed that while SAF levels were significantly lower than those found in dialysis patients they were still higher than levels found in control subjects [224]. While this study benefited from the presence of a control group it did not match for comparative renal function.

The reason for this decline post transplant is unclear as AGE deposition appears to be 'fixed' in the normal population with a half-life of approximately 13 years. Restoration of renal function could clear AGE products as they form and this could account for some of the difference in readings. Another possibility is that the removal of the metabolic and inflammatory stress of dialysis could be contributory. In PD this may be the removal of glucose exposure as cumulative glucose exposure is associated with greater SAF readings [30]. It is possible that stress induced by extra-corporeal therapy itself contributes to high AGE deposition in a manner that is yet to be defined. Studying the behavior of AGE deposition before and after transplantation has the potential to offer insights into the nature of dialysis induced metabolic stress and what the potential may be for reversing it's consequences.

1.13.1 Summary

Cardiovascular mortality reduces dramatically following renal transplantation although it does not normalize. The available evidence suggests that transplantation is associated with favourable remodeling of the heart although the effect is dependent upon a patients length of time on dialysis. In terms of the other non-classical risk factors contributing to adverse cardiovascular outcomes it would appear that transplantation either slows or stops progression, however this is an area that could benefit from further study both for understanding CVD in transplant recipients and providing insight into their contribution to morbidity and mortality in the dialysis population.

CHAPTER 2: THESIS AIMS

2.1 Formulation of the Hypothesis

As seen in chapter one it is increasingly clear from the evidence that HD causes a repetitive ischaemic injury, which is associated with adverse outcomes in terms of mortality and cardiac function. This injury is a result of underlying changes in the vasculature, which result in stiff, non-compliant vessels that are unable to respond to the considerable physiological stress placed on the patient by the dialysis procedure. It can be hypothesized that the adverse outcomes in dialysis patients are, at least in part, the cumulative result of the repeated stresses of HD causing fibrotic and other changes within the heart. Therefore the cardiovascular mortality occurring in dialysis patients needs to be addressed and solutions focused on the dialysis procedure itself should be considered. It is also important to further explore the nature of

ischaemic stress in HD, to gain further insight into the factors that influence it and to examine more closely the specific non-classical risk factors that contribute to this vulnerability to ischaemic stress.

We can also see from a number of pilot studies that it is possible to reduce the extent of dialysis induced cardiac injury may making relatively simple modifications to the dialysis procedure such as cooling the dialysate or switching to a more frequent schedule. Given that some interventions will be limited by patient tolerance and acceptability it is likely that further dialysis-based solutions will be required.

This thesis has therefore been planned to examine the following hypotheses

- *The accumulation of cardiovascular risk is in part a result of accumulated risk in chronic kidney disease and in part direct result of the dialysis procedure itself.*
- *Haemodialysis causes repetitive cardiac injury which over time leads to structural and functional changes that worsen cardiovascular mortality.*
- *Increasing the tolerance of the myocardium for ischaemic injury by utilizing remote ischaemic preconditioning can help to protect the heart from ischaemic injury.*
- *Reducing the inter-dialytic weight gain by reduction of dialysate sodium concentration will also protect the heart from dialysis stress.*

- *This is partially reversible upon transplantation as demonstrated by a reduction in the deposition of advanced glycation end-products.*
- *Following removal of dialysis, metabolic stress is reduced and this can be shown by a reduction in AGE deposition and shown by a reduction in skin autofluorescence.*

2.2 Research Questions

The aims of this thesis are to explore the following research questions:

- Further explore the relationship between dialysis, low blood pressure and dialysis induced cardiac injury
- Investigate the effect of HD of segmental and global longitudinal strain
- Examine whether the administration of remote ischaemic preconditioning has any effect on dialysis induced cardiac injury as measured by the effect on global and segmental longitudinal strain
- Explore the tolerability and safety of stepwise reduction of dialysate sodium
- Examine the effect of stepwise reduction of dialysate sodium on inter-dialytic weight gain
- Examine the effect of stepwise reduction of dialysate sodium on global and segmental longitudinal strain

- Examine the reversibility of non-classical risk factors by investigating the effect of transplantation upon advanced glycation end product deposition as measured by skin auto-fluorescence.

CHAPTER 3: MATERIALS AND METHODS

All methods used in more than one study are dealt with in detail in this chapter, and the relevant results chapters refer back to this. Techniques unique to a particular study are described in the appropriate results chapter.

3.1 Applications for Ethical Approval

Appropriate ethical approval was sought and granted for all aspects of the research detailed in this thesis. The author applied for appropriate ethics approval for the studies, and attended meetings of the research ethics committee to answer questions and address concerns. Approval from the Derby Hospitals NHS Foundation Trust Research and Development department was also sought.

Studies were carried out in accordance with the Declaration of Helsinki.

3.2 Patient Recruitment

All patients in the Derby-based studies were recruited from the Royal Derby Hospital haemodialysis unit, where the average number of prevalent HD patients is around 200. Since the cohort of patients available for study was limited, some patients took part in more than one study, however they did not participate in more than one study at a time and underwent an appropriate 'cooling off' period in between.

All patients were interviewed twice during the recruitment process. Inclusion and exclusion criteria for each study are included in individual chapters. At the initial visit, they were approached to gauge level of interest in study participation and given an approved patient information sheet. During the second visit, interest was confirmed and consent obtained from those individuals willing to take part. There was ample opportunity to ask questions during both visits and it was stressed that patients could withdraw at any time, without reason or detriment to their ongoing treatment.

The number of patients recruited to each study is detailed in the appropriate chapter.

3.3 Inclusion Criteria

In the dialysis studies the patients fulfilled the following inclusion criteria

1. Patients having HD treatment at least 3 times per week.

2. Willing and able to provide consent.
3. Male and female, age≥16 years old.
4. Exposure to HD for greater than 90 days

Exclusion criteria varied according to the study.

3.4 Determination of Left Ventricular Longitudinal Strain by 2-Dimensional Echocardiography

Myocardial fibres are arranged in layers of different orientation. This means that the pumping action of the heart is a complex 3-dimensional process that comprises longitudinal, radial and circumferential components and directions of movement [225]. To analyse myocardial deformation the current gold standard is tagged magnetic resonance imaging. This is an expensive tool and importantly is not available for dynamic intra-dialytic analysis of cardiac function as yet. In order to provide a dynamic quantification of the cardiac strain of HD, alternative tools must be used. The imaging modality most suited to dynamic intra-dialytic assessment is echocardiography as it is non-invasive and can be carried out with portable machines brought directly to the patient. Images can be subsequently stored and analysed by a number of different software packages.

2-dimensional speckle tracking echocardiography is increasingly being used for quantification of global, regional and segmental left ventricular mechanics using standard two-dimensional grey scale images [226]. Previous work from our group used tissue Doppler imaging to assess regional myocardial deformation. 2-D speckle tracking is angle independent and can analyse deformation in the longitudinal, radial and circumferential components, although can be sensitive to bad image quality [227]. It estimates motion by tracking the speckles in the image. The speckles appear as a result of interference generated when ultrasound waves scatter in tissue. This phenomenon occurs when sound waves are reflected by sub-millimetre structures smaller than the wavelength of ultrasound. Interference between waves either destroys or summates their amplitudes (destructive or constructive interference). This results in a pattern of bright and dark pixels. In the myocardium, the tissue structures causing interference are stationary within the tissue and randomly distributed. This produces a speckle pattern that is a stable, unique 'fingerprint' of that region that moves with the tissue. Previous studies have validated its accuracy against the reference standard of CMR [228, 229] and superiority to LVEF has been reported in a variety of clinical settings [230-232]. Junior echocardiographers are more able to acquire images of sufficient quality as it less noise sensitive than Doppler-based strain methods. The many advantages of strain by STE over strain by Doppler, have led to a rapid uptake of this technique in research and clinical practice.

Offline analysis provides quantification of left ventricular longitudinal strain and longitudinal strain rate and in theory reduces inter-observer variability. Using the two and four chamber views the endocardial border is traced manually as shown in figure 17. The software divides the left ventricular chamber into segments (12 in total – 6 in the 2 chamber view and 6 in the four chamber view) and uses speckle tracking to calculate strain values and rates for each segment.

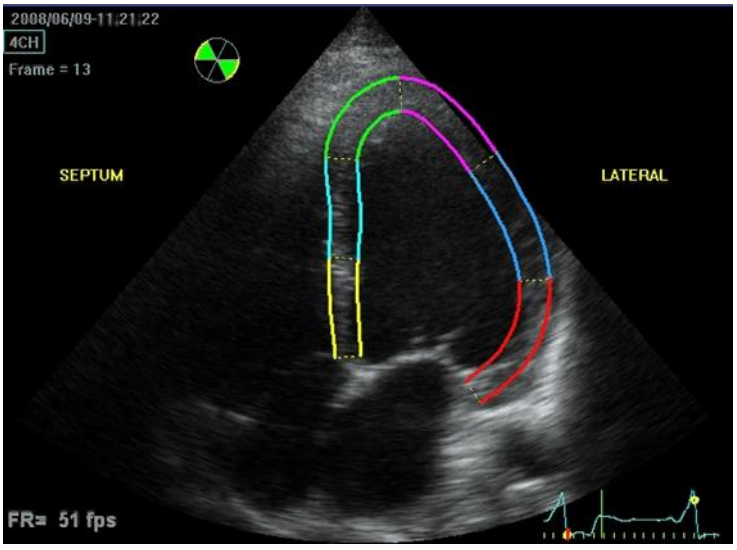

Figure 17: Tracing of the endocardial border in speckle-tracking analysis

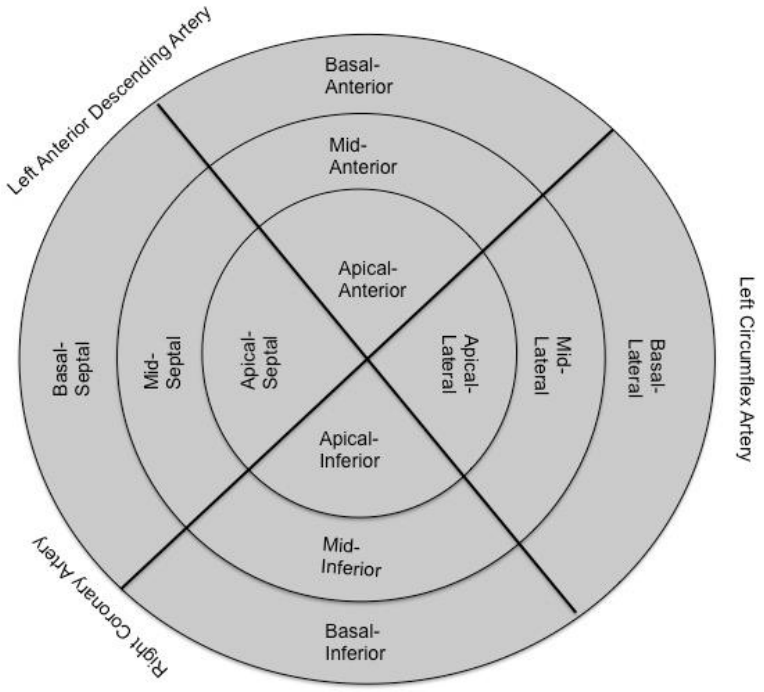

Figure 18: Schematic representation of left ventricular segments and the arterial supply [91]

3.4.1 Measurement of Strain

Deformation (or strain) is the fractional change in an objects dimension compared with the objects original dimension. Left ventricular global peak longitudinal strain is the ratio of the maximal change in myocardial longitudinal length in systole to the original length. Strain (either segmental or global) is represented by a negative value. The more negative the value is, the better the left ventricular function. Compared with left ventricular ejection fraction (LVEF) global longitudinal strain is considered to be a more sensitive and reproducible modality for assessing cardiac function [60, 233, 234]. In the general population, evidence suggests that GLS is a prognostic indicator. Figures 19 and 20 show how longitudinal strain curves are represented in the Echopac programme.

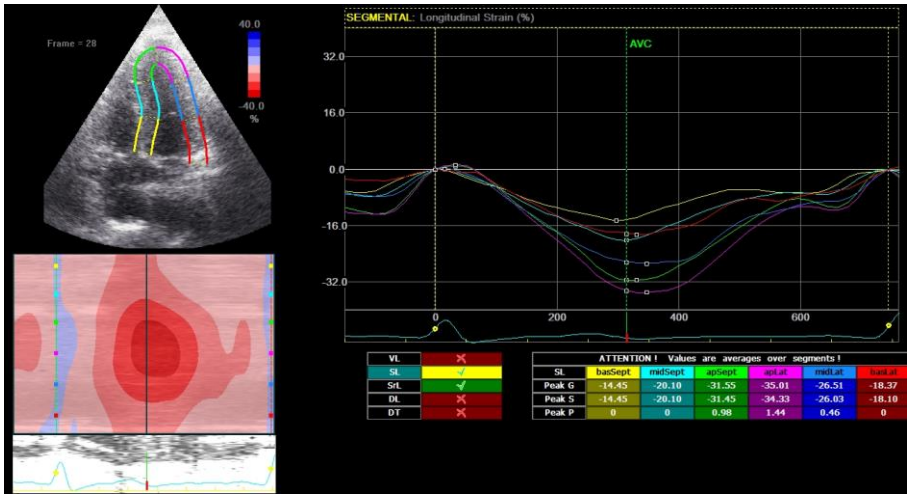

Figure 19: Longitudinal strain curve obtained from 2D speckle tracking analysis taken pre-dialysis

Figure 20: Longitudinal strain curve obtained from 2D speckle tracking analysis taken post-dialysis

The normal range to be found in the general population varies a little between studies but in terms of global longitudinal strain (GLS) it appears to be between -18 and -21. There is some variability to longitudinal strain (LS) measurements

depending on the ultrasound system and software used and this has been formally studied [226]. Table 3 below shows normal ranges according to age and gender for three different vendors. Our studies used vendor number one for analysis. The values obtained in this study were broadly in line with other similar studies in a normal population [225, 235].

