A Prospective Study of the Effects of Renal Transplantation on Uraemic Cardiomyopathy using Cardiac Magnetic Resonance Imaging

THE RETRACT STUDY

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Abstract

Background

There is an inverse graded relationship between cardiovascular risk and renal function, with the greatest risk observed in those with end-stage kidney disease. This relationship is attributed to the development of uraemic cardiomyopathy which is characterised by the development of myocardial fibrosis, left ventricular hypertrophy and both diastolic and systolic dysfunction. Following renal transplantation this risk reduces dramatically a finding which is often attributed to positive cardiovascular remodelling. At present, however, this has not been demonstrated in a prospective adequately powered study employing cardiac magnetic resonance imaging.

Objectives

To establish if the features of uraemic cardiomyopathy are reversible following renal transplantation.

Participants

50 renal transplant recipients recruited from local transplant waiting list. 20 transplant-listed control participants with end stage kidney disease not scheduled for live donor transplantation.

Methods

Participants had demographic and CMR parameters recorded at baseline and follow-up at 12-24 months. Renal transplant recipients were studied within a one-month period prior to transplantation. Measures of left ventricular mass, volume, systolic and diastolic function, myocardial fibrosis and biochemical parameters were all recorded both before and after transplantation.

Results

There were significant reductions in LVM observed in transplant recipients compared to control patients (between group mean difference -18.76g 95%CI [-30.46 - -7.05] P=0.002).

There was also a significant reduction in LVMI in the transplant group compared to controls (between group mean difference -11.37g/m^2 95%CI [-18.49 - -4.25] P=0.002). There were no significant differences observed in measures of either systolic or diastolic function. In terms of native T1 mapping times, there were significant reductions observed in transplant recipients compared to controls, in the global average of all segments in the mid ventricular slice (between group difference -34.37ms 95%CI [-61.01 - -7.74] P=0.01. There were no significant changes in T2 times between groups. Analysis of biochemical parameters indicated significant differences in multiple parameters including haemoglobin (18.39 g/l 95%CI[7.12-29.66]P<0.001), NTproBNP (x0.10[0.05-0.22]P<0.001), and marker of chronic kidney disease – mineral bone disorder; parathyroid hormone (x0.40 95%CI[0.17 – 0.93]p+0.035, phosphate (-0.28 mmol/L [-0.41 - -0.16]P<0.001) and Vitamin D (31.54 nmol/L 95%CI(16.03 -47.05)P<0.001).

Conclusion

The RETRACT study has shown that the features of uraemic cardiomyopathy are reversible following renal transplantation. This works adds to the current understanding of uraemic cardiomyopathy and provides a rationale for the development of targeted therapies aimed at reversing cardiovascular remodelling in those in whom transplantation is not a treatment option.

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Publications associated with this research

i. **Pickup LC**, Law JP, Radhakrishnan A, Price AM, Steeds RP, Smith T, Townend JN, Ferro CJ. Changes in left ventricular structure and function associated with renal transplantation, a systematic review and meta-analysis. ESC Heart Fail. 2021 Mar 15.

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v. **Pickup L**, Radhakrishnan A, Townend JN, Ferro CJ. Arterial stiffness in chronic kidney disease: a modifiable cardiovascular risk factor? Curr Opin Nephrol Hypertens. 2019 Nov;28(6):527-536. doi: 10.1097/MNH.0000000000000535. PMID: 31361609.

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xii. Radhakrishan A, Price AM, **Pickup LC,** Law JP, McGee KC, Fabritz L, Senior R, Steeds RP, Ferro CJ, Townend JN. Coronary flow velocity reserve and inflammatory markers in living kidney donors. Int J Cardiol. 2020 Dec 1; 320:141-147.

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Dedication

I would like to dedicate this thesis to my family; Laura, Leo, Lyra, Poppy, and Daisy without their patience and support this work would not have been possible.

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LIST OF ABBREVIATIONS

2D/3D	2/3 Dimensional
ACE	Angiotensin converting enzyme
ACR	Albumin creatinine ratio
AHA	American Heart Association
BNP	Brain natriuretic peptide
BSA	Body surface area index
CI	Confidence interval
c-FGF23	C-terminal FgF23
CKD	Chronic kidney disease
CKD-MBD	Chronic kidney disease – mineral bone disorder
CKD-EPI	Chronic Kidney Disease Epidemiology Collaboration
CMR	Cardiac magnetic resonance
COVID19	Corona virus disease 2019
DCM	Dilated cardiomyopathy
DNA	Deoxyribonucleic acid
ECG	Electrocardiogram
EDTA	Ethylenediamine tetraacetic acid
ERA-EDTA	European Renal Association - European Dialysis and Transplant Association
eGFR	Estimated glomerular filtration rate
ELISA	Enzyme linked immunosorbent assay
ESKD	End stage renal disease
FA	Flip angle
FGF23	Fibroblast growth factor-23
FOV	Field of view

GBCA	Gadolinium based contrast agents
GCS	Global circumferential strain
eGFR	Estimated glomerular filtration rate
GLS	Global longitudinal strain
GRS	Global radial strain
HD	Haemodialysis
HFpEF	Heart failure with preserved ejection fraction
HFrEF	Heart failure with reduced ejection fraction
HRP	Horseradish peroxidase
HLA	Horizontal long axis
Hs -Trop-T	High sensitivity troponin T
IVSd	interventricular septum diastole
i-FGF23	Intact FgF23
KDIGO	Kidney Disease Improving Global Outcomes
LGE	late gadolinium enhancement
LVM	Left ventricular mass
LVEDD	Left ventricular end diastolic dimension
LVMI	left ventricular mass indexed
LVEDV	Left ventricular end diastolic Volume
LVEDVI	Left ventricular end diastolic Volume indexed
LVESV	Left ventricular end systolic volume
LVESVI	Left ventricular end diastolic systolic indexed
LVSD	Left ventricular systolic dysfunction
MAP	Mean arterial pressure
MDRD	Modification of Diet in Renal Disease
MAPK	Mitogen-activated protein kinase signalling

MeSH	Medical subject headings
MOLLI	Modified look-locker inversion recovery
MRI	Magnetic resonance imaging
NHANES	National Health and Nutrition Examination Survey
NHS	National Health Service
NSF	Nephrogenic systemic fibrosis
NT-pro BNP	n-terminal pro B type natriuretic peptide
PTH	Parathyroid hormone
PWd	posterior wall diastole
PWV	Pulse wave velocity
RAAS	Renin angiotensin aldosterone system
SAX	Short axis stack
SCD	Sudden cardiac death
SCMR	Society of Cardiovascular Magnetic Resonance
STROBE epidemiology	Strengthening the reporting of observational studies in
SSFP	Steady state free precession
TE	Echo time
TI	Inversion time
TNF	Tumour necrosis factor
TR	Repetition time
TSE	Turbo spin echo
UK	United Kingdom
US	United States
USRDS	United States Renal Data System
VLA	Vertical long axis

Chapter 1: INTRODUCTION

This chapter was written by the research fellow. Two reviews have been published within the peer reviewed literature and have formed part of the basis of this chapter.

Both articles were written by the research fellow with all drafts and edits being the sole responsibility of the research fellow.

i. **Pickup LC,** Law JP, Townend JN, Ferro CJ. Sudden cardiac death in chronic renal disease: aetiology and risk reduction strategies. Nephrol Dial Transplant. 2019 Nov 20;36(8):1386-88.

ii. **Pickup L**, Radhakrishnan A, Townend JN, Ferro CJ. Arterial stiffness in chronic kidney disease: a modifiable cardiovascular risk factor? Curr Opin Nephrol Hypertens. 2019 Nov;28(6):527-536. doi: 10.1097/MNH.0000000000000535. PMID: 31361609.

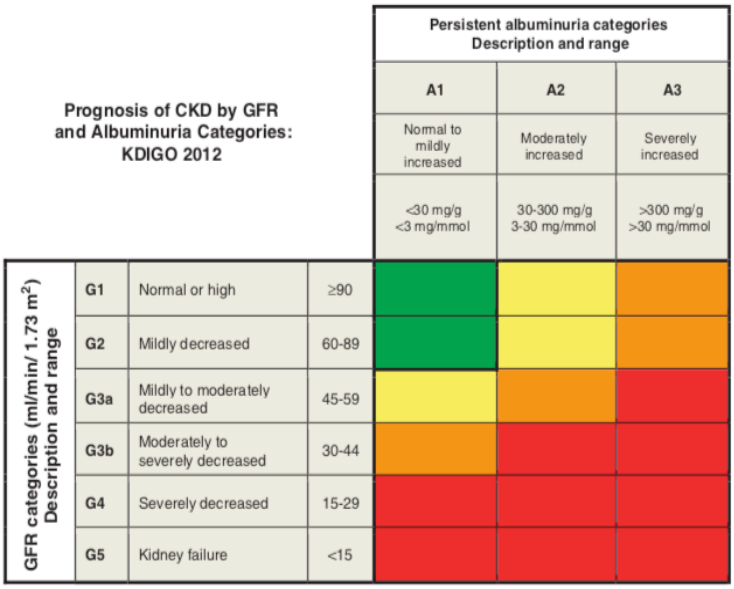
1.1 Chronic Kidney Disease

The definition of chronic kidney disease (CKD) has evolved over time. It is currently based on guidance from the Kidney Disease: Improving Global Outcomes (KDIGO) group (1). The diagnosis relies upon establishing markers of either kidney damage or reduced function for greater than three months which have implications for health regardless of the cause. Common markers of kidney damage include electrolyte disturbances, albuminuria, haematuria, abnormalities detected by either histology or imaging, and a history of transplantation. The most widely used measure of function is the estimated glomerular filtration rate (eGFR) where a measure below 60 mL/min/1·73 m^2 is regarded as a clinically significant (2). The Modification of Diet in Renal Disease Study (MDRD) equation and the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equations are currently the most commonly used internationally, and both were developed with creatinine assays traceable to isotope-dilution mass spectrometry (3, 4). Both use the same variables, standardized serum creatinine, age, and sex and have similar levels of accuracy in patients where eGFR<60 mL/min/1·73 m^2 (5) In those where eGFR is > 60 mL/min/1·73 m^2 the MDRD equation is recognised as being less accurate, and it is for this reason that the UK department of health recommends not reporting eGFR>90 mL/min/1·73 m^2 when using the MDRD equation (6).

Once a diagnosis of CKD has been established, measures of albumin creatinine ratio (ACR) and eGFR are then used in combination to risk stratify patients in-terms of their ongoing risk of progression of renal disease (**Figure 1.1**). Generally, in clinical practice it is this stratification based on eGFR which is used to categorise the stages of CKD, with stage 1 representing normal or high renal function with other markers of kidney damage to CKD stage 5 (eGFR <15mL/min/1.73 m^2) which is classified as end-stage renal disease (ESKD). At this point patients are deemed to be in "kidney failure" where renal function is insufficient to

sustain a person in the longer term. As a result, patients who progress to this stage of CKD will be considered for renal replacement therapy, in the form of either dialysis or renal transplant (7).

Figure 1.1 Prognosis of renal disease progression based on ACR and eGFR.



Green: low risk (if no other markers of kidney disease, no CKD); Yellow: moderately increased risk; Orange: high risk; Red, very high risk.

(Reproduced from Levin et al. (7))

1.2 Epidemiology of Chronic Kidney Disease

CKD is one of the most significant public health issues world-wide (8). In 2017, the global prevalence of CKD based on data from meta-analysis produced by Mills et al.(9) was estimated to be 11.1%. In 2019 the American Society of Nephrology, the European Renal Association and the International Society of Nephrology jointly published work stating that the number of individuals with CKD worldwide was 850 million (10). In the USA, where data is extensively compiled by United States Renal Data System (USRDS), it is estimated that currently the prevalence of CKD is 14%(11). A similar prevalence of 13·9% (95% confidence

interval (CI)12·2–15·7) was also reported for sub-Saharan Africa in a systematic review of 21 medium and high-quality studies reported by Stanifer et al. (12). Studies assessing prevalence across the Asian populations of Japan, Taiwan and China have reported overall prevalence rates of between 10.8% to 13% compared to rates of 16% reported in Australia. (13-15). However, it should be noted that true prevalence rates across Asia may be underestimated due to a lack of data in countries such as India where CKD prevalence is thought to be higher (16). Across Europe large variations in prevalence have been reported with rates as low as 3.31% (95% CI 3.30% - 3.33%) in Norway and as high as 17.3% (95% CI16.5% - 18.1%) in North-East Germany(17). In the United Kingdom (UK), national health service (NHS) data suggests that 1.8 million individuals have a confirmed diagnosis of CKD, it is also believed that there may be a further 1 million who remain undiagnosed due to the asymptomatic nature of the condition (6).

1.3 Socio- Economic Implications of CKD

Due to its high prevalence, CKD has significant economic implications for health providers across the world. The NHS spent an estimated £1.45 billion in 2009-10, on the treatment of CKD, the equivalent to £1 in every £77 of NHS expenditure. This financial burden is brought about by the direct costs of CKD treatment including medications to stop disease progression and renal replacement therapy, in addition there are also indirect costs which include the treatment of diseases associated with CKD, in particular cardiovascular disease (CVD) (6).

1.4 Aetiology of CKD

Chronic kidney disease does not reflect a single disease process but is the consequence of numerous conditions all of which have specific pathophysiological consequences for the kidney that ultimately lead to reduced function. At present, diabetes is overwhelmingly the most common cause of CKD worldwide, closely followed by hypertension. In the US 2021 39% of all patients developing ESKD were diabetic and 26% were associated with

hypertension (18). In 2018 Singapore, Malaysia, Qatar and Hong Kong all reported that greater than 50% of all incident cases of ESKD were due to diabetes (19). A similar pattern was also reported by the European Renal Association - European Dialysis and Transplant Association Registry (ERA-EDTA)(20), which includes data regarding patients receiving renal replacement therapy and renal transplantation across 34 European countries, including the United Kingdom (UK). This indicated that diabetes was associated with 20% of cases of ESKD and hypertension a further 12%.

1.5 CKD Morbidity and Mortality

CKD is the cause of considerable morbidity and mortality globally. The Global Burden of disease study (2017) (21) reported that CKD resulted in the loss of 7.3 million years of healthy life due to disability of which 40% were due to CKD stage 5. It was also estimated that 28.5 million years of life were lost due to 1.2 million deaths. (21). While excess mortality exists across the entire spectrum of CKD this risk is not uniformly distributed, with the highest burden of mortality observed as renal dysfunction progresses. Tonelli et al. (22) in a systematic review and meta-analysis examining CKD and mortality risk reported an exponential relationship between declining renal function and all-cause mortality. The predicted risk for death reported during the follow-up of 4.9 yrs. was 12% (95% CI [8 - 19]) when eGFR = 80mL/min/1.73 m^2, 17% (95% CI [11 - 25]) when eGFR = 60 mL/min/1.73 m^2, and 25% (95% CI [17 – 35]) for eGFR = 40mL/min/1.73 m^2. In addition, Neovius et al. (23) studied 3040 patients with CKD stage 4 and 5, and a further 725 on peritoneal dialysis (PD), 1791 on haemodialysis (HD) and 606 renal transplant recipients between 1999 and 2010. Here it was those receiving renal replacement therapy who have the highest mortality risk. Compared to CKD stages 4 and 5 PD patients had a hazard ration (HR) of 1.7 (95% CI [1.4 - 2.1]) and HD patients a HR 2.6 (95% CI [2.3 to 2.9]). Mortality risk was lowest in those who underwent renal transplantation HR 0.5 (95%CI [0.3 t- 0.7]).

1.6 Cardiovascular risk in CKD

The association between CVD and CKD was first described by Richard Bright in 1836 (24). Since this initial observation, there has been growing understanding of the complex interplay between CKD and CVD. In a review by Stevens et al. (25) using data from UK primary care it was reported that CVD was present in 15% of those with eGFR >60 ml/min/1.73 m^2 which increased to 51% in those with an eGFR <30 ml/min/1.73 m^2. In addition, the cardiovascular mortality associated with CKD also exhibits a similar inverse relationship. Thompson et al.(26) studied 81064 patients in Alberta, Canada between 2002-2009. In those with eGFR>60 ml/min/1.73 m^2 and no proteinuria cancer was the most common cause of death. However, as eGFR declined <60 ml/min/1.73 m^2 cardiovascular mortality became the most frequently observed. When adjusted for age and sex 33.3% (95%CI [32.5% - 35.0%]) of deaths were attributed to CVD in those with eGFR 45-59 ml/min/1.73 m^2. This proportion continued to increase as eGFR declined with 39.9% (95%CI [38.7% t- 41.1%]) of deaths due to CVD when eGFR<15-29 ml/min/1.73 m^2. It was also demonstrated in this cohort that as eGFR declined the observed mortality due to valvular heart disease and heart failure increased, whereas deaths due to ischaemic heart disease were not significantly different among those with normal renal function and across all stages of CKD (26). Due to this observed relationship, it was estimated that in 2017 in addition to the 1.2 million deaths caused directly by CKD, a further 1.4 million deaths were due to associated cardiovascular disease. As such CKD was responsible for around 8% of all cardiovascular deaths in 2017(21).

In addition to isolated measures of eGFR the presence of left ventricular hypertrophy (LVH) in CKD is also considered to be a powerful predictor of CVD risk. Stack and Saran et al. (27) in a prospective observation study of 2,584 patients newly commenced on dialysis showed that the presence of LVH was associated with an increased mortality in the first 6 months (relative risk (RR), 1.61; 95%CI [1.17 to 2.22]) which persisted at 2 years. Paoletti et al. (28) reported

that reduction in LVH following renal transplant was associated with reductions in both fatal and non-fatal cardiovascular events at 18 months compared to those in whom LVH remained unchanged (HR 0.41, 95% CI 0.22-0.79, P = 0.01). Rigatto et al.(29) in a retrospective cohort study review indicated that the presence of features of LVH on electrocardiogram (ECG) at one year following renal transplant was a risk factor for both death (RR 1.995%CI [1.22, 3.22]) and congestive heart failure (CHF) (RR 2.27 95%CI[1.08, 4.81]). In parallel with LVH the extent of myocardial fibrosis within the myocardium is also associated with cardiovascular mortality. Aoki et al. (30) demonstrated that in 40 patients receiving regular dialysis, those with greater than 30% myocardial fibrosis on biopsy had significantly less cumulative survival. Due to these associations between LVH and CVD, left ventricular mass (LVM) is viewed as a useful outcome measures and has been used in many renal clinical trials(31).

1.7 Cardiovascular mortality and CKD

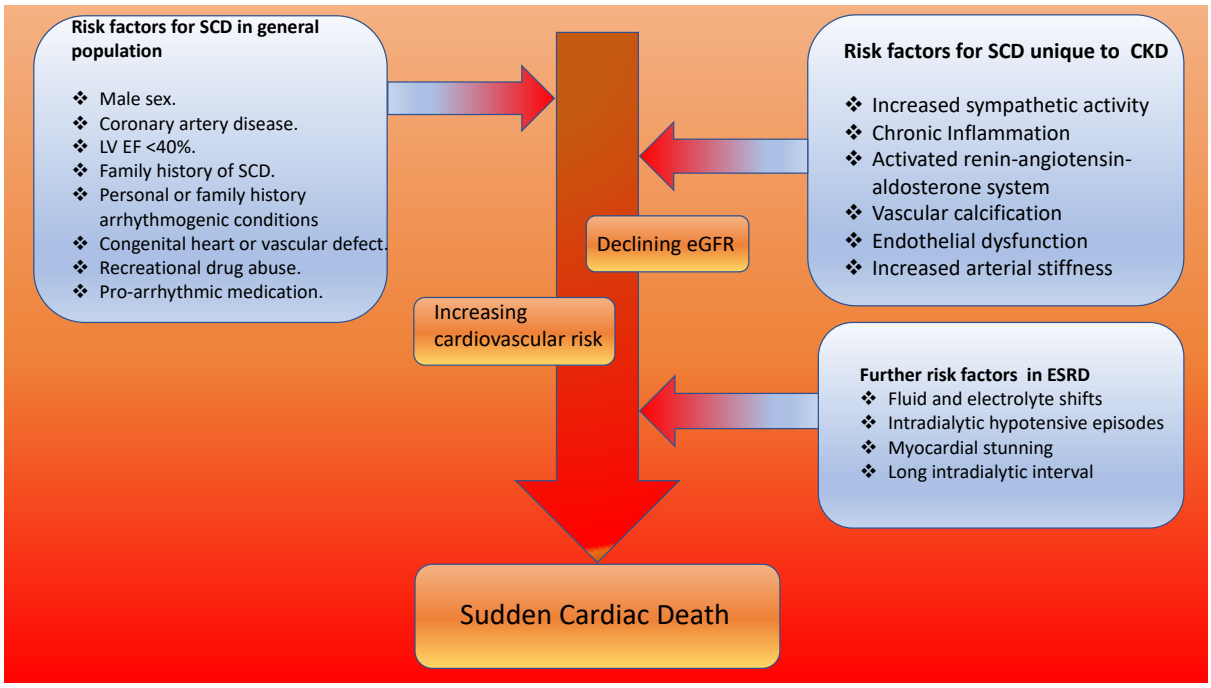
One of the unique features of CVD mortality observed in CKD is the large proportion attributed to sudden cardiac death (SCD). The term SCD is applies to deaths which are unexpected and can be ascribed to a cardiac cause. These events are defined as those that are either preceded by a witnessed collapse, occur within 1 hour of a sudden change in clinical condition, or happen not more than 24 hours since the deceased was known to be in their usual state of health (32). In the general population, most SCD events are attributed to coronary artery disease, as a result of either an acute coronary syndrome or left ventricular scarring leading to ventricular tachyarrhythmias. (32, 33). In patients with CKD, especially those with ESKD, the aetiology of SCD is more complex and almost certainly multifactorial. These factors include diffuse myocardial fibrosis and ventricular remodelling coupled with electrolyte disturbance, endothelial dysfunction, chronic inflammation, and fluid overload. It was previously believed this altered physiology created an unstable myocardium which increased the burden of malignant ventricular tachyarrhythmias(34). This assertion, however,

is now being questioned.

The association of SCD and CKD is well described in the sub-analyses of several randomised controlled trials. The Multicentre Automatic Defibrillator Implantation Trial (MADIT-II) examined the efficacy of implanted cardiac defibrillators (ICD) in those with prior myocardial infarction and left ventricular ejection fraction less than 30%. This highlighted that renal function was the most powerful baseline clinical parameter associated with SCD (35). ICD implantation however had no effect on all-cause mortality or SCD in those with an eGFR < 35 ml/min/1.73 m^2, suggesting that tachyarrhythmias were not the major underlying cause of SCD. (36) These findings were also confirmed by a recent trial that recruited 188 participants receiving regular HD with a left ventricular ejection fraction greater than 35%. Participants were randomised to either receive a prophylactic ICD or continued medical therapy and followed up for a median of 6.8 years (37).The cumulative SCD incidence at 5 years was 9.7% in the ICD group and 7.8% in the control group, resulting in a non-significant HR of 1.32 (95% CI, 0.53-3.29; P=0.55).

Both administrative databases and prospective cohort studies support the notion that the majority of cardiovascular deaths in patients with ESKD are due to SCD (38). The underlying mechanisms of SCD however are not well understood. Five studies using implantable loop recorders (ILR), enrolling 317 HD patients with mean follow-up ranging from 14 to 21 months, have been reported (39). Overall, there were 15 SCD associated with bradyarrhythmias, 2 associated with tachyarrhythmias and 3 with unclear ECG morphology. Most deaths occurred during the long intra-dialytic period. These data suggest that the mechanisms of SCD in patients with CKD may be diverse with only a minority of events due to ventricular tachyarrhythmias.

Figure 1.2 Causes of sudden cardiac death in CKD.



(reproduced from Pickup et al.(40))

1.8 Aetiology Cardiovascular disease in CKD

The inverse graded relationship between declining renal function and increasing cardiovascular risk is well established (41). Studies have demonstrated that cardiovascular risk begins to increase with only very minor reductions in eGFR at levels as high as 75 ml/min/1.73 m^2 when creatinine may still be normal (42) In addition structural changes within the cardiovascular system associated are also observed with only minor reductions in renal function <60 ml/min/1.73 m^2 (43) The burden of cardiovascular disease at very early stages of CKD is significant because while ESKD and the need for renal replacement therapy are the most recognisable outcome of kidney disease the prevalence of the earlier stages of the condition are highest(44). In fact, individuals with CKD are more likely to die of cardiovascular disease than they are to develop kidney failure. Cardiovascular risk however

remains highest for those with end-stage renal disease with more than half of all deaths in this group attributed to cardiovascular causes(45).

The aetiology of CVD in CKD is more complex than in the general population. This is due to the interplay of both traditional and non-traditional factors which drive the development of CVD in this group. CKD populations are often described as 'unique' as their increased risk of CVD is attributed to both atherosclerotic non-atherosclerotic factors. This idea is supported by epidemiological data showing that the increased mortality in CKD is not driven by an increasing prevalence of myocardial infarction and coronary events (46, 47). This is also supported by data from the Framingham study which is a predictive tool designed to detect CVD based on the presence of traditional risk factors. When applied to a CKD cohort of 577 women and 357 men, prediction of 5-year events was just 6% in men and 1.9% in women, at 10-year events were predicted in 13.9% of men and 4.8% of women (48).

The existence of "unique" risk factors in CKD is also supported by the lack of evidence for established CVD treatment strategies in CKD cohorts (49). This is particularly true of hyperlipidaemia. Yebyo et al.(50) performed a meta-analysis examining the effectiveness of statins for primary prevention in a general population. This included 94283 participants and showed statistically significant risk reductions of non-fatal CVD mortality (RR 0.80, 95%CI [0.71-0.91]), all-cause mortality (RR 0.89, 95%CI [0.85-0.93]). The 4S study also highlighted the benefit of statins in those with coronary disease, here Simvastatin reduced low density lipoprotein (LDL) cholesterol by as much as 35% with an associated 30% relative risk reduction of all-cause mortality compared to placebo over 5.4 years of follow-up (51). These significant benefits of lipid lowering strategies in CKD however are less well established.

Secondary analysis of the JUPITER study examined the use of statins for primary prevention in 3276 participants with moderate CKD defined as eGFR <60ml/min/1.73 m2 (52). This demonstrated that while cardiovascular event rates were higher among those with CKD, Rosuvastatin was associated with 44% reduction in all-cause mortality compared to placebo in this group. As renal function declines however, these benefits are less certain. The 4D study (53) assessed the efficacy of atorvastatin in 1255 patients with type 2 diabetes receiving HD. Here LDL cholesterol was reduced by a median level of 42% in those receiving atorvastatin compared to 1.9% reduction in the placebo arm of this study. This reduction, however, did not produce any statistically significant difference in the composite endpoint of cardiovascular death, nonfatal myocardial infarction, and stroke. The AURORA (54) study also reported similar findings with Rosuvastatin associated with significant reductions in LDL cholesterol in dialysis patients but no reduction in the composite endpoint of death from cardiovascular causes, nonfatal myocardial infarction, or nonfatal stroke. Meta-analysis of all the available studies was performed by Messow and Isles (55) of thirteen available randomised controlled trials which examined the effectiveness of statin therapy in patients with CKD stage 3 or less. This concluded that while statins reduce adverse cardiac events in CKD stage 3 and 4 and may reduce cardiovascular death in transplant recipients there was no evidence of benefit in CKD stage 5.

The effects of glycaemic control on cardiovascular risk in CKD is also not well established. The ACCORD trial examined 3636 participants with mild to moderate CKD and 6506 participants with normal renal function to compare the effects of either standard or intensive glycaemic control on cardiovascular outcomes (56). In patients with CKD, compared with standard therapy, intensive glucose lowering was significantly associated with a 31% higher

all-cause mortality (HR1.306 95%CI[1.065–1.60]) and 41% higher cardiovascular mortality (HR 1.412 95%CI [1.052–1.892]). No significant effects were found in patients without CKD. A recent meta-analysis conducted by Fox et al.(57) of 1,024,977 participants, including 128,505 with diabetes reported that in CKD cohorts when stratified for ranges of eGFR hazard ratios for all-cause mortality between those with and without diabetes were not significantly different. This suggests that while diabetes is an established risk factor for cardiovascular mortality in the general population, in those with CKD this relationship is more complex with renal function acting as a stronger predictor of cardiovascular outcomes.

1.9 Cardiorenal Syndrome

Cardiorenal syndrome encompasses several pathophysiological processes, whereby dysfunction in either the kidney or the heart leads directly to dysfunction in the other. The relationship between renal and cardiac dysfunction is well established, being first described in 1836 by Robert Bright (58). Since this initial observation, a greater understanding of the condition has developed. At present cardiorenal syndrome is classified into five sub-categories based on the Consensus Conference of the Acute Dialysis Quality Initiative (59).

Type one cardiorenal syndrome occurs where acute cardiac dysfunction leads to subsequent development of concomitant acute renal dysfunction. The most common example of this is observed following acute myocardial infarction, where an acute reduction in cardiac function is accompanied by acute kidney injury (60). In contrast, cardiorenal syndrome type two is associated with chronic cardiac dysfunction which ultimately causes impaired renal function over a long period of time. This occurs in conditions such as congenital heart disease, where cardiac dysfunction occurs before renal dysfunction, but gradually contributes to the development of chronic kidney disease(59).

In both cardiorenal syndromes type one and two, reduction in cardiac output leads to renal hypoperfusion. This activates the renin angiotensin aldosterone system (RAAS) and the sympathetic nervous system, which increases fluid retention in order to improve renal perfusion. This adaptive process leads to increased preload and worsening pump failure. Reduced renal perfusion however is not solely responsible (61). In cardiac dysfunction there is also increased central venous pressure. This has the effect of reducing the pressure gradient across the glomerulus, which further reduces glomerular filtration rate (62). This is supported by the ADHERE registry which demonstrated that in the context of acute heart failure syndromes increases in creatinine were similar in both, heart failure with preserved ejection fraction (HFpEF), and heart failure with reduced ejection fraction (HFrEF), suggesting that it is not just low cardiac output states which are responsible for declining eGFR (63).

Type three cardiorenal syndrome is considered a renocardiac syndrome, where acute reduction in renal function causes cardiac dysfunction. In the setting of acute kidney injury there is release of numerous inflammatory cytokines, including TNF-alpha, IL-1 and IL-6 which have deleterious effects on cardiac function (58). Type four cardiorenal syndrome describes chronic renal dysfunction leading to cardiac dysfunction. It is this process which results in the development of uraemic cardiomyopathy. The pathophysiology of this has been discussed extensively in section **1.15 (64)**.

Type five cardiorenal syndrome is the result of systemic pathologies which effect both cardiac and renal function simultaneously. There are numerous causes including, but not limited to, systemic lupus erythematosus, amyloidosis, sepsis, and toxins. The pathophysiology behind the observed renal and cardiac dysfunction is dependent on the underlying cause (65).

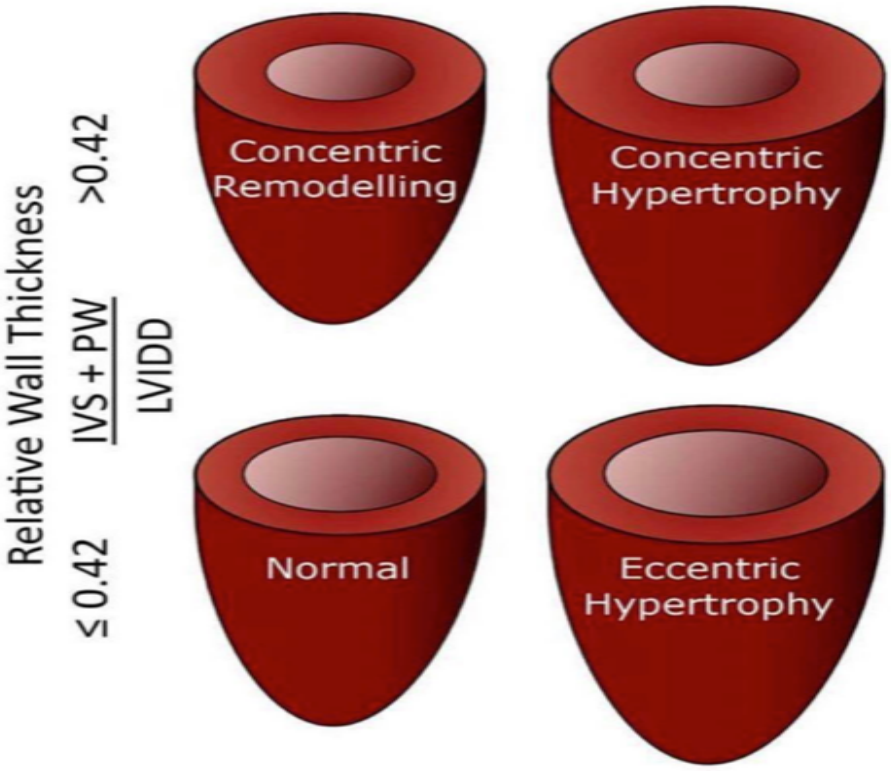
1.10 Uraemic cardiomyopathy

The changes observed in the cardiovascular system associated with CKD are collectively known as uraemic cardiomyopathy. The term uremic cardiomyopathy first came into use in the 1980s. The classical features of the condition are LVH, diastolic and systolic dysfunction, the development of diffuse interstitial myocardial fibrosis and increasing arterial stiffness.