Table 3. Effect of Age and Gender on Global Longitudinal Strain vs. Vendor

Age group	0–19 years	20–29 years	30–39 years	40–49 years	50–59 years	≥60 years	P value
V_1							
Overall	–22.1±2.4	–21.2±1.9	–21.1±2.1	–21.4±2.0	–21.0±2.2	–20.3±1.9	0.0218
Male	–21.7±3.1	–20.9±1.9	–20.6±1.9	–20.9±1.8	–21.0±1.9	–19.7±1.4	0.1982
Female	–22.4±1.6	–22.3±1.6	–22.8±1.8	–22.6±2.1	–23.3±1.9	–20.9±2.1	0.0348
P (M vs. F)	0.4292	0.0316	<0.0001	0.0178	0.0029	0.1381	
V_2							
Overall	–19.9±2.5	–19.0±2.1	–19.5±2.2	–18.2±2.5	–17.6±2.5	–16.7±2.1	<0.0001
Male	–19.4±2.7	–18.8±2.0	–19.1±2.3	–17.9±2.8	–16.9±2.3	–15.8±1.4	0.0019
Female	–20.5±2.2	–20.6±2.3	–20.2±2.0	–19.3±0.9	–20.4±1.5	–17.3±2.3	0.0002
P (M vs. F)	0.1349	0.0248	0.1083	0.4316	0.0294	0.0928	
V_3							
Overall	–21.4±1.7	–20.2±2.1	–20.4±2.3	–19.4±2.2	–18.5±2.6	–17.8±2.8	<0.0001
Male	–21.6±2.0	–20.2±2.0	–20.4±2.2	–19.8±2.3	–18.7±2.6	–16.3±3.1	<0.0001
Female	–21.2±1.5	–20.2±2.4	–20.4±2.8	–18.7±1.8	–18.3±2.8	–18.6±2.3	0.0141
P (M vs. F)	0.6076	0.9787	0.9201	0.1415	0.7374	0.0668	

Table 3: Normal range of GLS according to age and gender measured by three different ultrasound vendors [226].

Studies in dialysis patients have demonstrated a mean GLS well below that of the general population [55]. However segmental LS (based on division of the left ventricle into segments as per the American Heart Association model) also gives valuable information as many dialysis patients may suffer from perfusion defects in so called ‘watershed areas’ (segments with an anatomically defined higher degree of vulnerability to demand ischaemia in this portion of the uremic heart). Furthermore, there are reports of ‘compensatory’ hyperkinesis, wherein segments may demonstrate increased LS during periods of stress while segments associated with perfusion defects show reduced LS. This has

previous been observed in paediatric dialysis patients who also suffer from dialysis induced cardiac injury [236]. Compensatory hyperkinesis has been previously described following acute myocardial infarction where segments of the left ventricular wall develop wall motion abnormalities post infarct and compensatory function occurs in non-ischaemic segments. This effect usually reduces by about the third day post MI leaving the patient with an overall reduction in function [237]. The presence of 'compensatory' hyperkinesis would complicate assessment GLS as developing segmental abnormalities would be 'covered up'.

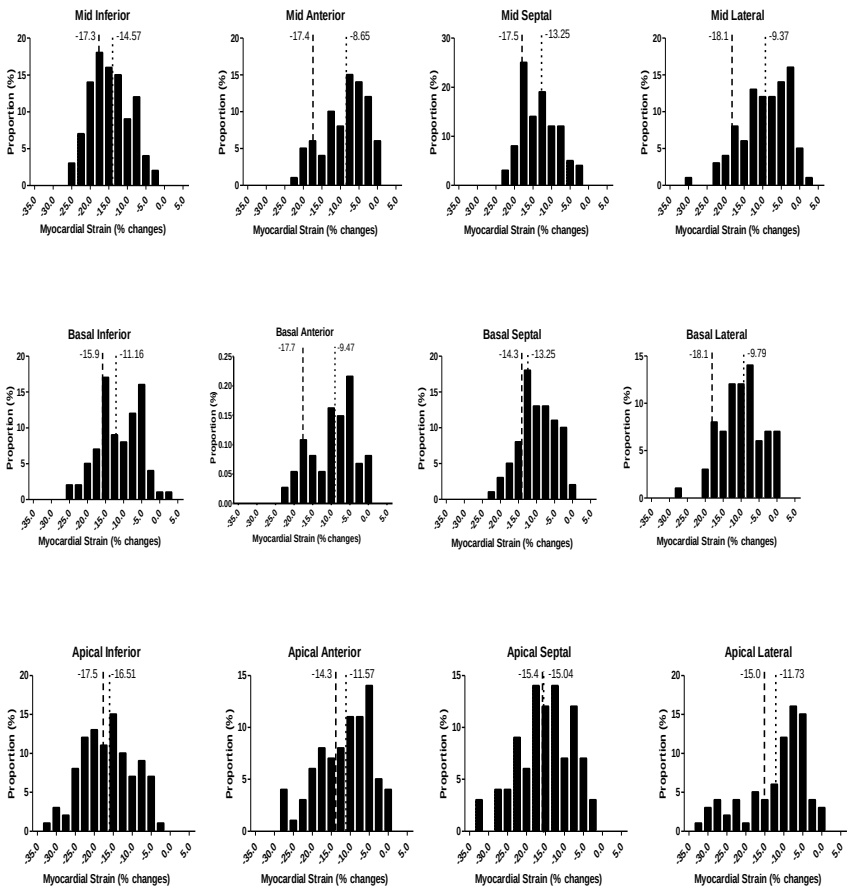

Figure 21: Distribution of myocardial strain for the 12 segments, and comparison to reference values for healthy individuals (light dash line: sample mean; dark dash line: reference values) [91]

As shown in figure 21, dialysis patients show reductions in LS in the majority of segments although the degree of difference is greatest in mid and basal segments.

3.4.2 Echocardiographic Assessment of Dialysis Induced Cardiac Injury

In theses studies left ventricular assessment was performed using 2D echocardiography. Images were recorded pre-dialysis and at 225 minutes into dialysis (peak stress). The images were obtained using commercially available equipment (3.6 MHz 3S probe, Vivid I®, GE medical systems, Sonigen, Germany). Standard apical 2 and 4-chamber views were recorded for off-line digital analysis to measure global and segmental longitudinal strain (using EchoPac, GE), as previously described in HD patients [91]**.** The region of interest (endocardial borders excluding papillary muscles) is traced for each image at the end-systolic frame. The speckle patterns on a frame-by-frame basis are tracked using the EchoPAC tracking algorithm. Three consecutive heartbeats are analyzed for each image, and the peak strain was measured across 12 left-ventricular segments. As well as producing a strain curve values of LS and strain rate are given for each left ventricular segment.

3.4.3 Definition of dialysis induced cardiac injury

Segmental values of left-ventricular longitudinal myocardial strain were reported. LS describes the fractional change in length of a myocardial segment and is expressed as a percentage change (either positive or negative depending on lengthening or shortening). The difference in values between the pre- and peak-HD myocardial strains were calculated for each segment and then and globally (average of all segments). If a segment underwent a greater than 30% reduction in longitudinal strain it was defined as a segment subject to dialysis induced cardiac injury. The rationale for selecting 30% came from extensive previous work on the correlation between the development of observable regional wall motion abnormalities on echo. Multiple studies have shown that the appearance of RWMA's during dialysis correlates to reduced myocardial blood flow [90] and negative outcomes [94, 95]. RWMA's have been validated against clinically significant outcomes.

3.5 Non-Invasive Haemodynamic Monitoring

NICOM™, (Cheetah Medical, USA), is a thoracic bioreactance monitor that employs delivery and detection of phase changes in alternating electrical current to derive cardiac output. This endows higher signal-to-noise ratio and greater precision than impedance cardiography. The monitor is validated in healthy volunteers, critical care settings [238] and chronic heart failure [239]. A recent study found the monitor comparable to expert dobutamine stress echocardiography to optimise cardiac resynchronisation therapy devices [240]. The bioreactance system (NICOM™) consists of applying four pairs of

adhesive electrodes to the patient's thorax, in the positions shown in the figure below. Within each sticker, one electrode is used by the high-frequency current generator to inject the high-frequency sine wave into the body, while the voltage input amplifier uses the other electrode. Two stickers are placed on the right side of the body, and two stickers are placed on the left side of the body. The stickers on a given side of the body are paired, as demonstrated in figure 22, so that the currents are passed between the outer electrodes of the pair and voltages are recorded from between the inner electrodes. A noninvasive CO measurement signal is thus determined separately from each side of the body, and the final noninvasive CO measurement signal is obtained by averaging these two signals [241].

Figure 22: Illustrating the data display and electrode placement of the NICOM system

Bioreactance works on the principal that changing aortic blood volume induces changes in intrathoracic volume and also changes in electrical capacitance and inductive properties. These changes result in phase shifts of the received

signal relative to the applied signal. Techniques for detecting these relative phase shifts are less susceptible to electrical noise than techniques using bioimpedence which detects changes in resistance to blood flow but is inaccurate in settings with significant electrical noise and body motion [241].

In these studies the NICOM was applied just prior to dialysis and calibrated against brachial blood pressure (if able to). BP was then measured every 30 minutes and readings for cardiac output, cardiac index, stroke volume and MAP were also recorded at 30-minute intervals. Post dialysis the files were downloaded into Excel spreadsheet format and average values for the 4-hour session derived.

3.6 Haematological and biochemical assays

Unless otherwise stated, all pre-dialysis blood tests were drawn immediately after insertion of access needles, and post-dialysis levels were taken from the arterial line 10 seconds after reduction of blood pump speed to 50 ml/min. Biochemical analysis was performed on a multichannel auto-analyser. Full blood count measurements were performed using a compact analyser.

3.7 Cardiac Biomarkers

In these studies, pre-dialysis blood samples were draw immediately after insertion of access needles and collected into lithium heparin and EDTA tubes. Biochemical analysis was performed on a multichannel autoanalyzer. Cardiac

troponin T was measured using a fourth-generation electrochemiluminescence assay (Elecsys, Roche Diagnostics, Lewes, United Kingdom). All treating physicians were blinded to troponin T levels measured for study purposes. However, clinically indicated Troponin T measurements (e.g. for episodes of acute chest pain) still proceeded during the study period.

3.8 Statistical analysis

All statistical analyses were performed by the author using either SPSS v21 or GraphPad Prism version 5 software (GraphPad Software, San Diego, USA). Continuous data were reported as mean +/- SD, or as median with 10^{th} and 90^{th} percentile when noted. Nominal data was expressed as numbers and percentages. Correlations between continuous variables within the data set were identified using a Pearson correlation co-efficient. Bonferonni correction was applied due to multiplicity (12 analyses for correlation were conducted), and as a result $P<0.004$ was considered statistically significant. Comparisons between patients were conducted using an unpaired T-test, with a two-sided P value of <0.05 considered statistically significant. Sample size calculations are provided in individual in the relevant study chapters.

3.9 Dialysis Details

All patients were over 18 years of age and were prevalent patients established on conventional HD therapy for at least 90 days. In these studies all patients

were receiving thrice-weekly dialysis with treatment duration of between 4-5 hours, with dialysate-flow rate of 500-800ml/min and blood-flow rate of 250-450 ml/min. Dialysate temperature and conductivity were fixed for the duration of the study period (with the exception of the dialysate sodium study where by definition conductivity was altered). Unless otherwise stated all dialysis described in this thesis was performed using Hospal Integra® monitors (Gambro-Hospal, Mirandola, Italy). Unless otherwise stated, low-flux polysulphone dialysers were either 1.8m^2 or 2.0m^2 as per individual patients' usual prescription (LOPS® 18/20, Braun Medical Ltd, Sheffield, UK).

CHAPTER 4: RESULTS EXPLORING THE RELATIONSHIP BETWEEN NADIR BLOOD PRESSURE AND DIALYSIS INDUCED CARDIAC INJURY

4.1 Introduction

Intra-dialytic hypotension (IDH) is a significant and common complication of hemodialysis (HD) treatment, occurring in approximately 20-30% of hemodialysis sessions [97]. Causes for IDH are multifactorial, with ultrafiltration rate, sodium removal, and levels of bacterial endotoxin amongst the factors contributing to its development [43, 98]. Hemodialysis-induced cardiac injury also appears to be related to circulatory stress and, at least in part, to hypotension. Previous studies have concluded that IDH is a determinant factor for myocardial stunning and is associated with increased mortality [93, 95, 99].

Recent important work by Flythe et al has clarified which of the many prevalent ways of defining IDH is actually associated with increased mortality. They identified that an intra-dialytic nadir systolic blood pressure <90 mmHg appears to represent a threshold of concern, with many of the other definitions (even those including a symptomatic element) not being associated with excess mortality [100]. These important findings have resulted in many nephrologists redefining what is an important blood pressure drop (although this was not a feature of the original publication), and potentially considering less severe reductions in blood pressure ‘safe’.

Given that both dialysis-induced cardiac injury and IDH are associated with increased mortality in HD patients, this study aimed to address the relationship between cardiac function, both in a rest and stressed state, and IDH. Furthermore, we sought to determine whether an intra-dialytic nadir systolic blood pressure below 90 mmHg was a potential threshold for identifying those at risk of dialysis induced cardiac injury.

4.2 Study Design

This study was a cross-sectional survey involving the dialysis unit at the Royal Derby Hospital in the United Kingdom. Ethics approval from was obtained from the Nottinghamshire Local Research Ethics Committee prior to commencement of the study. Patients with end-stage renal disease receiving conventional hemodialysis (Integra Hemodialysis Machines, Hospal, Mirandola, Italy) were recruited between 2008 and 2014. This study was in accordance with the Declaration of Helsinki. The primary outcome measures were the number of injured myocardial segments correlated with nadir blood pressure during dialysis. The lowest systolic blood pressure recorded throughout the hemodialysis process was identified as the intradialytic nadir systolic blood pressure.

Data collection

Patient baseline characteristics were recorded including age, gender, route of vascular access, and hemodialysis vintage (months). Standard

echocardiography was performed prior to as well as at peak (15 minutes before the end of hemodialysis) hemodialysis treatment to capture two and four-chamber views of the heart. Patients were placed in the left lateral decubitus position for this procedure. Blood was also drawn at the beginning and end of hemodialysis to determine Troponin-T blood concentrations. Lastly, blood pressure was measured every 15 minutes during hemodialysis using a CHEETAH NICOM device (Cheetah Medical, Tel Aviv, Israel).

Speckle-tracking Analysis

As previously described, the left ventricle in both the two-chamber and four-chamber echocardiograms were labeled into six segments per view (12 segments total) and analyzed for global and segmental LS by speckle-tracking using EchoPAC version 113 software (GE Healthcare, Tirat Carmel, Israel). Analyses were performed in a blinded fashion without access to the patient's clinical information. An injured segment was defined as a segment of the left ventricle with a >40% increase in LS, when comparing post-dialysis echocardiograms to pre-dialysis echocardiograms.

4.3 Results

165 patient-visits in 102 individual patients were completed for this study, with the characteristics of the cohort summarized in (**Table 4 below)**. Briefly, the

average age for the cohort was 60.76 +/- 14.80 years, with a mean vintage (number of months since dialysis treatment was initiated) of 35.3 +/- 41.3 months. 71.7% of the cohort was male, while the AV fistula was most prevalent mode of vascular access for these hemodialysis patients (91.9% usage rate).

Variable	Mean +/- SD	Median (10th,90th percentiles)
Age (years)	61.76+/-14.80	64 (38, 77)
Males	71.7%	
Females	28.3%	
Patients with AV fistula	94.6%	
Patients with AV grafts	5.4%	
Patients with lines	0.0%	
Mean vintage (months)	35.3+/-41.3	24 (5, 80)

Table 4: Basic characteristics of the study cohort (n=165 patient visits)

4.3.1 Cardiovascular characteristics

The baseline cardiovascular characteristics of the cohort, prior to examining for correlations, are summarized in **Table 5**. At the beginning of HD, patients had a global LS and Troponin-T concentration of -12.96 +/- 4.55% and 65.44 +/- 55.49 ng/mL, respectively. HD resulted in an increase of 8.51 +/- 31.54% in global LS, as well as an increase of 2.83 +/- 16.58 ng/mL in Troponin-T concentration. From a possible twelve segments labeled throughout the left ventricle, a mean of 3.03 +/- 2.36 segments were identified as injured by the end of HD treatment using the definition above. Lastly, the nadir systolic blood pressure from hemodialysis treatment for this study cohort was 105 +/- 27 mmHg.

Table 5: Cardiac measurements of the study cohort (n=102)

4.3.2 Correlations between nadir blood pressure and cardiovascular characteristics

Variable	Mean +/- SD
Number of injured segments during dialysis	3.03 +/- 2.36
Predialysis global longitudinal strain (%)	-12.94 +/- 4.55
Change in global longitudinal strain (%)	8.51 +/- 31.54
Nadir blood pressure (mmHg)	105 +/- 27
Pre-dialysis Troponin-T (ng/mL)	65.44 +/- 55.49
Change in Troponin-T (ng/mL)	2.83 +/- 16.58
Ultrafiltration volume (L)	2.03 +/- 0.94

A correlation was identified between intra-dialytic nadir systolic blood pressure and pre-dialysis global LS (r=-0.280, p=0.001) but not between intra-dialytic nadir systolic blood pressure and either the number of injured segments (r=-0.122, p=0.172) or the ultrafiltration volume (r=-0.062, p=0.451) (**Table 6**). Pre- dialysis Troponin levels were not correlated with either pre-dialysis global LS, the number of injured segments, nor ultrafiltration volume. Change in Troponin-T levels did correlate with pre-dialysis GLS (r=-0.23 p=0.008).

Variable	Nadir BP	Pre-HD GLS	Pre-HD Trop-T	Δ in Trop-T
Pre-dialysis GLS				
r value	-0.280	1.000	-0.174	-0.234
P value	0.001		0.054	0.008
Number of Injured segments				

r value	-0.122	0.042	-0.107	-0.058
P value	0.172	0.593	0.268	0.542
Ultrafiltration volume				
r value	-0.062	0.182	-0.145	0.030
P value	0.451	0.017	0.091	0.727

Table 6: Correlations with variables of cardiac injury within the study cohort (where BP=blood pressure, HD=Haemodialysis, GLS=Global Longitudinal Strain). Variable tested using Pearson correlation coefficient, n=102 patients.

4.3.3 Differential characteristics between patients with and without IDH

Based on recent literature suggesting that IDH should be defined as an intra-dialytic nadir systolic blood pressure <90 mmHg (14), hemodialysis patients from the study cohort were separated based on blood pressure. Patients with an intra-dialytic nadir systolic blood pressure of <90 mmHg were older (65.77+/- 12.89 vs. 59.95+/-14.77 years old), had a less negative global LS (-10.13+/-4.20% vs. -13.49+/-4.76%), and fewer of this group were comprised of males than patients with >90 mmHg (61.4% vs. 79.0%) (**Table 7**). There was no statistically significant difference in route of vascular access, vintage, number of injured segments during HD, change in global LS, Troponin-T concentrations, or UF volumes between the two groups.

Variable	BP<90	BP>90	P value

Age	65.77+/-12.89	59.95+/-14.77	0.018
Male (%)	61.40	79.00	0.021
AV Fistula (%)	92.90	95.20	0.572
Vintage (months)	42.04+/-54.35	38.37+/-42.21	0.656
Number of injured segments during dialysis	3.52+/-2.54	2.89+/-2.27	0.202
Predialysis global longitudinal strain (%)	-10.13+/-4.20	-13.49+/-4.76	<0.001
Change in global longitudinal strain (%)	7.37+/-43.12	-12.10+/-4.47	0.904
Nadir blood pressure (mmHg)	69+/-16	118+/-15	<0.001
Pre-dialysis cardiac Troponin-T (ng/mL)	62.38+/-38.07	64.09+/-53.78	0.913
Change in cardiac Troponin-T (ng/mL)	-2.46+/-21.12	3.23+/-16.64	0.269
Ultrafiltration volume (L)	2.13+/-0.75	2.06+/-0.97	0.698

Table 7: Comparison of patients suffering nadir systolic blood pressure of less than 90mmHg vs. group maintain systolic BP above 90mmHg (where total n=102).

4.4 Discussion

This study showed that in patients undergoing conventional HD, overall systolic function (as measured as pre-dialysis global LS) but not number of RWMA's developed post dialysis, or UF volume, correlated with intra-dialytic nadir systolic blood pressure. Usage of a working definition for IDH based on an intra-dialytic nadir systolic blood pressure of below 90 mmHg systolic does identify a high-risk group of HD patients with pre-existing cardiac dysfunction. However, dialysis induced cardiac injury still occurs commonly at BP levels higher than 90mmHg To our knowledge this is the first study to investigate the

relationship between dialysis-induced cardiac injury and intra-dialytic nadir systolic blood pressure using echocardiography.

Our results correlating intra-dialytic nadir systolic blood pressure with pre-dialysis global LS are consistent with findings from previous studies. Poldermans et al. who demonstrated that myocardial contractile reserve was more impaired in hypotension-prone hemodialysis patients than in hypotension-resistant patients and suggested this may contribute to the development of IDH [(242)]. Similar conclusions were made in a more recent study where hypotension-prone HD patients had diminished cardiovascular response to dobutamine-atropine stress compared to hypotension-resistant patients [(243)]. Others have also shown that a low cardiac index and a high total peripheral resistance are associated with increased risk of intra-dialytic morbid events such as intra-dialytic hypotension [(244)]. These data are consistent with the suggestion that IDH and cardiac remodeling both interact with one another in a positive feedback loop, resulting in poorer cardiovascular outcomes in HD patients, and worsening ability to maintain blood pressure during dialysis in the face of intermittent UF [(245)].

Results from this study suggest that intra-dialytic nadir systolic blood pressure greater than 90 mmHg does not exclude patients from developing dialysis-induced cardiac injury. Indeed there is no significant difference in the number of injured segments between patients with a nadir BP less than 90mmHg and

those with a nadir BP greater than 90mmHg. Therefore patients maintaining their BP above 90 during dialysis are still subject to dialysis induced cardiac injury leading to an inability to hold their blood pressure during the haemodynamic stress of dialysis. It is likely that these HD patients will develop contractile dysfunction over time, especially if appropriate measures to optimize the hemodynamic response to HD are not taken in the false belief that their blood pressure is safe above 90 mmHg.

Neither cardiac injury nor UF volume correlated with intra-dialytic nadir systolic blood pressure. The lack of a correlation between cardiac injury and intra-dialytic nadir systolic blood pressure in this setting may be due to cardiac injury correlating better with an overall change in perfusion pressure during a dialysis treatment, rather than just a single value above/below a threshold, as previously evidenced [93]. An alternative explanation for our results is that injury to other structures within the heart, such as the aortic and mitral valve, may be additional important determinants of IDH rather than just ventricular function alone [246]. Relative effects during dialysis on diastolic dysfunction and worsening myocardial perfusion may also be important [243, 247] but detailed analysis of serial diastolic dysfunction was beyond the scope of this particular study. With regards to a lack of correlation between UF volume and intra-dialytic nadir systolic blood pressure, UF rate may be a better prognostic factor for IDH than UF volume [248]. It should also be noted that some studies of HD induced left ventricular dysfunction have suggested that this dysfunction

develops early in HD and may be unrelated to changes in blood volume. The implication of these findings is that factors relating to the process of extra-corporeal therapy itself may have a role in the changes in cardiac function observed during dialysis [95, 249].

Other factors may also contribute to the development of IDH. Aside from pre-dialysis global LS, our sub-cohort of HD patients with an intra-dialytic nadir systolic blood pressure <90 mmHg differed by age and gender according to statistical analyses. This is consistent with the literature, identifying both older age (>60 years) and female sex as some of the risk factors associated with intra-dialytic hypotension [250].

The major limitation of this study is that a cross-sectional study design restricts our ability to make temporal relationships, especially as there is a natural history involved with initial cardiac injury sensitizing patients to further hypotensive episodes and begetting further injury [86, 94]. Additionally, blood pressure was measured every 15 minutes throughout hemodialysis treatment; usage of a device that continuously measures blood pressure would be beneficial in future studies to better understand if 'area under the curve' or episodic severity and duration were most important in driving negative cardiac events [251, 252].

For the purposes of this study we chose to focus exclusively on a nadir blood pressure below 90mmHg due to the recent work showing the relationship between this definition of IDH and mortality, however there are many other factors to explore in the relationship between dialysis induced injury and the haemodynamic changes of dialysis, our study is also limited by the lack of cardiovascular outcome data and demographic details (such as smoking history and diabetes status) on the cohort which may be helpful in determining association between nadir BP and cardiac events. Future work could include gathering mortality data on this cohort and exploring the relationship between stunning, nadir BP and mortality. It would also be useful to stratify the cohort according to blood pressure and determine if any other relationship between stunning, global longitudinal strain and different levels of BP can be elucidated.

4.5 Conclusion

Our findings confirm that dialysis induced cardiac injury remains a significant complication of the haemodialysis procedure. They also confirm that patients who are unable to hold their blood pressure above 90mmHg during dialysis have reduced contractile function, which is likely to be contributory to the increased mortality recently described. While a nadir systolic blood pressure of less than 90mmHg is associated with increased mortality, our study demonstrates that dialysis induced cardiac injury still occurs at nadir blood pressures greater than this. There should be continued focus on developing

strategies to improve the cardiac tolerability of haemodialysis at an early stage before irreversible contractile dysfunction and the consequent inability to maintain blood pressure during dialysis occurs.

CHAPTER 5: RESULTS EXPLORING THE EFFECT OF REMOTE ISCHAEMIC PRECONDITIONING ON DIALYSIS INDUCED CARDIAC INJURY

5.1 Introduction

In 1986 Murry et al first described the phenomenon of ischaemic preconditioning – the application of transient, non-lethal ischaemic insults to a target organ in order to protect from the effects of a larger ischaemic insult [119]. The smaller insult results in organ protection from the effects of the ischaemia-reperfusion injury where the restoration of circulation to an area of tissue depleted of oxygen and nutrients results in oxidative stress and an inflammatory response that causes oxidative damage [253, 254]. A great deal of research effort has focused on determining the mechanistic processes underpinning this phenomenon [137] and trying to harness the effects of ischaemic preconditioning to protect organ systems from ischaemia. Remote ischaemic preconditioning (RIPC), where the transient, non-lethal episodes are applied with a blood pressure cuff to a limb, distant from the heart, has emerged as a potential method of applying a preconditioning stimulus in a clinical setting without the need for invasive procedures [253].

While there is a great deal of pre-clinical data demonstrating the cardio-protective possibilities of RIPC [123], translation into the clinical arena has proved challenging with a number of clinical trials producing inconclusive or negative results**.** In designing clinical trials to assess the efficacy of RIPC difficulties arise because of the unpredictability of an ischaemic insult. HD

represents an attractive model to study RIPC in the clinical arena due to it providing a predictable ischaemic insult and the prevention of dialysis induced cardiac injury being an important therapeutic target.

We performed a pilot study to investigate whether the application of a RIPC stimulus produced a significant and sustained reduction in HD induced cardiac injury suitable for refinement and development into a therapeutic option to provide long-term cardio-protection in HD patients.

5.2 Methods

This study was a prospective semi-blinded randomized controlled trial (ISRCTN No. 22392984). Patients who fulfilled the inclusion criteria and provided informed consent underwent a screening echocardiogram at the first dialysis session of the week to establish a baseline level of cardiac injury prior to entry into the study. Basic demographic information, including concomitant medication, was collected and recorded in the database. The study design is summarized in the figure below.

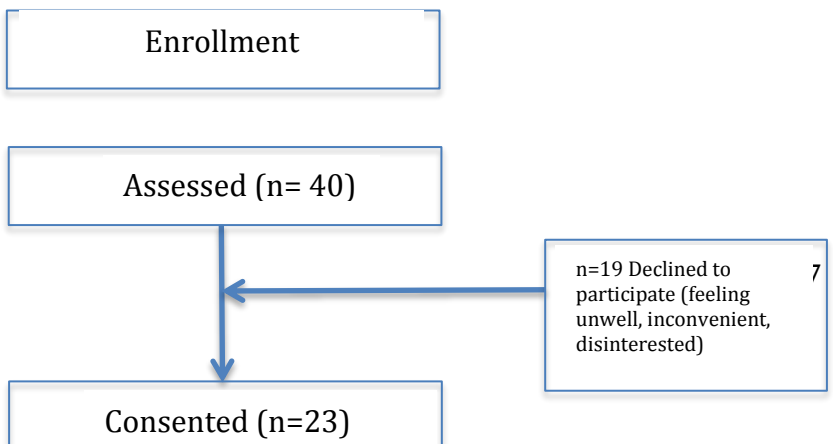

Figure 23: Schematic representation of study design

The purpose of the screening study was to confirm the presence of dialysis induced cardiac injury in the recruited patients. Following the screening study the patients were randomized on a 1:1 basis to receive either the full RIPC protocol (RIPC group) or a sham-RIPC protocol (control group). Randomisation was performed by an independent medical statistician with individual allocation in sealed envelopes opened just prior to the first study

visit. Patients were blinded to treatment group while the research team were unblinded due to the need to perform the intervention. The stimulus was administered by a member of the investigating team on the dialysis unit at the first study visit only and immediately prior to dialysis. The blood pressure cuff was applied to the patients leg and inflated to either 200mmHg (intervention group) or 40mmHg (control group). Each inflation lasted 5 minutes followed by 5 minutes of rest. The cuff was inflated a total of 4 times. The patients underwent further study at 48 hours and 28 days following administration of the RIPC stimulus. Study visits consisted of 2D echocardiography, blood tests and haemodynamic monitoring. Blood tests taken included full haematological and biochemical profile, CRP and pre and post dialysis Troponin T.

5.2.1 Primary endpoints and inclusion/exclusion criteria

The primary study endpoint was a change in the number of left ventricular segments showing evidence of dialysis induced cardiac injury. A broad and representative sample of current prevalent HD patients was enrolled in the study. Patients were eligible for inclusion if they were over 18 years of age and receiving three times weekly HD for more than 90 days. Exclusion criteria were exposure to HD for less than 90 days, NYHA Class IV heart failure and regular prescriptions for either Ciclosporin or ATP-sensitive potassium channel opening or blocking drugs.