1.11 Left Ventricular Hypertrophy

LVH is the most recognisable feature of uremic cardiomyopathy. LVH is classified as an increased LVM. This can be due to an increase in the wall thickness of the ventricle or due to dilation of the ventricular cavity. It is classified as being either concentric or eccentric, with the distinction being based on a calculation of relative wall thickness (RWT)(66) . RWT can be defined as the interventricular septum thickness, plus the posterior wall thickness divided by the left ventricular diastolic diameter. Where RWT is > 0.42 in the presence of increased LVM, LVH can be classified as concentric i.e. increasing wall thickness is the predominate cause of the increased mass. Where LVM is increased and RWT is <0.42 eccentric hypertrophy is present, here ventricular cavity enlargement is responsible for the observed increased mass(67). Where RWT is > 0.42 in the presence of normal LVM this is classified as concentric remodelling. Typically, in early stages of CKD eccentric hypertrophy is observed, however as renal function deteriorates the most commonly observed pattern of hypertrophy is concentric in nature (68).

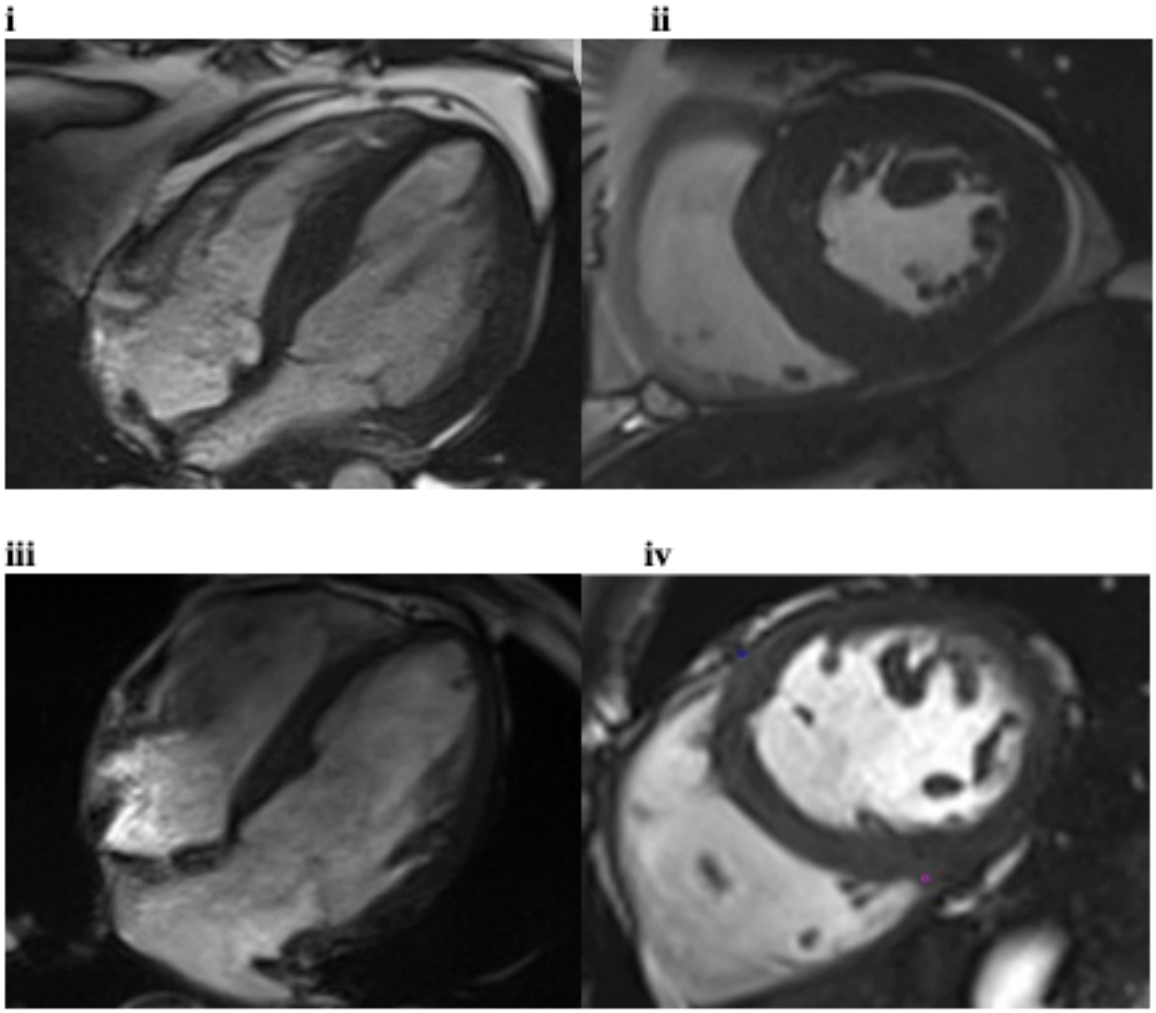
Figure 1.3 Classification of LVH based on current bristish sociaty of echocardiography guidance reporduced with permission



Reproduced from Harkness et al . (67)

The presence of LVH in CKD is clinically significant as it is a predictor of both morbidity and mortality as discussed previously in section **1.1.6.** It is also associated with progression to dialysis in those with CKD(69). Recent meta-analysis by Maki et al. (70) has also indicated that regression of LVM is an effective surrogate marker for improvements in mortality in CKD. In the general population rates of LVH are between 15 and 21% (71). In CKD cohorts LVH becomes more prevalent as renal function declines, with between 16 and 31% of individuals with an eGFR <30 ml/min estimated to have LVH. This increases to 60-75% prior to starting renal replacement therapy, rising further to 90% after the initiation of renal replacement therapy (72).

Figure 1.4 CMR images obtained from the RETRACT study.



(i) HLA (ii) sax image of a patient on HD with concentric LVH (LVMI 93 g/m^2). (iii) HLA (iv) sax image of a patient on peritoneal dialysis with normal left on cardiac magnetic resonance imaging (maximum wall thickness, 9 mm; LVMI 63 g/m^2)

1.12 Left Ventricular systolic and diastolic dysfunction

The development of LVH is an adaptive response to the metabolic and haemodynamic stresses associated with CKD, which ultimately leads to myocyte death and reductions in ventricular systolic function (73). The prevalence of left ventricular systolic dysfunction (LVSD) observed in CKD is considerably higher than in the general population with estimates varying from 15% for those with CKD stage 3 to as high as 56% in dialysis recipients (74, 75). The presence of LVSD is a further predicator of both morbidity and mortality in CKD. Recent work by Yu et al.(76) involving 76,688 participants indicated that reduced ejection fraction in a CKD cohort was associated with a significantly increased risk of cardiovascular mortality (HR 6.69 (95% CI [5.93-7.54]). Chisavu et al.(77) also reported increased mortality in those with LVEF < 50% (LVEF 40–49% 1.5-fold and LVEF < 40% 2.3-fold).

While traditionally the assessment of systolic function has relied on the calculation of ejection fraction, the development of advanced imaging techniques has now highlighted that in CKD changes in systolic function occur even in the presence a preserved ejection fraction. Hensen et al.(78) demonstrated that in those with CKD stage 3b or less 32% of participants exhibited reduced measures of global longitudinal strain (GLS) even in the presence of a normal ejection fraction. Furthermore, this work also indicated that these changes were associated with significantly increased risks of hospitalisation for heart failure and cardiovascular mortality.

Associated with the development of LVH and LVSD, there is also progressive diastolic dysfunction of the ventricle. Diastolic heart failure also known as heart failure with preserved ejection fraction is characterized by a clinical syndrome whereby there are features of heart failure in the presence of a preserved ejection fraction (>55%) and abnormal diastolic function. This occurs when a ventricle is unable to fill adequately at normal diastolic pressures. This is

observed in conditions where there is an increase in the stiffness of the ventricle leading to reduced relaxation and reduced end diastolic volumes.(79) In such conditions to achieve an end-diastolic volume sufficient to produce an adequate stroke volume the filling pressures of the left ventricle must increase. Transmission of this increasing pressure to the pulmonary capillary bed leads to pulmonary congestion and the classical features of heart failure. In CKD cohorts, the presence of heart failure with preserved ejection fraction is estimated to be as high as 35% (80). It is also associated with significant increases in mortality (HRs 1.59 (95% CI[1.48-1.70]) (76).

1.13 Myocardial Fibrosis

It is often reported in the literature that underlying mechanism behind the cardiovascular remodelling seen in uraemic cardiomyopathy is due to myocardial fibrosis. Initial work by Mall et al.(81) via post mortem analysis showed that in 91% of uraemic patients there was intermyocardiocytic fibrosis. Furthermore, in dialysis patients the severity of this fibrosis was related to dialysis duration. These changes observed remained present even in transplant recipients. Amann et al. (82) also reported post mortem findings which indicated that the volume and density of the myocardial interstitial tissue was increased in uraemic patients compared to controls.

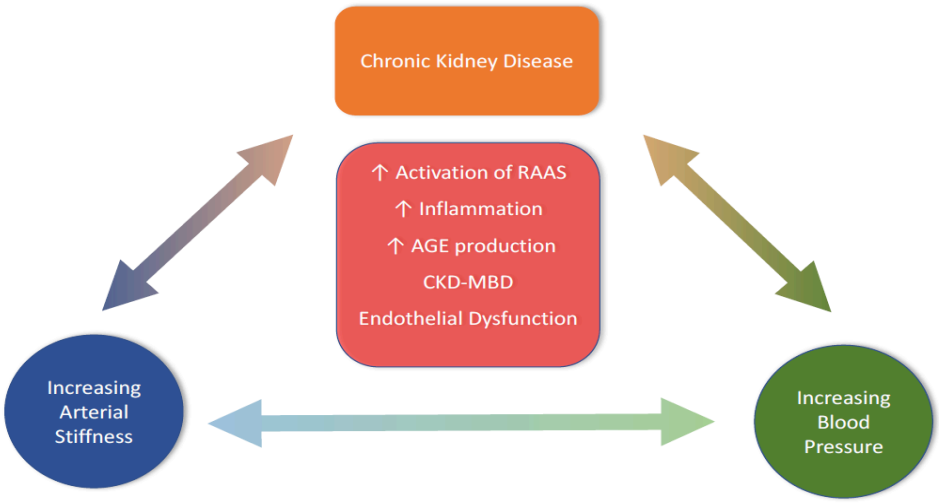
Work by Aoki et al(30) using endomyocardial biopsy also demonstrated that the histological features of the myocardium in uraemic cardiomyopathy included disarray of myocytes which were severely hypertrophic, there was also diffuse interstitial fibrosis which resembled the dilated phase of hypertrophic cardiomyopathy (30). Due to advances in imaging techniques, there is also now further data from cardiac magnetic resonance imaging (CMR) studies which have also implicated myocardial fibrosis in the development of UC. Rutherford et al. (83) demonstrated that native T1 mapping times are increased in ESKD compared to healthy

volunteers. Graham-Brown et al. (84)also reported similar findings with increased native T1 mapping timed being observed in those with ESKD. CMR data has also demonstrated that as CKD stage increases there are associated increases in native T1 time (85). Such findings suggest the fibrotic burden increases as renal function declines.

1.14 Arterial Stiffness

In parallel with increasing cardiovascular risk and left ventricular abnormalities, arterial stiffness increases as renal function declines (86-88). One of the major haemodynamic functions of the arterial system is to produce a steady state of perfusion to the organs of the body from the highly pulsatile flow generated by the left ventricle(89). This is achieved via distension of large elastic arteries, such as the aorta, which are able to store blood during systole and expel it during diastole (90). As a result of this buffering mechanism, uniform pressure is achieved in the capillary bed throughout the cardiac cycle. In conditions of increased arterial stiffness such as CKD there is a reduction in this Windkessel effect leading to higher pulse pressures(91) (92)

Figure 1.5 Pathophysiology of arterial stiffness in CKD



RAAS, renin-angiotensin-aldosterone system. CKD-MBD, chronic kidney disease mineral bone disorder.

In the context of CKD, the vasculature is affected by two distinct pathologies (93). Atherosclerosis is a disease consisting of fibro-atheromatous plaques affecting primarily the intimal layer of medium sized vessels, which can ultimately produce arterial occlusion. In contrast to non-uraemic individuals, those with CKD exhibit a greater burden of plaque calcification and increased thickness of both the media and intimal layers. Arteriosclerosis is a second commonly recognised pathology. In contrast to atherosclerosis, this is a disease primarily of the media layer in large conduit arteries(94). Concentric medial calcification, increased collagen content, hypertrophy and hyperplasia of smooth muscle combine to reduce the elastic properties of the arterial wall and increase arterial stiffness.

Blacher et al. (95), was the first study to examine whether increased arterial stiffness in CKD could be recognised as an independent predictor of cardiovascular events. This study showed that a pulse wave velocity (PWV) >12 m/s was associated with an odds ratio of 5.4 for all-cause mortality and 5.9 for cardiovascular mortality. This finding has subsequently been supported by further studies including data from the Chronic Renal Insufficiency Cohort (CRIC) which concluded that PWV is an independent predictor of progression to ESKD and all-cause mortality (96).

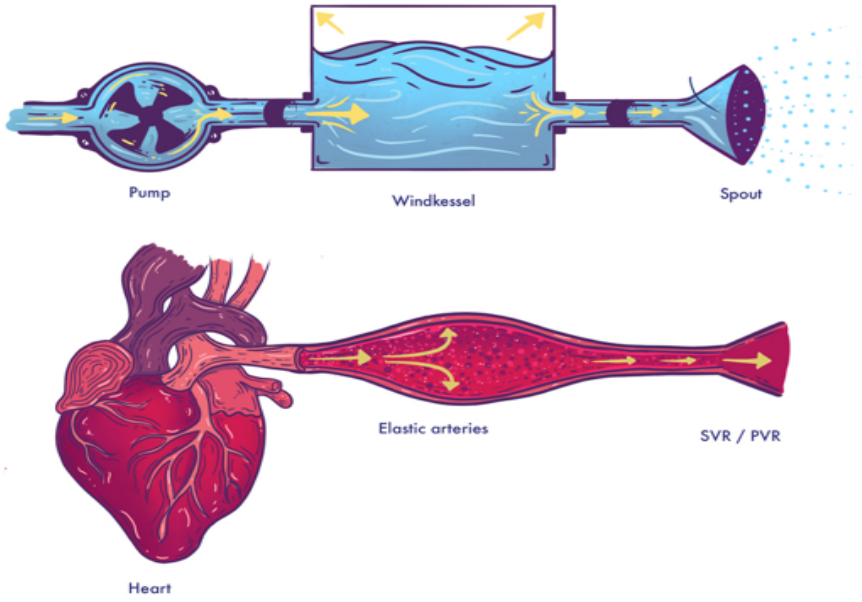
1.15 Uraemic Cardiomyopathy Differential Diagnosis

The above discussion highlights the clinical features of uraemic cardiomyopathy. However, it is also true that other forms of cardiomyopathy associated with development LVH may be present in those with renal dysfunction. Diabetes which is a leading cause of renal dysfunction, is also associated with the development of a cardiomyopathy (97). It is defined as the coexistence of cardiomyopathy and diabetes in the absence of other cardiac disease, most notably coronary artery disease. In the early stages it is characterised by the presence of diffuse fibrosis and increased left ventricular end diastolic pressure (98). In the late stages of the

condition there is progressive diastolic dysfunction associated with the development of LVH and further increases in end diastolic pressure (99). Typically, at this stage, patients may begin to exhibit clinical signs of heart failure (100).

In addition to diabetes, hypertension, which is both a cause and an effect of renal dysfunction may also result in the development of a cardiomyopathy. As with both diabetic and uraemic cardiomyopathy, features include LVH due to the development of diffuse myocardial fibrosis (101). Again, as hypertrophy worsens there is increased diastolic dysfunction, and ultimately the clinical features of congestive cardiac failure.

Figure 1.6 Pictorial representation of the windkessel effect.



(Reproduced from Pickup et al. (102))

1.16 Pathophysiology of Uraemic Cardiomyopathy.

The pathophysiological mechanisms behind the changes to the cardiovascular system in CKD are not fully understood but it is accepted that they are likely to be multifactorial. Such factors include mechanical stresses due to increased afterload as a result of hypertension and increased preload due to fluid overload (103). In addition, increased activity of the RAAS, over activity of the sympathetic nervous system, the presence of uraemic toxins and development of chronic kidney disease – mineral bone disorder (CKD-MBD) have all been implicated in the development of uraemic cardiomyopathy (104)

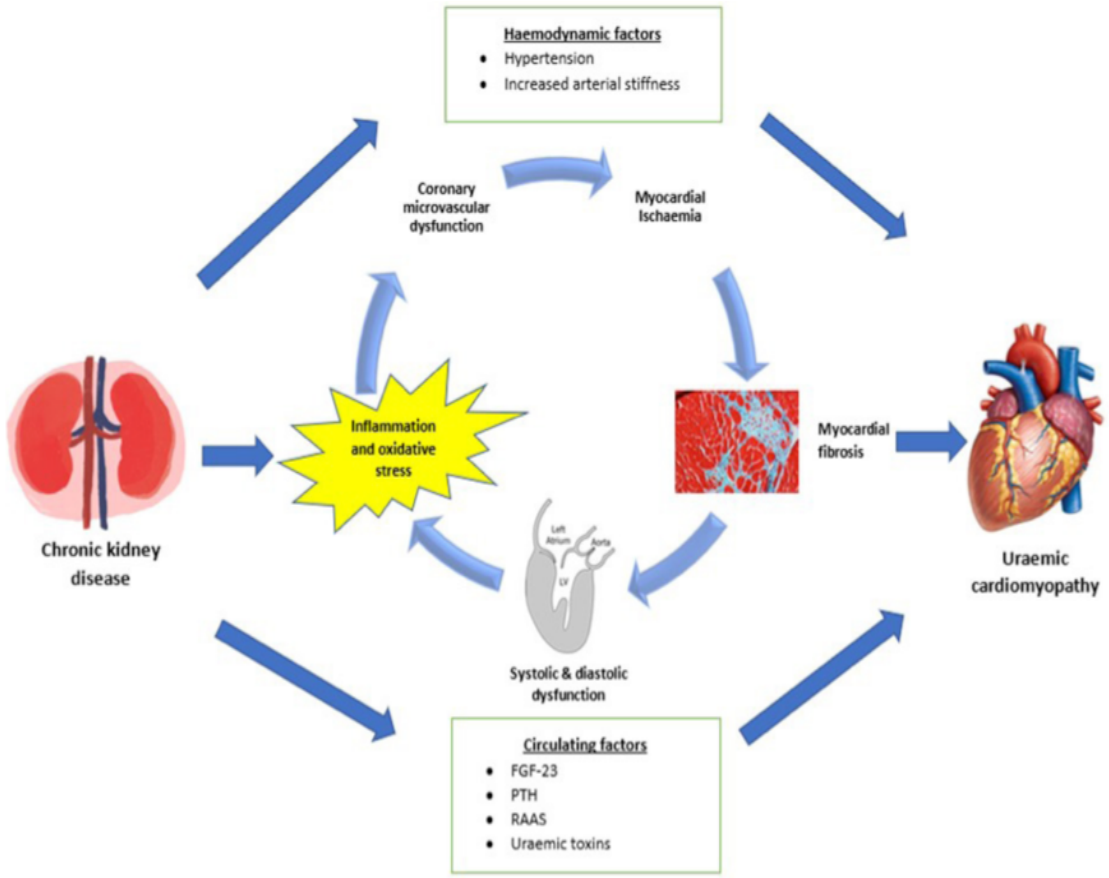


Figure 1.7 Pathophysiology of uraemic cardiomyopathy.

FGF-23, fibroblast growth factor-23; PTH, parathyroid hormone; RAAS, renin-angiotensin-aldosterone system. Reporduced from Radhakrishnan et al. (105)

1.17 Haemodynamic overload and mechanical stress

Haemodynamic factors play a key role in the development of LVH observed in uraemic cardiomyopathy (103). Such factors are those which alter either ventricular pre-load or afterload. Increased ventricular pre-load is directly linked to fluid status, which can become deranged in CKD, particularly in those who are receiving renal replacement therapy. In CKD plasma levels of both, brain natriuretic peptide (BNP) and n-terminal pro B type natriuretic peptide (NTproBNP) are known to significantly increase as eGFR levels fall. In a study of 229 adults Vickery et al.(106) indicated that mean BNP concentrations increased by 20.6% per 10-

mL/min1.73^2 reduction in estimated eGFR. Such increases in BNP can be considered a surrogate marker of increasing preload. Niizuma et al. (107) demonstrated that in 156 heart failure patients levels of BNP were significantly correlated (r=0.47 P=0.01) with measures of left ventricular end diastolic wall stress. The relationship between LVH and preload is further strengthened by the finding that in those patients with LVH levels of BNP are significantly higher compared to those without (106).

This relationship between LVH and preload has also been demonstrated in animals studies. In a pig model Fiorillo et al. (108) induced volume overload surgically via an aorto-caval shunt This induced volume overload resulted in the development of LVH within 96 hours, which was also associated with increases in markers of oxidative stress including lipid peroxidation products. There were also increases in ERK phosphorylation which has been implicated in the development of LVH in other conditions(109). Further work by Wang et al. (110) has also demonstrated that mechanical stretching of rat cardiac myocytes leads to increased gene expression of c-fos and tumour necrosis factor (TNF) - alpha, which are modulators of the hypertrophic response (110).

Increased afterload is also common in CKD and can be defined as the hydraulic load produced by the systemic circulation (111). This is reflected by the high prevalence of systolic hypertension in this group which is reported to be over 80% in CKD stage 4-5(47). The association between hypertension and LVH is well established with early work indicating that in those with hypertension, rates of LVH are as high as 56% in Caucasian populations and 71% in black populations(112). In addition to simply viewing afterload as a combination of systolic and diastolic blood pressure, the more recent view is that arterial stiffness expressed as PWV is also an important index of afterload (previously discussed in **section 1.2.4**). The

association between LVM and PWV is supported by meta-analysis of 23 available studies performed by van der Waaij (113). This work showed that with a reduction of 1.0m/s in PWV there was an associated reduction in LVMI of 6.9g/m. The association of afterload and LVH is also supported by animal studies. Nicks et al.(114) demonstrated that increased afterload via supra-renal aortic constriction in mice was associated with the development of significant LVH at one week. Villarreal and Dillman (115) also demonstrated that aortic banding in rats produced LVH which was also associated with increased expression of TGF-B and mRNA expression of fibronectin and collagen type I and III all of which have been implicated in the development of LVH.

1.18 Sympathetic nervous system activation

Overactivity of the sympathetic nervous system is associated with several chronic diseases including CKD (116). It is known to accelerate the progression of cardiovascular, renal, and metabolic pathologies. The most commonly observed consequence of increased sympathetic nervous system activity is systolic hypertension which is almost ubiquitously observed in advanced CKD. As has previously been discussed, the increased loading and mechanical stress associated this hypertension can lead directly to the development of LVH. However, it is also recognised that chronic activity of the sympathetic nervous at sub hypertensive levels can also produce changes in the cardiovascular system. In early work Laks et al. (117) demonstrated that dogs chronically infused with sub-hypertensive doses of norepinephrine developed increased LVM compared to controls. *In vitro* work by Simpson (118) also demonstrated that ventricular rat myocytes increased in size by 150% with the addition of L-norepinephrine. This response was attenuated by the administration of alpha-adrenoreceptor blocking agents rather than beta-adrenoreceptor blocking agents, suggesting that this hypertrophy is medicated by alpha receptors.

In addition to its effects on the myocardium, sympathetic nervous system activation is also implicated in the arterial stiffness seen in UC. Bevan (119) highlighted that in rabbit models vascular smooth muscle proliferation is mediated by the sympathetic nervous system. Holwereda et al. (120) also demonstrated that in humans sympathetic activity is correlated with increased measures of pulsed wave velocity and reduced carotid artery compliance. In addition, acute elevations in sympathetic activity without increased mean arterial pressure were also correlated to measures of PWV independent of age. Interestingly in younger participants elevated sympathetic activity was associated reduced carotid compliance, however, this relationship was not observed in older participants.

1.19 Activation of the Renin Aldosterone System

The RAAS is integral to the regulation of blood pressure, it also regulates fluid and electrolyte balance (97). This pathway involves the secretion of renin from the juxtaglomerular apparatus which converts angiotensinogen to angiotensin I. Angiotensin I is then converted in the lungs to angiotensin II by angiotensin converting enzyme. It is this angiotensin II which is a primary regulator of blood pressure. The role of overactivity of the RAAS system in the progression of CKD is now widely accepted as robust evidence from clinical trials has demonstrated the positive effects of RAAS blockade on declining renal function (98)

In addition to its role in the progression of CKD angiotensin II has also been implicated in the development of LVH and fibrosis which are the hall mark features of UC. Ichihara et al. (121) demonstrated that in a wild type mouse an infusion of angiotensin II caused the development of LVH whereas in mice lacking the angiotensin II receptor gene this was not observed. Sadoshima and Izum (122) also demonstrated that the cardiac effects of Angiotensin II are not purely mediated by afterload as a result of increased systolic blood pressure. Here Angiotensin II stimulated increased protein synthesis in neo-natal rat myocytes in culture, it also increased

deoxyribose nuclei acid (DNA) synthesis and cell number in cardiac fibroblasts. There was also rapid induction of genes involved in the hypertrophic response.

1.20 Coronary Microvascular dysfunction

Coronary microvascular dysfunction has been implicated in several diseases where LVH and underlying fibrosis is a pathological feature, including HCM and heart failure with preserved ejection fraction(123). Recently it has been proposed that the CKD may result in coronary microvascular dysfunction due to a combination of atherosclerosis, inflammation, and oxidative stress. The result of coronary microvascular dysfunction is then widespread myocardial ischaemia with associated diffuse interstitial fibrosis ventricular remodelling and ultimately LVH (105).

This notion that CMD is involved in the development of uraemic cardiomyopathy is supported by recent work from Radhakrishnan et al. (124) where coronary flow reserve was assessed using echocardiography in living kidney donors and healthy controls. This work demonstrated that even with small reductions in renal function there is significant reductions coronary flow reserve (CFR) (3.4 ± 0.7 vs 3.8 ± 0.6, mean difference 0.4 95% CI [0.03-0.8], P =0.036). In addition, a large retrospective analysis of 3946 patients using positron emission tomography demonstrated that coronary flow reserve reduced as renal function declined this relationship was however not linear as the largest reduction was observed in CKD stage 4, with no further significant decline in CKD stage 5 or those on dialysis (125).

1.21 Chronic Kidney disease – mineral bone disorder

The central role of CKD-MBD in the pathophysiology of uraemic cardiomyopathy has recently become increasing clear. CKD-MBD is the term used to describe the changes that occur in the cardiovascular and skeletal system associated with CKD(126). It is characterised by either one or a combination of abnormalities of calcium, phosphate, 1,25-dihyrdoxy vitamin D (calcitriol)

and parathyroid hormone (PTH) metabolism. (126)

1.22 Hyperphosphataemia

Serum phosphate levels even when still within normal range are associated with increased cardiovascular risk in both the general population and in those with CKD (127) In CKD mortality risk increases in a linear fashion with every 0.5mg/d increase in serum phosphate (128). This increase in mortality is accompanied by a process of cardiovascular remodelling. It is now recognised that the hyperphosphatemia which is commonly observed in more advance renal dysfunction has direct impacts on human smooth muscle cells. Jono et al. (129) identified that human smooth muscle cells cultured in media containing phosphate levels similar to that observed in CKD induced phenotypic changes which promoted mineral deposition in a dose dependant fashion. This effect of hyperphosphatemia was shown to be produced by the sodium-dependant phosphate cotransporter Pit-1. These in-vitro findings reflect further work which has demonstrated that higher phosphate levels are also associated with increases in ankle brachial pressure index in both CKD and those with normal renal function (130). In addition to arterial stiffness increased serum phosphate is also associated with the development of myocardial fibrosis and LVH. Amann et al. (131) highlighted that in a rat model of CKD, hyperphosphatemia is associated with the development of both interstitial myocardial fibrosis and increased arterial wall thickness. Chue et al.(127) also studied 208 CKD patients and demonstrated that as phosphate levels increased there was a concomitant significant increase in LVM.

1.23 Hyperparathyroidism

In CKD, hyperparathyroidism is common and has an increasing prevalence as eGFR declines. In those with eGFR> 60 mL/min/1·73 m^2 prevalence is <20% increasing to > 50% in those with eGFR< 30 mL/min/1·73 m^2(132). In CKD, as phosphate levels increase in conjunction with decreased vitamin D levels (calcitriol), there is decreased absorption of calcium within

the gut. Persistently reduced serum calcium levels, lead to increased synthesis of PTH and parathyroid gland hyperplasia, leading to the classical picture of secondary hyperparathyroidism (133). If this condition persists over time, it can ultimately progress to tertiary hyperparathyroidism(134).

As with other mediators of CKD-MBD, PTH is implicated in the phenotypical changes observed in uraemic cardiomyopathy. An in vitro study of neonatal rat myocytes demonstrated that PTH increased markers of the hypertrophic response, including phenylalanine incorporation and total protein content and induction of creatinine kinase (135). PTH has also been linked to increased expression of protein kinase C which is involved in the hypertrophic response through cardiac gene expression including C-fos (136). Amann et al. (131) also demonstrated that in a live rat model of CKD the administration of PTH and a high calcium diet was associated with the development of myocardial fibrosis, which suggests a role in the activation of myocardial fibroblasts. In addition to this, observational data from the fourth Tromsø study which included a sample of 2700 patients reported that when PTH was increased above the 95centile it was a positive predictor of increased LVM (137).

1.24 Fibroblast growth factor-23 and α-Klotho

While the elevated phosphate and PTH levels have numerous cardiovascular effects it is role of the phosphaturic hormones fibroblast growth factor-23 (FGF23) and α-Klotho, which are now believed central to the development of uraemic cardiomyopathy (138). As renal function declines FGF23 levels increase, with levels of up to 1000 times that of normal observed in ESKD (139). These increased levels are associated with an increased risk of heart failure (140-149)and all-cause mortality and cardiovascular mortality (142, 144, 148-156). Reduced levels of α-Klotho are also observed in early renal dysfunction and correlate with progression of CKD (157)

FGF23 was first discovered in 2000 as a growth factor secreted by osteocytes the main role of which is to increase urinary phosphate excretion. It is contains 251 amino acids with an N-terminal containing the FGF homology domain and a novel 71–amino acid C-terminus (158) There are 4 FGF receptors (FGFR1-4) which are transmembrane phosphotyrosine kinase receptors. When there is an increase in circulating phosphate levels, FGF23 acts on the proximal renal tubule to downregulate the sodium dependant phosphate cotransporter IIa/c which increases phosphate excretion. It also acts on the parathyroid gland to reduce parathyroid hormone secretion and vitamin D hydroxylation. These actions are achieved via the FGR-1(159).

FGF23 is the primary ligand for FGR-1, however, the cofactor, α-Klotho, is also required for its activation. The role of α-Klotho in the normal physiological function of FGF23 has been demonstrated in mouse models where α-Klotho deficient mice exhibit a similar phenotype to FGF23 deficient mice(160). In addition α-Klotho may also influence the organ specificity of FG23, as while FGR are expressed in many organs such as spleen adrenal glands and skin their lack of α-Klotho expression, suggests they are not a target for FGF-23 (158). This is also true of cardiac fibroblasts and myocytes suggesting that under normal physiological conditions the heart is not a target organ of FGF23 (138).

While α-Klotho dependent signalling is the mechanism by which FGF23 regulates phosphate levels it is now believed that the cardiovascular remodelling observed in uraemic cardiomyopathy is via a α-Klotho independent pathway. Faul et al. (138) showed a dose dependent increase in cell surface area of neonatal rat myocytes when exposed to FGF23. This was observed in the absence of α-Klotho indicating an alternative FGF23 signalling pathway

was present. Hu et al. (161) also reported that high levels of FGF23 correlate with myocardial remodelling in α-klotho deficient but not α-Klotho replete mice (162). It is hypothesised that FGR-4 is responsible for this signalling as it is present in cardiac myocytes and can bind with high affinity to FGF23 in the absence of α-Klotho. This is supported by the finding that in CKD patients there is a correlation between LVH and increased cardiac myocyte FGR-4 expression (163). This alternative FGF23 signalling was also suggested by the finding that rat myocytes exposed to FGF23 do not demonstrate increase levels of ERK which plays and integral role in the MAPK cascade which is the basis of intracellular signalling by FGR-1. Faul et al. (138) also reported that FGF23 induced LVH is attenuated by cyclosporin A, suggesting that the PLCγ-NFAT calcineurin signalling pathway rather than the MAPK cascade was responsible.

Further evidence of the role of FGF23 in uraemic cardiomyopathy was reported by the work of Gabner et al. (164) which showed that neonatal rat ventricular myocytes develop evidence of hypertrophy when exposed to FGF23 for just 48hrs. However, on removal of FGF23 stimulus LVH then regressed. Similar changes were also demonstrated *in vivo*. Mice with artificially elevated FGF23 levels initially were shown to develop LVH, which recovered following correction of FGF23 levels. These findings support the assertion that the cardiovascular remodelling in uraemic cardiomyopathy may be reversible. This is particularly important in the context of renal transplantation where restoration of renal function leads to significantly reduced FGF23 levels(165).

1.25 Evaluation of uraemic cardiomyopathy

1.25.1 Echocardiography

The first description of M-mode echocardiography was used in the study of preoperative mitral stenosis and regurgitation in 1953 by Inge Edler and Hellmuth Hertz (166). Since this initial work echocardiography has revolutionised the assessment of the cardiovascular system.

Multiple echocardiographic techniques are now employed in clinical practice. These include two- and three-dimensional (2D/3D) echocardiography, pulse, and continuous wave doppler, and colour doppler.

One of the first large scale studies using 2D echocardiographic to described the changes of uraemic cardiomyopathy in ESKD was conducted by Foley et al.(167). In this prospective cohort of 433 patients 36% were found to have LVSD, 32% left ventricular dilation, and 74% LVH. Following on from this early study, further echocardiographic techniques have been developed to allow assessment of uraemic cardiomyopathy even in its earliest stages. These include the use of tissue doppler velocities and trans-mitral pulse wave doppler velocity. Using this approach to quantify diastolic function Fashid et al. (168) examined 153 patients with CKD between 2007 and 2009. Results presented here again indicated that LVH was present in 75% of patients, however, this data also revealed that 85% of those studied also had some form of diastolic dysfunction based on echocardiographic criteria.