Statistical analysis

Statistical analysis was performed using GraphPad Prism V.6 and SPSS V.21. The primary analysis was intention-to-treat (ITT) including all randomised participants. An alpha-error at 0.05 was judged to be significant. Normally distributed data were presented as means±SDs; non-normally distributed data were presented as medians (interquartile ranges). Unadjusted data were analyzed using paired sample t tests for normally distributed variables, Mann–Whitney U tests for nonparametric variables, and chi-squared or Fisher exact tests for categorical variables. Differences between groups were also analysed using one-way analysis of variance (ANOVA) in the case of normally distributed data with Bonefferoni's test for multiple comparisons.

Sample Size Estimation

Primary Outcome = **Difference in number of injured cardiac segments**

Significance level= 0.05 Power= 80%

We have previously demonstrated a difference in this variable in 9 patients using an identical protocol when comparing standard and biofeedback dialysis [115] (wherein the mean number of affected segments was 5.25±1.6 per patient). A sample size of 9 per group would appear to be sufficient to detect a difference of 30%. We planned to recruit up to 20 patients to allow for drop out from death, transplantation or withdrawal of consent.

5.3 Results

In total 21 patients entered the study (flow of patients summarised in figure 23). Both groups showed evidence of moderate functional cardiac impairment at baseline with ejection fractions and global longitudinal strains (GLS) below the normal range. The level of functional impairment was comparable in both groups with a mean ejection fraction in the control group of 40.38±12.15 compared to 42.25±10.67 in the intervention group. All study participants showed evidence of dialysis induced cardiac injury at screening by demonstrating two or more segments with a greater than 30% reduction in LS between pre and peak dialysis echocardiograms. One patient was unavailable for further study before completion of the 28-day study period, due to transplantation. All other patients completed the full study period.

The mean age of the intervention group was slightly lower 56.2±14.9 vs. 62.2±14.2 years and the mean dialysis vintage was slightly higher (36.2months±34.3 Vs. 27.3±27.7). All patients were adequately dialysed according to their most recent Kt/V. Four of the control group had a previous diagnosis of ischaemic heart disease compared to one of the RIPC group, while five of the RIPC group were diabetic compared to three of the control group. The intervention was well tolerated. Three patients in the RIPC group reported symptoms of paraesthesia and discomfort during initial inflation of the BP cuff but all declined to have the cuff deflated and all patients completed the full four cycles of RIPC. There were no symptoms reported in the control group.

	Overall Cohort	Control	Intervention
Age/years	58.9±14.6	62.2±14.3	56.2±15
Gender (m/f)	18/4	9/2	8/2
Presence of Fistula	95%	100	91%
Dialysis Vintage/months	32±31	27±28	46±34
Diabetes	43%	36%	50%
IHD	24%	30%	18%
Kt/V	1.33	1.29	1.36

Table 8: Summary of baseline characteristics of overall cohort (n=21) and control (n=11) and intervention (n=10) groups.

A comparison of the measured cardiovascular parameters in each group at each time point is shown in table 8. The RIPC group showed a consistent reduction in the severity of dialysis induced cardiac injury, throughout the duration of the study. At the initial screening visit the intervention group had a mean number of 4.87±1.3 segments showing a 30% reduction in LS compared to 3.33±1.5 in the control group. Over the course of the study there was a significant difference in the number of segments undergoing a 30% reduction in LS in the RIPC group at all visits in comparison to the screening visit with

the number of injured segments falling to 2.2±1.5 (p=0.004) at the intervention. The reduction was greatest between the screening visit and the 48-hour visit (4.87±1.3 Vs. 1.62±1.3 p=0.0006) with the smallest reduction at 28 days (2.7±1.8 p=0.02). In the control group there were no significant differences in the number of affected segments between visits. All comparisons were made using a paired sample t-test.

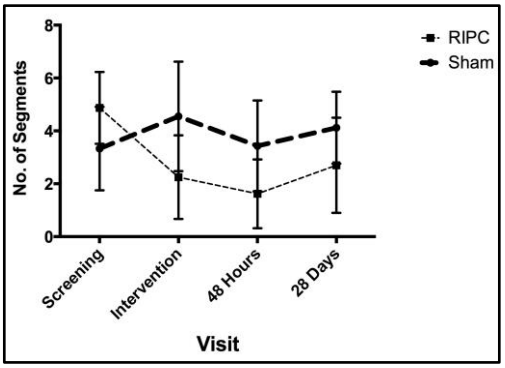

Figure 24: Number of segments showing a >30% reduction in longitudinal strain post dialysis in both groups and across all study visits

One-way ANOVA tests of repeated measures confirm a significant difference between the screening echocardiogram in the intervention group and at 28 days (F 6.44 p=0.0017) and a significant difference in mean injured segments between the two groups across the course of the study (4.23 p=0.007).

In both groups there was a reduction in global longitudinal strain (GLS) at peak dialysis during screening indicative of dialysis induced cardiac injury

causing a reduction in myocardial contractility. This reached significance in the RIPC group (-15.69%±-3 Vs. -13.04%±-3.4 p=0.04) although it did not reach significance in the control group (-14.23%±-4.1 Vs. -13.05%±-5 p=0.77). At all subsequent visits this difference in pre and post dialysis GLS in the RIPC group was abolished while in the control group there remained a consistent difference between pre and post dialysis GLS across all visits.

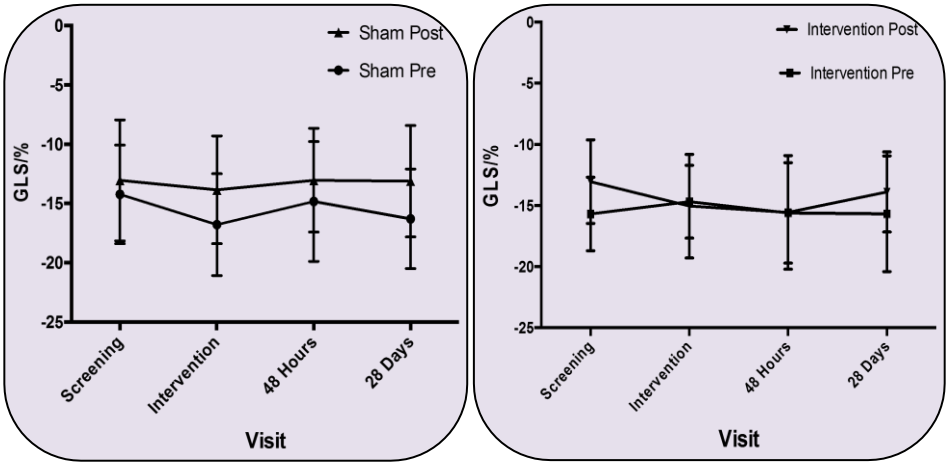

Figure 25: Comparison of GLS across each study visit in control group (left panel) and intervention group (right panel)

Ultrafiltration (UF) volumes and lower intra-dialytic blood pressure are the hallmarks of increased dialysis induced circulatory stress [87], and highly correlated to cardiac injury [93]. Randomisation, in this relatively small sample size did not result in equal exposure to these two factors between the groups. The RIPC group in fact experienced an even greater degree of circulatory stress (expected to be associated with *more severe* cardiac injury) than the control group. UF volumes and subsequent falls in intra-dialytic blood pressure

were in fact greater in the patients allocated to the RIPC group. The overall mean UF volume was 2.1L±0.9 vs. 1.3L±0.8 (p=0.0055). UF volumes held relatively constant across each study visit in both the intervention group (Screening 2.2L±0.6 Intervention 2.2±0.7 48 Hrs. 1.8±0.8 28 Days 2.04±1.1) and the control group (Scr 1.8L±0.5 Int 1.3±0.9 48 hrs 1.2±0.8 28 Days 1.7 ±0.5). The difference in BP between pre and post dialysis measurements (Δ SBP) was greater across all visits in the intervention group with the exception of the 48 hour visit (RIPC 12.4mmHg±21.2 Control 19.4±17.5). The greatest difference between the two groups occurred at the screening visit (23.8±27.4 Vs. 7.6±18.9). None of these differences reached significance.

	Sham Group				RIPC Group			
	Scr.	**Int.**	**48**	**28**	**Scr.**	**Int.**	**48**	**28**
Mean no. of Injured Segments	3.3±1.5	4.5±2.0	3.5±1.5	4.3±1.4	4.8±1.3	2.2±1.5**	1.6±1.3** *	2.7±1.8 *
Median no. of injured segments	3(2.1,4.5)	4(2.9,6. 1)	3(2.1,4.9)	4(3.2,5. 4)	4.5(3.7, 6)	2.5(0.9,3. 5)	1.5(0.5,2. 7)	2.5(1.3, 4)

Min-Max no. of injured segments	2-6	2-8	2-6	3-7	3-7	0-5	0-3	0-6
Pre-dialysis GLS (%)	-14.2±2.1	-16.8±4.6	-14.8±5.0	-18.0±5.2	-15.7±3.0	-14.8±3.1	-15.7±4.4	-15.6±4.5
Post-dialysis GLS (%)	-12.7±5.3	-13.8±4.5	-13.0±4.3	-12.1±3.9	-13.0±3.4	-15.0±4.2	-16.2±4.5	-13.8±3.0
Change in GLS (%)	2.9±2.9	3.3±4.4	1.3±2.7	3.4±3.6	2.8±2.0	-0.3±2.3	0.04±2.5	1.6±2.6
Pre-dialysis Ejection Fraction (%)	40.3±12.1	41.3±13.8	43.8±14.2	43.2±8.4	42.2±10.6	44.5±9.6	43.9±12.2	42.7±9.6
Pre-dialysis End Diastolic Volume (ml)	101.4±27.5	124±28	95.2±9.6	103.6±27.6	111.2±22.1	108.5±20.3	108±15.5	103.6±27.6
Pre-dialysis End Systolic Volume (ml)	62.2±23.1	73.3±13.8	53.4±13.1	62.4±33	62.6±21.4	61.3±22.5	61.7±18	60.2±22.
Ultrafiltration Volume (L)	1.9±0.49	1.3±0.9	1.21±0.8	1.7±0.5	2.2±0.61	2.2±0.7	1.8±0.8	2.0±1.0
Pre-dialysis Systolic BP (mmHg)	144.2±22.8	142.1±21.3	152.1±24.9	142.2±27.9	159.4±25.2	146.1±21.1	147.2±17.5	147±27.5

Table 9: Cardiovascular and haemodynamic characteristics across each study visit

Finally Troponin T levels were measured across the three study visits taking place following the intervention as pre-dialysis troponin t levels and the change in troponin t during dialysis have previously been shown to correlate with severity of dialysis induced cardiac injury. There were no significant differences between pre-dialysis troponin t levels across the three visits,

however interpretation is greatly hampered by the lack of a baseline troponin t value. The graphs below demonstrate the trend in pre-dialysis troponin t. It is likely that the sample size was too small to adequately show an effect on troponin t levels.

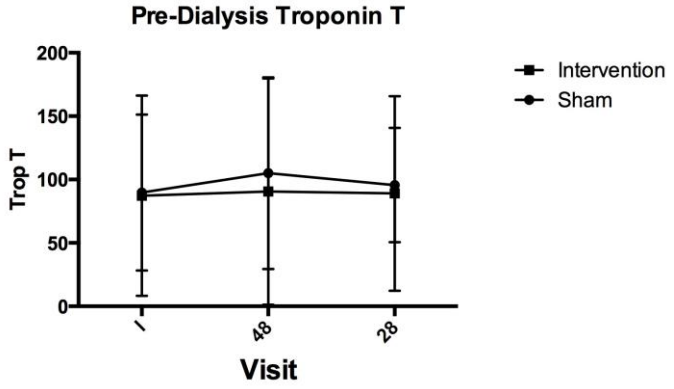

Figure 26: Trend in pre-dialysis Troponin T across each study visit

5.4 Discussion

This initial randomized controlled trial demonstrates that a single application of remote ischaemic preconditioning is both effective at reducing dialysis induced cardiac injury and is well tolerated in the dialysis population.

We have demonstrated that applying RIPC using a single ‘dose’ of 4 cycles of cuff inflation and deflation has cardio-protective effects that may last for up to 28 days. The number of segments undergoing a reduction in segmental longitudinal strain greater than 30% was reduced by more than half across each study visit as compared to the screening visit. In contrast the number of

affected segments in the control group remained constant throughout the study period. Given that outcome in dialysis patients is related to severity of cardiac injury [95] this finding is potentially very significant as any reduction in severity of dialysis induced cardiac injury may improve outcome. The intervention group also demonstrated a relative preservation of global longitudinal strain post dialysis compared to controls. Previous studies have demonstrated that a less negative GLS is associated with worse outcomes in haemodialysis patients and is an independent predictor of all-cause mortality [55, 255]. These findings are consistent with a recently reported small study of a similar RIPC intervention, which demonstrated some effect on cardiac biomarkers of myocardial injury, but did not include echocardiographic study [14].

The results are more impressive considering the relative haemodynamic stress the two groups were subject to. Ultrafiltration volume is a key factor associated with the presence of myocardial stunning. An ultrafiltration volume of 1.5L is associated with an odds ratio of 11.6 for the presence of dialysis induced cardiac injury [93]. The RIPC group consistently had higher inter-dialytic weight gains than the patients in the control group, and therefore had to be subjected to dialysis sessions with higher UF volumes and lower intra-dialytic blood pressure. No changes were made to the target weight of patients during this study, and this would appear to be an artifact of randomization in

small sample size. It would be expected that the patients who had been allocated to the RIPC group would be subject to more severe cardiac injury.

The dose of RIPC chosen for this study was four 5-minute cycles. The number of inflation-deflation cycles used in RIPC differs in the literature. In one of the first translational studies in humans, Loukogeorgakis et al. used three 5-minute cycles to demonstrate protection against endothelial ischaemia-reperfusion injury [124]. This protocol has been used in subsequent cardiac trials with mixed results [139, 141]. More recently a study of ninety-four patients undergoing ad-hoc PCI suggested a one-cycle protocol of RIPC applied to the upper arm reduced Troponin I release [168]. Further trials, which have been suggestive of a benefit in the setting of acute myocardial infarction, have used a four-cycle protocol [169, 170]. The dose, and need to repeat the application, of RIPC clearly now need to be refined in this particular target patient group.

Despite strong pre-clinical evidence showing a beneficial effect with pre-conditioning, clinical trials have not produced consistent definitive positive results. The most recent, and largest trials looking at the use of RIPC following cardiac surgery have been particularly disappointing showing no impact of RIPC application on any significant clinical outcomes [142, 143]. The circumstances in which to use this intervention is clearly more difficult to define and access in clinical use compared to deliberately induced experimental ischemia. The predictable nature of HD to deliver (usually) sub lethal

predictable cardiac ischaemia, provides an attractive model to study RIPC, and provides an opportunity to study dose-response relationships. Furthermore the dialysis population contains many elderly and diabetic patients; groups where the efficacy of conventional RIPC protocols has been questioned [167, 256].

In this study there was evidence of an effect lasting up to 28 days from a single application. It is well recognized that RIPC results in two distinct windows of protection lasting at least 48 hours [257]. It is not clear whether the effect of RIPC is truly elongated in dialysis patients and further work is needed to define the duration of effect in this patient group in order to guide the design of suitably robust clinical trials. There is a need for further refinement of the therapy, prior to large scale definitive testing.

5.4.1 Study limitations

This initial pilot study did have a number of limitations. The sample size was small and the level of dialysis induced cardiac injury was unequal at screening. Future work should be done on larger sample sizes to increase the chances of the groups being more equally matched. We were also unable to perform any adjustment for potential confounding variables due to the small sample size, illustrating a further benefit of studying a larger sample size. In addition to the above the authors cannot exclude the possibility that the repeated insults of the dialysis procedure itself results in a degree of preconditioning

and that the greater degree of dialysis induced cardiac injury in the intervention group resulted in more ‘natural’ preconditioning. However the reduction of injured segments across the entire study period suggests that even if we assume that some preconditioning has occurred there appears to be at least some additive effect from a single dose of preconditioning. Further work would be required to define the dose-response curve and explore the long-term consequences of preconditioning. A further limitation is the lack of clinical outcomes due to the short duration of the study meaning it is not possible to determine whether the biological effect demonstrated here has any long-term clinical consequence.

Coronary angiography results were not available for the majority of patients within the study, so the extent and relative contribution, of atherosclerotic coronary artery disease is unknown. While we cannot exclude the possibility of significant large vessel disease in these patients the predominant pathophysiological process in dialysis patients appears to be small vessel disease not amenable to angiographic intervention [88] and previous studies have demonstrated that HD is capable of generating significant cardiac ischaemia in patients with demonstrably normal coronary arteries (adults and children) [90, 92].

Our study only examined the effect of the intervention upon global longitudinal strain and therefore may not apply to circumferential and radial strain.

However there is evidence from studies of HD patients that longitudinal segmental strain is affected by dialysis and circumferential and radial are not [52]. Certainly reduced contractile function, identified by longitudinal strain, is highly predictive of cardiovascular mortality in HD patients [52]. It is possible that longitudinal strain is a more sensitive method of detecting dialysis-induced injury given the relative positioning of the longitudinal myocardial fibres, predominantly near the endocardium, with increased ischaemic vulnerability as the blood supply needs to traverse the characteristically hypertrophied myocardium.

5.5 Conclusion

This initial randomized controlled trial of RIPC in HD demonstrates that a single application of 4-cycle RIPC provides cardio-protection for up to 28 days following administration. Further studies are now needed to develop RIPC as a therapeutic option including studies of the dose response effect.

CHAPTER 6: RESULTS EXPLORING THE EFFECT OF LOWERING DIALYSATE SODIUM ON INTER-DIALYTIC WEIGHT GAIN AND DIALYSIS INDUCED CARDIAC INJURY

6.1 Introduction

One of the main determinants of dialysis induced cardiac injury is the level of circulatory stress the patient is subject to. High inter-dialytic weight gains (IDWG) drive large falls in systolic blood pressure and are independent determinants of cardiac injury [93]. Multiple studies have found high weight gains and the consequent higher ultrafiltration rates to be associated with increased mortality [201, 258]. Therefore the reduction of IDWG provides a plausible therapeutic target for attenuating the ischaemic insult and improving long-term outcomes.

Dialysate sodium levels have attracted interest as a potentially influential factor in the accumulation of fluid between dialysis sessions. Since the development of ultrafiltration via transmembrane pressure, and the ability to remove large amounts of fluid in a relatively short time span, patients have generally been dialysed at higher dialysate sodium levels to avoid disequilibrium and symptoms such as profound hypotension, cramps and headache [178]. This does not take into account an individuals serum sodium and there remains the possibility of creating a gradient across the dialysis membrane enabling transfer of sodium to the patient during dialysis [259]. This could have the effect of driving thirst and water retention and increasing IDWG.

While factors influencing salt and fluid accumulation are undoubtedly complex, previous studies have indicated that reduction in dialysate sodium results in

reduction of IDWG [185, 186, 207, 208] although the effect of this on dialysis induced cardiac injury has not previously been examined. To add to the uncertainty large population based studies have demonstrated higher mortality rates at facilities using a lower dialysate sodium prescription [209]. A more individualized approach to dialysate sodium prescription may be required.

We tested one approach to the dialysate sodium prescription by examining whether a controlled, stepwise reduction in dialysate sodium was safe and tolerable to patients, and whether it had any effect on IDWG or dialysis induced cardiac injury.

6.2 Methods

Ethical permission was granted by the Nottingham Research and Ethics Committee. Non-diabetic patients were recruited from three haemodialysis units. The inclusion criteria were patients over 18 receiving thrice-weekly dialysis for longer than 90 days and whose mean inter-dialytic weight gain was greater than 1.5kg. Potential participants were excluded if they were diabetic, had been exposed to haemodialysis for less than 90 days, if their mean inter-dialytic weight gain was lower than 1.5kg or if they had severe heart failure (NYHA grade IV).

Diabetic patients were excluded as previous work has suggested that the principal contributing factor to IDWG in this population is hyperglycaemia

rather than sodium retention [206]. The study design is summarized in Figure 27.

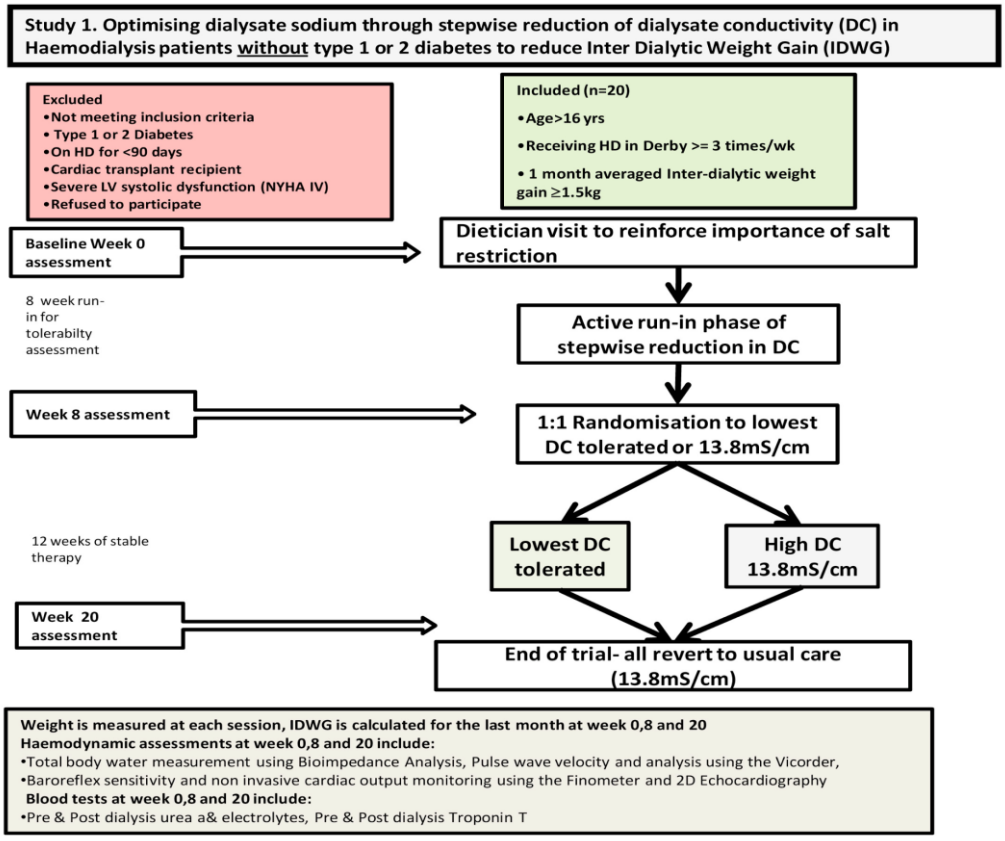

Figure 27: Study design schematic

In total 19 patients were recruited to take part in the study and 15 patients completed the full study period with 3 patients withdrawing from the study due to transplantation and 1 due to an intercurrent illness prior to administration of the intervention and therefore unrelated to the study. None of the enrolled patients were active in any other concurrent clinical trials.

6.2.1 Randomization

Following completion of the initial eight-week period, each patient was then randomized to either remain at their lowest tolerated sodium or to return to the standard treatment of 140mEq/L. Patients returning to standard treatment had their dialysate sodium increased over the course of 1-2 weeks (depending on how low they had tolerated reduction). Patients were dialysed on lowest tolerated versus standard treatment for a further 12 weeks following randomization. Randomisation was performed by and independent medical statistician who retained the master list. Patients were blinded to treatment allocation while investigators were aware due to the need to programme dialysis machines and monitor patients for adverse events. Allocation of

groups occurred via sealed envelopes opened following the completion of the eight-week study.

6.2.2 Study Visit

Prior to study entry, each patient was visited by a unit dietitian to reinforce standard dietary advice regarding salt and fluid intake. Following this participants underwent a baseline study visit consisting of pre-dialysis and peak stress echocardiography, pre and post dialysis full blood count, biochemical profile and troponin T and continuous haemodynamic monitoring using the NICOM Cheetah thoracic bioimpedance system. In addition they completed questionnaires regarding thirst symptoms [260] and dietary intake of sodium [261]. Demographic data was collected, and mean inter-dialytic weight gain over the previous month was calculated along with mean systolic and diastolic blood pressure. The patients underwent further study visits at 8 and 20 weeks.

6.2.3 Initial Eight-Week Period

Following the initial study visit all patients underwent a stepwise reduction in their dialysate sodium. Dialysate sodium was lowered by approximately 2mEq/L every two weeks for a total of eight weeks. Patients were carefully monitored by the nursing staff for adverse signs. The greatest reduction possible under this protocol was from 140mEq/L to 132mEq/L. If it appeared that the patient was no longer tolerating the sodium reduction the patient

reverted to the lowest sodium at which they were asymptomatic and continued at this level until the end of the eight-week period. This stepwise reduction regime has been previously demonstrated to be safe and well-tolerated [186]. Following this initial 8-week period the patients were then monitored for a further 12 weeks. These lengths of time were selected to allow for the 'lag phenomenon' which refers to the delayed effect in blood pressure reduction after removal of fluid overload that occurs from 4 weeks [262]. The further advantages of this study design include;

1. The longest length of assessment of the altered dialysate sodium concentration.
2. The avoidance of selecting a cohort of those who are inherently able to tolerate lower conductivities by randomisation.

6.2.4 Assessment of Thirst and Tolerability

As stated above, patients were carefully monitored by nursing staff and adverse symptoms recorded. As well as the routine blood pressure monitoring undertaken during dialysis sessions patients were asked on a weekly basis by nursing staff if they had symptoms of nausea, headache, cramping or dizziness. Patients also filled out the Dialysis Thirst Inventory questionnaire (Appendix 1) in order to undertake ongoing monitoring of symptoms of thirst.

Assessment of Dietary Sodium Intake

The impact of dietary sodium intake on blood pressure in the general population has been widely described and though the evidence in dialysis

patients is not as strong [263] it is an accepted part of practice to advise patients on the benefit of a low salt diet. Therefore in order to ensure that any impact of lowering dialysate sodium was not due to alterations in dietary intake (perhaps prompted by recruitment to the study) patients completed a food frequency questionnaire [261] to monitor any changes in dietary sodium. These questionnaires were completed at baseline, eight and twenty weeks.

Sample Size Calculation

Primary Outcome = **Difference in number of injured cardiac segments**

Significance level= 0.05 Power= 80%

We have previously demonstrated a difference in this variable in 9 patients using an identical protocol when comparing standard and biofeedback dialysis [115] (wherein the mean number of affected segments was 5.25±1.6 per patient). A sample size of 9 per group would appear to be sufficient to detect a difference of 30%. We planned to recruit up to 20 patients to allow for drop out from death, transplantation or withdrawal of consent.

Statistical Analysis

Statistical analysis was performed using GraphPad Prism V.6 and SPSS V.21. The primary analysis was intention-to-treat (ITT) including all randomised participants. An alpha-error at 0.05 was judged to be significant. Normally distributed data were presented as means±SDs; non-normally distributed data

were presented as medians (interquartile ranges). Unadjusted data were analyzed using paired sample t tests for normally distributed variables, Mann–Whitney U tests for nonparametric variables.

6.3 Results

Baseline characteristics of the two groups are summarized in Table 9. In total 19 patients entered the study, 10 of which were randomized to low dialysate sodium and 9 of which were randomized to the standard sodium prescription. Four patients withdrew for reasons unrelated to the study, 3 due to transplantation and 1 due to illness developing after the baseline study but prior to randomization. In total, 8 patients completed the high sodium protocol and 7 the lowest tolerated sodium protocol.

After eight weeks there was a significant difference in IDWG measured in Kg of 2.25±0.7 Vs. 1.61±0.7 (p=0.017) across the whole cohort. This was also true when using IDWG indexed to dry weight (IDWG%) where the mean weight gain was 2.89%±0.9 Vs. 2.07%±0.9 (p=0.012). On dividing the groups into the standard and low sodium dialysate groups, the trend of declining IDWG indexed to weight gain continued up until 20 weeks in the low sodium group while the standard sodium group saw an increase in IDWG between 8 and 20 weeks.

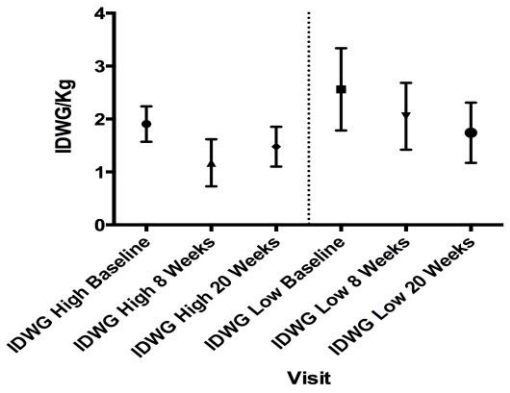

Figure 28: Trends in IDWG in both groups across each all study visits

Analysis of echocardiography showed no difference in the number of segments showing a 30% reduction in longitudinal strain at baseline between the high and low sodium groups 3.56±2 Vs. 3.78±2.3. At 8 weeks there was no difference in the number of affected segments between the high and low groups 3.57±0.9 Vs. 3.86±1.3. Figure 29 illustrates the number of affected segments in both groups across the study period. Overall there was no significant difference in either group between the beginning and end of the study period.