In addition to the use of doppler techniques echocardiographic measures of myocardial strain have been shown to useful in the assessment of diseases where there is diastolic dysfunction, including HCM, amyloidosis, and uraemic cardiomyopathy(169, 170). Kramann et al.(171) used rat models of uraemic cardiomyopathy to assess echocardiographic changes 4-6 weeks after the induction of kidney disease (171). Echo parameters obtained showed that global radial and circumferential strain parameters decreased significantly before standard measures such as fractional shortening showed any reduction. In addition, peak global and radial strain (Pearson correlation coefficient (PCC)=0.701 [P<0.001] and 0.678 [P<0.001]) and peak systolic radial and circumferential strain (PCC=0.613 [P<0.001] and 0.611 [P<0.001]) showed highly significant correlations with interstitial fibrosis as assessed by histology. In the same

article 171 ESKD participants also underwent speckle tracking echocardiography this showed that less negative measures of peak longitudinal strain (i.e., worsening cardiac function) were associated with an increased cardiovascular mortality.

Echocardiography has also been employed in numerous studies to evaluate LVM which is an established predictor of mortality in uraemic cardiomyopathy. Both M- Mode echocardiography and 2D echocardiography can be used to calculate LVM. The advantage of M-Mode is that it allows better endocardial border definition due to higher frame rates. However, accurate orientation of the echo beam is required to obtain accurate results. Alternatively 2D echocardiography, images the entire ventricle, allowing the presence of any asymmetry and regional wall motion abnormality to be fully appreciated. Accurately delineating endo and epicardial borders however can be more problematic due to lower lateral resolution and lower frame rates (172).

When using echocardiography to derive LVM there are several equations which have been proposed all of which are based on similar mathematical principals. The first attempt at standardising LVM calculation was from Troy et al.(173) which was based on M-mode image acquisition. Currently the methodology which is advocated the British Society of Echocardiography was proposed by Devereux et al. (174) and is given below.

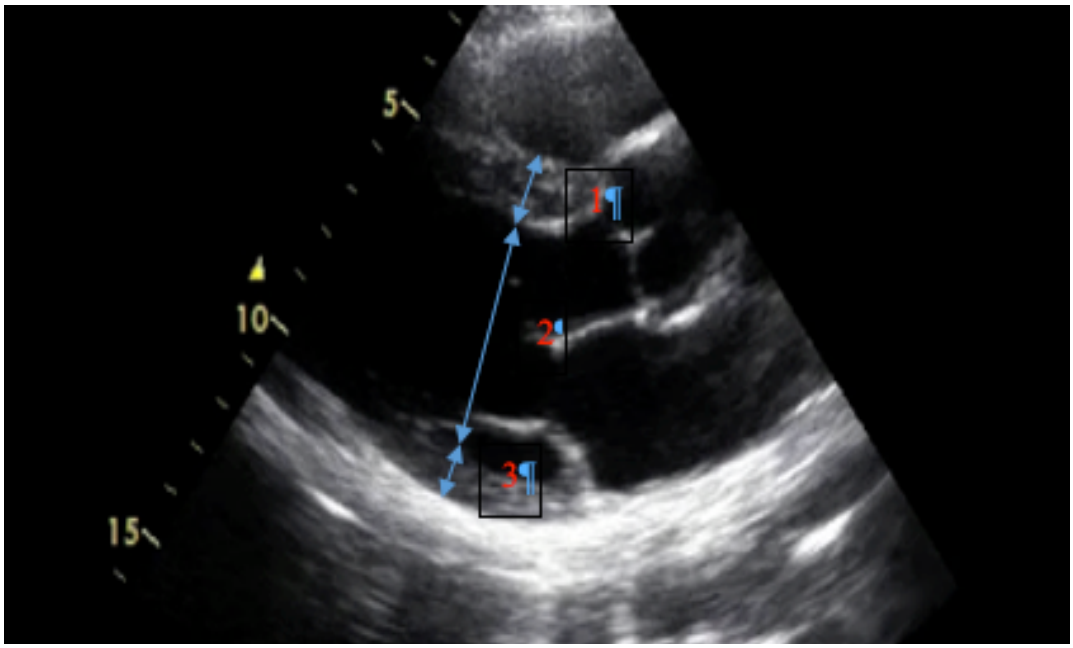
$$0.8[1.04[([LVEDD + IVSd + PWd]\ 3 - LVEDD3)]] + 0.6$$

LVEDD left ventricular end diastolic dimension

IVSd interventricular septum diastole

PWd posterior wall diastole.

Figure 1.8 Echocardioghic image - parasternal Long axis view.



Parasternal long axis view depicting

1. IVSd interventricular septum diastole

2 LVEDD left ventricular end diastolic dimension

3. PWd posterior wall diastole

The use of this linear approach to calculate mass is problematic. In ventricles where there is considerable asymmetric geometry there can be underestimation of the LVM. This is particularly true of conditions such as apical hypertrophic cardiomyopathy. In addition, the development of such formulae is often based on a small number of post-mortem findings, in the case of the above this was created using just 52 patients. As a result of such concerns Bachenberg et al. (175) assessed the accuracy of these formulae in assessing LVM by examining 93 patients who had undergone echocardiography within 30 days of death and subsequently underwent post-mortem examination. Result showed only a modest correlation (r=0.58) between the calculated LVM and actual LVM measured at post-mortem. All formulas

also showed increasing deviations as the LVM increased. As such in conditions such as uraemic cardiomyopathy where there may be considerable LVH the use of such methods may introduce considerable inaccuracy. A further consideration in CKD is also fluid status, particularly in dialysis recipients where interobserver variability of LVM measures using echocardiography are reported as being very high (40.1 ± 22.1g) (176)

1.25.2 Magnetic resonance Imaging

While echocardiography is undoubtable a useful tool in the assessment of patients with uraemic cardiomyopathy, it is now accepted that the gold standard cardiac imaging technique in chronic kidney disease and particularly ESKD is CMR (177). The accuracy of CMR to measure LVM has been demonstrated in both human and animal studies. Farber et al.(178) in a novel study directly measured the mass of 58 explanted hearts during orthoptic heart transplantation. These hearts were then prepared and suspended in saline and their mass evaluated using a 1.5T scanner. This demonstrated that there were strong correlations between 3D derived LVM (301 ± 93 g) and the directly measured LVM (313 ± 96 g; $r = 0.95$, $p < 0.001$). In a review of the subject Myersen et al. (179) also reported good agreement between CMR and direct measurements of LVM in human studies where the SD of the difference was ≈8 g (95% CI, ≈15 g) and in canine studies the SD of the difference was 10 g (95% CI, ≈19 g)

CMR has also been shown to have excellent reproducibility when compared to echocardiography. Stewart et al. (180) measured LVM with both CMR and echocardiography in 35 dialysis dependant patients. The results indicated that intra-observer variability was higher for echocardiography (30.9 ± 14.7 g) compared to CMR (8.8 ± 7.1 g). Inter-observer variability was also higher for echocardiography relative to CMR (40.1g ± 22.1g Vs. 15.7g ± 13.2g). Grothues et al. (181) also studied 60 patients (20 heathy, 20 LVSD, 20 LVH) each

undergoing 2 CMR studies and echocardiograms where LVM, volume and ejection fraction were measured. This indicated that there was good interstudy reproducibility across all parameters for CMR (r=0.94-0.99), which was much less for echocardiography (r=0.65-0.98). The calculated coefficients of variability were also substantially lower for CMR than echocardiography for all parameters. The obtained data also demonstrated that to detect a 10g change in LVM for those with LVH only 15 participants would be required using CMR compared to 152 for echocardiography.

In addition to accurately measuring the structure and function of the heart CMR also allows clinicians to obtain information about the myocardium. Myocardial fibrosis is a hallmark feature of uremic cardiomyopathy and is known to be related to an increased risk of arrhythmias, heart failure and sudden cardiac death (84). Historically the assessment of myocardial fibrosis was done via endomyocardial biopsy which is an invasive procedure with an associated risk of morbidity and mortality, it is also subject to error associated with sampling where the presence of fibrosis is not uniformly distributed (182). Due to these difficulties CMR methods have been applied, in order to non-invasively characterise changes in the myocardium associated with CKD.

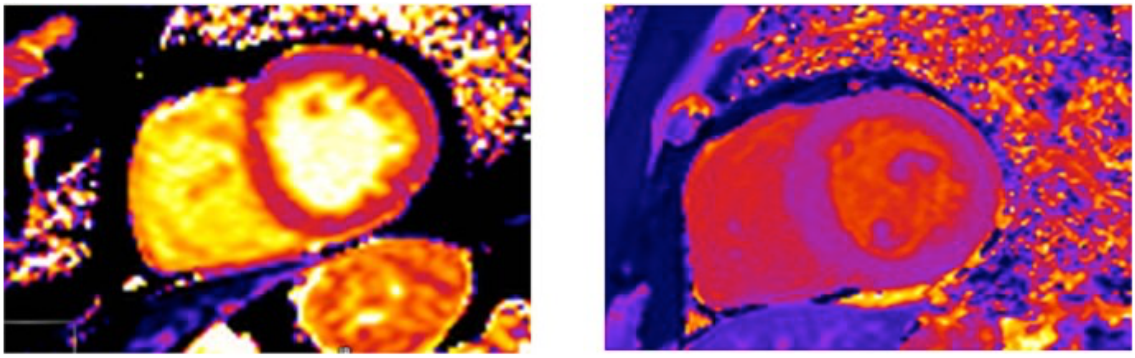
The use of late gadolinium enhancement (LGE) imaging in magnetic resonance imaging (MRI) imaging was initially developed to detect infarcted myocardium via delayed contrast wash-in and wash-out in tissues with increased extra-cellular space(84). This use of LGE has subsequently been expanded to use in other disease states including CKD with LGE reported in up to 28% of those with ESKD(183). Due to the diffuse nature of fibrosis in CKD, LGE assessment is, however, now considered to be a insufficiently sensitive measure as it relies on changes in relative signal intensity which may be difficult to detect (184). Price et al.(185)

studied 159 pre-dialysis patients who had undergone LGE imaging. LGE was present in 34% of subjects with multiple patterns of LGE demonstrated which did not correlate to cardiac structure and function. in addition, over 7 years of follow-up LGE was not associated with adverse cardiac outcomes. In addition, since 2006 there are safety concerns regarding the administration of Gadolinium based contrast agents (GBCA) in ESKD due to the associations with the development of nephrogenic systemic fibrosis (NSF) (186).

Due to these difficulties, tissue characterisation techniques in ESKD have focused on developing native or non-contrast methods. Native T1 mapping time measures the longitudinal relaxation times of hydrogen ions after applying inversion magnetisation pulses (187). Several techniques have been developed but the general principal of native T1 mapping is to acquire multiple images with different TI weightings. The data for each pixel across the images is then used to create inversion recovery curves. In this way the T1 relaxation times can be established (187). The most commonly used method of native T1 mapping in clinical practice is the Modified Look Locker sequence (MOLLI) first described by Messroghli et al. (188).

Several studies have examined the application of T1 mapping in CKD. In terms of its relationship to histological findings, Hakamori et al. (189) showed that in 36 subjects with dilated cardiomyopathy (DCM) native T1 mapping was significant correlated with biopsy proven collagen fraction ($r=0.77$ $p<0.05$). Rutherford et al. (83) compared T1 values of 33 incident HD patients and 28 age- and sex-matched healthy volunteers. Results indicated that in global, septal, and mid septal T1times were significantly higher in the HD group (Global 1171 ± 27 ms vs. 1154 ± 32 ms; septal T1 HD 1184 ± 29 ms vs. 1163 ± 30 ms; and mid septal T1 HD 1184 ± 34 ms vs. 1161 ± 29 ms). T1 times were also noted to be correlated with LVMI ($r=0.452$ $P=0.008$). Graham-Brown et al. (84) also reported that septal T1 times were

significantly higher in 35 HD patients compared to 22 healthy controls (1270 vs. 1085 ms). Edwards et al. (190) also highlighted that septal T1 times are higher in patients with CKD stage 2-4 compared with healthy sex matched controls (986 ± 37 vs. 955 ± 30 $p<0.05$). Hayer et al. (191)also demonstrated that T1 times increased incrementally through the stages of CKD from 2-5 (966+/-33ms 994+/-33ms $p<0.001$). Contti et al. (192)also found significant reductions in T1 times observed at 6 months following renal transplant (1331 ± 52 ms vs. 1298 ± 42 ms $p=0.001$).



A **B**

Figure 1.9 Example of T1 mapping analysis performed in CVI42.

T1 mapping of the SAX image of the mid left ventricular cavity (A) (Global T1 time 1385 ms) and (B) (Global T1 time 1170 ms) of 2 peritoneal dialysis patients consistent with high and low levels of cardiac fibrosis respectively (reproduced from Law et al. (103))

It is also possible to further characterise the myocardium using T2 mapping. Multiple sequences have been proposed including T2 turbo spin multi-echo, T2 prepared state free procession as well as gradient spin echo mapping sequences. T2 values are directly related to myocardial water content, and as such were first used in the detection of myocardial oedema related to acute ischaemia (193). T2 mapping has also been used in the characterisation of the myocardium in CKD. Arcari et al. (194) performed CMR studies including T1 and T2 mapping on 154 CKD patients, 163 hypertensive patients, 158 patients with hypertrophic cardiomyopathy and 133 normotensive controls. The finding here demonstrated that in all these conditions associated with LVH T1 mapping times significantly higher than control participants. It also highlighted that in CKD T2 times were significantly higher than controls. There was also a stepwise increase in T2 times as CKD stage progressed. In addition, only in CKD participants was there a significant correlation between T2 times and LVM (r=0.231,

P<0.01). This finding suggested that intramyocardial fluid may play a key role in the LVH observed in uraemic cardiomyopathy.

1.26 Kidney Transplantation

The first ever kidney transplantation took place in a dog in 1902 where a kidney was attached to the carotid artery; it survived 2 days. Although never formally published the first ever human to human kidney transplant is attributed to Dr Levi Hammond in the USA 1911, this however was not successful (195). A second attempt in 1933 by Yurii Voronoy using a cadaveric organ was noted to have produced urine prior to failing at 48hrs. The first successful kidney transplant took place within the USA in 1954. This initial procedure was conducted between identical twins and was a success with the recipient living a further nine years following transplantation before dying of a heart attack. While this initial procedure was a success further transplantation between non-genetically identical persons produced far fewer encouraging results. This was due primarily to the lack of effective immunosuppression regimes to prevent graft rejection. As a result, a major challenge for the field of transplant medicine has been the development of effective immunosuppression regimes (196).

In the UK today renal transplantation is considered the gold standard of care for renal replacement therapy. Since the first renal transplant was conducted in Edinburgh in 1960 the number of transplants performed each year across twenty centres has continued to increase. In 2021 there were 3,525 adults active on the kidney transplant waiting list. There were 3190 kidney only transplants performed between 2019/20 of these 1326 were from deceased brain-dead donors, 915 were from deceased circulatory dead donors and 949 were from living donors. The national rate of graft survival in 2010 at five years for living donation was 93% and 86% for deceased donation (197).

1.27 Renal Transplantation and Cardiovascular Disease

The current gold standard for the treatment of those with ESKD is renal transplantation (72). Following transplantation all-cause mortality and CVD mortality fall dramatically (198). The USRDS report (2018) examining mortality in ESKD reported that adjusted mortality was 160.8 per thousand patient years for HD patients, 131.7 for PD patients and 48.9 renal transplant recipients(11) . In addition, significant reductions in the proportion of renal transplant recipients dying of cardiovascular causes compared to both HD and PD were observed. The most dramatic reductions were observed in mortality related to SCD, with 44% of all HD patients, 40 % of all PD patients and 21% of renal transplant recipients dying of SCD. It should however be noted that both overall mortality and cardiovascular mortality remain considerably higher in transplant recipients than age and sex matched controls (199).

Following transplantation, improved cardiovascular mortality observed is often attributed to a regression in the cardiovascular remodelling associated with uraemic cardiomyopathy (200). In a recent study comparing arterial stiffness in renal transplant recipients and CKD patients demonstrated that at 12 months post-transplant recipients have a significantly lower arterial stiffness than those with CKD, 10.5m/s vs11.0m/s p=0.008. Meta-analysis data comparing pre- and post-transplant arterial stiffness has also reported reduction in aortic PWV of 1.14m/s p=0.02 (201). This was based on 225 transplant recipients across seven studies; however, very high levels of study heterogeneity were reported (I^2=83%), none were controlled, or observer blinded. Furthermore, only two of these studies examined changes in PWV corrected for mean arterial pressure which produced conflicting findings (202). At present therefore the available data suggesting that arterial stiffness reduces following transplantation is weak. In addition to arterial stiffness, it is also suggested within the literature, that the LVH associated with uraemic cardiomyopathy is reversed following renal transplantation (73, 203). This assertion however is not based on robust evidence. **Chapter 2** of this thesis will therefore address this question.

1.28 Summary

Over half of deaths in ESKD are due to cardiovascular disease. The majority of these deaths are not due to myocardial infarction as a result of coronary atheroma, but due to heart failure and sudden cardiac death. This unique burden of cardiovascular disease is attributed to the development of uraemic cardiomyopathy, which is characterised by LVH and increased arterial stiffness. The gold standard treatment for ESKD is renal transplantation, this is associated with considerable reductions in cardiovascular mortality. In the literature it is often cited that renal transplantation produces significant structural remodelling in the cardiovascular system which is responsible for this reduction. The current evidence-base, however, is of poor quality with an over reliance on echocardiography. It is therefore the aim of the RETRACT study to address these shortcomings within the literature by examining the effects of renal transplantation on cardiovascular structure and function employing CMR.

Chapter 2: CHANGES IN LEFT VENTRICULAR STRUCTURE AND FUNCTION ASSOCIATED WITH RENAL TRANSPLANTATION: A SYSTEMATIC REVIEW AND META-ANALYSIS

This chapter was previously published in European Society of Cardiology Heart Failure.

The initial idea for this review was conceived by the research fellow The literature search and all analysis were also performed independently by the research fellow. All drafting of the manuscript and edits following peer review were carried out the research fellow.

Reference

Pickup LC, Law JP, Radhakrishnan A, Price AM, Steeds RP, Smith T, Townend JN, Ferro CJ. Changes in left ventricular structure and function associated with renal transplantation, a systematic review and meta-analysis. ESC Heart Fail. 2021 Mar 15.

PROSPERO registration number: CRD4201811

Abstract

Objective

This meta-analysis set-out to examine if the changes associated with uraemic cardiomyopathy in end-stage renal disease are reversed by renal transplantation.

Method

MEDLINE, Embase Open Grey, and the Cochrane Library databases were searched from 1950 to February 2018. Studies were included if they used any imaging modality to examine LVMI, both before and after successful renal transplantation. Study methodological quality was assessed using the Newcastle Ottawa Scale. Meta-analyses were performed where possible and when inappropriate a narrative review of the data was presented.

Results

Twenty-five studies including 1738 renal transplant recipients were included. Twenty-three included studies used echocardiography and two used CMR as their principal imaging modality. The methodological quality of the evidence was graded as poor. Four studies recruited control groups, two using CMR and two using echocardiography. Meta-analysis of these studies indicated that there was no difference in the LVMI of control patients and transplant recipients at follow-up (Standard Mean Difference -0.09 [95%CI -0.44 to 0.26] p=0.63). There was also no difference observed in left ventricular ejection fraction in the two controlled studies where this was reported (Mean Difference, 0.39 % [CI -4.09 to 4.87%] p=0.86).

Conclusion

The current evidence does not support the notion that uraemic cardiomyopathy is reversible by renal transplantation. However, the evidence is limited by methodological weaknesses, which should be considered when interpreting these findings.

2.1 Introduction

Over half of deaths in end stage chronic kidney disease (ESKD) are due to cardiovascular disease; the age corrected relative risks are extreme reaching over 100 fold in younger subject (204). Most of these deaths appear not to be due to myocardial infarction as a result of coronary atheroma as occurs in the general population, the majority are due to heart failure and sudden cardiac death (205-207). Consistent with this observation, treatments for traditional cardiovascular risk factors such as hypertension and cholesterol lowering are relatively ineffective in this population (207-209). These observations can be explained by the near universal syndrome of uraemic cardiomyopathy in patients with ESKD (104, 177). Hypertrophic and fibrotic changes are widely held to account for the high incidence of arrhythmic and heart failure events in this group.

Uraemic cardiomyopathy is characterised by the presence of increased LVM and hypertrophy (LVH), ventricular dilatation along with systolic and diastolic dysfunction. Histologically, the myocardium exhibits disarray of myocytes which are severely hypertrophied in addition to the presence of diffuse interstitial fibrosis (30). As renal function declines these features become more prevalent and are present in up to 90% of those requiring renal replacement (72). Such cardiovascular changes are strongly linked to cardiovascular outcomes with the presence of LVH associated with increased mortality in both transplant recipients and those requiring HD.

The current gold-standard for the treatment of patients with ESKD is renal transplantation(72). The associated improvement in eGFR reduces cardiovascular risk below that of those who remain on waiting lists (210). However, CVD risk still remains higher than healthy individuals of the same age and sex with transplant recipients displaying a three-fold increased risk (198). The restoration of renal function associated with successful renal transplantation improves

many of the factors thought to cause uraemic cardiomyopathy. Indeed, uraemic cardiomyopathy is often said to be reversed by renal transplantation (73, 203). However, this assertion is largely based on the reduction of LVMI reported small echocardiographic studies (73, 203). However, echocardiography has proven unreliable to accurately measure LVMI, with a strong tendency to overestimate, in situations of fluid overload, particularly in patients on HD (180). As a result, CMR is considered an accurate and volume-independent methodology for assessing cardiac dimensions, is now accepted as the gold standard imaging modality for patients with EKRD (177).

The aim of this study was to perform the first systematic review and meta-analysis to establish if the features of uraemic cardiomyopathy are reversible following successful renal transplantation.

2.2 Methodology

A PRISMA compliant systematic review was conducted (211). Published and unpublished articles and conference proceedings registered on or before 1 February 2019 were searched. The electronic databases used to search the published literature were MEDLINE, EMBASE, Open Grey and the Cochrane Library (clinical trials database and database of systematic reviews). All searches were limited to adult human studies. Reference lists of all pertinent review papers and eligible studies were reviewed. The search terms used (medical subject headings (MeSH) and keywords) are presented for the MEDLINE search in Supplementary **Figure2.1**. These were modified for the specific databases searched.

Inclusion Criteria

All full-text articles assessing changes in LVMI to either body surface area or height, before and after successful renal transplant using any form of imaging technique were included. Single-subject case reports, comments, letters, editorials, protocols, guidelines, or review

papers were excluded. Studies were also excluded if participants received more than one organ type.

2.2.1 Study Selection

Two reviewers (L.P, J.L.) independently reviewed all titles and abstracts generated from the search strategy. Following this initial screening process, the full texts of eligible articles were reviewed independently by each author against the predefined eligibility criteria

2.2.2 Critical Appraisal

All papers were critically appraised independently by two reviewers (L.P, A.R.). Methodological appraisal was conducted using the Newcastle-Ottawa scale (212). A maximum score of nine points can be awarded based on participant selection, comparability and study outcome including follow-up. Scores are defined as poor (0-3) fair (3-6) and good (6-9). Any disagreements between the two reviewers were adjudicated by a third reviewer (J.L) to gain a consensus through discussion

2.2.3 Outcome measures and data extraction

Two reviewers (L.P, J.L.) extracted all data into a pre-constructed table. Information gathered included: number of participants, age range, sex distribution, dialysis modality, immunosuppression regime and time to follow-up after transplantation.

The primary outcome measure was LVM and LVMI. Secondary outcome measures included: left ventricular dimensions, measures of diastolic and systolic function

2.2.4 Statistical Analysis

Statistical analysis was conducted using Review Manager 5.0 for Apple (Nordic Cochrane Centre, Copenhagen, Cochrane Collaboration, 2008). Statistical heterogeneity was assessed by χ^2 and I^2. If χ^2 was greater than P=0.10 and the I^2 statistic indicated that heterogeneity was present (>20%), a random-effects statistical model was adopted to calculate mean difference

(MD) or standardised mean difference (SMD) between groups. When $\chi^2$and I^2 values demonstrated low heterogeneity a fixed-effects model was adopted (213). Where this was not possible due to insufficient data a narrative approach was adopted.

2.3 Results

2.3.1 Search Strategy Results

A total of 2356 potentially relevant citations were identified, with 25 being eligible for inclusion. The results of the search strategy are summarised in **Figure 2.1**. The characteristics and outcomes of the 25 included studies are presented in **Figure 2.3**.

Figure 2.1 Prisma search strategy.

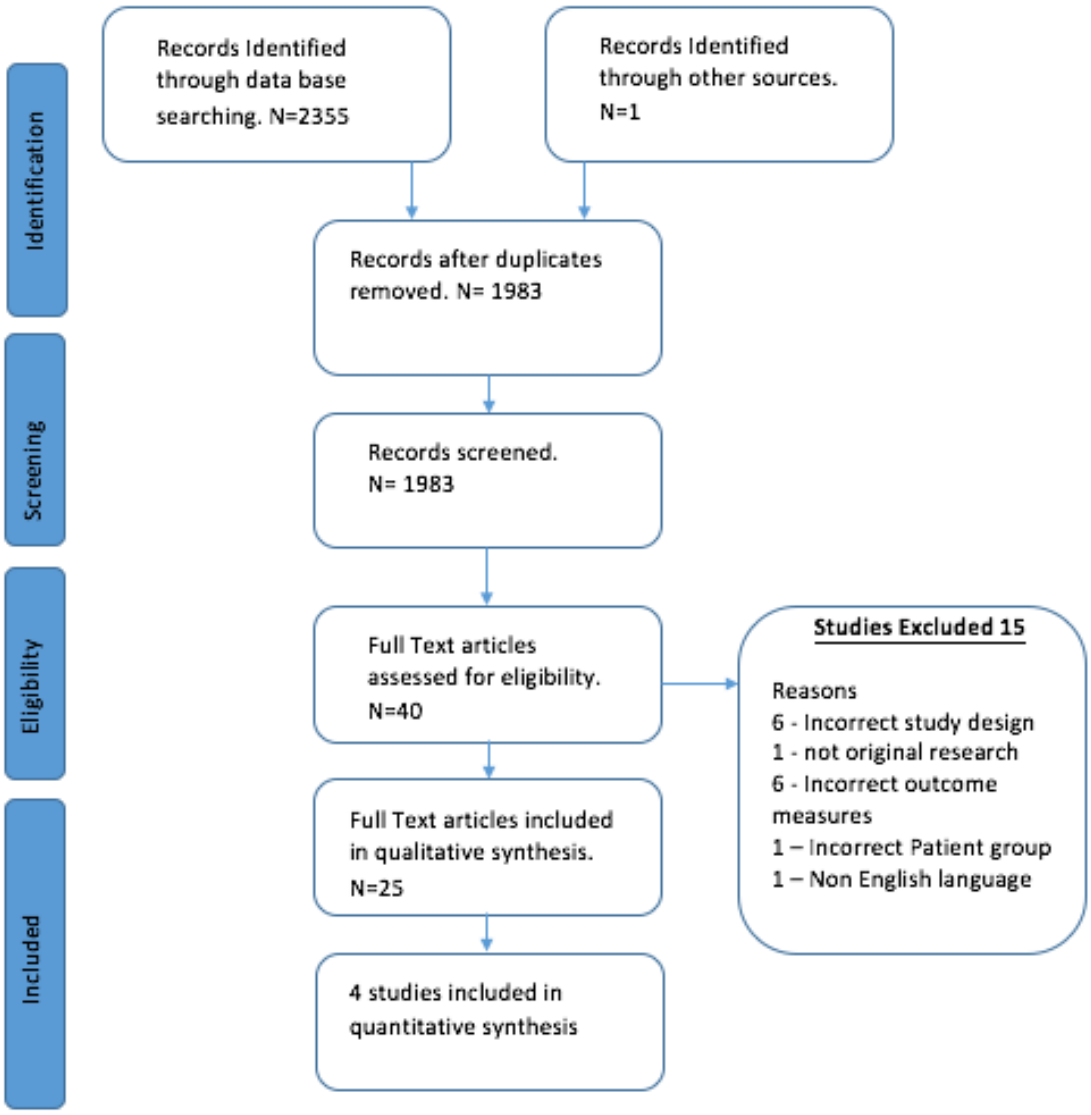


Figure 2.2 Example of search strategy employed for Ovid Medline.

1. humans$.mp.
2. adult$.mp.
3. male$.mp.
4. female$.mp. [
5. exp ADULT/
6. 1 or 2 or 3 or 4 or 5
7. cardiac imaging$.mp.
8. echocardiography.mp.
9. magnetic resonance imaging$.mp.
10. exp Heart Ventricles/dg [Diagnostic Imaging]
11. Magnetic Resonance Imaging/
12. exp ECHOCARDIOGRAPHY,
13. 7 or 8 or 9 or 10 or 11 or 12
14. kidney transplant$.mp.
15. exp Kidney Transplantation/
16. exp Kidney Failure, Chronic/
17. exp Renal Insufficiency, Chronic/
18. 14 or 15 or 16 or 17
19. exp Heart Ventricles/
20. exp HYPERTROPHY, LEFT VENTRICULAR/
21. exp Cardiomegaly/
22. exp Ventricular Function, Left/
23. exp Ventricular Dysfunction, Left/
24. left ventricular hypertrophy$.mp.
25. left ventricular mass$.mp.
26. 14 or 15 or 16 or 17 or 18 or 19 or 20 or 21 or 22 or 23 or 24 or 25
27. 6 and 13 and 18 and 26

2.3.2 Methodological Appraisal

A total of 23 studies used echocardiography. The methodological quality of these was generally poor (**Figure 2.3**). Only two studies (214, 215) classified as fair and none classified as good. Two studies recruited control groups, which consisted of individuals receiving HD (214, 215). Assessor blinding was only employed in four studies (216-219) and none used a sample size calculation.

Two studies employed CMR both of which were classified as methodologically good (220, 221). In both, recipients and controls were recruited from local transplant waiting lists. Prasad et al. (220) used a sample size calculation. This, however, was designed to detect changes in Adiponectin levels rather than LVMI. Assessor blinding was employed in both cases (220, 222).

The length of follow-up across all the studies varied from one week to five years, the most common follow-up time-point being 12 months.

2.3.3 Study Population

In total, 1738 renal transplant recipients were included of which 1205 were male and 533 females. The pooled weighted mean age was 43 years (range 16-85 years). Thirteen studies reported type of transplant with a total of 870 live donors and 364 deceased donor recipients (214, 218, 220, 223-232). Twenty studies included information regarding renal replacement therapy of recipients, with 1090 receiving HD and 161 receiving PD (217-221, 223, 226-230, 232-238). In total, 127 control patients were recruited in four studies, 102 of whom were receiving renal replacement therapy (214, 215, 220, 221).

Aetiology of ESKD was reported in 10 studies (214, 215, 217, 220, 223, 224, 227, 234, 236, 238) with glomerulonephritis being the most commonly reported cause. Four studies excluded

patients with either ischaemic heart disease or congestive cardiac failure (216, 217, 223, 236) and a further two (238, 239) only included patients who were asymptomatic from any cardiovascular disease. Prasad et al. (220) reported that 10% of transplanted patients had undergone prior coronary revascularisation. Vaidya et al. (219) reported that 43% of their cohort had a prior diagnosis of coronary artery disease and McGregor et al. (235) indicated that 84% of participants were classified as having a DCM at baseline.

Figure 2.3 Selected Data for all studies included in systematic review.

.		Subjects	Age (Years)	Follow-up	Primary outcome measure findings	NOS	Comments
Echocardiographic Studies							
Hamidi et al. (2018)	2D	25 recipients on HD.	44.64 ± 13.91	1 month	Significant reductions in LVMI (g/m^2) -73.82 ± 11.6; $P < 0.001$, and relative wall thickness (-0.056 ± 0.023; $P = 0.021$).	Poor	Prospective Blinded Non-controlled Iran
Hewing et al. (2016)	2D	31 recipients.	44y range: 19-85	Median 19 months [range13-32]	Significant reduction in LVMI (g/m^2) 111. 2 [IQR 88.7-150.6] to 103.8 [IQR 78.4-113.8] p=0.001. No change observed in LV diastolic function.	Poor	Retrospective Non-blinded Non-controlled Germany
An et al. (2015)	2D	767 recipients.	45.0 ± 11.5	1 week 1 year 5 years	Significant reductions in LVMI (g/m2) at 1 and 5 years compared to pre-transplant and 1week p<0.001. (Baseline 129.1 (IQR 103.0 -161.6) 1 wk. 130.4 (IQR 103.7, 161.6) 1yr 119.9 (IQR 96.5, 150.4) 5yrs 110.0 (IQR 90.4, 137.2) P <0.001.	Poor	Retrospective Non-blinded Non-controlled Korea
Deng et al. (2013)	2D	48 recipients with no history of MI, cardiomyopathy, CHF, arrhythmias, or OSA.	Range (36-67)	6 months	Significant reduction in LVMI (g/m^2) from 104.00±16.47 to 95.50±21.44 p=0.043.	Poor	Prospective Blinded Non-controlled USA
Salerno et al. (2013)	2D	104 recipients assigned to two alternative immunosuppression strategies; CNI+EVE (28) or CNI +MMF (76).	CNI + EVE 47.5 ± 13.1 CNI + MMF 47.8 ± 12.1	36 months	No significant difference between immunosuppression groups. Both showed significant reductions in LVMI at 3 years in everolimus group (126.5±46.4 to 121.9±39.4 g/m^2), in the mycophenolate group (116.6±38.3 to 113±28.9 g/m^2; $P < 0.05$).	Poor	Retrospective, Non-blinded Non-controlled Italy
Vaidya et al. (2012)	2D	105 recipients with ≥1 year of CKD prior to Tx.	53.8 ± 12.3	Mean 2.2 years	57 participants had significant LVMI g/m^2 decrease (mean difference −37.2±31.3 g/m^2) and 48 had no significant regression (mean difference 15.7±17.1 g/m^2). The extent of the LVM before transplant was the only predictor of LVM regression (odds ratio 1.50, 95% CI (1.26 to 1.80).	Poor	Retrospective Non-controlled Non-Blinded
Souza et al. (2012)	2D	40 live donor recipients. Consecutive recruitment 2008-2010.	31.6 ± 12.7	1 month 3 months 6 months	Significant reduction in LVMI (g/m^2) from baseline 131.48±38.93, to 1 month 126.41±29.45 p<0.05, to three months 128.81±30.71and 6 months 113.03 ± 29.99 (p=0.02 comparison between 6 months and baseline. No significant difference between other time follow-up times and baseline).	Poor	Prospective, Non-blinded, Non-controlled. Brazil

Namazi et al. (2010)	Not stated	47 recipients with no history of cardiovascular disease	Range 23-56	4 months	Significant reduction in LVMI(g/m^2) from baseline 120 (SD not given) to 110 (SD not given) p=0.002.	Poor	Prospective Non-blinded Non-controlled Iran
Keven et al. (2008)	2D	28 recipients on HD and 23 matched controls on HD	34 ± 9	12 months	No significant change in LVMI (g/m^2) between transplant132±38 and HD145±38 p<0.05.	Fair	Prospective Non-blinded Controlled Turkey
Iqbal et al (2008) * Group 1	2D,	22 recipients.	31± 9	3 months	LVMI (g/m^2) reduced at 3 months from 379±114 to 248±58 g/m^2 ($P < 0.001$).	Poor	Retrospective Non-blinded Non-controlled Bangladesh
Group 2		30 recipients.	31± 8	3months 6 months 12 months	LVMI (g/m^2) reduced significantly from baseline 275 ± 91 at 3 months 191±38, 6 months 173±39 and 12 months159 ± 26 g/m^2; p < .001.		
Hernández et al. (2007)	2D	60 participants with stages 4–5 CKD patients without ischaemic heart disease or congestive cardiac failure. The cohort was divided based on the presence of LVH at the start of the study.	LVH Group 52 ± 12 No LVH Group 48 ± 12	Prior to starting dialysis - median 8 months. During dialysis - median 3 months. After transplant median 19 months.	52% (23) of participants with no LVH at baseline developed LVH or > 20% increase in LVMI at follow-up. 22% (8) participants with LVH at bassline showed regression to normal at follow-up.	Poor	Prospective Non-Blinded Non-controlled Spain
Montanaro et al (2005)	Not Stated	23 recipients without diabetes.	43 ± 10	24 months	LVMI (g/m2) reduced significantly at 24 months from 161.4±48.2 to 122.1±27.7 (p<0.007).	Poor	Retrospective Non-blinded Non-controlled USA.
Ferreira et al (2002)	2D	24 recipients on HD.	33.5 ± 10.0	3 months 6 months 12 months	LVMI (g/m2) reduced significantly at 12 months from 164.6±47.0 to 130.5 ± 39.8 (p=0.004). The incidence of LVH decreased from 75 to 52.1% 12 months post-transplant.	Poor	Prospective Non-blinded Non-controlled Brazil
Sahagun-Sanchez et al. (2001)	2D	13 recipients on HD	33.64 ± 10.13	3 months 4 months	Reduction in LVMI (g/m^2) from baseline 102.8±27.7 to 3 months 83.5±18.1 and 4 months 71.5±16.2 p=0.001.	Poor	Prospective Non-blinded Non-controlled Mexico
McGregor et al. (2000)	2D	67 recipients on HD.	38.3 (18.7-64.5)	4 months	No significant change in LVMI (g/m^2) from baseline 143 (range 61-48) to 4 months 145 (range 62-37) p=0.71).	Poor	Prospective Non-blinded Non-controlled UK

Hernandez et al. (1997)	2D	38 non-diabetic recipients on HD, stratified according to genotype either DD or ID+II of intron 16 of the ACE gene.	DD group 46.2±.1 ID+II group 45.2±2.9	6 months 12 months	LVMI increased significantly at 12 months in those with DD genotype 166.6±10.4 to 201.5±21.6 p<0.05. There was no change in LVMI in the ID+II groups 181.3±9.1 to 176.9±9.4 p>0.05.	Poor	Prospective Non-blinded Non-controlled Spain
Palfrey et al. (1995)	2D	102 recipients.	37±12	12 months	LVMI (g/m^2) reduced significantly from baseline 158±39 to one year 132±39 (p<0.001).	Poor	Prospective Non-blinded Non-controlled Canada.
De Lima et al. (1994)	2D	Transplant Group 17 live donor recipients previously on HD for at least 1 year. Control Group 36 ESKD patients on HD for at least 1 year.	Transplant Group 44 ± 13 HD Group 40.5 ± 10	Minimum 15 months.	No change in LVMI in recipients (156.7±51.3 vs. 132.9±31.0 g/m^2, p>0.05). or controls (170.6±50.8 vs. 155.6±43.1 g/m^2, p>0.05.	Fair	Prospective Non-blinded Controlled Brazil
DeCastro et al. (1993)	2D	23 nondiabetic recipients on HD.	39.1±13.7	1 Year	LVMI (g/m^2) decreased from 157.78±53.5 to 108.1±19.5 (P-value not stated).	Poor	Prospective Non-blinded Non-controlled Italy
Huting. (1992)	2D	24 recipients on HD.	47 ± 12	Mean 41± 30 months	No significant change in LVMI (g/m^2) from baseline 175±48 to follow-up 171±49; p=0.05.	Poor	Prospective Non-blinded Non-controlled Germany
Larrsson et al. (1986)	M-Mode	27 recipients with juvenile onset diabetes.	33 range (27-45)	6 months 13 months 44 months	LVMI (g/m^2) decreased from baseline 176±51, to 6 months 143±44, 13 months 133 ±44 and 44 months 111±22 p<0.01.	Poor	Prospective Non-blinded Non-controlled Sweden
Ikaheimo et al. (1982)	M-Mode	13 recipients on HD.	31 (20-50)	Baseline echo performed both before and after HD session prior to transplant. 9 months	LVMI (g/m^2) decreased from baseline pre-HD session 197.7±44.8 and post-HD session143.5±47.3; to 143.5±47.3 p=0.001 after transplant.	Poor	Prospective Non-blinded Non-controlled Finland
Cuerto-Garcia et al. (1981)	M-Mode	18 non-diabetic recipients.	28.8 (SD/range not given)	31.16±23.59 weeks	LVM (g) decreased significantly from baseline 286.6±64.4 to follow-up 182.7±55.7 p<0.0005.	Poor	Prospective Non-blinded Non-controlled Mexico
CMR Studies							
Prasad et al. (2018)		39 live donor transplant recipients and 43 patients who remained on the transplant waiting list.	Transplant Group 46.5 ± 12.4	12 months	No difference in LVMI change at one year between recipients −1.98±5.5 and waiting list patients −0.36±5.7 g/m^2 p=0.44.	good	Prospective Blinded Controlled UK

		Control Group 55.5 ± 11				
Patel et al. (2008)	25 transplant recipients and 25 patients on the transplant waiting list.	Transplant Group 45.9 ±14.4 Control Group 52.7 ± 10.4	12 months	No significant difference in LVMI change (%) at 12 months between recipients and those who remained on the waiting list, 2.75±9.1 *vs* 3.6±16.7 p=0.10.	Good	Prospective Blinded Controlled UK

Abbreviations
CNI: Calcineurin inhibitor, D: deletion, EVE: Everolimus, HD: haemodialysis, I; insertion, LVMI Left ventricular mass index, LVM: Left ventricular mass, LVH: left ventricular hypertrophy, LVEF: left ventricular ejection fraction, OSA: obstructive sleep apnoea, PD: peritoneal dialysis SD: standard deviation TX: Transplant.

2.3.4 Left Ventricular Mass Index

Nineteen echocardiography studies reported the mean changes in LVMI following transplantation for their entire cohort with 16 reporting significant reductions in LVMI at follow-up. The magnitude of change observed varied greatly between studies. Iqbal et al. (231) in a cohort of 22 participants reported the largest reduction in LVMI of 131g/m^2 three months after transplantation. Three studies found no significant change in LVMI (215, 239).

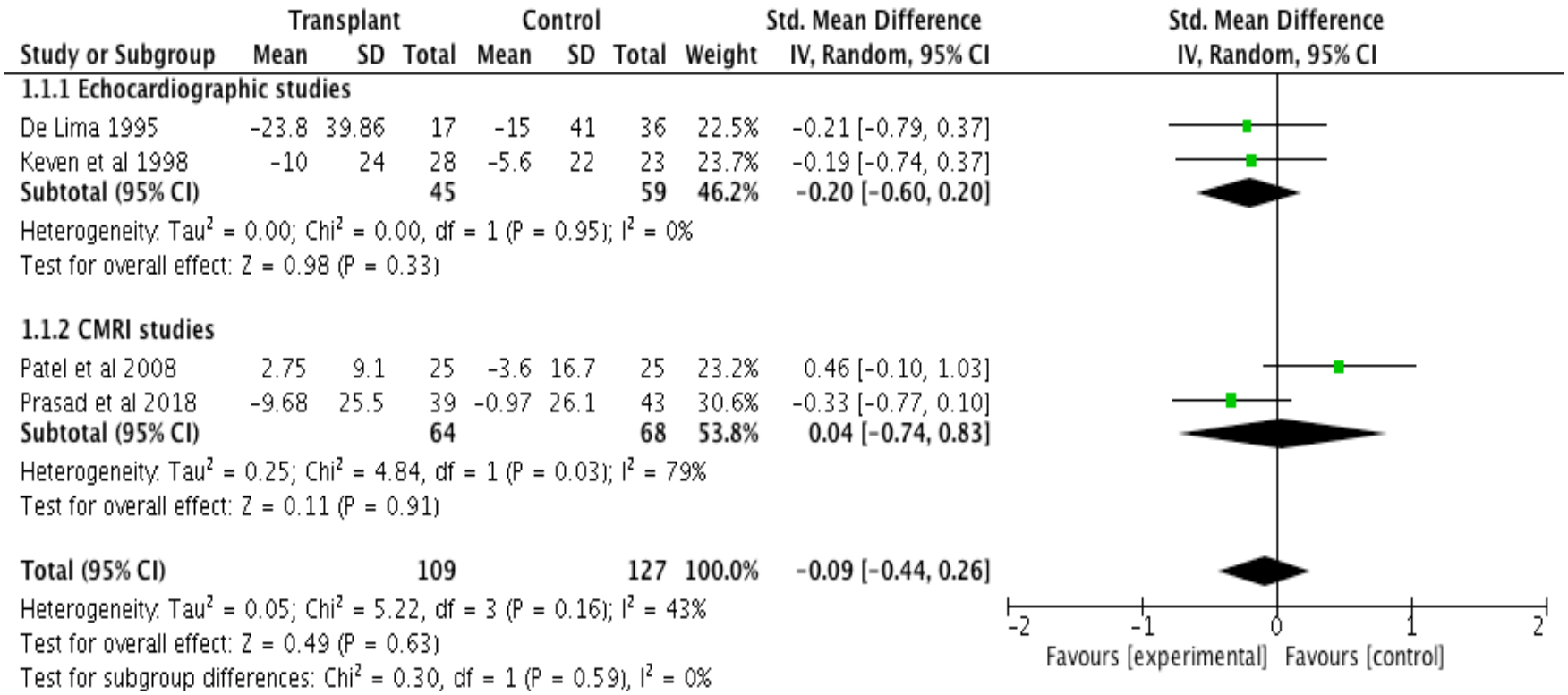
Four echocardiography studies only presented changes in LVMI based on predetermined sub-groups and not for the whole study group (218, 219, 232, 237). Two studies examined the effect of baseline LVMI on subsequent changes (219, 232). In their cohort Vaidya et al. (219) reported pre-transplantation LVMI was the only predictor of subsequent regression following transplantation (odds ratio 1.50, 95% CI: 1.26-1.80). Hernandez et al. (232) studied 60 patients with initial LVMI shown to be independent predictor of subsequent change in LVMI. Salerno et al. (237) examined changes in LVMI in patients treated with either everolimus or mycophenolate mofetil. While the reduction seen in both groups was significant from baseline to follow-up (everolimus 126.5 ± 46.4 to 121.9 ± 39.4 g/m^2; $p<0.05$, Mycophenolate 116.6 ± 38.3 to 113 ± 28.9 g/$m^{2;}$ $p<0.05$) there was no difference between the groups. Hernandez et al. (218) studied the effect of angiotensin converting enzyme (ACE) polymorphisms. Those with an unfavourable genotype (highest ACE activity) had a significant increase in LVMI after transplantation (23.3 ± 7.9%; $p<0.05$) whereas in those with a more favourable genotype no change was observed (-0.08 ± 4.9%; $p>0.05$).

Both included CMR studies and two using echocardiography studies recruited a control group. The two echocardiographic studies showed no significant change seen in LVMI following transplantation compared to the control group (214, 215). Keven et al. (214) studied 28

transplant recipients and 23 HD patients with follow-up at one year. There was a significant reduction of LVMI in transplant recipients from baseline to follow-up, however, the magnitude of change observed was not significantly different from that observed in the control group (132 ± 38 g/m^2 vs 145 ± 38 g/m^2; p>0.05). De Lima et al. (215) studied 36 HD patients and 17 transplant recipients and at mean follow up of 30 ± 8 months. In both groups no significant change was observed in LVMI from baseline to follow-up (LVMI g/m^2 Transplant group 156.7 ± 51.3 to 132.9 ± 31.0; p>0.05, HD group 170.6 ± 50.8 to 155.6 ± 43.1; p>0.05) The two CMR studies reported no significant overall change in LVMI following transplantation compared to the control group (220, 221). However, the trends in mean change were conflicting. Patel et al. (221) observed an increase in LVMI in transplant recipients and a decrease in the control group of HD patients (Recipients +2.9g/m^2 [95%CI -6.3 – 11.9]; Controls -3.6g/m^2 [95%CI -20.3 – 13.1] p=0.1). Prasad et al. (220) reported a reduction in LVMI in both recipients and controls who remained on the waiting list. (Recipients -1.98 ± 5.5 g/m^2, Controls -0.36 ± 5.7 g/m^2, p=0.44).

A meta-analysis of the four studies reporting change in LVMI in transplant recipients and controls was conducted (**Figure 2.4**). At total of 236 participants were included in this analysis, the overall SMD was -0.09 [95%CI -0.44 to 0.26] p=0.63), suggesting no difference between transplant and control groups. However, heterogeneity was moderate (I^2=43%). Subgroup analysis is also presented based on imaging modality. The two echocardiographic studies (214, 215) (standard mean difference -0.20 [95%CI -0.60 – 0.20] p=0.33) and the two CMR studies (220, 221) (SMD 0.04 [95% CI: -0.74 - 0.83]) showed no mean change in LVMI. There was no significant difference between the findings of the two imaging modalities (p=0.59). However, heterogeneity in the echocardiographic sub-analysis was low (I^2=0%) but substantial (I^2=79%) in the CMR sub-analysis

Figure 2.4 Meta- analysis of changes in LVMI following renal transplantation. Sub-group analysis presented based on imaging modality.

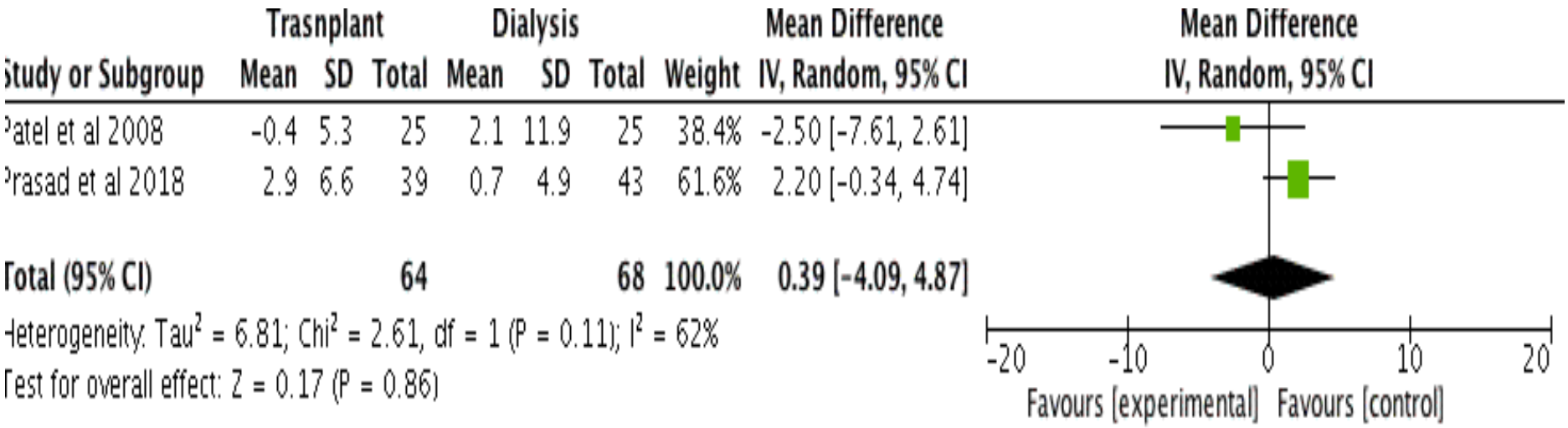


2.3.5 Systolic Function

Thirteen studies reported changes in left ventricular ejection fraction (LVEF), 11 using echocardiography (216, 217, 220, 221, 223-225, 227, 229, 230, 236, 238, 239) and the two using CMR (220, 221). None of those using echocardiography were controlled studies. Seven studies reported statistically significant increases in LVEF following transplant, six of which had recruited individuals with normal mean LVEF prior to transplant. Deng et al. recruited participants with a mean LVEF of 40 ± 11% which increased to 60 ± 14% ($p<0.05$).

The two CMR studies both reported no statistically significant changes in LVEF at follow-up. Meta-analysis of these two studies included 64 transplant recipients and 68 participants receiving regular dialysis and showed no change in LVEF compared to controls (MD: 0.39 % [CI -4.09 - 4.87 %] p=0.86) with high heterogeneity (I^2=62%) (**Figure 2.5).**

Figure 2.5 Meta-analysis of CMR studies representing change in LVEF after renal transplant.



2.3.6 Left Ventricular Dimensions

The most commonly reported measure was left ventricular internal diameter in diastole in 13 non-controlled echocardiographic studies with all but three reporting a significant reduction (223-225, 227-232, 234, 235, 238, 239). Both CMR studies reported left ventricular end diastolic volume (LVEDV) with conflicting results. Prasad et al. (220) reported a reduction in LVEDV compared to controls (−21.23 ± 37.0 ml vs 0.84 ± 38.3ml; p=0.02) whereas Patel et al. (221) reported no significant difference in mean percentage change (3.4 ± 31.5% vs 0.1 ± 19.5%; p=0.64).

2.3.7 Diastolic Dimensions

The most commonly reported parameter of diastolic dysfunction was E/A ratio in eight studies with three reporting statistically significant changes following transplantation (215, 216, 223). One controlled study, De Lima et al. (215) reported a small reduction (1.42 ± 0.6 to 1.10 ± 0.4; $p<0.05$) at one year follow-up whereas Deng et al. (216) reported a small increase in E/A ratio (1.04 ± 0.57 to 1.21 ± 0.52; p=0.001). An et al. (223) reported that recipients with moderate diastolic dysfunction (grade II) pre-transplantation showed significant reduction at 12 months (baseline 1.13 vs 0.98; $p <0.05$) whereas those with mild dysfunction (grade 1) only exhibited a significant change at the five-year follow-up (baseline 0.72 vs 0.81 at five years; $p<0.05$). Other parameters reported included left atrial size which was reported in two studies both of which reported significant reductions (230, 231). Mitral valve deceleration time was also reported in four studies(216, 224, 225, 227) with one study reporting a small significant increase (225) and one a small significant decrease (216). Neither of these changes represented a change in the grade of diastolic function observed.

2.4 Discussion

Reversing uraemic cardiomyopathy is potentially the key to reducing excessive cardiovascular morbidity and mortality associated with ESKD. Although no targeted therapy has been shown to achieve this, it is generally taken for granted that restoration of kidney function by successful

kidney transplantation reduces LVMI and left ventricular volumes, and improves left ventricular diastolic and systolic function, the key clinical features of uraemic cardiomyopathy. However, in this first systematic review and meta-analysis, we have shown that the available evidence does not currently support the notion that uraemic cardiomyopathy is improved by successful kidney transplantation or is indeed reversible.

In this review, we have shown that the majority of uncontrolled echocardiographic studies reported significant reductions in LVMI following transplantation. Making conclusions based on this data is, however, problematic. Echocardiography is unreliable when measuring LVMI, with a strong tendency to overestimate in situations of fluid overload, especially in patients on HD where large volume fluctuations are common (180). As a result, CMR, an accurate and volume-independent method for assessing cardiac dimensions is generally accepted as the gold-standard imaging modality for patients with ESKD (177). In the two CMR studies included in our review neither found a significant change in LVMI, however the trends observed were conflicting. Furthermore, in a meta-analysis of the four available studies with control groups, two echocardiographic and two CMR, renal transplantation was not associated with any reduction in LVMI, and sub-group analysis indicated that this finding was not affected by imaging modality. In addition, we did not find any convincing evidence that successful renal transplantation improves either systolic or diastolic left ventricular function. It would, therefore, appear that the assumption that the features of uraemic cardiomyopathy are reversed by successful renal transplantation is not supported by the current published literature.

However, before concluding that uraemic cardiomyopathy is irreversible it is important to examine the quality of the evidence available. Studies were generally classified as poor with

only two rated as good and two as fair using the Newcastle-Ottowa scoring system. The majority were opportunistic, unblinded, with little attempt to reduced risk of systematic bias. Only four studies, comprising a total of 109 transplant recipients, recruited a suitable control group (214, 215, 220, 221). A further limitation was the lack of sample size justification with only one study providing a power calculation (220), which was aimed at detecting changes in circulating adiponectin levels rather than any cardiac parameter. The fact that only two CMR studies have been conducted, with a total of only 64 transplant recipients, is also a major weakness of the current evidence base. The meta-analyses also demonstrated high heterogeneity suggesting that the currently available studies do not reliably answer the question of whether uraemic cardiomyopathy is reversible.

While there are weaknesses in the evidence base it may also be true that uraemic cardiomyopathy is not reversible. Following renal transplantation many traditional risk factors for cardiovascular disease persist and, in some cases, may develop *de novo (240).* Hypertension, dyslipidaemia, and diabetes are all recognised complications of both steroids and calcineurin inhibitors routinely administered following transplant. In addition, there is also persistence of non-traditional risk factors including persistent uraemia, proteinuria, and chronic inflammation as examples (240). As transplantation cannot fully reverse these factors, the same may be true of uraemic cardiomyopathy.

Recent evidence from the developing field of tissue characterisation in CMR has begun to produce encouraging results suggesting that diffuse interstitial myocardial fibrosis may regress following transplantation (241). Contti et al. (192) reported significant reductions in T1times, 6 months following renal transplant in 44 recipients (1331 ± 52 ms vs. 1298 ± 42 ms p=0.001). More recent work, however, has suggested that both T1 and T2 times remained unchanged 2 months following transplant in 24 recipients (85). This finding may be explained

by the relatively short follow-up time, but further work is needed to assess changes in T1 times associated with transplantation.

Our study has several strengths in that it included data from both echocardiography and CMR studies, which enabled all relevant data pertaining to the subject to be incorporated. The number of studies identified ensured that there was a significant pooled sample size on which conclusion could be based. There were however significant limitations. While there were an appropriate number of studies included in the systematic review the number suitable for meta-analysis were small. There were also moderate levels of heterogeneity noted among the studies when meta-analysis was undertaken. Subsequent sensitivity analysis suggested that this was being driven by the conflicting findings of the CMR studies. Such heterogeneity can make the interpretation of any findings problematic. However, we took the view that demonstrating this variability between studies highlights the need for further work to be conducted in this area.

2.5 Conclusion

Reversing uraemic cardiomyopathy, and its individual components, is a potential target for reducing the increased cardiovascular morbidity and mortality associated with CKD and especially ESKD. This syndrome has generally been assumed to be reversible by renal transplantation. This study has highlighted that at present it is not clear if this is indeed the case. Whether this is due to a lack of methodologically robust studies and an over reliance on echocardiographic data or whether uraemic cardiomyopathy is indeed not reversible cannot be conclusively deduced from the current data. Our study highlights the need for adequately powered and controlled studies to answer this fundamental question and provide further insights into other potential strategies to reverse uraemic cardiomyopathy and improve the increased cardiovascular risk associated with ESKD.

Chapter 3: METHODOLOGY

3.1 Extent of personal Contribution

The RETRACT study was a 2-year longitudinal, blinded end point study. It was supported by the British Heart Foundation via a Clinical Research Training Fellowship (FS/18/29/33554). The application was developed by Professor Charles Ferro, Professor John Townend, and I. Ethical approval was granted from West Midlands-Solihull Research Ethics Committee, following an application via the Integrated Research Application System which was completed and presented by myself. All regulator aspects and day to day running of the study were conducted by myself.

I screened, approached, and recruited all the participants in the study. I arranged all follow-up visits. As part of each visit, I completed blood pressure measurements and venepuncture, CMR image acquisition was conducted by myself with the assistance of a qualified radiographer based at the Institute of Translational Medicine Imaging Centre. Serum and Plasma was stored at the Welcome Trust Clinical Research Facility. Following storage subsequent analysis including ELISA for FGF23 and Klotho was conducted with the assistance of Dr Jonathan Law. All CMR analysis including all mass and volumetric assessments, feature tracking, and T1/T2 mapping assessment was conducted by me. Secondary analysis for reproductivity was conducted by Dr Anna Price. All Statistical analysis and data interpretation was conducted by me under the supervision of the medical statistics department based at the Queen Elizabeth Hospital Birmingham

3.2 Study Design

The RETRACT study had a prospective, longitudinal, parallel group design. It compared changes in LVM/LVMI over a 12–24-month follow-up period in kidney transplant recipients and similar participants on the renal transplant waiting list.

3.3 Ethics

The study was approved by the West Midlands Research Ethics Committee (Ref: 18/WM/0287) and was registered with the U.S. National library of medicine (ClinicalTrials.gov Identifier: NCT03892343). The conduct and reporting of this study was guided by the STROBE 150 Statement (Strengthening the Reporting of Observational Studies in Epidemiology)(242) Written informed consent was obtained from all subjects. The study was also approved by the local research and development department at University Hospitals Birmingham Foundation Trust

3.4 Participants

Transplant recipients were recruited from a single transplant centre in the UK (Queen Elizabeth Hospital, University Hospitals Birmingham NHS Foundation Trust). In this unit, patients who are scheduled to receive a live donor kidney transplant are given an operation date 6 weeks in advance. Patients were initially identified by local transplant coordinator nurses within this window. At this stage all patients were screened by the research fellow using locally available electronic resources. If patients were deemed suitable for the study based on the predefined inclusion/exclusion criteria they were then approached either via telephone or to face to face during a clinical appointment. At this stage prospective participants were provided with a detailed information sheet regarding all aspects of the study. Participants were then given a minimum of 48hrs to review this information, after which they were then contacted again by the research fellow where they could be formally recruited into the study. All patients signed written consent before any aspect of the study was conducted.

Control patients were recruited from the same renal transplant centre in the UK. Prospective participants were identified from the local transplant waiting list, they were not scheduled for, or currently being considered for a live donor transplant. Participants were screened using locally available electronic resources to ensure that they satisfied the inclusion exclusion criteria for the study. If suitable for the study, they were then contacted via either telephone or at a clinical appointment where then were provided with the study information sheet in the same way as for the transplant recipient group. Prospective participants were then given a minimum of 48 hours before being recontacted and recruited into the study if they consented to do so.

3.4.1 Inclusion Criteria

1. Patients registered on the kidney transplant waiting list at the Queen Elizabeth Hospital, University Hospitals Birmingham NHS Foundation Trust.
2. Aged over 18 years

3.4.2 Exclusion Criteria

1. Previous history of being unable to tolerate MRI scanner.
2. Contraindication to MRI – e.g., metal fragments in eye.
3. Non-Uraemic myocardial disease

3.4.3 Sample Size Consideration

The primary endpoint of the study was change in LVM. Using effect sizes and variances established from the Birmingham Cardio-renal group's previous work (change in LVM 7g, SD of change 10g) it was calculated that by studying 50 transplanted subjects and 25 non transplanted controls, there would be 80% power to detect a reduction in LVM of 7 g with an alpha value of 0.05. (43, 243)This effect is clinically important as a fall in LVMI of one SD has been shown to be associated with a 38% reduction in cardiovascular mortality. (244) We

will aim to recruit a total of 100 patients (60 transplanted + 40 controls allowing for an overall 10% drop-out rate and 25% of controls being subsequently transplanted during the two years of follow-up (43, 245).

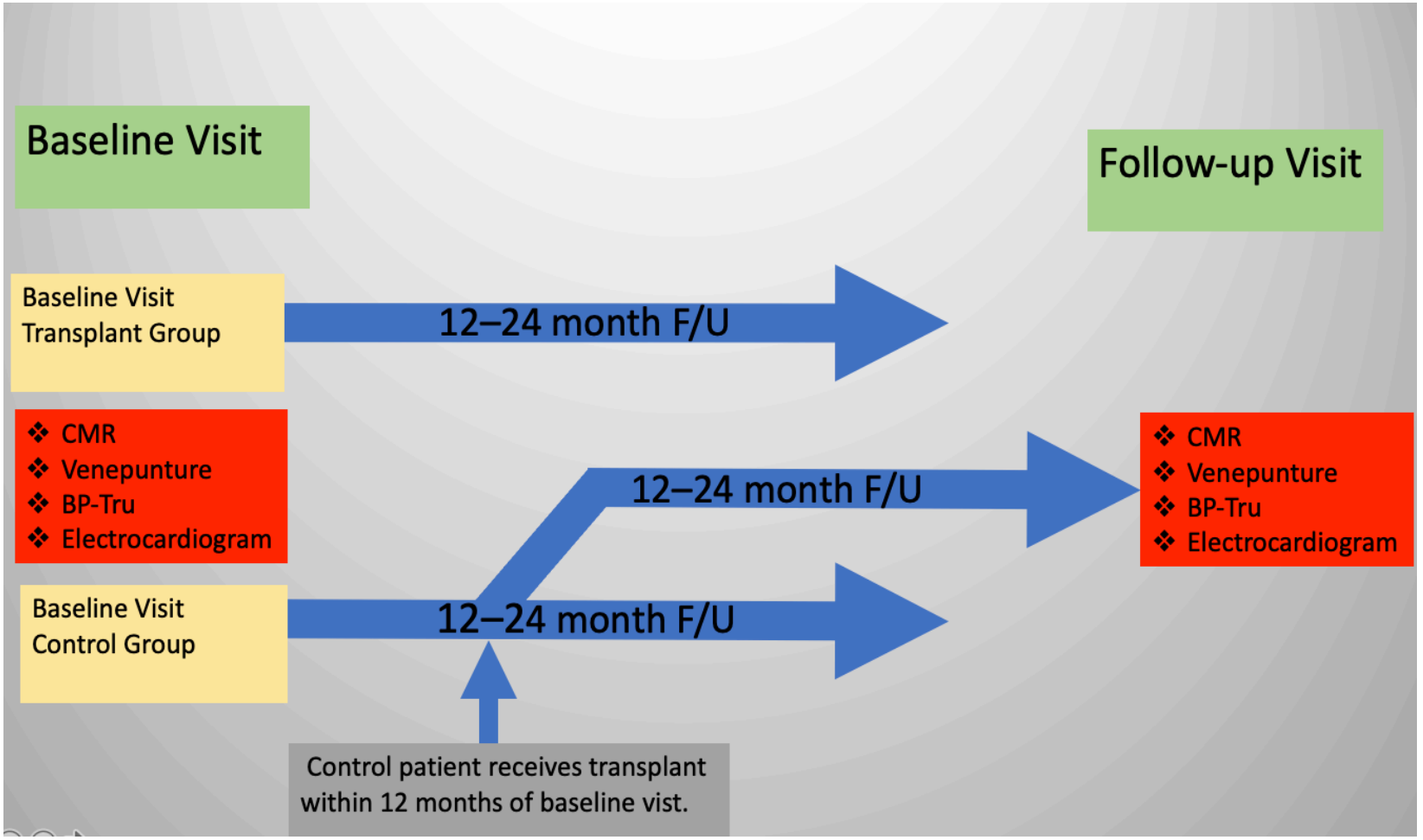
3.5 Study Protocol

The study protocol is set out in **Figure 3.1.** At baseline all participants under-went all investigations. These investigations were then repeated at between 12-24 months for both groups. This range for follow-up times was a pragmatic consideration due to the effects of Corona Virus Disease 2019 (COVID19). The effects of COVID 19 are discussed fully in **section 3.9.**

In addition to standard follow-up, it was also recognised that there would participants who would be required to change study group during the follow-up period. The circumstances in-which this was permitted were:

1. Control group participants receiving a renal transplant during the first year of follow-up. Participants would then be moved to the transplant group with follow-up taking place 12-24 following transplantation.
2. In cases where a renal transplant was deemed to have immediately failed during the perioperative period participants were transferred to the control group.

Figure 3.1 Study Flow Diagramme.