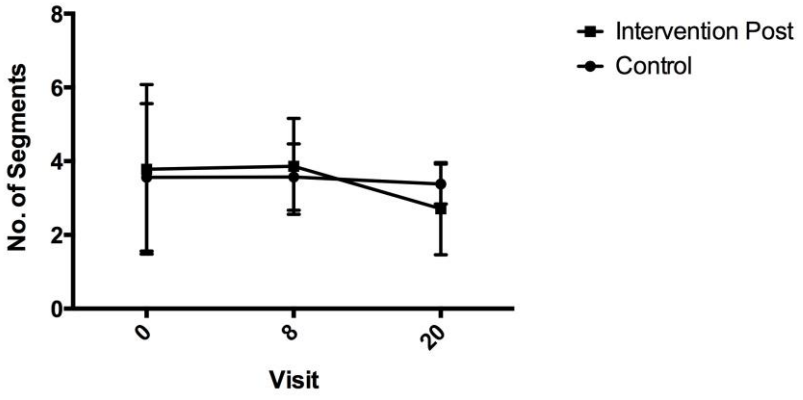

Figure 29: Trends in number of injured left ventricular segments in both groups across all study visits

At baseline the control group had a higher mean global longitudinal strain of -16.11±-2.92 versus -13.44±-3.79 in the intervention group. Trends in GLS are shown in figure 30. Over the course of the study there was little change in GLS in the control group at 8 weeks (-16.10±-2.66) or 20 weeks (-15.05±-2.62). In the intervention group there was little difference at 8 weeks (-14.18±2.74) but there was a non-significant trend towards an improved GLS at 20 weeks (-15.23±3.3).

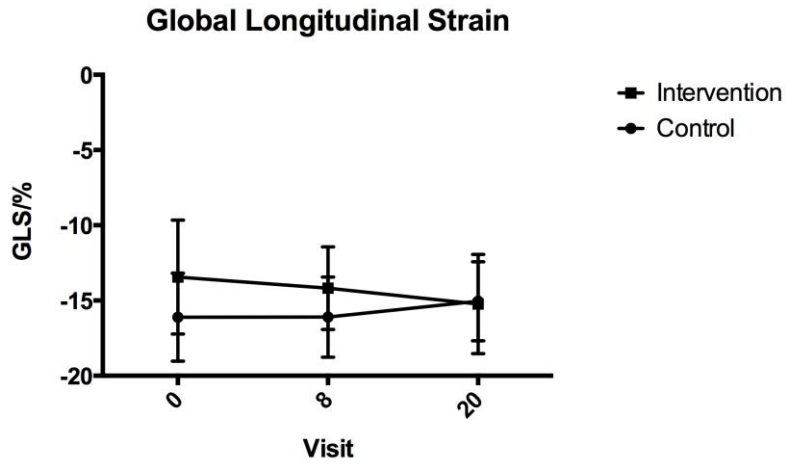

Figure 30: Trends in global longitudinal strain in both groups over each study visit

Over the initial eight weeks of sodium reduction 4.68% of treatments were complicated by symptoms ascribed to sodium reduction. The most common reason for stopping the stepwise reduction of sodium was a hypotensive episode during dialysis. The lowest sodium reduction achieved was 132 in two patients. The mean lowest tolerated dialysate sodium in the whole cohort was 134mEq/L±2.2 and 135mEq/L±1.9 in the group who were randomized to low

sodium. Following randomization there was no reported difference in the number of sessions complicated by hypotension between the two groups and no significant adverse events related to the study.

At the beginning of the study the mean pre-dialysis serum sodium level across the whole cohort was 138.6mEq/L±4.5 (137.1mEq/L±5.2 in the intervention group and 139.6mEq/L±3.1 in the control group). At eight weeks this had fallen slightly to 136mEq/L±4.5 across the cohort (135mEq/L±4.6 in the intervention group and 138mEq/L±4.3 in the control group). At twenty weeks the pre-dialysis serum sodium of the intervention group had continued to fall to 134mmol/L±6.7, while in the control group the pre-dialysis serum sodium had increased back to the pre-study value of 139mEq/L±2.1. These results are shown in figure 31.

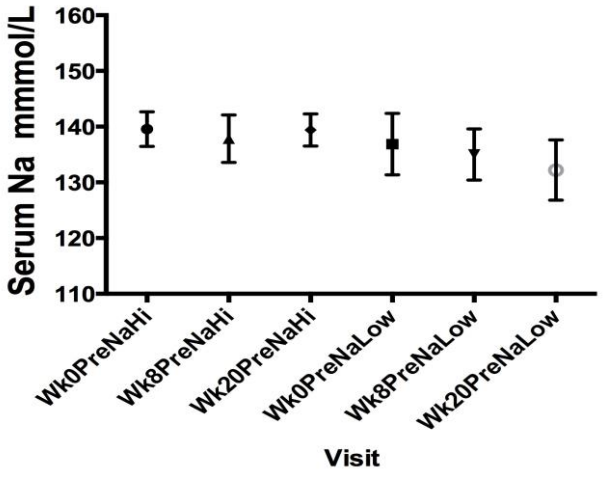

Figure 31: Trends in pre-dialysis serum sodium over each study visit

There were no significant changes in mean pre-dialysis systolic or diastolic blood pressure in either group between baseline and eight weeks. In the intervention group the mean pre-dialysis systolic BP at baseline was 142.9mmHg±57.1 compared with 140.7±21.3. Diastolic BP in this group was 75.1mmHg±21.4 at baseline compared with 76.4mmHg±20.6. In the control group there were also no significant differences in the diastolic blood pressure between eight and twenty weeks.

	Control Group	Intervention
Age	65.4±10.8	54.1±16.3
Gender (M/F)	5/9	9/1
Dialysis Vintage (months)	35.1±17.7	76.4±100.7
LVEF (%)	61.14±4.3	62.25±3.7
Pre Systolic BP (mmHg)	147.9±19.2	142.9±22.6
Post Systolic BP (mmHg)	136.4±23.6	135.2±19.8
Pre Dialysis GLS (%)	-16.11±2.92	-13.59±4.06
No. of Injured Segments	3.55±2.06	3.75±2.37
Pre Dialysis Na.	139.6±3.1	136.9±5.5
Pre Dialysis Trop T	54.1±44.6	55.4±20.7
IDWG (Kg)	1.90±0.33	2.56±0.77
IDWG%	2.46±0.56	3.29±1.01

Table 10: Baseline characteristics of both control and intervention groups at commencement of study

Assessment of symptoms of thirst was undertaken using the dialysis thirst inventory showed a baseline mean thirst score of 17.4±5.3 in the high sodium group vs. 17.3±5.7 in the low sodium group (where the score is out of a total of 35). At eight weeks there was a trend towards a reduced thirst score across the cohort with the standard sodium group having a mean score of 14.7±10.2 and the low group a mean of 15.8±5.8. At 20 weeks the standard sodium group demonstrated a trend back towards a higher thirst score with a mean of 17.4±11.6 versus 15±6.7. None of the differences between the groups reached statistical significance.

6.4 Discussion

This study demonstrates that stepwise reduction of dialysate sodium achieves a consistent reduction in IDWG without adverse effects either on haemodynamic tolerability of dialysis or on intra-dialytic cardiac function. To our knowledge this is the first study to examine the effect of reducing dialysate sodium on intra-dialytic cardiac tolerability.

The overall effect of dialysate sodium concentration on patient outcomes continues to be an area of considerable uncertainty. A number of studies have now reported decreased IDWG with dialysate sodium reduction protocols. A quality improvement study conducted by Mendoza et al in 2011 dialysed patients in 12-week periods at 140mEq/L, followed by 12 weeks at either 136mEq/L or 134mEq/L and then 12 weeks at 140mEq/L. During the lower

sodium phase there was a statistically significant fall in IDWG, IDWG% and systolic blood pressure [208]. Lambie et al showed that a similar stepwise reduction in dialysis conductivity (and thus dialysate sodium) was well tolerated and resulted in a reduction in IDWG [186]. In a further study, a more gradual reduction in conductivity over a period of 7 weeks (0.1mS/cm) again resulted in a reduction in IDWG [264].

The reduction in IDWG is likely to be related to a change in sodium gradient. Sodium is removed on dialysis via both diffusion and convection. The amount of sodium removed by diffusion will depend upon the difference between the dialysate and plasma sodium concentrations. If the sodium gradient is positive then the plasma sodium transfer could potentially increase resulting in an increase in thirst and IDWG [178]. A positive sodium gradient has been associated with increased IDWG and IDWG% [183, 264].

There were no statistically significant differences in dialysis induced cardiac injury although there was a modest trend towards improved cardiac tolerability. Previous studies have shown that IDWG is associated with higher mortality in dialysis patients [201] and it has been suggested that this is due to increased dialysis induced cardiac injury accelerating the development of fixed cardiac dysfunction and therefore heart failure. The development of regional wall motion abnormalities [93] correlates to areas found to have a reduction in myocardial blood flow during dialysis [90]. While the improvement in cardiac

tolerability in this study is only modest it is still of clinical significance. In their study, Assa et al demonstrated that survival is affected by the number of RWMA's that develop during dialysis [95]. This suggests that even a small reduction in affected segments may have a survival benefit.

There has been recent evidence that facility level reductions of dialysate sodium are associated with a greater risk of death [209]. In this paper the authors suggested that a contributing factor to this elevated mortality might be the use of lower sodium concentrations at a facility level causing greater haemodynamic instability and dialysis-induced cardiac injury. Our study suggests that this is not the case if the sodium is reduced in a gradual, stepwise process and allowances are made for the individual level of tolerance.

In our study there was no change in systolic blood pressure during the course of the study period. Previous trials looking at dialysate sodium reduction have demonstrated decreases in systolic blood pressure as an effect of the intervention [208, 264] although other studies have found sodium reduction alone insufficient [185]. The relative contribution of dietary sodium intake may be important. The benefits of reduced dietary sodium intake in the general population remain unclear [174]. While prescribing a low salt diet in dialysis patients continues to be a mainstay of the dialysis prescription the evidence for the benefits is not strong [263]. In this study patients received further

education regarding the need to limit salt intake in their diet and were interviewed at each study visit to ensure there was no change in their diet. Dietary sodium advice in combination with a dialysate sodium prescription that is specific to a particular patient appears to offer the best opportunity to affect a patient's IDWG.

In the intervention group there was a fall in serum sodium observed throughout the study. There is evidence that low plasma sodium is associated with a higher risk of death in dialysis patients [209, 211]. In these instances hyponatraemia was defined as below 135mEq/L. The intervention group cohort in our study had a mean serum sodium of 134mEq/L. While no causal association between hyponatraemia and mortality in dialysis patients has been clearly established caution in lowering dialysate sodium and careful monitoring of serum sodium levels should be maintained in these patients.

Our study did have some limitations. Firstly the loss of patients due to transplantation led to a smaller number of patients completing the full 20 weeks. This may have led to an underestimation of any improvement in cardiac tolerability seen by the end of the study. Bioimpedence measurements may have provided more detailed information regarding hydration status than indexed IDWG and can be of use as part of a comprehensive evaluation leading to estimation of target weight, however there is no consensus as yet to what an ideal hydration level would be for dialysis patients and where target

weight should be set. At present IDWG is the method for determining fluid removal in day-to-day clinical practice. A further limitation is a lack of clinical outcomes in this study. While there were no admissions due to episodes of heart failure and no deaths during the study period, data collection around anti-hypertensive use and measures of performance status such as exercise tolerance would have been a useful additions to the study.

6.5 Conclusion

The stepwise lowering of dialysate sodium levels according to individual tolerance is a safe and effective method to reduce inter-dialytic weight gain. It does not have any negative effects on cardiovascular tolerability and it may have resulted in a modest improvement in the patient's ability to tolerate the haemodynamic stresses of dialysis.

CHAPTER 7: RESULTS EXPLORING TISSUE ADVANCED GLYCATION END PRODUCT DEPOSITION AFTER TRANSPLANTATION

7.1 Introduction

Across the spectrum of patients with chronic kidney disease (CKD) the prevalence of cardiovascular disease (CVD) is higher than the general population. The risk is greatest in patients with end-stage renal disease (ESRD) who are receiving dialysis. Following a kidney transplant this risk reduces but remains elevated with the greatest excess morbidity and mortality seen amongst young patients [213]. It is increasingly appreciated that the cardiovascular risk associated with CKD and transplant recipients is disproportionate and cannot be ascribed solely to traditional factors such as smoking, dyslipidaemia and hypertension. The effect of a number of complex 'non-traditional' factors unique to transplant and CKD patients appear to be of paramount importance in understanding the pathology of CVD in renal patients and is increasingly the focus of research efforts.

Advanced glycation end-products (AGEs) are a group of compounds formed by the reaction of free amino groups on proteins, lipids and nucleic acids with reactive carbonyl groups on reducing sugars. AGEs accumulate as a consequence of several metabolic reactions including the Maillard reaction [265], are increased by oxidative and carbonyl stress and are regarded as a marker of cumulative metabolic stress. A further source of AGEs is food, particularly if cooked at high temperature. Reduced renal clearance of plasma AGEs in renal failure is probably an additional important factor in their accumulation [266].

AGEs are implicated in the progression of a number of chronic diseases. They are independent predictors of CVD and death in people with diabetes and heart failure [222, 267]. Several proposed mechanisms of cellular damage link AGE deposition to increased cardiovascular risk. AGE formation leads to changes in the structure and function of many proteins. For example, cross-linking of collagen proteins causes rigidity and loss of tissue elasticity [33]. The presence of AGE has been shown to correlate with increased pulse wave velocity (PWV), a marker of arterial stiffness related to diminished arterial elasticity in diabetics [268]. In patients with ESRD, pulse wave velocity is itself a predictor of morbidity and mortality [269].

Furthermore, AGEs deposit in the basement membranes of the vessel walls and bind to receptors for AGE (RAGE). Intracellular pathways are activated leading to the production of cytokines and the defective mediation of vasodilatation through impaired release of nitric oxide. This dysfunction of the endothelial layer compromises its pivotal role in vascular function. These structural and functional alterations lead to a loss of the protective capabilities of the endothelium and the triggering of a cascade that potentially leads to atherosclerosis [270]. AGEs also induce oxidative stress. Their attachment to sites on various cells results in the depletion of anti-oxidant defence mechanisms and the generation of free radicals [271, 272].

Direct measurement of AGEs in blood is technically demanding and previously a skin biopsy was required to determine tissue AGE accumulation. More recently, it has become possible to measure skin AGEs indirectly based on measurement of skin autofluorescence (SAF), a method that utilises the correlation between collagen linked fluorescence and AGE content observed in skin biopsies.

In ESRD, AGE accumulation is elevated in both diabetic and non-diabetic patients suggesting that hyperglycaemia alone does not account for the increase [273, 274] AGEs as measured by SAF have also been shown to be higher in patients on haemodialysis and peritoneal dialysis compared to non-uraemic control subjects [30]. SAF has been shown to be independently associated with higher cardiovascular morbidity and mortality in populations on dialysis [37]. In kidney transplant recipients, the risk factors associated with cardiovascular diseases (recipient age, impaired renal function, hypertension, the presence of diabetes, proteinuria, hyperlipidaemia, obesity, transplant ischaemia and use of calcineurin inhibitors) overlap with those associated with accumulation of AGEs [275].