3.5.1 Withdrawal

It was anticipated that drop-out from the study would be low as both transplant recipients and control participants are seen regularly in clinic for follow-up. This allowed the study to be completed with minimal inconvenience to participants. Specific withdrawal criteria were however established as the following

i. Pregnancy.
ii. Repeated non-attendance.
iii. Subject decision to withdraw.
iv. Inability to tolerate MRI scanning.
v. Subsequent reason for MRI to be contraindicated.

3.5.2 Clinical Assessment

All participants in the study underwent a clinical assessment at each study visit the details are recorded below.

3.5.3 Demographic Information

All participants recruited to the study had basic demographic information recorded at both baseline and follow-up. This included measures of height and weight current and previous medication usage. In addition, details of previous medical history were recorded.

3.5.4 Office Blood Pressure

Office blood pressure was recorded using a BpTRU device. This uses the same oscillometric method employed by standard automated blood pressure monitors. Previous work has demonstrated that this method of office blood pressure measurement is well correlated with daytime average ambulatory blood pressure measures(246) A standard method of using the device as previously described in the literature was adopted (246).

3.5.5 Electrocardiogram

Where possible an electrocardiogram (ECG) was performed at both baseline and follow-up. A standardised protocol was adopted as previously described in the literature (247). ECGs were assessed for evidence of structural heart disease, ischaemia, and arrhythmias. Where 12 lead ECG assessment could not be undertaken analysis of a three lead ECG during MRI scanning was undertaken.

3.5.6 Laboratory Analysis

At both baseline and follow-up, routine clinical blood and urine samples were taken. Standard clinical phlebotomy blood tubes containing either ethylenediaminetetraacetic acid or serum separating gel were used for the following:

i. eGFR – measured by the CKD-epi equation.
ii. Full blood count,
iii. Urea and electrolytes
iv. Liver function tests
v. Bone profile
vi. PTH
vii. Vitamin D
viii. Urate
ix. Magnesium
x. Total cholesterol (non-fasting)
xi. Protein Creatinine ratio.

All samples were processed at the local laboratory within the Queen Elizabeth Hospital laboratory.

In addition, samples of urine serum and plasma were also processed and stored at the Welcome trust clinical research facility to allow subsequent analysis to be completed. Serum and plasma were centrifuged at 4°C at 1500g for 15 minutes. The plasma and serum supernatant were then aliquoted and stored at -80°C. Urine was centrifuged twice at 4°C for 15min at 1500g then one aliquot of 2ml was stored at -80°C. (**Appendix A)**.

3.5.7 Fibroblast Growth Factor 23

FGF23 and levels were analysed at both baseline and follow-up. Analysis was performed using human plasma collected and stored as described above. Analysis was performed using the Human FGF23 carboxyl-terminal 2nd generation enzyme-linked immunosorbent assay (ELISA) kit manufactured by Immutopics, Inc, San Clemente, California (Catalogue number: 60-600) which measures C-terminal FgF23 (c-FGF23). This method of analysis was chosen as it has been previously used by the Birmingham Cardiorenal group, thus allowing direct comparisons with previous work. In addition, previous work has also indicated that c-FGF23 assays are be more able to detect bioactive FGF23 fragments than Intact FGF23 (i-FGF23) assay. c-FGF23 has also been shown to have stronger correlation with eGFR and graft failure following transplantation than i-FGF23 (248).

The c-FGF-23 assay used was a two-site sandwich ELISA. This technique uses two purified goat polyclonal antibodies which detect epitopes within the carboxyl-termina portion of FGF-23. One of these antibodies is biotinylated for capture and the other antibody is conjugated with the enzyme horseradish peroxidase (HRP) for detection. Any FGF-23 within a sample is effectively sandwiched between these antibodies. This enzyme complex is then incubated in the presence of substrate solution, following which levels of absorbance can be measured in a microtiter reader. Sample analysis was performed within the laboratories at the Institute of

Biomedical Research at the University of Birmingham. All samples were fully anonymised and tested in a random order.

Prior to analysis all reagents were brought to room temperature. All analysis was performed in duplicate. For each assay five FGF-23 standards and all participant samples were prepared.100 μL of either FGF-23 standard solution or participant serum and 50μL of the working antibody solution containing biotinylated antibody and HRP antibody were then pipetted into a pre-designated well. The plate was then incubated at room temperature for 3 hours on a horizontal rotator.

Each well was then aspirated and washed five times with 350 μL of working wash solution. 150 μL of ELISA HRP substrate was then pipetted into each of the wells. The plate was then incubated again at room temperature on a horizontal rotator for 30 minutes. The absorbance at 620nm was then measured within five minutes in a microtiter plate reader. 50 μL of ELISA stop solution was then added into each well and mixed on a horizontal rotator for one minute. The absorbance at 450nm was then read within five minutes in a microtiter plate reader.

Following this procedure, the average absorbance of duplicate samples was calculated. A standard curve was then generated from the absorbance of the six standards provided measured at 450nm. From this the concentrations of FGF23 could be calculated. Where samples were found to fall between the values of the fifth and sixth standard a second standard curve was generated using values calculated at 620nm. Where samples were found to have higher readings than the highest standard value at 620nm a dilution of 1:10 was performed and the sample re-assayed to obtain corrected values

3.5.8 α-Klotho

Soluble α-klotho levels were analysed using the human soluble α-klotho kit manufactured by Immuno-Biological Laboratories Co, Ltd, Japan (Catalogue number: JP27998). As with the kit used for FGF-23 analysis kit is a solid phase sandwich ELISA using the same principal described above.

All α-klotho analysis was performed at the Institute of Biomedical Research at the University of Birmingham. All samples were fully anonymised and analysed in a random order.

Prior to analysis all reagents were brought up to room temperature. In order to allow generation of a calibration curve for each analysis it was first necessary to prepare seven standards. This was done by diluting the 12000 pg/mL standard provided within the analysis kit to create 7 points of known dilution, Tube-1 6,000 pg/mL Tube-2 3,000 pg/mL Tube-3 1,500 pg/mL Tube-4 750 pg/mL Tube-5 375 pg/mL Tube-6 187.5 pg/mL Tube-7 93.75 pg/mL.

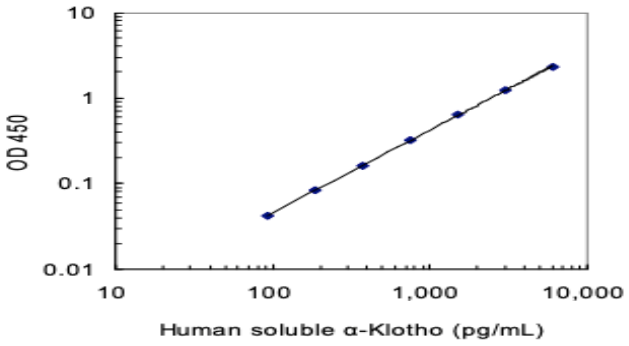
Participant serum samples was prepared by initially diluting them 2-4-fold with EIA buffer. 100 µL of either participant serum or standard were then added to the 96 well plate in pre-mapped locations. The plate was then incubated at room temperature with the plate lid in place. Each well was then washed 4 times using wash buffer provided. All excess liquid was the removed. 100 µL of prepared labelled antibody was then added into each of into the wells. The plate was then incubated at room temperature for a further 30 minutes. The wells were then washed five times using the same process as above. 100 µL of TMB solution was then added to each well and incubated at room temperature shielded from light for 30 minutes. Following this stop solution was added. The absorbance of each sample was then measured at 450nm.

A standard curve was then created by plotting the concentration of each standard against the absorbance recorded. The absorbance of each sample was the plotted along this curve to establish the actual values of α-Klotho.

Figure 3.2 Example of standard curve used to establish α-Klotho levels

Example of standard curve and measured value

Conc. (pg/mL)	Absorbance (450nm)
6,000	2.307
3,000	1.245
1,500	0.635
750	0.324
375	0.166
187.5	0.087
93.75	0.046
0 (Test Sample Blank)	0.004



(Taken from product literature)

3.6 Cardiac Magnetic Resonance Imaging

Magnetic resonance imaging was performed using a 3 tesla (3T) (Siemen's Skyra) located at the Institute of Translational medicine at University Hospital Birmingham. Where this scanner could not be used the 1.5 tesla (Siemen's Avanto) situated in the Queen Elizabeth Hospital imaging department was used.

3.6.1 Imaging Protocol

Prior to taking part in the study participants were first pre-screened to ensure that there were no contraindications to undergoing an MRI scan. Immediately prior to scanning participants were then required to complete a safety questionnaire used in routine clinical practice. This was then reviewed by a qualified radiographer to confirm suitability for scanning.

Height and weight of all participants was recorded immediately before scanning. Blood pressure was recorded immediately before scanning using the BP-tru device as previously

described above. Venepuncture was then performed as described above. Once positioned on the scanner a 3-lead electrode was then placed on bony prominences to allow ECG gating for image acquisition.

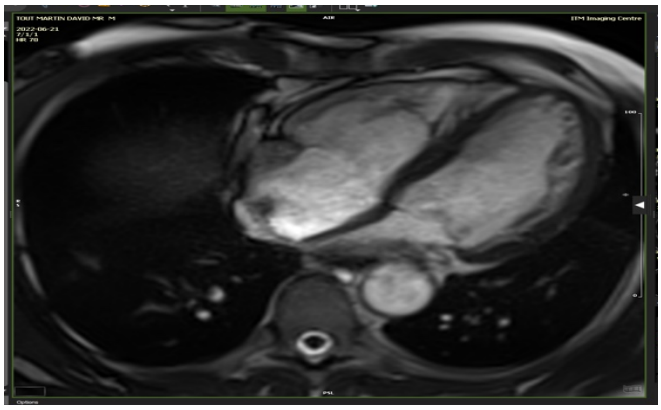
3.6.2 Image Acquisition

Images were obtained in the following sequence:

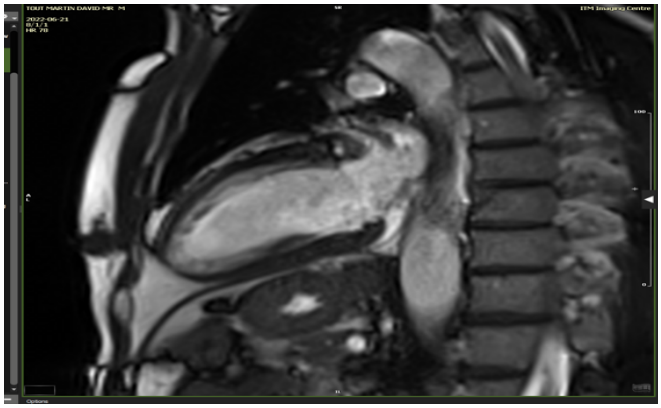
Initially multi plane localisers were acquired, with the heart adjusted to isocenter of the scanner. An axial dark blook half fourier single-shot turbo spin-echo (HASTE) was then obtained from immediately above the aortic arch to the diaphragm. Two, chamber, horizontal longa axis (HLA) and vertical long axis (VLA) in addition to SAX localisers were then obtained.

Following the acquisition of localiser images cine images were then obtained. Images obtained were two, three and four chamber, left ventricular outflow tract and, SAX. The following scan parameters were used: echo time 1.69 (TE); repetition time 45.48ms (TR); flip angle (FA); 65; field of view (FOV) 340mm with a slice thickness of 7mm with a 3mm gap over 25 phases per cardiac cycle. These image parameters were chosen as they are in line with previous studies performed by the Birmingham Cardio Renal Group. All images were acquired at the end of expiration.

A



B



C

Figure 3.3 Example of CMR imge aquasition.

A, HLA, B, 2 Chamber C, VLA Cine images acquired using 3T scanner

3.6.3 T1 mapping

MOLLI sequences are widely used for T1 mapping in a variety of conditions (249). It is known to have high levels of precision when estimating T1 times. There are a number of variants of the MOLLI sequence which have been employed since it was initially developed. Currently the MOLLI 5(3)3 is the most commonly used in clinical practice due to the reduced scanning times with little impact on image quality.(188) Due to these advantages an end-expiratory breath hold SSFP motion corrected MOLLI 5(3)3 sequence was used. Standard parameters employed were TE 1.12ms, TR 280.56ms, TE 1.12ms, FOV 360mm and FA 35.

T 1 mapping images were acquired in the short axis view for the basal and mid ventricular slices The basal slice was defined at the first slice with ventricular myocardium visible throughout the cardiac cycle without the presence of the aortic outflow tract. The mid ventricular slice was then taken as that slice which was two slices more proximal to the ventricular apex, where papillary muscle was present. Once image position was established this was then used for both T1 and T2 mapping..

Prior to image acquisition radio frequency and volume selective shimming were performed to optimise image quality. ECG gating was also monitored to ensure accurate triggering of the MOLLI sequence. Source images were also reviewed to look for the presence of artefact to allow repetition if considered non-diagnostic.

3.6.4 T2 Mapping

T2 relaxation time is a marker of myocardial oedema (250). T2 weighted imaging was the first technique developed to detect the presence of myocardial oedema, however, image acquisition is challenging and allows only qualitative analysis. Due to these challenges T2 mapping was developed to allow quantitative assessment of myocardial water content(250). T2 mapping sequences were bright blood T2 preparation pulse-based sequences. Three single shot T2 weighted

SSFP images were acquired at the following T2 preparation times 0ms, 30ms and 55ms. Image position was as previously described for T1 mapping.

3.6.5 CMR Analysis

All analysis was performed in a single study core laboratory by a single assessor (LP), blinded to participant status (transplant recipient/ control) and study stage (baseline/follow-up). In order to achieve this on completion of all studies all identifiable data was removed from each study and replaced with a randomly generate code. All studies were then analysed. Following completion of all analysis studies were then decoded to allow statistical analysis to take place.

3.6.6 Ventricular assessment

In order to calculate ventricular volumes and masses segmentation of the left and right ventricle was performed using the SAX images acquired. The basal slice was first identified as that slice in which 50% of the blood pool was surrounded by ventricular myocardium as has previously been described by the Society of Cardiovascular Magnetic Resonance (SCMR) (251). The endocardium and papillary muscles were delineated using a semi-automated thresholding method. The epicardium was initially draw manual and subsequently smoothed automatically. Analysis was performed in end-diastole and end-systole, LVM, volume and stroke volume were then automatically produced by the post-processing software. All values were indexed to body surface area as calculated using the Mosteller formula. Maximal wall thickness was also calculated automatically using the American Heart Association (AHA) 17 segment model during the end-diastolic phase.

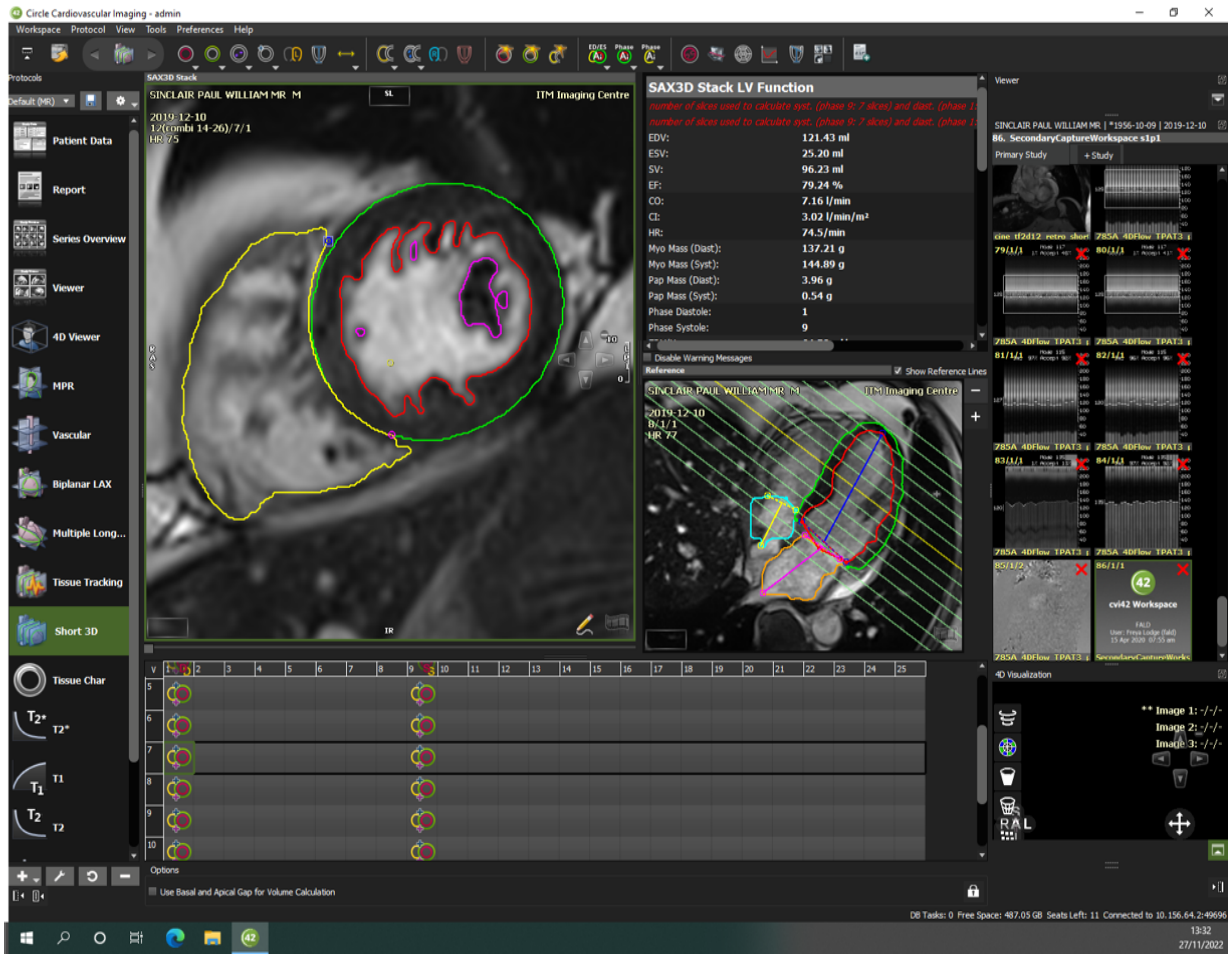


Figure 3.4 Screen shot of ventricular analysis performed using CVI42 reporting programme.

Main image shows the segmentation of the left and right ventricle. Below main image are thumbnail images for each of the SAX slices at each of the different phases of the cardiac cycle.

3.6.7 2D and 3D Feature Tracking

Myocardial strain is more sensitive than ejection fraction as a measure of systolic function and is able to identify sub-clinical ventricular dysfunction in a range of cardiomyopathies (252). Both measures of 2D and 3D strain as assessed by CMR have been shown to have high inter and intra observer reliability(253, 254).

Cvi42 produces 2D and 3D feature tracking data using a previously validated algorithm which generates an incompressible deformable model which is tracked throughout the cardiac cycle. Using both the 2D and 3D features of cvi42 the global longitudinal strain (GLS), global circumferential strain (GCS) and global radial strain (GRS) were calculated. In addition, the peak systolic strain rate S', early diastolic strain rate E' and late diastolic strain rate A' for each parameter were also calculated. Only global values were presented due to the lack of reproducibility of segmental analyses (24)

3.6.8 Tissue Characterisation

Modified look locker (MOLLI) native T1 mapping was performed the basal and mid-levels of the SAX. Parameters for these images were as previously described. Initially the 8 motion corrected images were reviewed to ensure these was no significant motion artefact that would render the final map inaccurate. The final T1 colour map was created with thresholds for different levels of T1 set by the research fellow.

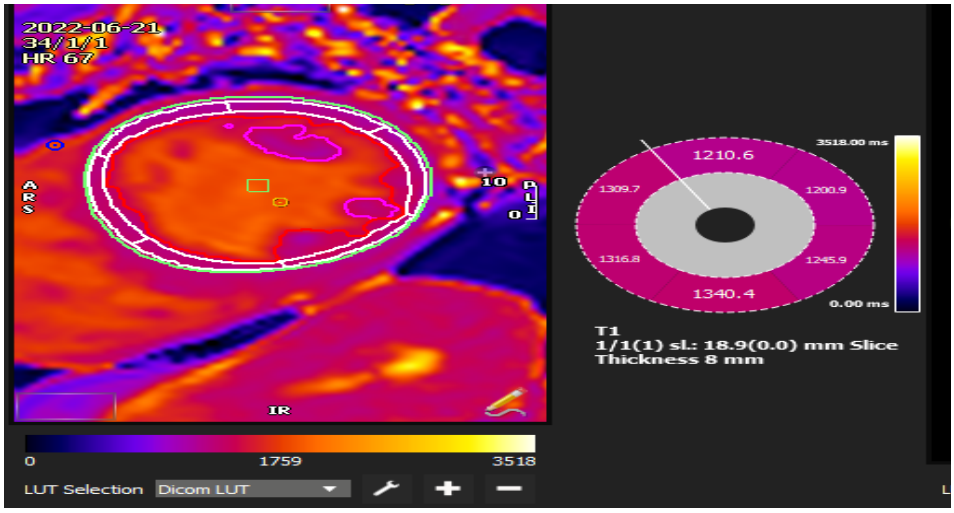
Each short axis slice was divided into segments as previously described by the AHA. In order to orientate the segments, it was necessary to identify the anterior RV insertion point on each slice. The following T1 times were calculated for each SAX slice:

I. Individual segmental T1 times
II. Global T1 time -average of all segments

III. Septal T1 time – average of 2 septal segments

The generation of T2 maps and subsequent analyses was the same as that for native T1 maps

Figure 3.5 Example of native T1 and T2 analysis using CVi42 reporting software.



A. *Analysis of left ventricular T1 times using colour map. Both and endocardial and epicardial borders have been identified. To right of image is the automatically calculated T1 values for AHA segments.*

3.6.9 Interstudy variability

In order to test inter-observer variability of LVM, T1 and T2 mapping times, 10 CMR scans were chosen at random. These were then assessed, by a second observer, using the same techniques and post processing software as previously described. All scans were fully blinded. In order to examine intra-observer variability 10 blinded CMR studies were analysed by the research fellow one month apart, again LVM T1 and T2 mapping times were calculated. It was also necessary to establish inter-study variability. This was established by 10 participants undergoing a repeated CMR within one week of their initial scan in order to recalculate LVM as previously described.

3.6.10 T1 mapping stability assessment

During the course of the study, it was necessary to ensure the stability of T1 mapping times over time. This was done using the Eurospin TO5 phantom. The specifics of the phantom scanning protocol are given in **Appendix B.**.

3.7 Statistical Analysis

Statistical analysis was overseen by the medical statistics team at University Hospitals Birmingham. All analysis was performed using SPSS©, version 23 (IBM, Armonk, New York, US). All variables were tested for normality using the Kolmogorov-Smirnov test. Where a variable was normally distributed values were reported as mean ± standard deviation. Categorical data was presented as total number (%)

Where data was non-normally distributed it was first log10 transformed then tested for normality as described above. Where this data was normally distributed a parametric test was then used. Test data were then anti-logged and data were reported as multipliers. Baseline and follow-up data were presented as geometric mean (95%CI). Where data remained non-normally distributed after log10 transformation a non-parametric test was applied.

For all between group differences (Transplant recipients vs. controls) an independent sample t-test was used for all continuous variables. Where within group changes were analysed (i.e., baseline to follow-up) a paired student t-test was used. Where the groups were compared at baseline and follow-up and data was considered non-parametric a Mann Whitney test was applied. Differences between categorical variables were assessed using chi-square tests. Missing data was handled with a listwise deletion approach.

In order to examine the relationship between variables univariate liner regression was initially performed. Following this univariate analysis, multivariate linear regression was then

performed. Variables were selected if univariate analysis was considered statistically significant ($P<0.05$) or if they were deemed clinically significant. Prior to analysis collinearity was established by assessing the variance inflation factor between independent variables in the model. Normal distribution of residuals was assessed visually through the production of a Q-Q plot.

3.7.1 Subgroup analysis

Two independent sub-group analyses were also undertaken for transplant recipients. Recipients were first stratified based on the presence of LVH at baseline. The definition of LVH was based on values previously published by Kawel-Boehn et al(255).

Transplant recipients were also stratified based on the achieved renal function at follow-up. An eGFR of 50 ml/min/1.73 m^2 was chosen. This was decided as the median eGFR of the transplant group was 52 ml/min/1.73 m^2. Adopting 50 ml/min/1.73 m^2 ensured an even division between the two groups, thus ensuring meaningful results.

3.8 Impacts of COVID-19 ON RETRACT study design.

3.8.1 Length of follow-up

The RETRACT study was heavily impacted by the occurrence of the global COVID-19 pandemic. The first wave of COVID-19 began in in March 2020 until May 2020. The second wave of COVID-19 took place from September 2020, until April 2021. Associated with this were national lock downs from March 2020, until June 2020, and September 2020 until July 2021. During these periods there was national guidance for those considered clinically vulnerable to shield themselves to avoid infection. This group classified as extremely clinically vulnerable included both those with CKD and those who had received a previous renal transplant. In addition, from March 2020 to June 2021 and January 2021 until February2021 the renal transplant programme at university hospitals Birmingham was suspended.

These constraints on research activity during the timeline of the study therefore made it impossible to be able to follow-up patients within the twelve-month timeline that was initially set out in the research protocol. As such the research team decided that follow-up would be changed to between 12 and 24 months. This was a pragmatic consideration to allow meaningful data to be obtained. This change was approved by the local research and development department and submitted to the research ethics committee as Amendment 002.

3.8.2 Data Acquisition

3.8.2.1 24-hour blood pressure monitoring

Due to limitations set by infection control during the study it was not possible to undertake 24-hour blood pressure monitoring using reusable devices and blood pressure cuffs.

3.8.2.2 Measures of Arterial Stiffness (pulse wave velocity)

It was initially the intention was to study PWV in all participants. The initial study design was for all patients to initially be reviewed in the Centre for Rare Disease at the Queen Elizabeth Hospital, where this could be conducted. During the COVID19 pandemic this area of the hospital was repurposed for vaccine research as such this area was not an appropriate environment for immunosuppressed patients. In addition, the research team took the decision that participants should not be exposed to multiple areas of the hospital particularly any area where there were in-patients. It was, therefore, decided where possible patient appointments would take place within the Institute of Translational Medicine Imaging Centre. This is a standalone MRI scanner which was only for outpatient use. This was deemed more suitable to minimise the risk of COVID19 exposure as all staff wore PPI and only single patients were allowed into the building at any one time. The facility was also fully cleaned both before and after each patient appointment. As such participant came into contact with no other individuals other than clinical staff who were preforming regular lateral flow tests as part of the hospital

guidelines. Due to pressure on appointment times, it was not possible to perform PWV in this facility. This pragmatic step was taken to ensure that the primary endpoint of LVM could be obtained.

3.8.2.3 Biochemical Analysis

In some case it was necessary to perform patient visits outside of working hours as patients did not want to attend the hospital when they perceived there would be the most exposure to other people. During these visits there was no access to the Welcome Trust laboratory processing facilities. As a result, in a number of patients it was not possible to perform analysis of FGF23 and α-Klotho.

3.8.2.4 CMR acquisition

In a small number of participants, it was not possible to gain access to the 3T CMR scanner to accommodate participant availability again due to the requirement to accommodate scanning outside of normal working hours. In this situation the 1.5T scanner was used as an alternative in a small number of participants. This was deemed an acceptable alternative as it would ensure the collection of LVM/LVMI data as well as feature tracking data. Due to the substantial differences in native T1 and T2 times between the two imaging modalities it was not possible to include these participants in the tissue characterisation data.

Chapter 4: LEFT VENTRICULAR MASS AND VOLUMETRIC ANALYSIS

Abstract

Background

Renal transplantation is the gold standard treatment for those with ESKD. It is associated with improvements in both major cardiovascular events and cardiovascular mortality. It is widely reported that this benefit is due to regression of uraemic cardiomyopathy the features of which are increased LVM, diffuse interstitial myocardial fibrosis and left ventricular dysfunction. At present however chapter 2 of this thesis has shown that this is not supported by the current evidence base.

Objectives

To establish if the increased LVM component of uraemic cardiomyopathy is reversible following renal transplantation.

Participants

50 renal transplant recipients recruited from local transplant waiting list. 20 transplant-listed control participants with ESKD not scheduled for live donor transplantation.

Methods

Participants had demographic and CMR parameters recorded at baseline and follow-up at 12-24 months. Renal transplant recipients were studied within a one-month period prior to transplantation. LVM, LVMI, left ventricular end diastolic volume (LVEDV), left ventricular end diastolic volume indexed (LVEDVI), left ventricular end systolic volume (LVESV) and left ventricular end systolic volume indexed (LVESVI) were all recorded at both study visits. Analysis was performed to compare the both the transplant and control groups. Secondary analysis was performed with transplant recipients stratified based on post-transplant improvement in eGFR (eGFR<50 mL/min/1.73m^2 vs. eGFR>50mL/min/1.73m^2) and the presence or absence of LVH at baseline.

Results

There were significant reductions in LVM observed in transplant recipients compared to control patients (Between group mean difference -18.76g 95%CI (-30.46 - -7.05) P=0.002). There was also a significant reduction in LVMI in the transplant group compared to controls (Between group mean difference -11.37g/m^2 95%CI (-18.49 - -4.25) P=0.002). When stratified for the presence of LVH there were significant reductions in LVM and LVMI(LVM mean difference-21.83g 95%CI [-33.96-9.71] P=0.001 LVMI mean difference -13.74g/m^2 95%CI [-20.89- -6.59 P=0.001) Analysis of transplant recipients based on post-transplant eGFR indicated that there were no significant between group changes. Within group analysis highlighted that in those transplant recipients with eGFR<50 mL/min/1.73m^2 there were no significant changes in any of the parameters. In those with higher levels of eGFR, however, there were significant reductions from baseline to follow-up in all parameters (LVM mean change -10.77g±23.84 P=0.022, LVMI mean change -8.02g/m^2±13.90 P=0.004)

Conclusion

The RETRACT study has shown that LVM and LVMI reduces significantly following renal transplantation compared to a similar group of control participants. It has also shown that these changes observed are greater in participants with LVH at baseline.

4.1 Introduction

A well-established inverse graded relationship exists between renal function and cardiovascular risk, even with only minor reductions in renal function (43). This relationship is not linear in nature; the highest risk is predominantly observed among individuals with ESKD, especially those undergoing regular renal replacement therapy. In young adults with ESKD, the risk of cardiovascular mortality can be up to 500 times greater than that of age- and sex-matched controls (204).

In conjunction with the escalating cardiovascular risk, there are discernible structural changes within the cardiovascular system that are detectable with only minor reductions in renal function (43, 177). These changes include vascular remodelling due to arterial calcification. In parallel there is also development of diffuse interstitial myocardial fibrosis, which is associated with LVH and both systolic and diastolic dysfunction (256). Collectively this cardiovascular remodelling is known as uraemic cardiomyopathy.

In those with ESKD these changes are almost ubiquitously observed and serve as powerful predictors of cardiovascular mortality. Early research by Silberberg et al. (257) highlighted that among dialysis patients, both all-cause mortality and cardiovascular mortality were significantly higher in those within the highest quartiles of LVMI compared to those in the lowest quartiles. Further investigations by Zoccali et al. (258) underscored that, after the initiation of dialysis, a monthly increase in LVMI of 1 g/m² was linked to a 62% escalation in the risk of fatal and nonfatal cardiovascular events. In addition to LVM measures of left ventricular geometry are also of prognostic significance in CKD Fitzpatrick et al.(259) demonstrated that increasing left ventricular volumes are associated with mortality and hospitalisations for heart failure in those with mild to moderate CKD. Inoue et al. (260) also reported that increased left ventricular end diastolic diameter was associated with increased all-cause mortality in a HD cohort.

Currently, renal transplantation is the gold standard treatment for individuals with ESKD (199). Transplantation significantly reduces cardiovascular risk compared to those who remain on transplant waiting lists(210). Nevertheless, it is important to note that the risk remains elevated in comparison to the general population. It is commonly suggested that this risk reduction is a consequence of cardiovascular remodelling and resolution of the features of uraemic cardiomyopathy, including LVH. Despite several attempts to investigate this assertion, recent meta-analysis presented in **chapter 2** has shown that the existing body of evidence is insufficient to confidently draw such conclusions (261).

The RETACT study is the first robust prospective clinical study using CMR, which sets out to investigate if the cardiovascular remodelling associated with uraemic cardiomyopathy, is reversible following renal transplantation. The following chapter will focus on mass and volumetric analysis.

4.2 Brief Methods

RETRACT was a longitudinal, controlled, blinded endpoint study. It prospectively registered with Clinical trials.gov (NCT03892343). Recruitment began in 2018 and completed in 2021. Follow-up of all participants was completed in August 2022. The study methodology is detailed in full in **chapter 3.**

4.3 Brief Hypotheses

1. The restoration of renal function after successful renal transplantation is associated with decreased LVM and LVMI
2. In those with the most prominent features of uraemic cardiomyopathy LVH at baseline there will be the greater reductions in LVM/LVMI following transplantation.
3. Higher levels of renal function following transplantation will be associated with greater reductions in LVM and LVMI.

4. Any changes in LVM observed after transplantation will not be totally explained by changes in blood pressure.

4.4 Primary and Secondary Endpoints

Primary endpoint:

i. LVM

ii. LVMI.

- *Secondary endpoints:*

i. Left ventricular end diastolic volume (LVEDV)

ii. Left ventricular end diastolic volume indexed to BSA (LVEDVI)

iii. Left ventricular end systolic volume (LVESV)

iv. Left ventricular end systolic volume indexed to BSA (LVESVI)

v. Systolic blood pressure

vi. Diastolic blood pressure

4.5 Stratification of transplant recipients.

It was decided that secondary analysis would be performed examining the changes observed in transplant recipients stratified based on renal function at follow-up, and the presence of LVH at baseline. LVH was classified based on recently published references values (255). At follow-up a value of eGFR50 mL/min/1.73m² was used to stratify transplant recipients (transplant recipient stratification is discussed in in section 3.7.1).

4.6 Results

4.6.1 Transplant and control participants

In total 50 transplant recipients and 20 control recipients were included in the final analysis.

A study timeline showing recruitment and follow-up is shown in **Figure 4.1**

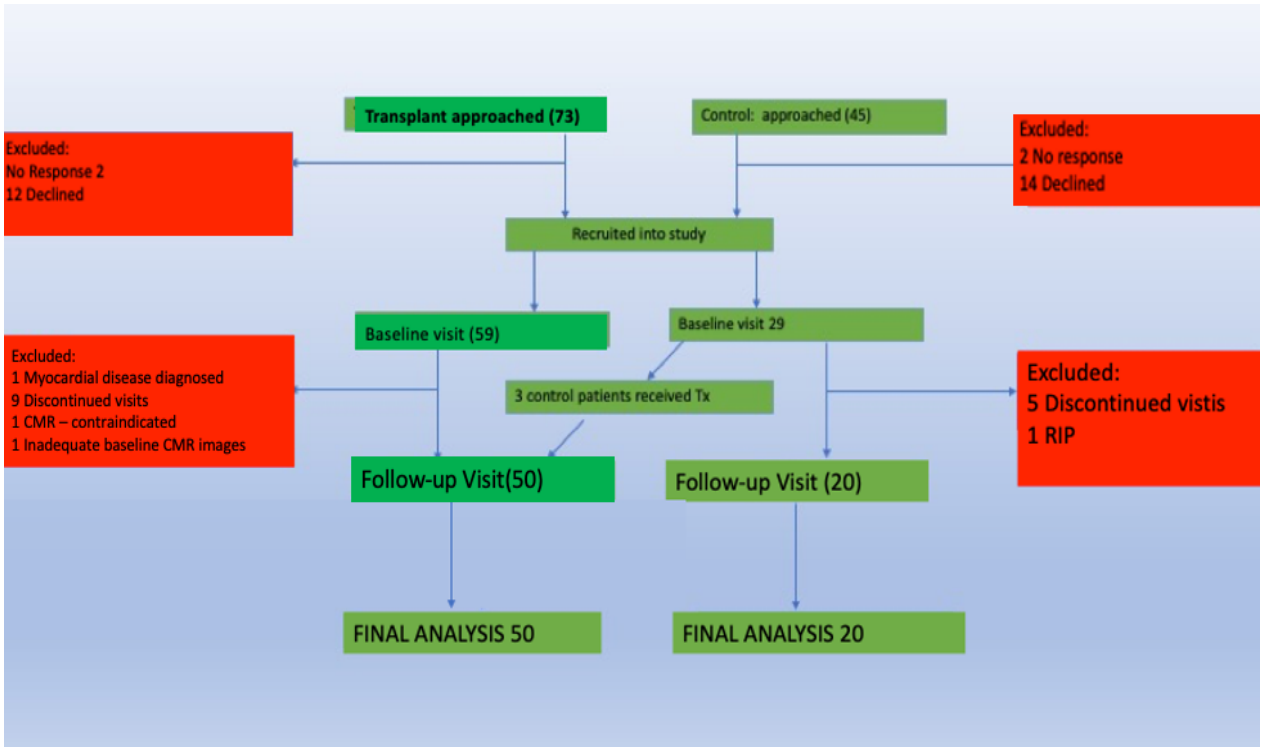


Figure 4.1 Study recruitment.

In the transplant group one participant was diagnosed with hypertrophic cardiomyopathy and excluded from analysis, one participant was unable to undergo CMR due to claustrophobia, one participant had inadequate baseline data due to poor CMR image quality and 6 participants declined follow-up.

In the control arm 3 participants underwent cadaveric renal transplant and were subsequently included as transplant recipients at follow-up. 5 participants declined follow-up and 1 participant died during the follow-up period from a condition unrelated to ESKD.

Figure 4.2 Demographic and clinical characteristics at baseline.

	Transplant recipient N=50 (%)	Controls N=20 (%)	P Value
Age (yrs)	47 ± 13	52±12	P=0.156
Male sex	31 (62)	13 (65)	P=0.81
Length of follow-up (days)	447±111	504±147	P=0.130
Race or ethnic group			
White	42 (84)	16 (80)	P=0.66
Asian	7 (14)	4 (20)	P=0.51
Black	1 (2)	0	
Body Surface Area	1.88 ±0.25	1.95±0.20	P=0.272
Dialysis Modality			
PD	16(32)	13(65)	0.011
PD vintage (Yrs)	1.99 ±1.93	1.71±1.58	0.717
HD	17(34)	0	*
HD vintage (Yrs)	2.01 ±2.90	*	*
Risk factors			
Hypertension	39 (76)	17 (85)	P=0.50
Diabetes	5 (10) YES	1 (5)	P=0.51
Cardiovascular disease	4 (8)	1 (5)	P=0.67
Hypercholesterolaemia	19 (38)	13 (65)	P=0.04
Previous Transplant	8 (16)	1 (5)	P=0.21
Current Smoker	3 (6)	3 (15)	P=0.22
Ex-smoker	12 (24)	7 (35)	P=0.60
Medication			
Statin	19 (38)	13 (65)	P=0.04
Antihypertensive agent			
ACE inhibitor	11 (22)	4 (20)	P=0.85
ARB	5 (10)	1 (5)	P=0.50
Beta-Blocker	17 (34)	5 (25)	P=0.49
Alpha-blocker	14 (28)	7 (35)	P=0.56
Ca2+ Channel Blocker	27 (54)	17 (85)	P=0.01

**Length of follow-up was considered time between renal transplant and second CMR scan in transplant recipients. In control patients' length of follow-up was the time between CMR scans Data were analysed using either a student t-test for continuous data or a chai-square tests for categorical data. Data are presented as mean ± SD or n (%).*

(ACE angiotensin converting enzyme, ARB angiotensin receptor blocker)

Demographic data are presented in **Figure 4.2**. The groups were considered similar at baseline. There were no significant differences in terms of age, gender, ethnicity, or body surface area at baseline. There was also no significant difference in the length of follow-up between the two groups.

In terms of past medical history, there were a greater number of control participants diagnosed with hypercholesterolemia (transplant group n=19 (38%) vs. control group n=13 (65%), P=0.04). There were also significantly more participants receiving regular PD in the control group (13(65%) vs16(32%) P=0.011) there was no difference in PD vintage observed between the two groups. There were no patients receiving regular HD in the control arm whereas 17(34%) were on regular HD in the transplant group. When comparing medication usage, more participants in the control group were using statins, there was also a higher prevalence of calcium channel blocker usage in the control group (transplant group n=27(54%) vs. control group n=17 (85%), P=0.01).

Changes in blood pressure parameters are known to have a significant impact on LVM. Changes in systolic blood and diastolic blood pressure are presented in **Figure 4.3**.
Analysis indicated that there was no significant difference in blood pressure between the two groups at either baseline or follow-up. In addition, the magnitude of change between the two groups was also not significantly different (systolic blood pressure mean difference 1.06 mmH^20 95%CI [-10.17 - 12.29] P=0.85, diastolic blood pressure mean difference -1.00 mmH^20 [-8.02 - 6.02] P=0.78)

Figure 4.3 Systolic Blood pressure analysis at baseline and follow-up.

	N	Baseline	Follow-up	Within group change	P Value	Between group change	P Value
Systolic Blood pressure							
Transplants	50	132.31±18.58	130.00±16.48	-2.31 (-8.12 - 3.50)	0.43		
Controls	20	138.72 ±15.07	135.35±22.27	-3.37 (-14.23 -7.49)	0.52	1.06 (-10.17 - 12.29)	0.85
Diastolic blood pressure							
Transplants	50	81.05±11.10	81.20±10.39	0.15 (-3.92 - 4.22)	0.94		
Controls	20	84.55±9.98	85.70±11.80	1.15 (-3.63 - 5.93)	0.62	-1.00 (-8.02 - 6.02)	0.78

*Data are displayed as mean±SD at baseline and follow-up for the whole cohort. Within-group differences were determined by paired samples t tests. Between-group differences were determined using independent samples t-tests. Between group differences are displayed as the mean difference in values (95% CI). ** Denotes significant difference (p=<0.05) between groups at baseline or follow-up.*

The impact of renal transplantation on cardiac masses and volumes for both transplant recipients and controls are presented in **Figure 4.4**. Analysis between the groups for the primary endpoints, LVM and LVMI, demonstrated significant reductions in both parameters in the transplant group compared to controls (LVM mean difference -18.76g, 95%CI [-30.46 - -7.05], P=0.002, and LVMI g/m^2 mean difference -11.37g/m^2, 95%CI [-18.49 - -4.25], P=0.002). Furthermore, significant reductions were observed in all other measured parameters within the transplant group when compared to controls, except for LVEDV.

Upon analysing the changes observed within each group, it was evident that the parameters within the transplant group exhibited significant changes from baseline to follow-up (LVM mean change -7.23g 95%CI [-13.80 - -0.66] P=0.032; LVMI mean change -5.47g/m^2 95%CI [-9.40 - -1.54], P=0.007). Conversely, in the control group there was a significant increase in LVM (LVM mean change 11.52g 95%CI [2.37 – 20.67], P=0.016), as well as in LVMI (LVMI mean change 5.90g/m^2 95%CI [0.04 – 11.77], P=0.049).

Figure 4.4 Left ventricular mass and volumetric analysis at baseline and follow-up.

	N	Baseline	Follow-up	Within group change	P Value	Between group change	P Value
LVM(g)			**				
Transplant	50	129.75±36.65	122.51±28.42	-7.23(-13.80 - -0.66)	0.03		0.002
Control	20	147.27±47.18	158.80±50.37	11.52(2.37 – 20.67)	0.02	-18.76 (-30.46 – -7.05)	
LVMI (g/m^2)			**				
Transplant	50	69.42±19.18	63.95±12.52	-5.47(-9.40 - -1.54)	0.01		0.002
Control	20	74.91±21.37	80.81±23.76	5.90(0.04 – 11.77)	0.05	-11.37(-18.49 – -4.25)	
LVEDV (mL)			**				
Transplant	50	162.78±45.86	146.00±34.28	-16.78(-26.78 - -6.78)	<0.001		
Control	20	166.74±47.61	166.98±38.95	0.23(-12.84 – 13.31)	0.97	-17.01 (-34.61 – 0.58)	0.06
LVEDVI			**				
(mL/m^2)	50	86.90±23.62	76.56±16.32	-10.3(-16.02 - -4.66)	<0.001		0.03
Transplant	20	84.69±19.04	85.24±16.69	0.55(-5.79 – 6.88)	0.86	-10.88(-20.61– -1.16)	
Control							
LVESV (mL)							
Transplant	50	60.77±24.30	52.43±21.23	-8.35(-16.02- -4.66)	0.004		0.04
Control	20	61.39±25.55	63.59±23.70	2.20(-6,00 – 10.39	0.58	-10.54(-20.61 – -0.47)	
LVESVI (mL/m^2)	50						
Transplant	20	32.39±12.53	27.41±10.07	-4.98(-8.04 – -1.92)	0.002		
Control		31.01±10.83	32.40±11.24	1.39 (-2.71 – 5.49)	0.49	-6.37(11.77 – -0.96259)	0.02

*Data are displayed as mean ± SD at baseline and follow-up for the whole cohort. Within-group differences were determined by paired samples t tests. Between-group differences were determined using independent samples t-tests. Between and within group differences are displayed as the mean difference in values (95% CI). ** Denotes significant difference (p=<0.05) between groups at baseline or follow-up*

LVM left ventricular mass, LVMI left ventricular mass indexed, LVEDV left ventricular end diastolic volume, LVEDV left ventricular end diastolic volume indexed, LVESV left ventricular end systolic volume, LVESVI left ventricular end systolic volume indexed.

Figure 4.5 Individual cases plotted from baseline to follow-up for both transplant and control group showing changes in LVM (g).

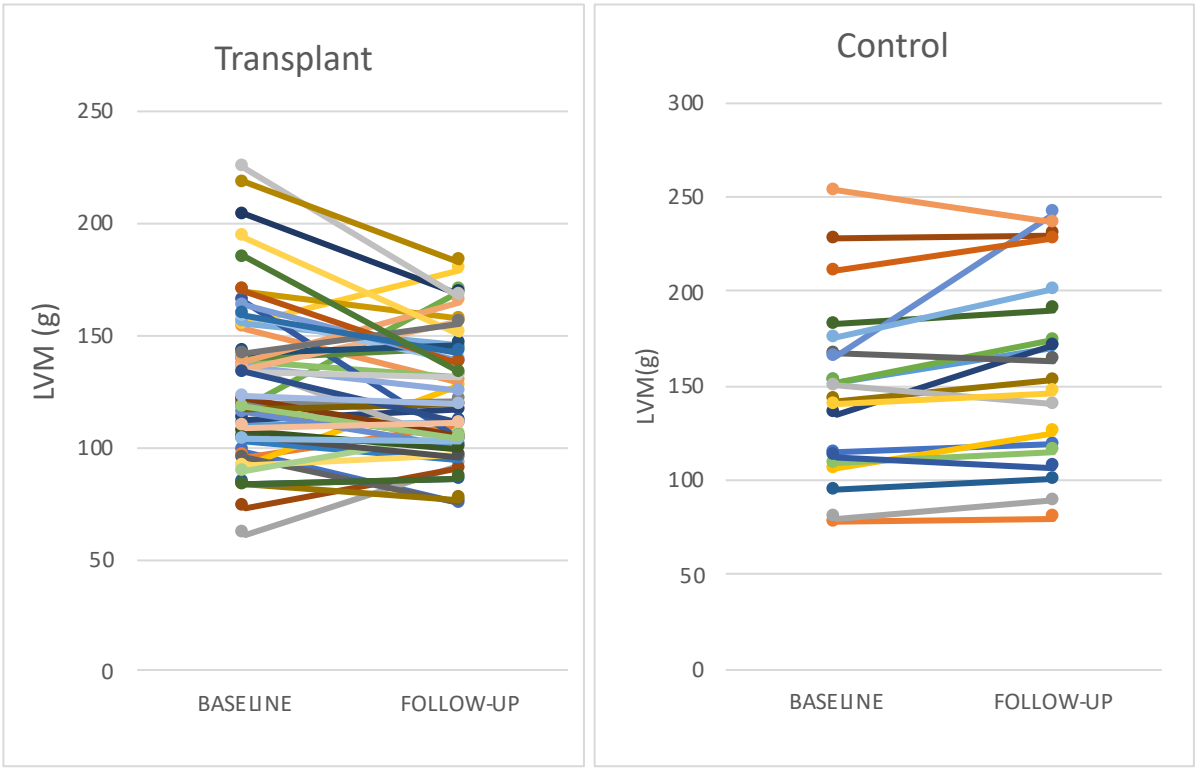
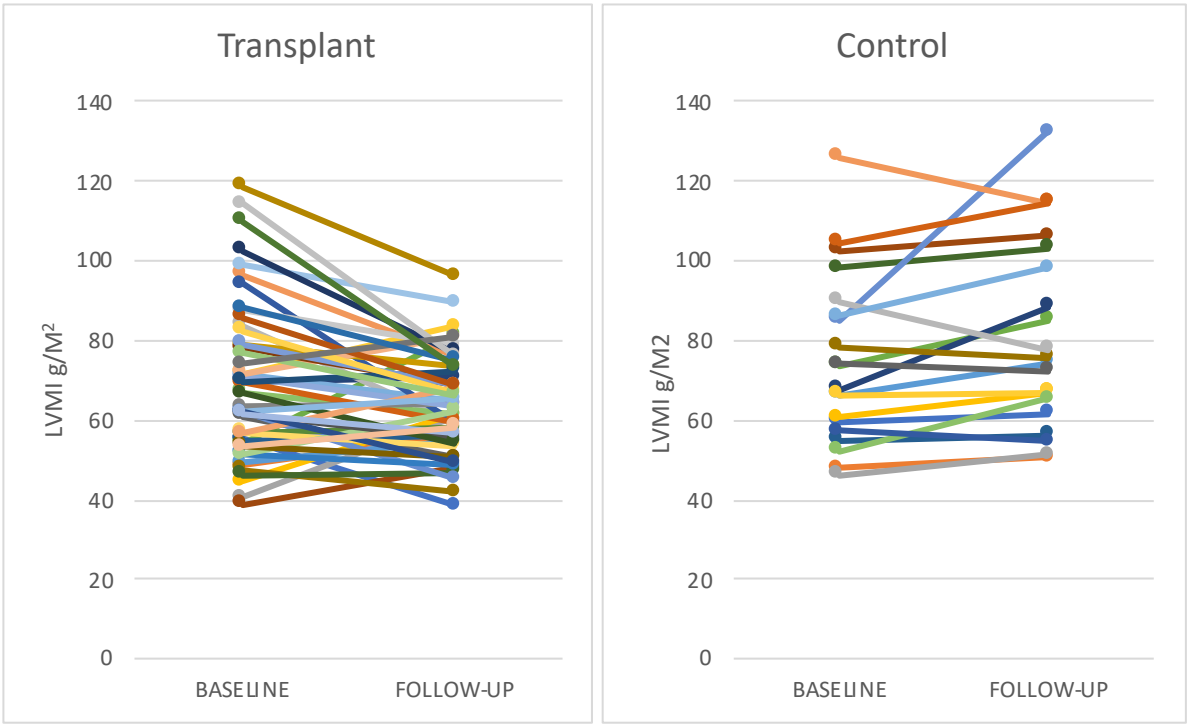


Figure 4.6 Individual cases plotted from baseline to follow-up for both transplant and control groups showing changes in LVMI (g/m2).



4.6.2 LVH Subgroup Analysis.

Transplant recipients were also stratified based on the presence of LVH at baseline as previously described in **Section 3.7.1**.

Demographic data for the two groups is presented in **Figure 4.7.** This indicated that age and length of follow-up was similar in both groups. There was a significant difference in the sex distribution between the two groups (84% LVH group, 48% non LVH group P=0.01). There were also differences observed in the use of alpha blockers (53%vs13% P=0.001) and calcium channel blockers (74% and 10% P<0.001).

When transplant recipients were stratified based on the presence of LVH at baseline there were significant reductions in all parameters in the LVH group compared to the non-LVH group; LVM (mean difference-21.83g 95%CI [-33.96-9.71] P<0.001), LVMI (mean difference --13.74g/m^2 95%CI [-20.89- -6.59] P<0.00), LVEDV(mean difference -23.81(-43.46 - -4.16)P=0.02), LVEDVI (mean difference -14.63 mL/m^2 95%CI[-27.45 - -1.81] P=0.03), LVESV (mean difference -15.67mL 95%CI[-26.37 - -4.97]P<0.001) LVESVI (mean difference -8.96mL/m^2 [-14.77 – -3.15]P<0.001).

Percentage change in LVM from baseline to follow-up in in those with LVH at baseline showed a decrease of -12.14% ±11.53%, in those without LVH at baseline there was an increase of 2.72%±18.80%. The differences in percentage change between the groups were statistically significant (P=0.03). Percentage change in LVMI from baseline to follow-up in in those with LVH at baseline showed a decrease of -14.54% ±12.67%, in those without LVH at baseline there was an increase of 1.71%±22.85%. The differences in percentage change between the groups were statistically significant (P=0.04).

Within group analysis from baseline to follow-up in the LVH group indicated that there were significant reductions in LVM (mean change -20.77g 95%CI[-30.97 – -10.57] P=0.001)and LVMI (mean change -13.99g/m^2 95%CI [-20.29 - -7.69] P=0.001) It was also noteworthy that this change was also accompanied by significant reductions in measures of LVEDV (mean change, -31.54ml 95%CI[-52.63 - - 10.46]P=0.001) LVEDVI (mean change, -19.41ml/m^2 95%CI[- 31.35 - - 7.46)P=0.001], LVESV (mean change -18.05ml 95%CI [-28.80 - -7.32] P=0.002) LVESVI (mean change -10.54ml/m^2 95%CI[-16.35 - -4.72]P=0.001).

In those without LVH there were no significant within group changes observed for either the parameters of LVM/LVMI or left ventricular volumes.

Figure 4.7 Demographic and clinical characteristics stratified by the presence of LVH.

	LVH N=19 (%)	No LVH N=31 (%)	P Value
Demographics			
Age (yrs)	45±14	49±12	0.33
Male sex	16(84)	15(48)	0.01
*Length of follow-up (days)	435±107	459±117	0.45
Race or ethnic group			
White	15(79)	27(87)	0.62
Asian	4(21)	3(9)	0.26
Black	0	1(3)	*
Body Surface Area	1.90±0.26	1.84±0.22	0.42
Risk factors			
Hypertension	17(89)	22(71)	0.25
Diabetes	1(5)	4(13)	0.45
Cardiovascular disease	2(11)	2(6)	0.60
Hypercholesterolaemia	6(32)	13(41)	0.36
Previous Renal Transplant	3(16)	5(16)	0.97
Current Smoker	2(11)	1(3)	0.29
Ex-smoker	5(26)	7(22)	
Medication			
Statin	6(32)	13(42)	0.36
Antihypertensive agent			
ACE inhibitor	3(16)	8(26)	0.76
ARB	2(11)	3(9)	0.92
Beta-Blocker	7(37)	10(32)	0.74
Alpha-blocker	10(53)	4(13)	0.002
Calcium Channel Blocker	14(74)	3(10)	<0.001

Data were analysed using either a student t-test for continuous data or Fisher exact tests for categorical data. A 2-tailed P<0.05 was considered statistically significant. Data are presented as mean±SD or n (%). *Length of follow-up was considered time between renal transplant and second CMR scan in transplant recipients. *(ACE angiotensin converting enzyme, ARB angiotensin receptor blocker)*

Figure 4.8 Left ventricular mass and volumetric analysis at baseline and follow-up for transplant recipients stratified by presence of absence of LVH at baseline.

	N	Baseline	Follow-up	Within group change	P Value	Between group change	P Value
LVM(g)		**	**				
LVH	19	159.04±31.05	138.27±24.58	-20.77(-30.97 – -10.57)	0.001		<0.001
No LVH	31	111.79±27.19	112.86±26.51	1.06(-6.42 – 8.55)	0.91	-21.83(-33.96-9.71)	
LVMI(g/m^2)		**	**				
LVH	19	86.85±16.37	72.86±9.77	-13.99(-20.29 - -7.69)	0.001		<0.001
No LVH	31	58.74±11.40	58.49±10.84	-0.25(-4.52 – 4.03)	0.77	-13.74(-20.89—6.59)	
LVEDV (mL)		**	**				
LVH	31	193.33±37.57	161.79±34.62	-31.54(-52.63 - - 10.46)	0.001		0.02
No LVH		144.05±40.42	136.32 ±30.75	-7.73 (-17.10 – 1.64)	0.10	-23.81(-43.46 - -4.16)	
LVEDVI (mL/m^2)		**	**				
LVH	19	105.68±20.62	86.27± 17.72	-19.41(- 31.35 - - 7.46)	0.001		0.03
No LVH	31	75.39±17.20	70.61± 12.26	-4.78 (-9.97 – 0.41)	0.07	-14.63(-27.45 - -1.81)	
LVESV (mL)		**	**				
LVH	19	79.05±23.47	60.99± 24.63	-18.05(-28.80 - -7.32)	0.002		<0.001
No LVH	31	49.57±17.11	47.18± 17.24	-2.39(-8.02 – 3.24)	0.39	-15.67(-26.37 - -4.97)	
LVESVI (mL/m^2)		**	**				
LVH	19	42.98±11.36	32.44± 12.00	-10.54(-16.35 - -4.72)	0.001	-8.96 (-14.77 – -3.15)	<0.001
No LVH	31	25.90±8.04	24.32± 7.31	-1.57(-4.64 – 1.50)	0.30		

*Data are displayed as mean ± SD at baseline and follow-up for the whole cohort. Within-group differences were determined by paired samples t tests. Between-group differences were determined using independent samples t-tests. Between and within group differences are displayed as the mean difference in values (95% CI). ** Denotes significant difference (p=<0.05) between groups at baseline or follow-up*

LVM left ventricular mass, LVMI left ventricular mass indexed, LVEDV left ventricular end diastolic volume, LVEDV left ventricular end diastolic volume indexed, LVESV left ventricular end systolic volume, LVESVI left ventricular end systolic volume indexed..

Figure 4.9 Individual cases plotted from baseline to follow-up for transplant recipients, showing changes in LVM(g) stratified according to presence of LVH at baseline.

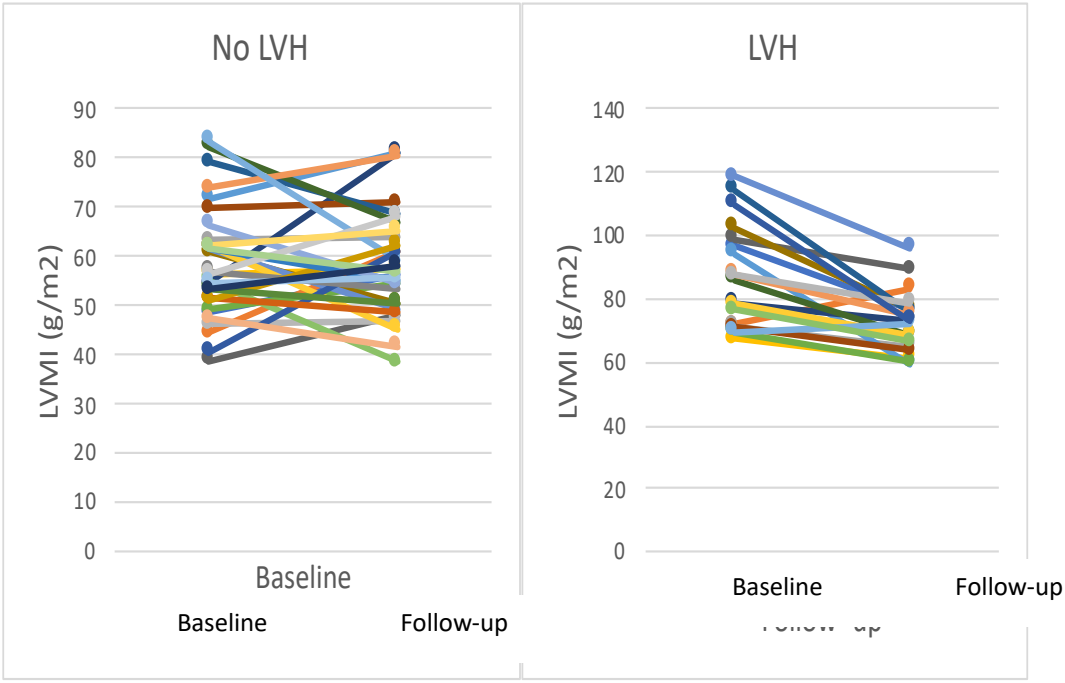


Figure 4.10 Individual cases plotted from baseline to follow-up for all transplant recipients showing changes in LVMI(g/m2) stratified according to presence of LVH at baseline.

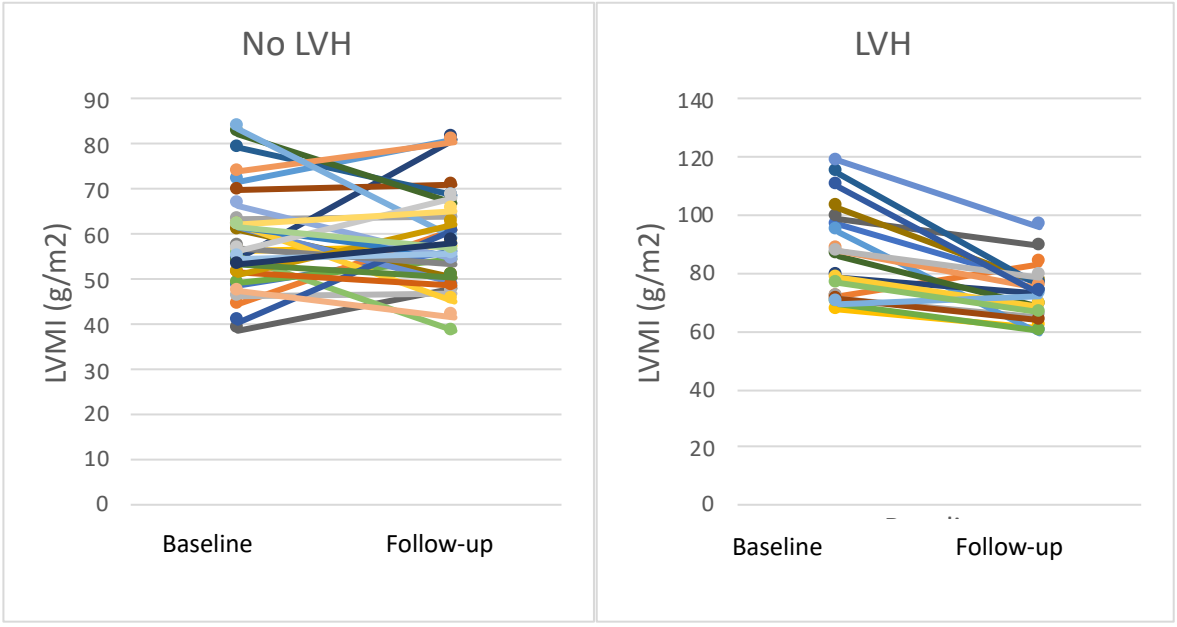


Figure 4.11 Box and whisker plot showing percentage change in LVM from baseline to follow-up.

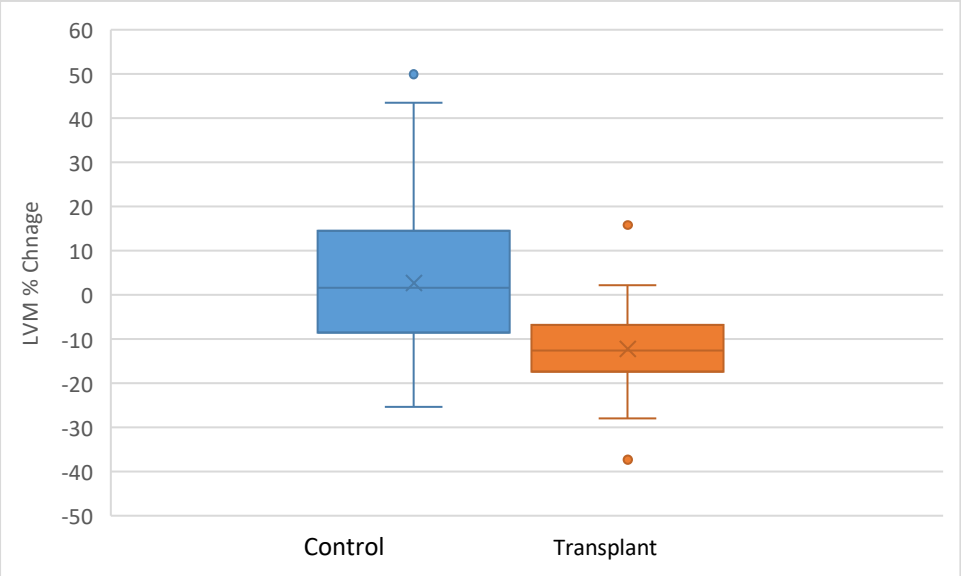
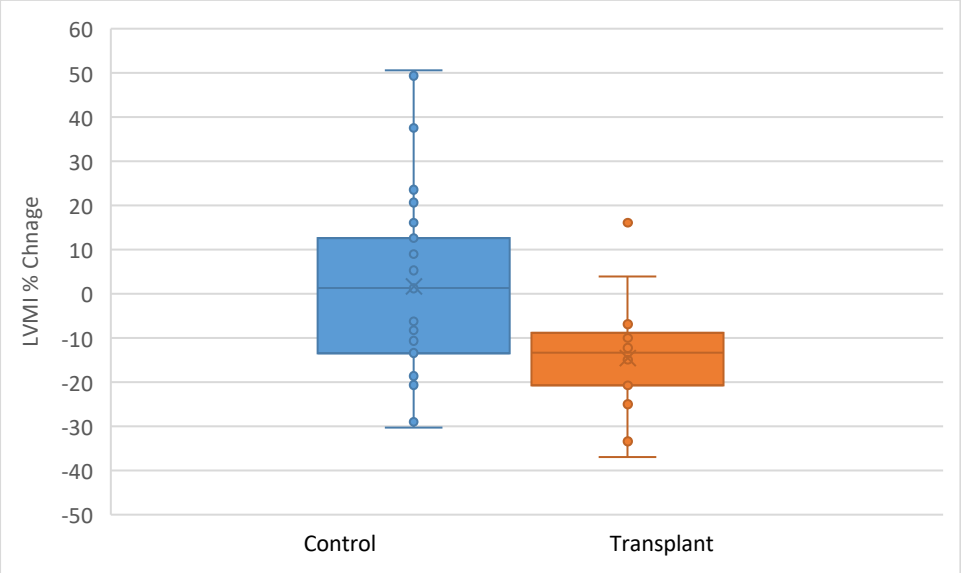


Figure 4.12 Box and whisker plot showing percentage change in LVMI from baseline to follow-up.



4.6.3 eGFR Subgroup Analysis

Transplant recipients were stratified based on eGFR at follow-up as previously described in **Section 3.7.1**. Demographic data is presented for the two groups in **Figure 4.13** which indicated that, sex distribution and length of follow-up was similar in both groups. Those with eGFR<50 mL/min/1.73m² had a higher mean age (50.90yrs 9.90 vs. 43.00yrs ±14.05 P=0.03) There were also differences observed in the prevalence of hypercholesterolaemia and statin use (57% vs 33% P= 0.02).