SAF measured in kidney transplant recipients has been compared to healthy controls, but has not been compared to values in a CKD population with similar renal function [224]. Moreover, it is currently unknown if transplantation has any effect on SAF levels in patients with ESRD. We aimed to profile SAF values in a group of patients at least six months post kidney transplant and compare

the values obtained with those in patients with CKD stage 3 or on dialysis to gain insights into the effects of kidney transplantation on tissue AGE accumulation. We have also followed a small cohort of patients prospectively to investigate the effect of transplantation on SAF levels in dialysis patients.

7.2 Methods

7.2.1 Study Population

We studied 66 kidney transplant recipients at least six months post transplantation who were followed up at the Royal Derby Hospital. For comparison we used data from 1707 patients with CKD stage 3 who are part of a large community based cohort study [(276)] and 115 patients on dialysis (53 haemodialysis and 62 peritoneal dialysis) at the Royal Derby Hospital [(30)].

Appropriate ethical approval was obtained. Past medical history, including the presence of diabetes and ischaemic heart disease, previous dialysis modality, renal replacement therapy vintage and drug history were recorded. Haematological and complete biochemical profiles were time-averaged over six months for the transplant recipients.

Patients receiving chronic haemodialysis were dialysed thrice weekly for 4 hours with low-flux polysulphone dialysers, either 1.8 or 2.0 m^2 (LOPS 18/20; Braun Medical Ltd., Sheffield, UK). For all treatments, dialysate contained 138 mmol/L sodium, 1 mmol/L potassium, 1.25 mmol/L calcium, 0.5 mmol/L

magnesium, 32 mmol/L bicarbonate, 1 g/L glucose, and 3 mmol/L acetate. All treatments were of 4-hour duration, and circuit's anticoagulated with unfractionated heparin. Dialysate flow was 500 ml/min and conductivity was set at 13.6 mS/cm.

Patients receiving peritoneal dialysis (PD) utilised lactate/bicarbonate-buffered, 1.36 or 3.86% glucose-containing solutions (Physioneal®, Baxter), Extraneal® (7.5% icodextrin, Baxter) or Nutrineal® (1.1% amino acids, Baxter) as prescribed for routine clinical care. PD glucose exposure was generated from historical PD prescription data and review of all historical Baxter home delivery records.

7.2.2 Skin Autofluorescence

Skin autofluorescence was measured in arbitrary units (AU) using a previously validated cutaneous AF device that utilises a UV source at a specific range of wavelengths (AGE Reader®, DiagnOptics, Groningen, The Netherlands). The AF reader illuminates a patch of skin of approximately 1cm^2, guarded against surrounding light, with an excitation light source between 300 to 420 nm (peak excitation approximately 350nm). Only light from the skin is measured with a spectrometer in the 300- to 600 nm range, using 200µm glass fibre [(28)]. The non-dominant forearm is placed on the device away from any areas of bruising or increased skin pigmentation [(277)]. All readings are taken within 1cm of each other if possible and at room temperature. The AF reader is connected to a

personal computer and software analyzes the degree of autofluorescence and compares it to previously obtained normal values for each age group [28]. Measurements were obtained at a routine outpatient appointment. Three readings were recorded and the average calculated.

7.2.3 Comparison

Comparison of SAF values was made between the three groups: kidney transplant recipients (n=66), patients with CKD stage 3 (n=1707) and patients on haemodialysis (n=53) or peritoneal dialysis (n=62). Specific comparison was then made between the transplant cohort and a CKD stage 3 comparator group extracted from the larger CKD cohort and matched, on a two to one basis, for age, presence of diabetes and estimated glomerular filtration rate (eGFR).

7.2.4 Statistical Analysis

Group data are presented as mean ± SD unless otherwise stated. All data were tested for normality using QQ plot. Analysis was performed using SPSS v20.0.1 (SPSS Inc, Chicago, IL). Categorical data were compared using Chi-square test, continuous data using paired or unpaired Students t-test or one-way ANOVA with Tukey's correction as appropriate. Multivariable linear regression analysis, using the forward stepwise method, was used to identify independent determinants of higher SAF.

7.3 Results

Comparison of demographic data between the groups is shown in table 10. All groups were comparable in terms of ethnicity (predominantly Caucasian due to technical limitations of measuring skin autofluorescence in individuals with darker skin pigmentation). The haemodialysis cohort was older and had a lower proportion of patients with diabetes.

	Transplant	CKD	Dialysis	
			HD	PD
Mean Age (Yrs.)	53.3±12.4	58.9±9.8	66.2±13.5	59.7±15.7
Male (%)	54.2	52.3	67.9	76.9
RRT Vintage (months)	143±103	———	53±33.1	24.5±10.0
Transplant Age (months)	93.6	———	———	———
Diabetes (%)	25.7	24.7	13	28.8
Mean eGFR (mL/min)	45.9±12.7	47.5±10.3	———	———

Table 11: Participant Demographic Details (n=66). Data are mean ± SD. CKD, chronic kidney disease; HD, haemodialysis; PD peritoneal dialysis; eGFR, estimated glomerular filtration rate.

The mean skin autofluorescence values are shown in figure 32. Mean SAF in transplant recipients (2.81±0.64 arbitrary units or AU) was significantly lower than in patients on haemodialysis (3.73±0.88AU) and peritoneal dialysis (3.57±0.75AU) ($p<0.001$) but was not different from that observed in CKD stage 3 (2.79±0.66AU; $p=0.42$) although it must be noted that adjustment for age was not performed while making these comparisons.

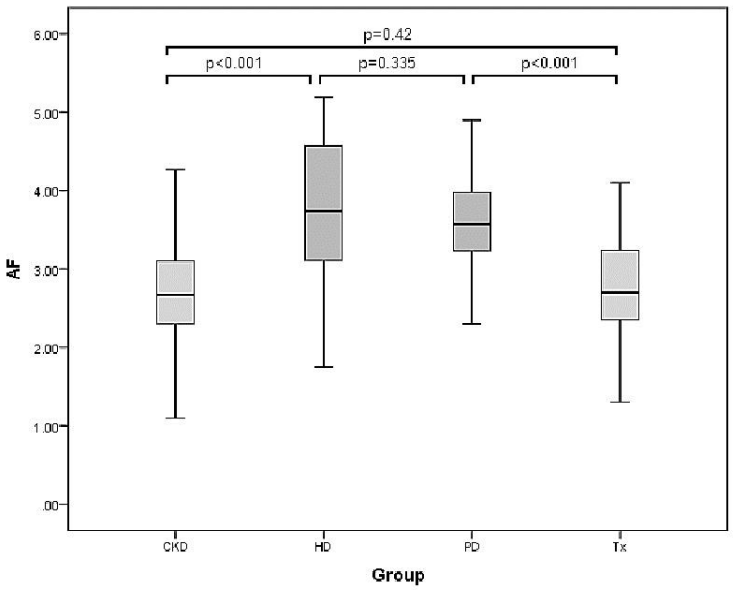

Figure 32: Comparison of mean skin autofluorescence in different patient groups between CKD stage 3, HD, PD and Tx patients

The transplant cohort was also compared to 106 patients with CKD stage 3 matched for age, presence of diabetes and eGFR. There was again no difference in mean SAF (2.81±0.64AU versus 2.79 ±0.64AU; p=0.56).

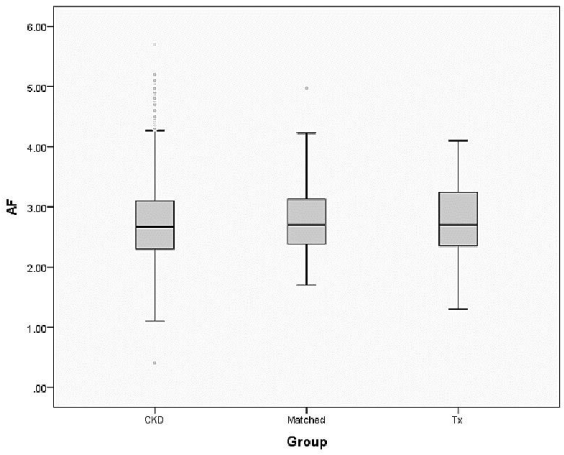

Figure 33: Comparison of mean skin autoflourescence between CKD stage 3, matched CKD stage 3 controls and Tx patients

A small cohort of patients (n=6) had SAF values recorded during their time on dialysis and after kidney transplant. All patients except one showed a decline in SAF after transplantation as shown in figure 34. The mean SAF was significantly lower after transplantation, dropping from 4.0 to 3.4 at a mean time 20 months post transplant.

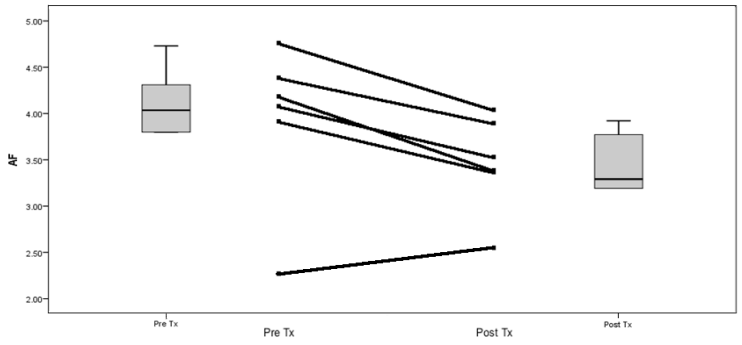

Figure 34: Change in mean Skin autofluorescence in dialysis patients post transplantation. Each line represents an individual patient on dialysis (Pre Tx) and after transplantation (Post Tx). AF, autofluorescence (skin); Tx, transplant.

The results of univariate analysis examining associations between potentially important variables and SAF in transplant recipients are shown in table 11. The most significant correlation with SAF in the transplant group was patient age (r=0.316 p=0.010). The presence of diabetes also correlated with SAF just reaching statistical significance (r=0.239 p=0.05). In PD patients there was a positive correlation with chronological age and dialysis vintage (r=0.4 p=0.004 and r=0.34 p=0.1 respectively), while in HD patients there was again

a positive correlation with chronological age (r=0.46 p=0.0001). Estimated GFR and renal replacement therapy vintage did not correlate significantly. When subject to multivariable analysis the only independent determinant of SAF in the transplant cohort was chronological age (B=0.020, β=0.364, p=0.007) although the small sample size and small number of positive correlations should be noted in interpretation of the multivariable.

	r	p value
Age	**0.32**	**p=0.01**
Gender	**-0.04**	**p=0.72**
Diabetes	**0.24**	**p=0.05**
NODAT	**0.04**	**p=0.75**
RRT Vintage	**0.05**	**p=0.59**
Tx Vintage	**-0.13**	**p=0.30**

Table 11: Correlations between potentially important variables and SAF in transplant recipients (n=66)

7.4 Discussion

Our results across four groups (transplant, haemodialysis, peritoneal dialysis and CKD stage 3 patients) confirm that SAF is elevated in patients with chronic kidney diseases compared to previously obtained normal values. The highest level is seen in patients on dialysis who demonstrate SAF levels that are comparable with diabetics suffering from macro- and microvascular complications [274, 278, 279]. We know from previous studies that even the

relatively low SAF levels in CKD patients are raised compared to general population [276, 280]. We observed SAF values in kidney transplant recipients that were similar to a CKD comparator group. Interestingly, the values observed in transplant recipients were significantly lower than those seen in patients on dialysis and our data from a small prospective cohort of patients suggest that SAF values decrease after kidney transplantation.

To our knowledge this is the first time a direct comparison of SAF values has been made between a group of transplant recipients and patients with CKD well matched for chronological age and renal function. SAF levels have previously been shown to be lower in transplant patients as compared to dialysis patients [224] and they have also been shown to be useful in predicting graft dysfunction and renal outcomes [223, 281]. In a population with CKD stage 3, SAF was also elevated and independently associated with multiple CV and renal risk factors [276].

The lower SAF values seen in transplant recipients suggest that following transplantation AGE accumulation regresses and returns to levels seen in earlier stages of CKD. The results from the small number of participants who had pre and post transplant values imply that this process begins relatively soon after transplantation (mean age of transplant was 20 months) although the numbers were too small to draw firm conclusions. It has been reported that the advanced glycation of proteins occurs slowly in normal aging, and causes cross-linking of collagen [33]. The process is accelerated in diabetes

and possibly in renal failure, but relatively little is known about how AGEs are removed from tissue once deposited. We are not aware of any previous reports of serial measurements SAF in patients following a fall in SAF for any reason. It is not known how fast SAF or tissue deposition of AGEs would fall after transplantation as there are no data on SAF level measured shortly after transplantation, but we are surprised by the relatively short period needed for SAF to return to that of matched CKD controls. Interestingly, the only patient in our cohort whose SAF did not fall post transplantation was measured at 6 month post transplant compared to a mean of 20 months in the whole group.

In transplant recipients, rates of cardiovascular morbidity and mortality decline to levels similar to those seen in CKD, although they remain proportionally higher than the general population [213]. While the reason for this improvement is not fully understood, the removal of the metabolic and haemodynamic stress involved in the dialysis process is likely to be a significant factor. We speculate that the lower SAF levels seen in transplant recipients are a result of this reduced metabolic stress, and offer a partial explanation for the lower cardiovascular event rate.

Our results show that the only independent determinant of SAF levels in transplant patients is chronological age. It has previously been reported that in general population and patients with CKD stage 3 SAF increases with ageing [276, 280]. There was a weaker correlation between diabetes and SAF

levels similar to that seen in the CKD population [276]. Previous work published by our group reported a correlation between dialysis vintage and SAF level in PD patients still on dialysis [276] but this effect appears to have been abolished by kidney transplantation. There was no correlation between eGFR and SAF level in the transplant group, unlike that reported in previous work performed in CKD [224] and there was no effect from haematological or biochemical parameters or the choice of immunosuppression.

Our study is limited by the relatively small sample size in the transplant group and the small sample with prospective pre- and post transplant SAF measurements. Our transplant recipients were studied at different time point post transplantation. We did not find any significant association of SAF with transplant age or renal function, which might be attributable to our small sample size. We also did not measure other variables that may potentially play a role in AGE accumulation such as immunosuppresive therapy burden, episodes of rejection following transplantation and elevated systolic blood pressure.

7.5 Conclusion

In summary, structural and functional changes caused by deposition of AGEs are increasingly recognised as potential contributors to the pathogenesis of cardiovascular disease in CKD. The highest SAF levels are seen in patients on dialysis who are subject to the greatest metabolic stress. Following a

kidney transplant, SAF levels reduce to levels seen in CKD with similar renal function and this may be a contributing factor in the concurrent reduction in cardiovascular morbidity and mortality. Further work is needed to fully elucidate the natural history of AGE levels as measured by SAF in the transplant population. The relationship between metabolism and vascular structure and its contribution to cardiovascular risk in CKD is of paramount importance and further understanding may identify potential strategies to modify risk in this vulnerable patient group.