This stratified analysis indicated that there were no significant differences observed between the two groups for any of the parameters under study (LVM mean difference -8.41g 95%CI [-21.64 – 4.82] P=0.207 and LVMI mean difference -6.06g/m² 95%CI [-13.92 – 1.79] P=0.127. All data are presented in **Figure 4.14.** This analysis did however show that there was a trend towards greater reductions in all the parameters in those with higher renal function.

Paired analysis of changes within each group indicated that for those with an eGFR <50 mL/min/1.73m² at follow-up, there was no significant differences in any of the indices studied. In contrast, longitudinal changes for those with eGFR ≥50 mL/min/1.73m² indicated significant reductions in all parameters (LVM mean change -10.77g 95%CI [-19.84 - -1.70] P=0.002; LVMI mean change 8.02g/m² 95%CI [-13.30 - -2.73], P=0.004). This finding suggests that the achieved renal function following transplant was having a significant impact on cardiovascular remodelling.

Figure 4.13 Demographic and clinical characteristics stratified by eGFR at follow-up.

	eGFR<50 N=21	eGFR>50 N=29	P Value
Demographics			
Age (yrs)	50.90±9.90	43.00±14.05	P=0.03
Male sex	11(52)	20(69)	P=0.23
Length of follow-up (days)	452±98	439±120	P=0.69
Race or ethnic group			
White	18(86)	24(83)	P=0.78
Asian	2(9)	5(17)	P=0.44
Black	1(5)	0	*
Body Surface Area	1.89±0.27	1.88±0.24	P=0.82
Risk factors			
Hypertension	15(71)	24(83)	P=0.24
Diabetes	4(19)	1(3)	P=0.07
Cardiovascular disease	4(19)	0	*
Hypercholesterolaemia	12(57)	7(24)	P=0.02
Previous Renal Transplant	4(19)	4(14)	P=0.61
Current Smoker	1(5)	2(7)	P=0.75
Ex-smoker	7(33)	5(24)	
Medication			
Statin	12(57)	7(33)	P=0.02
Antihypertensive agent			
ACE inhibitor	5(24)	6(21)	P=0.79
ARB	2(10)	3(10)	P=0.92
Beta-Blocker	8(38)	9(31)	P=0.43
Alpha-blocker	5(24)	9(31)	P=0.57
Calcium Channel Blocker	9(43)	18(62)	P=0.18

*Data were analysed using either a student t-test for continuous data or Fisher exact tests for categorical data. A 2-tailed P<0.05 was considered statistically significant. Data are presented as mean±SD or n (%). *Length of follow-up was considered time between renal transplant and second CMR scan in transplant recipients*

(ACE angiotensin converting enzyme, ARB angiotensin receptor blocker)

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Figure 4.14 Left ventricular mass and volumetric analysis at baseline and follow-up for transplant recipients only, stratified by eGFR at follow-up.

	N	Baseline	Follow-up	Withing Group Change	Sig	Between group change	P value
LVM(g)							
eGFR<50	21	127.18±35.21	124.83±29.64	-2.35(-12.22 – 7.52)	0.62		
eGFR≥50	29	131.60±38.17	120.84±27.90	-10.77(-19.84 - -1.70)	0.02	-8.41(-21.64 – 4.82)	0.21
LVMI(g/m^2)							
eGFR<50	21	67.56±18.22	65.61±12.49	-1.95(-7.99 – 4.08)	0.51		
eGFR≥50	29	70.77±20.05	62.75±12.62211	-8.02(-13.30 - -2.73)	0.004	-6.06(-13.92 – 1.79)	0.13
LVEDV (mL)							
eGFR<50	21	159.21±43.32	149.88±37.30	-9.33(-23.30 – 4.64)	0.18		
eGFR≥50	29	165.36±48.20	143.19±32.29	-22.17(-36.53 - -7.82)	0.004	-12.85(-32.99-7.30)	0.21
LVEDVI (mL/m^2)							
eGFR<50	21	84.53±22.46	79.48±18.05	-9.33(-23.30 – 4.64)	0.20		
eGFR≥50	29	88.61±24.67	74.44±14.91	-22.17(-36.53 - -7.82)	<0.001	-9.13(-20.46 – 2.20)	0.11
LVESV (mL)							
eGFR<50	21	62.44±26.43	55.51±25.10	-6.93(-15.33 – 1.46)	0.10		
eGFR≥50	29	59.56±23.04	50.19±18.06	-9.37(-17.25 - -1.48)	0.02	-2.44(- 13.85 – 8.98)	0.67
LVESVI (mL/m^2)							
eGFR<50	21	33.07±13.47	29.37±12.02	-3.71(-8.34 – 0.93)	0.11		
eGFR≥50	29	31.89±12.02	25.99±8.32	-5.90 (-10.17 - -1.63)	0.01	-2.19(-8.42 – 4.03)	0.48

*Data are displayed as mean ± SD at baseline and follow-up for the whole cohort. Within-group differences were determined by paired samples t tests. Between-group differences were determined using independent samples t-tests. Between and within group differences are displayed as the mean difference in values (95% CI). ** Denotes significant difference (p=<0.05) between groups at baseline or follow-up*

LVM left ventricular mass, LVMI left ventricular mass indexed, LVEDV left ventricular end diastolic volume, LVEDV left ventricular end diastolic volume indexed, LVESV left ventricular end systolic volume, LVESVI left ventricular end systolic volume indexed.

Figure 4.15 Individual cases plotted from baseline to follow-up for all transplant recipients showing changes in LVM(g) stratified according to eGFR at follow-up.

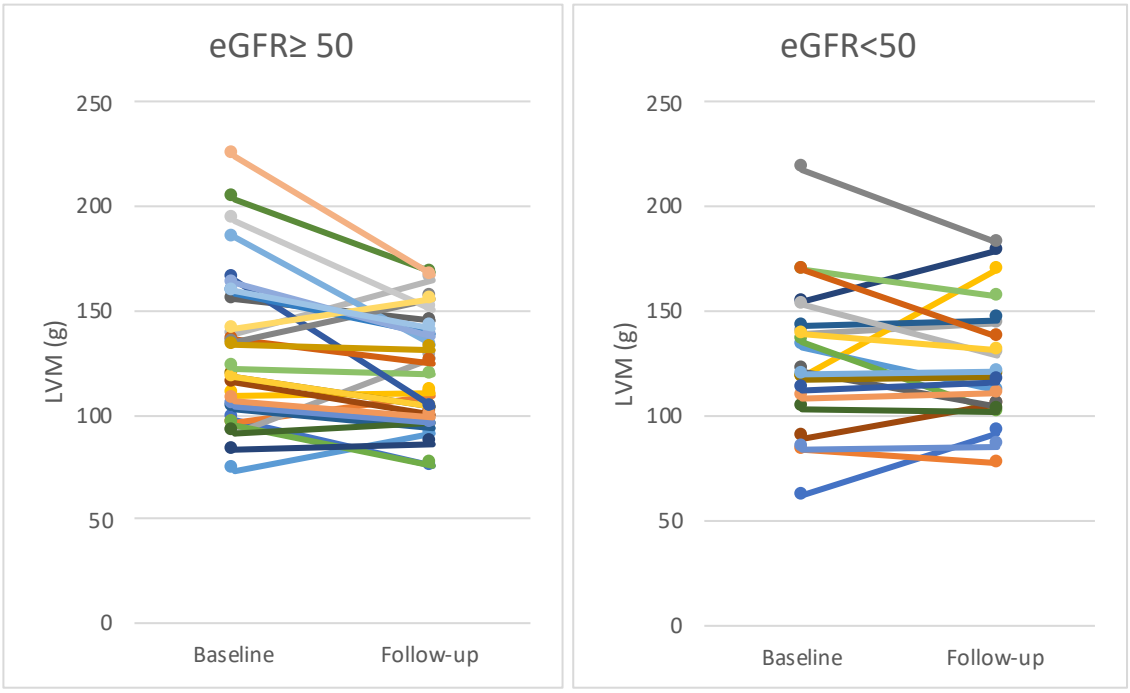
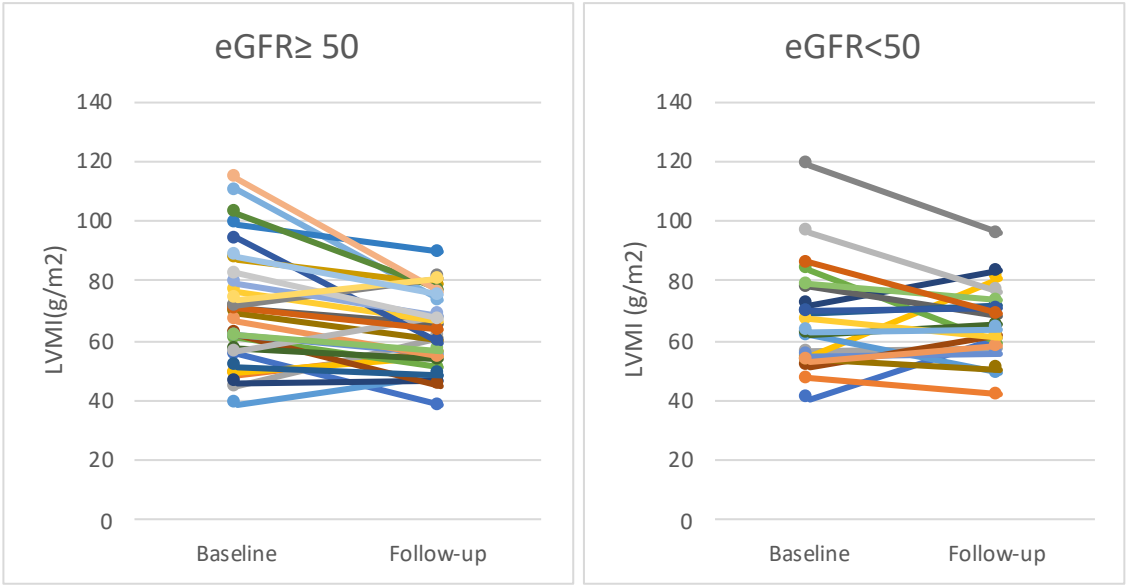


Figure 4.16 Individual cases plotted from baseline to follow-up for all transplant recipients showing changes in LVMI(g/m2) stratified according to eGFR at follow-up.



4.7 Reproducibility

Intra-observer and interobserver variability as measured by intraclass correlation was low (intra-observer variability 0.99, 95%CI [0.97–0.99] inter-observer variability 0.99, 95%CI [0.99–1.00], intra-study variability 0.96 95%CI [0.85 -0.99] P=<0.001. Bland-Altman plots are presented in **Figures 4.17, 4.18, 4.19**.

Figure 4.17 Bland-Altman plot for inter-observer reproducibility of LVM.

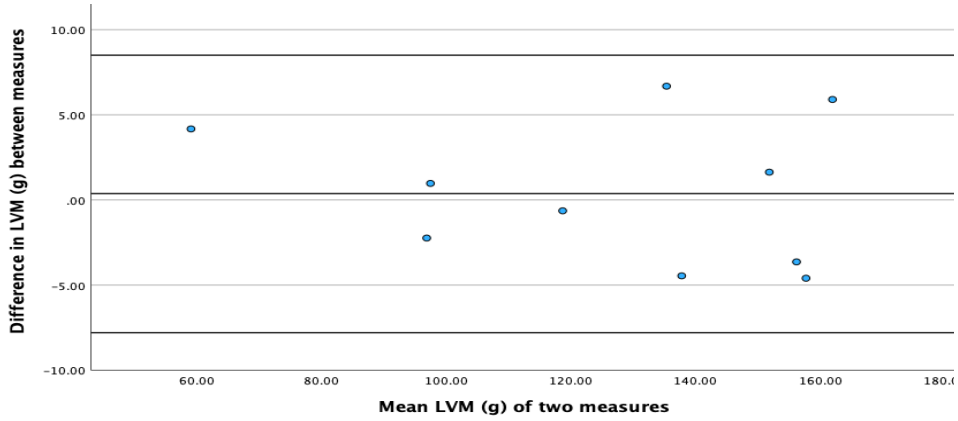


Figure 4.18 Bland-Altman plot for intra-observer reproducibility of LVM.

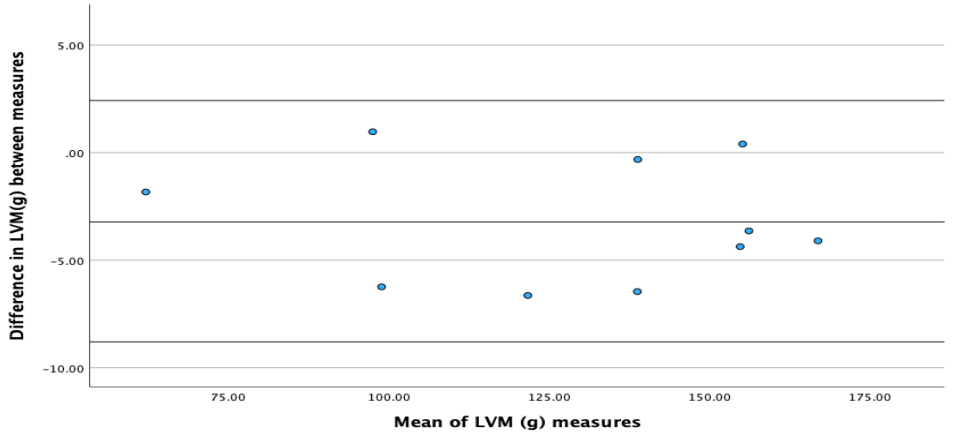
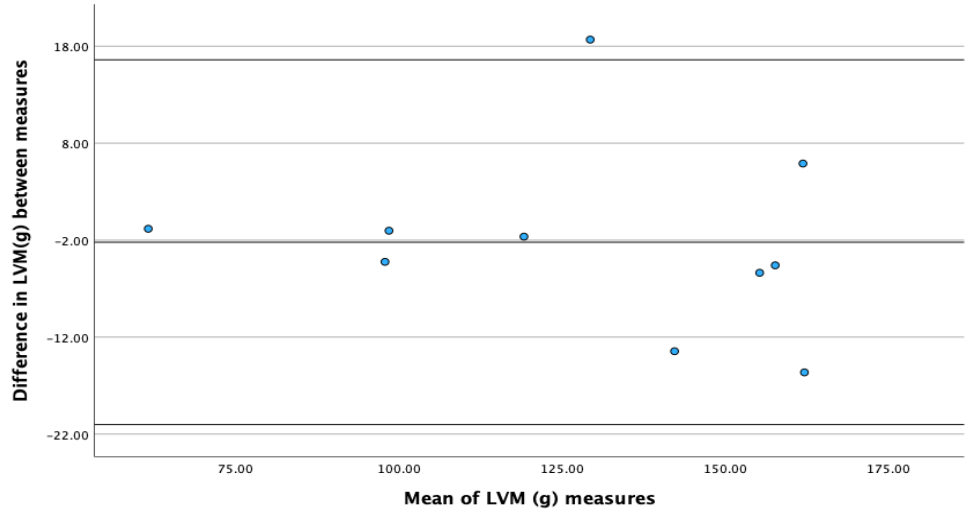


Figure 4.19 Bland-Altman plot for intra-study reproducibility of LVM.



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4.8 Discussion

This chapter set out to examine four hypotheses identified in **section 4.3**. The current data has identified that following renal transplantation:

i. The restoration of renal function after successful renal transplantation was associated with decreased LVM and LVMI

ii. In those with LVH at baseline there were greater reductions in LVM/LVMI following transplantation.

iii. Higher levels of renal function (eGFR>50 ml/min/1.73 m^2) following successful transplantation were associated with greater reductions in LVM and LVMI.

iv. There were no significant differences in blood pressure changes between the two groups. There were also no longitudinal changes within either groups.

4.8.1 Transplant recipients and controls

The current data clearly demonstrates that renal transplantation results in a substantial decrease in the primary endpoints of LVM and LVMI when compared to a similar group of control participants with ESKD. Moreover, there were also significant reductions in LVEDVI, LVESV, and LVESVI in the transplanted group compared controls. These findings indicate that the adverse cardiovascular remodelling observed in ESKD is reversed by renal transplantation.

The RETRACT study is one of the first prospective, blinded, controlled studies examining changes in LVM following renal transplantation. LVM was selected as the primary outcome in the study due to its proposed association with cardiovascular morbidity and mortality in those with ESKD and after renal transplantation (257). Stack and Saran et al. (27) highlighted that the presence of LVH, in patients newly commenced on dialysis, was significantly associated with mortality at six months (RR 1.61 95% CI [1.17-2.22]), with this risk persisting

at two years of follow-up. Rigatto et al. (29) also reported that the presence of LVH at one year following renal transplantation was associated with both mortality (RR1.9 95%CI [1.22-3.22]) and heart failure (RR 2.27 95%CI [1.08-4.81].

This proposed association between LVM, and mortality has more recently been the subject of debate within the literate. Initial meta-analysis by Badve et al. (262) concluded that there was no clear and consistent association between intervention induced LVM change and mortality. As a result, it was suggested that evidence for LVM as a valid surrogate end point in CKD is currently lacking. Many of the trials included in this analysis, however, were of short duration with small sample sizes. There was also, either an uncertain, or high risk of bias in those trails included. As such this data must be interpreted with caution. A further meta-analysis performed more recently, has refuted these findings (70). It is the opinion here that this data is more robust given that only studies with a follow-up greater 12 months were included, in addition the levels of heterogeneity of the studies within the meta-analysis was low. This work reported that in studies with longer than 12-month follow-up in which there was a reduction in LVM in a treatment group compared to controls the RR of all-cause mortality was 0.72 95% CI [0.57 - 0.90] P = 0.005.) This study, therefore, supports the notion that reduction in LVM is an appropriate surrogate marker for mortality in those with CKD.

While there are conflicting opinions on the relationship between LVM and mortality, it is often cited that the significant improvements in cardiovascular mortality following renal transplantation are due to regression in LVH (198, 263). The data presented in **chapter 2** has however, demonstrated that the assumption that LVH regresses after transplant is not based on robust evidence (261) As such the current data which was powered to detect a clinically significant change in LVM of 7g adds significantly to the current understanding of

cardiovascular remodelling following transplantation. This current finding, therefore, support the hypothesis that such remodelling may be contributing to the improvements in mortality observed. It is, however, acknowledged that the RETRACT study was not designed to assess improvements in mortality rates in transplant recipients.

The fact that LVM is reversible following renal transplantation is important. While renal transplantation is the gold standard treatment for ESKD, there remain a large proportion of patients who are clinically unsuitable for transplantation or who are unable to find a suitable donor. The burden of cardiovascular disease in this group remains unacceptably high. At present there is considerable focus on developing targeted therapies for the treatment of uraemic cardiomyopathy, particularly targeting FGF23 and α-Klotho (264). The current data lend further support to this developing area of research as it clearly establishes that uraemic cardiomyopathy is a reversible process.

Blood pressure data were also presented for the two groups. Blood pressure changes are a confounding factor when examining interventions aimed at inducing cardiovascular remodelling in those with CKD. Often it is not possible to establish if an intervention is having a direct effect on the myocardium, or if changes are being produced indirectly via changes in blood pressure. Such difficulties were demonstrated by the SpiroCKD study which aimed to look at the effects of reduced aldosterone levels on LVM in those with CKD 2-3(243). This study reported a reduction LVM of 14g in those receiving spironolactone compared to 3g in placebo group. This change, however, was also accompanied by significant reduction in blood pressure in the treatment arm of the study making interpretation of data problematic. The current data suggests that the changes in LVM were not associated with significant changes in blood pressure either within, or between the two groups. Neither group had significantly

elevated blood pressure at either baseline or follow-up. This relationship is further discussed in **chapter 8** where liner regression analysis was employed to understand this interaction in greater detail.

The current data has also shown that there were significant reductions in measures of both diastolic and systolic volumes. This finding supports the previous work of Prasad et al. (220) who reported similar findings in transplant recipients, compared to control participants following transplantation. Hayer et al. (85) also reported significant reductions in diastolic volumes two months following transplantation. Hypervolaemia is known to be common in ESKD and is a particular problem in both HD and PD cohorts (265) Hypervolemic states produce increased preload which can induce ventricular dilation, diastolic dysfunction, hypertension and the development of LVH (266). Ventricular dimensions represent an indirect marker of fluid status and are therefore clinically significant as hypervolaemia is strongly associated with both all-cause mortality and cardiovascular mortality in ESKD (267). The current finding of reduced left ventricular volumes following transplantation is further evidence of the beneficial effects of renal transplant on both cardiovascular structure and consequently cardiovascular mortality.

It is important to acknowledge that volume status can also affect the diagnostic accuracy of imaging modalities. This issue is a particular problem when using echocardiography, where large variations in volume status, such as those seen in HD and following transplantation, are associated with high levels of interobserver variability(177). This problem was overcome in the current study by employing CMR which is considered the gold standard for cardiovascular imaging in CKD. Previous work by Hunold et al.(176) demonstrated measures of LVM both before and after dialysis underwent only small changes (3.7±2.0%), whereas changes in

LVEDV were significantly higher (20.4% ±11.4) as such it was concluded that measures LVM using CMR were largely independent of loading conditions. As such the current data is considered robust.

4.8.2 eGFR subgroup analysis

Subgroup analysis stratified by renal function did not highlight any between group changes in the data.

Within group analysis, however, demonstrated that in those with eGFR<50 there were no significant changes in any of the parameters studied. In those recipients where was eGFR>50 there were significant reductions in all the parameters including LVM and LVMI suggesting that post-transplant cardiovascular remodelling is influenced by the magnitude of eGFR improvement. The relationship between renal function and LVM has previously been examined in CKD by Izamura et al. (268) where declining renal function across the stages of CKD was associated significant stepwise increases in LVM. Following transplantation Dzemidzic et al. (269) also demonstrated that following transplantation positive cardiovascular remodelling correlated significantly with reductions in serum creatinine.

The finding that LVM/LVMI are associated with changes in renal function has not previously been investigated in a study which has employed CMR. This aspect of the RETRACT study is therefore a clinically important finding which contributes significantly to the understanding of renal function on cardiovascular remodelling.

When interpreting this data, it must also be acknowledged that renal function is a continuous variable. As a result, performing this binary stratification will result in an oversimplification

of any relationships which may be present. This issue is addressed in **chapter 8** where linear regression will further address the relationship between eGFR and LVM/LVMI.

4.8.3 LVH subgroup analysis

LVH is considered the predominant feature of uraemic cardiomyopathy. As such, those patients presenting with LVH represent a specific group most effected by the condition. It was therefore hypothesised that this cohort may benefit the most from transplantation, in terms of cardiovascular remodelling.

This idea is supported by our data which has indicated that when LVH was present at baseline there were significant reductions in both LVM and LVMI at follow-up. When LVH was not present the reverse was true, with no significant changes in either parameter. When examining percentage in LVM and LVMI it was also of note that in those with LVH at baseline there were significant reductions in both parameters, however the reverse was true in those where LVH was not present. The current findings are supported by the earlier work of Hernandez et al. (232) where it was reported that there were significant correlations between pre-transplant LVMI and post-transplant changes in LVMI. These data must also be interpreted with some caution given the possibility of the phenomena of regression to the mean which can produce similar findings.

It was also an interesting finding that in those with LVH at baseline, reductions in left in LVM and LVMI occurred in conjunction with similar changes in ventricular volumes. This finding was unsurprising as the development of hypertrophy is well recognised in other volume loaded conditions, such as in aortic and mitral regurgitation. Increased ventricular dilation produces increased ventricular wall stress. This increased wall stress directly stimulates sarcomere replication which in turn results in the development of LVH. This compensatory mechanism

can be explained by Laplace's law which states (pressure x radius)/ (2xwall thickness) (270). It has also been demonstrated previously that this process is reversible following the correction of volume overload. Vollema et al (271) reported that following surgical aortic valve replacement for aortic regurgitation LVM declined by 28.8%±24.8. Shafii et al.(272) also demonstrated a similar pattern of results following mitral valve repair or replacement. As such the current data which has shown reductions in both LVM and LVEDV mirrors these findings.

4.9 Limitations

There are several limitations to the RETRACT study. It was not possible to recruit the predetermined number of participants as initially defined by the Power calculation of 50 transplant recipients and 25 control patients. This was primarily due to the difficulties of conducting research during the COVID-19 pandemic. While numbers of control participants were therefore slightly lower than those projected, the author believes that the data presented does make a significant contribution to the current evidence. This is supported by the work of Myerson et al. (179) who concluded that a clinically significant change of 10g in LVM can be accurately detected based on 13 participants using CMR.

The current data has indicated that changes in LVM were also accompanied by changes in left ventricular volumes. While previous work has noted that changes in LVM are largely independent of loading status it would have been useful to perform body composition analysis in conjunction with CMR. This would have allowed the contribution of hydration status to the changes observed to be fully investigated. This analysis was not within the scope of the current work due to funding constraints. As such this is an avenue for future work in this area.

The measurement of blood pressure was also considered a limitation. In the current data this was assessed using BPTru. This approach was required as it was not possible to perform

ambulatory monitoring due to infection control constraints during the COVID-19 pandemic. Using BPtru was considered the best alternative to ambulatory blood pressure as it has been shown to correlate with ambulatory recordings more favourably than standard office blood pressure(246) HD recipients were also studied on a day following dialysis in order to avoid recording artificially elevated values due to fluid overload. Even with this approach it is acknowledged office blood pressure in ESKD may be subject to inaccuracies (72).

Follow-up of participants was also significantly affected by COVID-19 due to restrictions on research activity. As previously stated, this forced the research team to change the initial research protocol allowing follow-up to take place at between 12-24 months. While this created slightly differential follow-up between the groups, subsequent analysis has shown that there was no statistical difference between follow-up times in the two groups of the study. It is possible that a longer follow-up time may influence the magnitude of change observed. In order to fully understand this relationship, it would be beneficial to repeat the current analysis at a five-year interval.

A further limitation of the current data is that it does not include morbidity and mortality outcomes. A such it is not possible to conclude what effects the current patterns of change will have on mortality. Further longitudinal research of this cohort should therefore be considered to understand the relationship between cardiovascular remodelling and mortality.

4.10 Conclusion

The RETRACT study has shown that following renal transplantation there is a significant reduction in LVM and LVMI. This study is the first to employ a rigorous research methodology to address this question. This research supports the idea that renal transplantation can reverse uraemic cardiomyopathy. Due to this observed reversibility future research efforts

should be focused on targeted therapies aimed at improving features of uraemic cardiomyopathy in those where renal transplantation is not an available treatment option.

Chapter 5: SYSTOLIC FUNCTION AND DIASTOLIC FUNCTION ANALYSIS

Abstract

Background

Systolic and diastolic dysfunction are common in those presenting with uraemic cardiomyopathy. They are powerful predictors of cardiovascular morbidity and mortality in those with ESKD. At present there is conflicting evidence regarding the effect of renal transplantation on ventricular function.

Objectives

To establish if indices of left ventricular systolic and diastolic function improve following renal transplantation.

Participants

50 renal transplant recipients recruited from local transplant waiting list. 20 control participants with ESKD not scheduled for live donor transplantation.

Methods

Participants had CMR parameters of systolic and diastolic function recorded at both baseline and follow-up of 12-24 months.

Results

There was no significant between group reductions observed for transplant recipients in any of the parameters examined (between group changes; left ventricular stroke volume (LVSV) -7.29mL95%CI [-18.26 - 3.67] P=0.19, ejection fraction (EF) 3.01%95%CI [-0.76 - 6.77] P=0.26, contraction fraction 2.17%95%CI [-1.10 – 5.45] P=0.45, left ventricular global functional index (LVGFI) 2.17% [-1.10 – 5.45] P=0.19). When stratified by the presence of LVH at baseline there were significant between group differences in LVSV (-12.93ml 95%CI [-24.8- -1.05] P=0.03 and LVSVI (-8.44ml/m^2 95%CI [-14.85- -2.03] P=0.001). There were no significant between group differences when stratified by eGFR at follow-up. Feature

tracking analysis suggested that there were no changes seen following renal transplantation when compared to controls for markers of either systolic or diastolic function.

Conclusion

The RETRACT study has shown that in a group with normal measures of systolic function at baseline there is no significant improvement compared to control participants.

5.1 Introduction

The phenotypic changes seen in uremic cardiomyopathy are almost ubiquitously observed in patients with ESKD effecting up to 70% of this group (167, 261). The main changes observed include LVH, myocardial fibrosis and both systolic and diastolic dysfunction (256). In those receiving HD it has been reported that LVSD occurs 10-30 times more often than in the general population with rates observed between 13 and 48% (273, 274).The finding of LVSD is important in this cohort as it has significantly implications for prognosis. Jokie et al. (275) reported that LVSD (EF<50%) had a positive predictive value of 42% for cardiac death. In addition, Parfrey et al. (276) highlighted that median survival after initiation of dialysis in those with LVSD was 38 months. Once clinical features of heart failure develop the age and sex adjusted 1 year mortality rate is 11 times in this group, than those without(76)

The gold standard for assessment of cardiac structure and function in those with ESKD is CMR. This is because in a patient cohort where large volume shifts can take place, CMR is considered to produce data which is independent of these changes (177). Traditionally the standard assessment for quantifying left ventricle systolic function is EF% which is calculated based on the percentage change between measures of end-diastolic and end-systolic volumes. While it is often cited within the literature that systolic function improves after transplantation, this relationship is not currently fully understood. Multiple non-controlled echocardiographic studies have suggested that ejection may fraction improve following transplantation (216, 217, 223, 225, 229, 236, 239). These results however have not been reproduced by studies which have employed CMR as their imaging modality (220, 221). The meta-analysis presented in **Chapter 2** has demonstrated that at present the literature does not support the notion that EF improves following transplant. The results presented also highlighted that the current evidence base is of low quality with high levels of heterogeneity. As such drawing firm conclusions regarding changes in systolic function after transplantation is problematic.

While EF is the most widely used parameter in clinical practice there are circumstances where EF can be considered an incomplete measure of systolic function and associated cardiac output. In conditions where LVH predominates there can be progressive changes in left ventricle cavity size. As a result of this issue, contraction fraction which is a novel index of both left ventricular structure and function has been developed (277). Contraction fraction is the ratio of left ventricular stroke volume to myocardial volume. It has previously been shown to differentiate between pathological and physiological hypertrophy in addition to being a predictor for cardiovascular events in previously healthy individuals (278) This measure, however, has not been applied to those with ESKD or to a cohort of renal transplant recipients.

In addition to contraction fraction a further index developed to examine both systolic function and left ventricular structure is the left ventricular global functional index (LVGFI) (279). This combines the left ventricular stroke volume with and systolic and diastolic volumes as well as LVM. This measure has been shown to be a powerful predictor of lifetime cardiac events and incident heart failure in a multi-ethnic population (279). As is the case with contraction fraction this parameter has not been applied to either ESKD or renal transplant recipients.

A further technique used in CMR is that of feature tracking. This technique allows the assessment of global longitudinal, radial, and circumferential strain of the left ventricle. This method is reported to allow the detection of alterations in myocardial function prior to changes in the EF. Global longitudinal strain (GLS) is established as an independent predictor of prognosis in multiple myocardial conditions including uraemic cardiomyopathy. Rankin et al.(280) highlighted that among 215 patients with normal ejection fraction and ESKD reduced GLS was an independent predictor of mortality HR 1.08 (95% CI [1.01–1.16]). GLS has also been reported to improve following renal transplantation. Barbosa et al. (281) reported that in 44 renal transplant recipients

GLS improved from 13.4% ± 3.0 at baseline to 15.2% ± 2.7 at follow-up ($p < 0.001$). Kim et al.(282) also demonstrated that in 488 patients GLS improved significantly following transplant These studies, however, were not considered methodologically robust as they a not adequately powered, were non-blinded and did not employ a comparable control group. Further robust studies are therefore required to establish changes in myocardial strain after transplant.

Diastolic dysfunction is common in CKD. Yu et al.(76) reported that in a CKD cohort of 14249 individuals with prevalent heart failure, 70% was due to diastolic dysfunction. The aetiology of this dysfunction is multifactorial, which includes haemodynamic changes due to increased afterload as a result of increasing arterial stiffness, and Increased preload in response hypervolaemia(103). Metabolic and electrolyte disturbances are also believed to have direct effects on the myocardium which leads to the development of diffuse interstitial fibrosis (138). Following transplantation there is currently no consensus regarding changes in diastolic dysfunction, the current data is reviewed in **Chapter 2.**

It was the aim of this section of the RETRACT study to examine parameters of both systolic and diastolic function to establish the effects of renal transplantation.

5.2 Methods

The study methodology was as described previously in **Chapter 3**.

5.3 Hypothesis

Following renal transplantation:

i. Left ventricular EF will improve significantly in transplant recipients compared to control participants.

ii. Novel markers of left ventricular function; Contraction Fraction and LVGFI will improve significantly following transplant.

iii. Indices of left ventricular systolic strain will improve significantly.

iv. Indices of left ventricular diastolic strain will improve significantly.

5.3.1 Primary Endpoints

i. Left Ventricular Ejection Fraction

5.3.2 Secondary Endpoints

i. Stroke Volume.

ii. Contraction Fraction.

iii. Left ventricular global function index.

iv. GLS.

v. GCS.

vi. GRS.

vii. Early and late diastolic longitudinal strain rate.

viii. Early and late diastolic radial strain rate.

ix. Early and late diastolic circumferential strain rate.

5.4 Results

5.4.1 Transplants and controls

Demographic data for the cohort are discussed in presented in **Figure 4.2**.

When examining parameters of systolic function there was no significant different changes observed between the groups for either LVSV (-7.29ml 95%CI (-18.26 - 3.67) P=0.19) or LVSVI (-5.01ml/m^2 95%CI (-10.91- 0.90) P=0.95). This data did suggest there was a trend for a greater reduction in transplant recipients in these parameters than controls, this is in keeping with the previous data presented in **chapter 4** which highlighted greater reductions in both LVEDV and LVESV in transplant recipients than controls.