CHAPTER 8: CONCLUSION

8.1 Summary of Results

The advent of HD for treatment of ESRD is a historic medical achievement that has extended the lives of millions of people in the last half century. However, as dialysis continues to expand worldwide and the social and economic costs of ESRD, there remain numerous challenges confronting health care providers. HD patients are burdened by disproportionately elevated levels of cardiovascular morbidity and mortality. In the general population the strategies to address CVD focus on risk factors such as hypertension and elevated lipids. In the dialysis population these strategies have so far proven ineffective. This is partly due to a difference in the pathophysiology of vascular disease in this population where a multitude of complex factors such as vascular calcification, endothelial dysfunction and autonomic dysfunction affect the ability of the vasculature to respond to conditions of stress.

The concept of dialysis induced end-organ damage has only recently been appreciated. It has long been noted anecdotally that on commencing dialysis many patients suffer a sharp decline in functional status. Acceptance that the repeated circulatory challenge of dialysis plays a role in accelerating end-organ damage is now widespread. Much of the current research has focused

specifically on the effect on the heart due to the high rates of heart failure and sudden cardiac death. However it should be noted that injury is not likely to be confined to one organ system and other vulnerable vascular beds such as those supplying the brain, kidney and gut are also affected. Recognition of the potential detrimental effects of dialysis has renewed focus on the dialysis process itself and if any alterations in procedure can mitigate its effect.

There is still much to be learnt regarding the causes and effects of dialysis induced cardiac injury. Earlier studies suggested that falls in systolic blood pressure were an important determining factor in its development. We proposed a study to further define potential 'safe' levels of IDH. Recent work in which only a fall in nadir blood pressure to below 90mmHg was found to be associated with mortality gave our study extra urgency as it may have led physicians to believe that patients who did not exhibit a fall of this nature were 'safe' from dialysis induced injury. This led us to further explore the relationship between a nadir of 90mmHg and cardiac injury. From our findings there was no link between a nadir BP of less than 90mmHg and a greater degree of injury, instead it would seem that injury still occurs at levels of blood pressure much higher than this. Our findings suggest that the greater degree of mortality in the nadir90 group may be because they have poorer cardiac function. These patients may have already developed the fixed contractile dysfunction caused by cumulative ischaemic insults. This study also provides further evidence for appreciating the importance of biological processes as a

continuum and that in applying thresholds of care (particularly on large numbers of individuals) care needs to be taken that they fit the biological basis of disease.

Knowing that the consequences of ischaemia-reperfusion include myocardial dysfunction led us to investigate interventions that may reduce the impact of this injury and thus reduce cardiac injury associated with dialysis. We tested the technique of remote ischaemic preconditioning which has been extremely successful in experimental models in reducing injury due to ischaemia but has proven difficult to translate effectively into clinical situations. In our small clinical trial, patients receiving a single application of RIPC showed a reduced level of cardiac injury. This effect persisted for up to 28 days despite the intervention group having comparatively higher ultrafiltration requirements and larger falls in systolic blood pressure, factors which would suggest that they were perhaps vulnerable to more severe injury. This small pilot study offers promise that RIPC could be used therapeutically in this population.

We investigated the possibility that the dialysis process could be modified to ease the haemodynamic stress of dialysis and therefore reduce cardiac work. Given that high inter-dialytic weight gains have been strongly associated with the development of dialysis induced cardiac injury we targeted an intervention that previous work suggested was an effective method of reducing these fluid gains. Reduction of dialysate sodium in a stepwise fashion was well tolerated

and safe in this study cohort. Furthermore it significantly reduced IDWG in line with findings in previous studies while having no adverse intra-dialytic cardiac effects. By the end of the twenty-week study there may even have been a modest improvement in the degree of cardiac injury in those patients randomized to their lowest tolerated sodium level.

Finally, we investigated the evolution of one of the non-classical risk factors for CVD post renal transplantation. The aim was to determine the potential plasticity of some of the changes to the vasculature that occur during CKD and attempt to gain insight into the relative contribution of dialysis treatment to metabolic stress. We found that patients who had undergone renal transplantation has SAF readings comparable to those of matched cohort of patients with CKD stage 3 and that these readings were much less than patients on HD. We also found in the small number of patients for whom we had pre and post transplant readings, SAF (and therefore presumably AGE deposition) declined quickly post transplantation suggesting that AGE deposition begins declining relatively soon after transplantation and thus it's effect on the vasculature is not entirely fixed and is reversible.

In conclusion, HD induced circulatory stress is a major contributor to end-organ damage and poor outcomes in the dialysis population. While falls in UF rate and systolic blood pressure may be significant determining factors, dialysis induced injury can occur at any level of systolic blood pressure and it

is unlikely that there is one safe threshold above which any individual patient might be considered ‘safe’. We found that the application of a single dose of RIPC reduced the level of dialysis-induced injury for up to 28 days. This is probably due to the effect it has on mitigating the ischaemic reperfusion injury. We also found that stepwise reduction in the concentration of dialysate sodium was well tolerated and effective in reducing IDWG. This intervention was not associated with any adverse cardiac effects during dialysis and may have led to a small improvement in cardiac tolerability. Lastly, we found that at least one of the risk factors for vascular disease in CKD patients is at least in part reversible with deposition of tissue AGE reduced in transplant patients as compared to those on dialysis possibly due to both restoration of normal physiological function and removal of the metabolic stress of dialysis and the uraemic state. This finding suggests that some of the changes that occur in the vasculature are ‘plastic’ and thus can be mitigated with via transplantation or from a better understanding of the processes that cause them.

8.2 Limitations

Each study has some limitations that should be discussed in turn. In study one the focus was exclusively on the relationship between systolic blood pressure below 90mmHg and cardiac tolerability of dialysis. The main limitation was the cross-sectional nature of the study meaning it was not possible to draw long-

term conclusions. The study did not consider other definitions of IDH and stratify the cohort accordingly, nor did it examine mortality data.

The study of RIPC was limited by the relatively small sample size. There was also a lack of information of the patients current coronary circulation with none of them having undergone recent coronary angiography. The randomization resulted in an uneven distribution amongst the groups in the levels of dialysis induced cardiac injury and UF rate. A larger sample size may have smoothed out the differences in groups following randomization and allowed a more direct comparison. Furthermore, there remains a possibility that patients suffering a dialysis induced cardiac injury already undergo an element of preconditioning from this insult that is difficult to quantify but may itself be cardioprotective. The effect seen may therefore be additive rather than solely as a result of the preconditioning stimulus.

The study looking at the stepwise reduction of dialysate sodium was also limited by a small sample size that was further reduced by drop out due to transplantation. Thus it became underpowered to determine changes in number of injured segments. While there is a consistent and clear reduction in IDWG seen there remains controversy about the best way to determine a patients dry weight and thus the study would possibly have benefited from bioimpedence measurements to determine dry weight and change in intra and extracellular fluid volume. Estimation of the sodium mass balance via online

measurement would also have been helpful, unfortunately this technology was unavailable on the machines being used to dialyse the majority of patients in this study. Despite the absence of bioimpedence and mass balance measurement it should be noted that the fall in inter-dialytic weight gain as indexed to dry weight is very much in line with many previous studies in this population.

Finally, the study of AGE deposition in transplant recipients was limited by a small sample size particularly in patients who had a pre and post transplant reading. While it was of interest seeing that transplant recipients had SAF reading comparable to CKD stage 3 (which is in line with their cardiovascular mortality rates) it would have been helpful to have a study formally examining the evolution of SAF reading before and after transplant.

8.3 Future Work

It should be noted that all of these studies should be considered as initial ‘scoping’ studies designed to detect whether or not the individual areas of interest warranted further more definitive study. Firstly, it is necessary to more fully elucidate the factors that lead to dialysis induced cardiac injury. A further exploration of the relationship between blood pressure falls, cardiac injury and mortality would be useful as would determining further risk factors for development of injury. The relationship between UF volume and rate, blood pressure fall and cardiac injury is not definitive and it may be that other yet to

be determined factors associated with extra-corporeal therapy are of equal importance. Examining whether there is a connection between dialysis induced injury and severity of coronary artery disease is also necessary as it will assist the nephrology community in the development of adequate therapeutic options.

In terms of the interventions tested a study expanding on the potential of RIPC is the next stage in developing it as a viable therapeutic option. A formal dose response study is needed to ascertain fully the length of any effect and whether more frequent applications of RIPC result in an even more potent effect.

A longer-term prospective study looking at the effect of an individualized reduction of dialysate sodium is indicated. The study should look at whether this reduction in IDWG can be sustained over a longer period and whether this has only long-term effect on cardiac structure and function. A number of studies looking at the effect of dialysate sodium concentration are ongoing around the world at time of writing. One study is underway with two groups randomized to a blanket high and low sodium level [(282)]. This study will use MRI to determine if there is any long-term effect on left ventricular remodeling due to the reduced weight gains associated with low dialysate sodium. While this study will undoubtedly provide useful information on the long-term effects of sodium reduction and confirm whether the positive effects on IDWG can be

sustained, the evidence regarding adverse outcomes in facilities that reduce dialysate sodium across their whole patient group indicates that a more individualized approach is sensible, particularly given that we have demonstrated that this method of reducing dialysate sodium is safe and well tolerated and has no adverse cardiac consequences in the short term.

Dialysis based interventional clinical studies are often rather difficult to complete and the generalizability of results limited by patient selection bias. However the sodium intervention in particular is well suited to evaluation in registry based cluster randomized controlled trials. In these trials groups of subjects (i.e. whole dialysis facilities) are randomized rather than individual patients. Outcomes can then be measured via analysis of the registry data that is collected as standard in most dialysis units. The advantages of the cluster-randomized trial are its allowance for complete coverage of a dialysis unit including the inclusion of ‘marginal’ patients that are often excluded from studies (i.e. frail patients, patients with dementia, those whose first language is not English). This increases the generalizability of the results. These studies are also cost effective partly due to the simplified nature of data collection. Dialysis facilities provide an almost perfect forum for a cluster-based trial as the population is unlikely to be lost to follow up and data is already continuously collected in this patient population.

Finally, the effect of transplantation on the cardiovascular system needs further robust evaluation. There are numerous avenues for research in this area including the use of sophisticated imaging techniques to quantify structural and functional cardiac remodeling following transplant and long term studies following the progression (or regression) of numerous non-classical risk factors including pulse wave velocity and skin autofluorescence. These studies promise valuable insights into the not just the consequences of transplantation but also the whole evolution of cardiovascular disease in CKD and the factors which influence it.

LIST OF ABBREVIATIONS

KDOQI	
CKD	Chronic Kidney Disease
eGFR	Estimated Glomerular Filtration Rate
RRT	Renal Replacement Therapy

ESRD	End Stage Renal Disease
HD	Haemodialysis
PD	Peritoneal Dialysis
FFR	Fractional Flow Rate
SAF	Skin Autofluorescence
AGE	Advanced Glycation End Products
LVH	Left Ventricular Hypertrophy
LAVI	Left Atrial Volume Index
CMR	Cardiac Magnetic Resonance Imaging
BNP	Brain Naturitic Peptide
RWMA	Regional Wall Motion Abnormality
UF	Ultrafiltration
IDH	Intra-dialytic Hypotension
IDWG	Inter-dialytic Weight Gain
MI	Myocardial Infarction
IPC	Ischaemic Pre-conditioning
RIPC	Remote Ischaemic Pre-conditioning
NO	Nitrous Oxide
MPTP	Mitochondrial Permeability Transmission Pore
AKI	Acute Kidney Injury
BP	Blood Pressure
IMB	Ionic Mass Balance
VC	Vascular Calcification

PWV	Pulse Wave Velocity
baPWV	Brachial-Ankle Pulse Wave Velocity
NICOM	Non-Invasive Cardiac Output Monitoring
MAP	Mean Arterial Pressure
GLS	Global Longitudinal Strain
LS	Longitudinal Strain
AV	Arteriovenous

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APPENDIX I: THE DIALYSIS THIRST INVENTORY

Thirst is a problem for me

Never ☐ Almost Never ☐ Occasionally ☐ Fairly Often ☐ Often ☐

Score_____

I am thirsty during the day

Never ☐ Almost Never ☐ Occasionally ☐ Fairly Often ☐ Often ☐

Score_____

I am thirsty during the night

Never ☐ Almost Never ☐ Occasionally ☐ Fairly Often ☐ Often ☐

Score_____

My social life is influenced because of my thirst feelings

Never ☐ Almost Never ☐ Occasionally ☐ Fairly Often ☐ Often ☐

Score_____

I am thirsty before dialysis

Never ☐ Almost Never ☐ Occasionally ☐ Fairly Often ☐ Often ☐

Score_____

I am thirsty during dialysis

Never ☐ Almost Never ☐ Occasionally ☐ Fairly Often ☐ Often ☐

Score_____

I am thirsty after dialysis

Never ☐ Almost Never ☐ Occasionally ☐ Fairly Often ☐ Often ☐

Score_____

Total Score_____(7-35)

APPENDIX II: THE FOOD FREQUENCY QUESTIONNAIRE

Please circle the appropriate response:

1. How often do you add salt to your food at the table?

Never Once a week or less 2-3 times per week

Once per day More than once per day

2. How often do you add salt in cooking?

Never Once a week or less 2-3 times per week

Once per day More than once per day

3. How often do you eat the following foods?

a) Salted snacks, e.g. crisps, salted nuts, Bombay Mix, salted crackers

Never Once a week or less 2-3 times per week

Once per day More than once per day

b) Hard cheese, e.g. Cheddar, Red Leicester, Cheshire, Edam

Never Once a week or less 2-3 times per week

Once per day More than once per day

c) Processed Cheese, e.g. Cheese Slices, Cheese Spreads such as Dairylea, Laughing Cow

Never Once a week or less 2-3 times per week

Once per day More than once per day

d) Tinned, packet or fresh soup (not home-made)

Never Once a week or less 2-3 times per week

Once per day More than once per day

e) Ready-prepared meals, e.g. fresh or frozen meals such as Lasagne, Sausage and Mash, Stew and Dumplings, Fish Pie, Curry and Rice

Never Once a week or less 2-3 times per week

Once per day More than once per day

f) Tinned or processed meats, e.g. ham, bacon, sausage, corned beef

Never Once a week or less 2-3 times per week

Once per day More than once per day

g) Tinned fish in brine

Never Once a week or less 2-3 times per week

Once per day More than once per day

h) Smoked fish

Never Once a week or less 2-3 times per week

Once per day More than once per day

i) Tinned beans or vegetables in salt water

Never Once a week or less 2-3 times per week

Once per day More than once per day

j) Stock cubes or ready-made cooking sauce, e.g. jar or packet such as pasta sauce, Indian, chilli con carne, sweet and sour, white wine sauce

Never Once a week or less 2-3 times per week

Once per day More than once per day

k) Breakfast cereals

Never Once a week or less 2-3 times per week

Once per day More than once per day

4. On average, how many slices of bread do you eat <u>each day?</u>

_____ slices per day