This data also indicted that there were no differences between the groups in EF (3.01%95%CI [-0.76 - 6.77] P=0.12), contraction fraction (2.93% 95%CI [-4.26 -10.62] P=0.45) or LVGFI (2.17 95%CI [-1.10 – 5.45)] P=0.19). This data indicated a trend towards a small improvement in EF in transplant recipients, both contraction fraction and LVGFI reduced in both groups.

Longitudinal paired analysis was also conducted. In transplant recipients from baseline to follow-up there were significant reductions in LVSV (mean change-9.6 mL 95%CI [3.69 – 15.65] P=0.002) and LVSVI (mean change -6.10mL/m^2 95% [-9.39 - -2.80] P=<0.001). There were no significant changes observed in the other parameters.

The same analysis repeated for control participants indicated that there were statistically significant reductions in contraction fraction (mean change -6.23% 95%CI [-12.17 - -0.29], P=0.041). The other parameters did not change significantly.

Figure 5.1 Indices of left ventricular function in transplant and control participants at baseline and follow-up.

	N	Baseline	Follow-up	Within group change	P Value	Between group change	P Value
EF (%)							
Transplant	50	63.39±7.00	64.82±7.27	1.44(-0.50 - 3.37)	0.142		
Control	20	63.94±7.17	62.37±8.56	-1.57(-5.27 - 2.13)	0.39	3.01(-0.76 - 6.77)	0.12
LVSV (ml)							
Transplant	50	103.25±26.80	93.58±19.09	-9.67(3.69 – 15.65)	0.002		
Control	20	105.70±28.92	103.32±25.2	-2.38(-7.00– 11.76)	0.60	-7.29(-18.26 - 3.67)	0.19
LVSVI (mL/m^2)							
Transplant	50	55.14±13.57	49.04±9.41	-6.10(-9.39 - -2.80)	<0.001		
Control	20	53.90±12.61	52.81±10.92	-1.09(-5.79 - 3.62)	0.63	-5.01(-10.91- 0.90)	0.95
CF (%)							
Transplant	50	85.04±14.48	81.74±13.75	-3.30 (-7.62 – 1.03)	0.132		
Control	20	77.96±15.73	71.73±16.23	-6.23(-12.17 - -0.29)	0.04	2.93(-4.26 -10.62)	0.45
LVGFI (%)							
Transplant	50	44.47±6.09	43.95±6.22	-0.52(02.26 – 1.23)	0.556		
Control	20	42.39±6.99	39.70±0.07	-2.69(-5.66 -= 0.28)	0.07	2.17(-1.10 – 5.45)	0.19

Data are displayed as mean ± SD at baseline and follow-up for the whole cohort. Within-group differences were determined by paired samples t tests. Between-group differences were determined using independent samples t tests. Results are displayed as the mean difference (95% CI).

LVSV- left ventricular stroke volume LVSVI - left ventricular stroke volume indexed to BSA, EF - Ejection fraction CF- contraction fraction LVGFI – left ventricular global functional index.

Figure 5.2 Individual cases plotted from baseline to follow-up showing change EF for trasnsplants and controls.

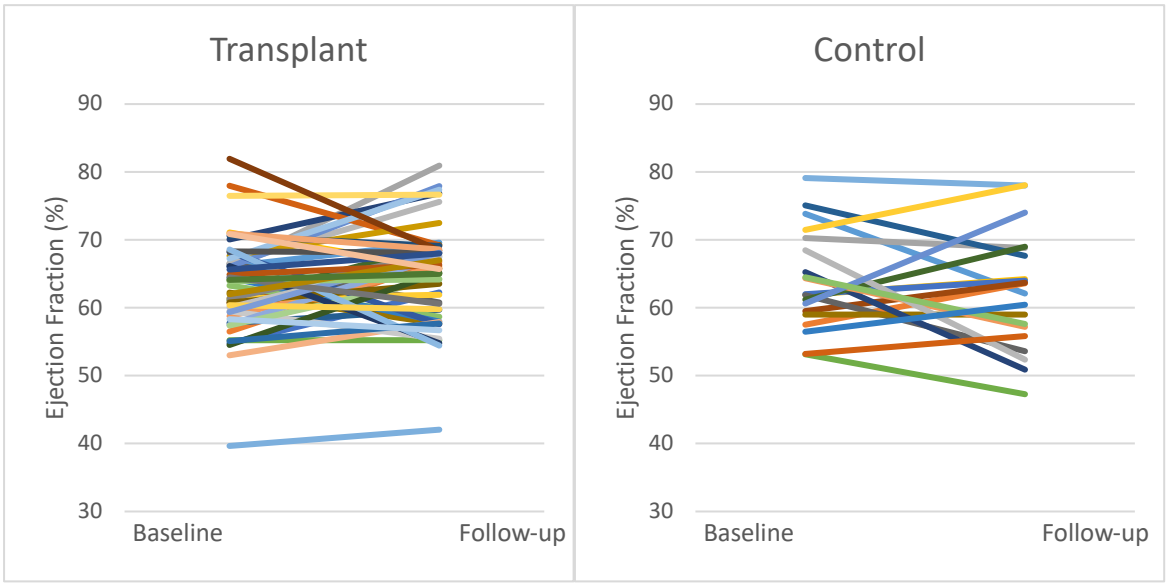
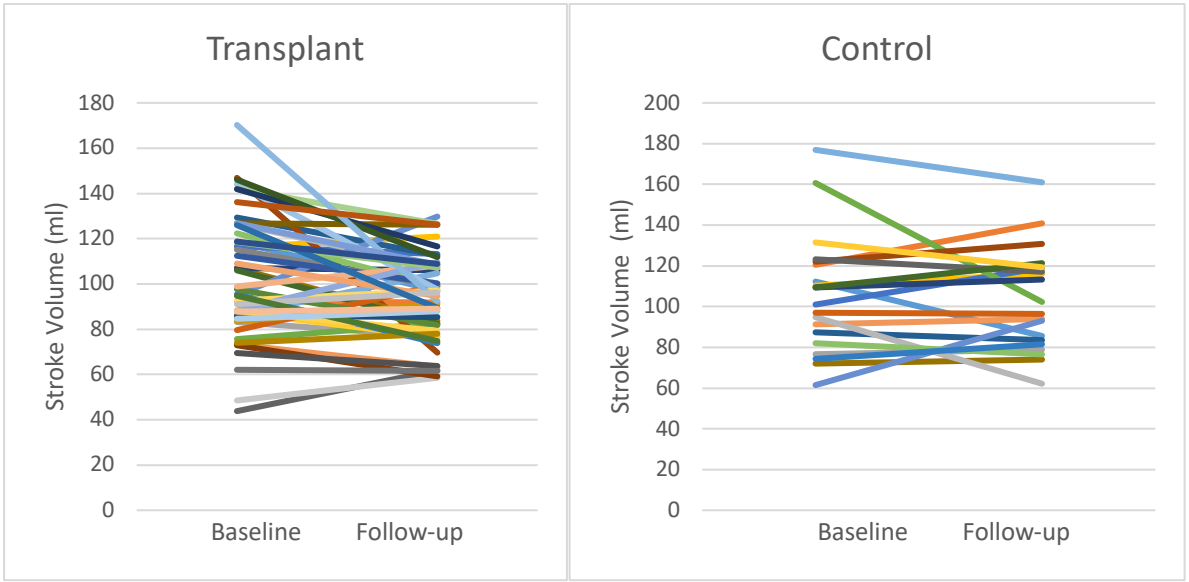


Figure 5.3 Individual cases plotted from baseline to follow-up showing change in stroke volume (ml) for trasnsplants and controls.



5.4.2 LVH Subgroup Analysis

Demographic data stratified by presence of LVH at baseline is presented in **Figure 4.2.**

At follow-up there were significant reduction observed in the LVH group compared to the no LVH group in both LVSV-12.93ml95%CI [-24.8- -1.05] P=0.03 and LVSVI -8.44ml/m^2 95%CI [-14.85- -2.03] P=0.01. There were no significant differences between the groups for the other parameters studied.

Longitudinal analysis of those patients with LVH showed that there were significant reductions in EF (3.52% 95%CI [1.21- 5.83] P<0.001), LVSV (-17.69ml 95%CI [-28.15 - -7.23] P=<0.001 and LVSVI (-11.33ml/$m^2$95%CI [-17.53 - -5.13] P<0.001). Examining the cases individually only one transplant recipient had EF<50% at baseline which showed a small improvement (39% to 42%). It was therefore not possible to perform a sub-analysis of those presenting with LVSD at baseline.

Longitudinal analysis of the patients who initially presented without LVH showed that there were no significant changes in any of the parameter's studies.

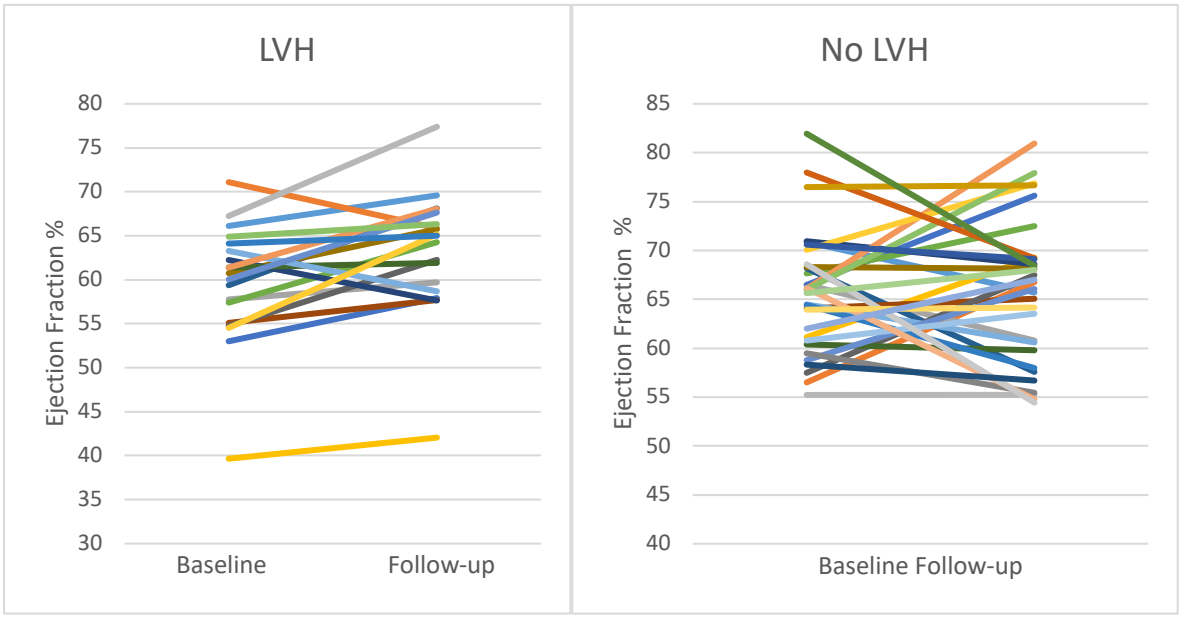
Figure 5.4 Indeces of left ventriucalr function in trasnplant receipients startified by LVH at baseline.

	N	Baseline	Follow-up	Within group change	P Value	Between group change	P Value
EF % LVH No LVH	 19 31	** 59.69± 6.79 65.66± 6.20	 63.21±7.12 65.81±7.30	 3.52(1.21- 5.83) 0.16(-2.62-2.94	 <0.001 0.91	3.36 (-0.54 - 7.27)	0.09
LVSV (mL) LVH No LVH	 19 31	** 118.50±20.01 93.91± 26.37	** 100.81± 16.63 89.15±19.39	 -17.69(-28.15 - -7.23) -4.76(-11.87-2.35	 <0.001 0.18	-12.93(-24.8- -1.05)	0.03
LVSVI (mL/m^2) LVH No LVH	 19 31	** 64.84± 11.55 49.19± 11.17	** 53.52±9.17 46.30±8.60	 -11.33(-17.53 - -5.13) -2.89(-6.41- 0.64)	 <0.001 0.10	-8.44(-14.85- -2.03)	0.01
Contraction Fraction (%) LVH No LVH	 19 31	** 79.82± 14.62 88.23± 13.65	 77.93±14.36 84.07±13.04	 -1.89(-7.65- 3.87) -4.16(-10.41-2.09)	 0.51 0.18	2.27(-6.71 -11.25)	0.61
LVGFI LVH No LVH	 19 31	** 41.68 ±6.43 46.17± 5.28	 41.80±5.15 45.27±6.52	 0.12(-2.09 - 2.33) -0.91(-3.47-1.65)	 0.91 0.48	1.03(-2.60 - 4.66)	0.57

D*ata are displayed as mean ± SD at baseline and follow-up for the whole cohort. Within-group differences were determined by paired samples t tests. Between-group differences were determined using independent samples t tests. Results are displayed as the mean difference (95% CI).*

LVSV- left ventricular stroke volume LVSVI - left ventricular stroke volume indexed to BSA, EF - Ejection fraction CF- contraction fraction LVGFI – left ventricular global functional indexed to BSA

Figure 5.5 Individual cases plotted from baseline to follow-up showing change EF in trasnsplants startified by LVH at baseline.



5.4.3 eGFR subgroup analysis

Demographic data for the cohort stratified by renal function at follow-up are presented in **Figure 4.13**.

The levels of change observed between the groups was not significantly different for any of the parameters under study (EF -0.86% 95%CI [-4.81 – 3.10] P=0.36).

Longitudinal analysis indicated that in those patients that showed improvement in eGFR>50 mL/min/1.73m^2 there were significant reductions in both LVSV (mean change -12.22 mL95%CI [-21.44--3.01] P=0.01) and LVSVI (mean change -7.99ml/m^2 95%CI [-12.94--3.03] P=0.003) There were no significant changes observed in the other parameters.

In those participants where renal function was eGFR<50 ml/min/1.73m^2 there were significant reductions in LVSVI (-3.49 95%CI [7.53 - 0.56] P=0.09). There were no significant changes observed in the other parameters.

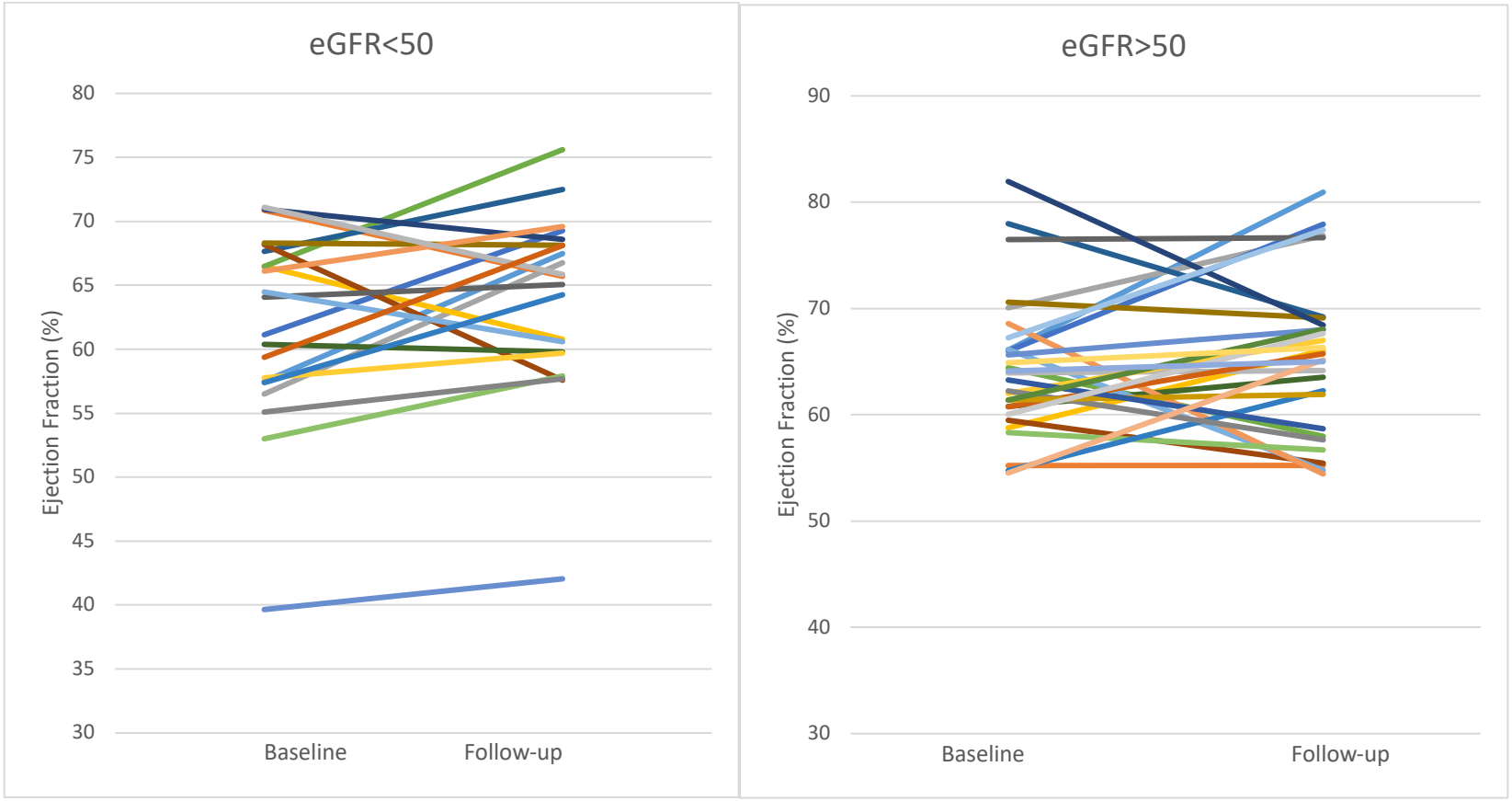
Figure 5.6 Indeces of left ventriucalr function in trasnplant receipients startified by eGFR at follow-up.

	N	Baseline	Follow-up	Within group change	P Value	Between group change	P Value
EF (%)							
eGFR<50	21	62.02±7.57	63.95±7.09	1.93(-0.75– 4.62)	0.445		
eGFR>50	29	64.38±6.51	65.46±7.46	1.08(-1.77 – 3.92)	0.445	-0.86(-4.81 – 3.10))	0.36
LVSV (mL)							
eGFR<50	21	100.53±24.59	94.38±18.27	--6.15(-0.92 – 13.21)	0.09		
eGFR>50	29	105.22±28.56	93.00±19.97	-12.23(3.01 – 21.44)	0.011	-6.08(-18.20 - 6.04)	0.32
LVSVI (mL/m^2)							
eGFR<50	21	53.36±12.40	49.87±8.94	-3.49(-7.54 – 0.56)	0.003		
eGFR>50	29	56.43±14.44	48.45±9.85	-7.99(-12.94 - -3.03)	0.003	-4.50(- 11.12 -12.12)	0.18
Contraction Fraction (%)							
eGFR<50	21	84.56±14.36	81.04±13.33	-3.52(-10.44 – 3.40)	0.301		
eGFR>50	29	85.38±14.80	82.24±14.25	-3.14(-9.03 – 2.76)	0.285	0.38(-8.47- 9.24)	0.75
LVGFI (%)							
eGFR<50	21	44.15±6.85	43.30±5.75	-0.85(-3.53 – 1.83)	0.516		
eGFR>50	29	44.69±5.59	44.42±6.60	-0.27 (-2.72 – 2.17)	0.82	0.58(-3.00 - 4.15)	0.43

Data are displayed as mean ± SD at baseline and follow-up for the whole cohort. Within-group differences were determined by paired samples t tests. Between-group differences were determined using independent samples t tests. Results are displayed as the mean difference (95% CI).

LVSV- left ventricular stroke volume LVSVI - left ventricular stroke volume indexed to BSAEF - Ejection fraction CF- contraction fraction LVGFI – left ventricular global functional indexed to BSA

Figure 5.7 Individual cases plotted from baseline to follow-up showing change EF in trasnsplants startified by eGFR at follow-up.



5.4.4 Systolic Strain

Initial analysis of feature tracking data indicated that none of the data was normally distributed. It was therefore necessary to use non-parametric analyses as described in **Section 3.7** to assess the differences between participants at baseline and follow-up. In the transplant group 5 patients and in the control group 1 patient was excluded from the analysis due to lack of endocardial border definition long in long axis images.

When comparing transplant recipients and control participants there were no significant differences between the two groups at either baseline or follow-up. In addition, there was also no significant differences observed between the magnitude of change in each group for any of the parameters studied.

When stratified based on the presence of LVH at baseline there were again no differences between the groups at baseline. A significant difference was observed at follow-up in measures of GCS (P=0.02), however there were no differences between the magnitude of change seen between the two groups.

When stratified by eGFR the similar pattern of results was again observed, with no significant differences observed in any of the measures observed.

Figure 5.8 Feature tracking analysis of systolic function in transplant and control participants.

	N	Baseline	Between group P Value	Follow-up	Between group P Value	Median change	Between group P Value
GLS Transplant Control	 44 19	 -15.00(-15.98 – -13.13) -14.00(-15.70 – -12.50)	 0.26	 -13.70(15.85 – 11.83) -12.10 (-14.00 – -9.20)	 0.92	 0.55(-1.28 – 2.18) 1.20 (-0.40 – 3.90)	 0.64
GRS Transplant Control	 44 19	 29.55(24.40 – 35.43) 27.70(23.10 – 34.50)	 0.46	 32.75(27.93 – 39.75) 30.80(25.20 – 34.90)	 0.24	 2.80(-3.78 – 8.83) 1.50(-3.20 – 3.50)	 0.93
GCS Transplant Control	 44 19	 -19.70(-21.58 – -16.8) -19.50(-20.80 – -15.90)	 0.48	 -19.55(-21.48 – -17.65) -19.20 (-21.10 – -15.80)	 0.68	 -0.35(-2.08 – 1.45) 0.00(-2.60 – 1.90)	 0.93

Figure 5.9 Feature tracking analysis of systolic function in transplant recipients stratified by LVH at baseline.

	N	Baseline	Between group P Value	Follow-up	Between group P Value	Median change	Between group P Value
GLS NO LVH LVH	 27 18	 -15.20 (-16.65 – 13.35) 13.55 (-15.8 – 10.90)	 0.087 .	 13.40 (-16.52- -11.45) -13.45 (-14.37 - -10.87)	 0.26	 0.30 (-1.90 – 2.84) 0.90(-0.90 – 2.55)	 0.44
GRS NO LVH LVH	 27 18	 31.40 (28.05 – 36.28) 24.20 (19.70 – 30.60)	 0.018	 33.85 (29.65 – 40.70) 30.10(25.90 -39.65)	 0.22	 1.50 (-4.90 – 12.00) 4.45 (-2.70 – 9.33)	 0.49
GCS NO LVH LVH	 27 18	 -19.85 (-21.43 - -17.5) -17.2 (-21.0 - -11.50)	 0.209	 -20.05 (-21.72 - -18.32) -18.15 (-20.05 – 14.87)	 0.021	 -1.0 (-3.0 – 1.70) 0.0 (.2.35 – 1.67)	 0.84

Data
are presented as median (IQR).
Statistical significance was established using non-parametric Mann-Whitney U test
GLS- global longitudinal strain
GRS- global radial strain
GCS- global circumferential strain

Figure 5.10 Feature tracking analysis of systolic function in transplant recipients stratified by eGFR at follow-up.

	N	Baseline	Between group P Value	Follow-up	Between group P Value	Median change	Between group P Value
GLS							
eGFR<50	20	-14.10(-16.20-11.73)		-13.30(-15.35- -10.85)		0.90(-0.98-2.63)	
eGFR>50	25	-15.20(-15.95-13.23)	0.555	-13.75(-16.08- -11.80)	0.377	0.20(-1.88-2.58)	0.334
GRS							
eGFR<50	20	28.20(20.13-34.38)		30.70(27.10-38.23)		1.50(-4.40-8.55)	
eGFR>50	25	30.15(27.20-36.88)	0.248	34.60(28.00-39.95)	0.346	3.20(-2.78-10.18)	0.860
GCS							
eGFR<50	20	-18.20(-21.10- -16.80)		-19.20(-20.78- -17.68)		-0.90(-3.08-2.13)	
eGFR>50	25	-20.25(-21.82 - -16.87)	0.396	-19.35(-22.38- -16.28)	0.944	0.15(-1.95-1.65)	0.451

Data are presented as median (IQR).
Statistical significance was established using non-parametric Mann-Whitney U test
GLS- global longitudinal strain
GRS- global radial strain
GCS- global circumferential strain

5.4.5 Diastolic strain

Analysis of diastolic parameters was also undertaken. This was performed using the 3D stain methodology which has previously been described in **Section 3.6.7**. Early (e') and late (a') diastolic strain rates were analysed. Data are presented in **Figures 5.11 5.13 5.15.** Data was stratified as previously described in **chapter 3.** None of the data analysed followed a normal distribution as such non-parametric analysis was conducted as previously described.

Analysis of the transplant and control participants indicated that at baseline there was no significant difference between the groups for any of the parameters under study. In addition, the diastolic function of both groups was considered within normal range when compared to previously published data using CMR 3D tracking (254). Repeat analysis also indicated that the groups remained similar with no significant differences between them at follow-up. Again, the values obtained were considered to represent normal diastology.

5.4.6 LVH subgroup analysis

This highlighted a trend towards reduced diastolic function in those with LVH at baseline. There was only a significant difference observed in early diastolic radial strain rate (no LVH group -1.85IQR (-2.37 - -1.52) vs LVH group -1.35 IQR (-1.97 - -1.00) P=0.04). At follow-up there was only significant difference detected in early global circumferential strain rate no LVH group 0.95IQR(0.90 – 1.13) vs. LVH group 0.85 IQR(0.57 – 1.00) P=0.013.

5.4.7 eGFR subgroup analysis

Analysis stratified by renal function indicated that failed to show that here were any differences at either baseline or follow-up. Both groups demonstrated normal diastolic function at both time points. Again, the magnitude of changes observed were small. Longitudinal within group analysis again failed to show any significant changes from baseline to follow-up in either group.

Figure 5.11 Diastolic strain rate analysis in transplant and control groups at baseline and follow-up.

	N	Baseline	Follow-up	Within group change	P Value	Between group change	P Value
GLS e' Transplant Control	 45 19	 0.70 (0.40 – 0.80) 0.50 (0.40 - 0.90)	 0.329	 0.60 (0.50 – 0.88) 0.50 (0.40-0.60)	0.092	 0.00(-0.10 -0.1) 0.00(-0.10 -0.1)	0.743
GLS a' Transplant Control	 45 19	 0.60 (0.60 -0.80) 0.50 (0.40 - 0.80)	 0.318	 0.60 (0.50 – 0.70) 0.50(0.40 – 0.70)	 0.521	 0.00(-0.15 - 0.1) 0.00((-0.20 – 0.10)	0.988
GCS e' Transplant Control	 45 19	 0.90 (0.70 – 1.10) 0.60 (0.40 -0.90)	 0.144	 0.90 (0.73 – 1.00) 0.80 (0.70 – 1.1)	 0.551	 0.00(-0.20 – 0.20) 0.00(-0.10 -0.40)	0.279
GCS a' Transplant Control	 45 19	 0.80(0.60 – 0.90) 0.60 (0.40 – 0.90)	 0.130	 0.70 (0.60 – 0.80) 0.70 (0.60 – 0.90)	 0.794	 0.00(-0.15 - 0.1) 0.10 (0.00 -0.20)	0.069
GRS e' Transplant Control	 45 19	 -1.80(-2.23 - -1.16) -1.40(-.80 - -1.10)	 0.095	 -1.80 (-2.48 - -1.50) -1.70 (-2.50 - -1.30)	 0.939	 -0.15(-.60 – 0.40) -0.70(-1.0 – 0.10)	0.177
GRS a' Transplant Control	 45 19	 -0.70(-1.13 - -0.50) -0.50(-0.90 - -0.40)	 0.223	 -0.70 (-1.00 - -0.60) -0.60 (-0.90 - -0.50)	 0.245	 0.00 (-0.50 – 0.30) -0.10 (-0.50 – 0.30)	0.988

Data are presented as median (IQR). Statistical significance was established using non-parametric Mann-Whitney U test. GLS- global longitudinal strain GRS- global radial strain GCS- global circumferential strain

Figure 5.12 Individual cases plotted from baseline to follow-up showing change in early diastolic radial strain rate for transplant and control participants.

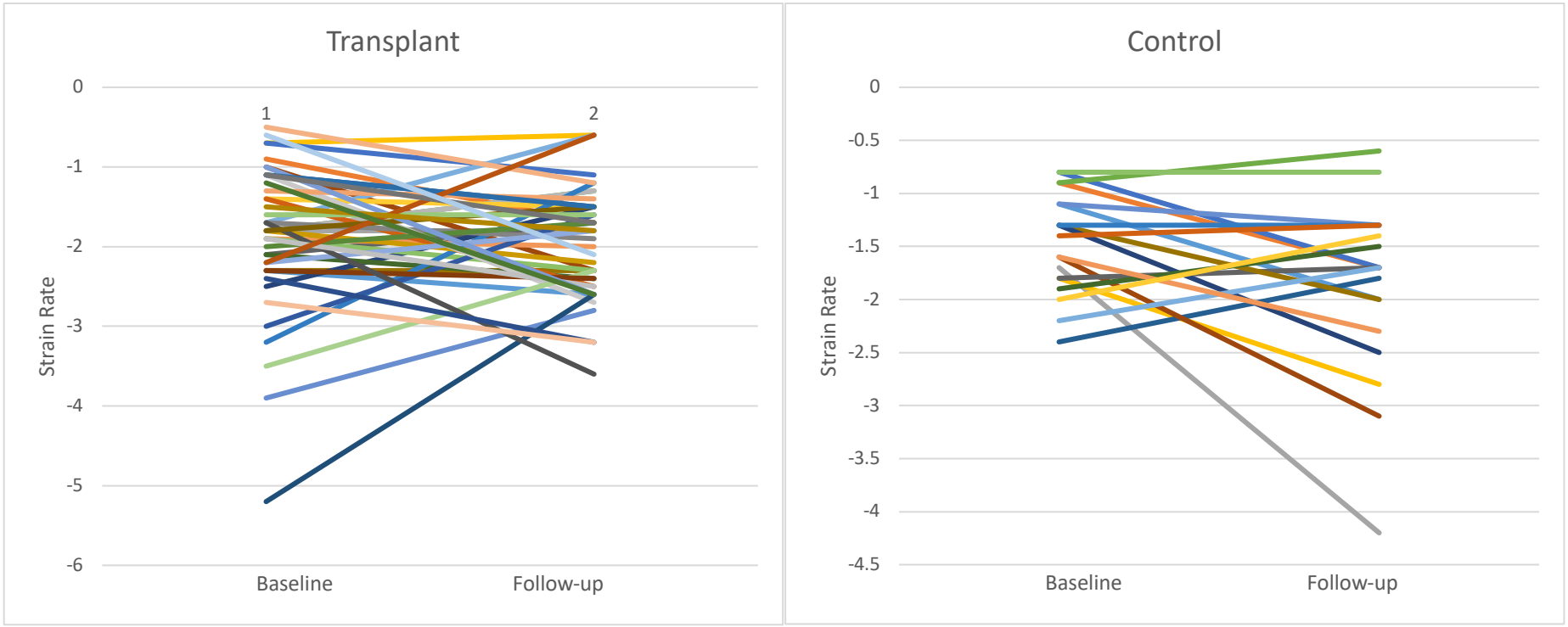


Figure 5.13 Diastolic strain rate analysis of transplant recipients stratified by LVH at baseline.

	N	Baseline	Follow-up	Within group change	P Value	Between group change	P Value
GLS e' NO LVH LVH	 27 18	 0.70 (0.53 -0.98) 0.50 (0.40 – 0.80)	 0.87	 0.60 (0.60 – 0.90) 0.50(0.45 – 0.80)	 0.49	 0.00(-0.10 – 0.10) 0.00(-0.12 – 0.13)	 0.833
GLS a' NO LVH LVH	 27 18	 0.65 (0.60 -0.80) 0.60 (0.50 – 0.70)	 0.120	 0.60 (0.50 – 0.70) 0.60(0.40 – 0.60)	 0.125	 0.00(-0.10 – 0.10) -0.05(-0.20 – 0.02)	 0.465
GCS e' NO LVH LVH	 27 18	 0.90 (0.80 – 1.18) 0.80 (0.50 – 1.10)	 0.079	 0.95(0.90 – 1.13) 0.85(0.57 – 1.00)	 0.013	 0.01(-0.20 – 0.30) 0.00(-0.12 - 0.13)	 0.475
GCS a' NO LVH LVH	 27 18	 0.80 (0.60 – 1.00) 0.70 (0.50 – 0.80)	 0.158	 0.70 (0.60 – 0.83 0.70 (0.48 – 0.83)	 0.348	 0.01(-0.20 – 0.00) 0.00(-0.15 0.23)	 0.452
GRS e' NO LVH LVH	 27 18	 -1.85(-2.37 - -1.52) -1.35 (-1.97 - -1.00)	 0.037	 -1.80(-2.52 - -1.58) -0.70 (-0.90 - -0.57)	 0.189	 -0.10(-0.60 – 0.50) -0.40 (– 0.65 – 0.05)	 0.353
GRS a' NO LVH LVH	 27 18	 -0.80 (-1.10 - -0.52) -0.60 (-0.70 - -040)	 0.19	 -0.80 (-1.00 - -0.55) -0.70(-0.90 - -0.57)	 0.636	 0.05(-0.50 – 0.45) -0.10(-0.50 – 0.05)	 0.138

Data are presented as median (IQR). Statistical significance was established using non-parametric Mann-Whitney U test. GLS- global longitudinal strain GRS- global radial strain GCS- global circumferential strain

Figure 5.14 Individual cases plotted from baseline to follow-up showing change in early diastolic longitudinal strain rate in transplants startified by LVH at baseline.

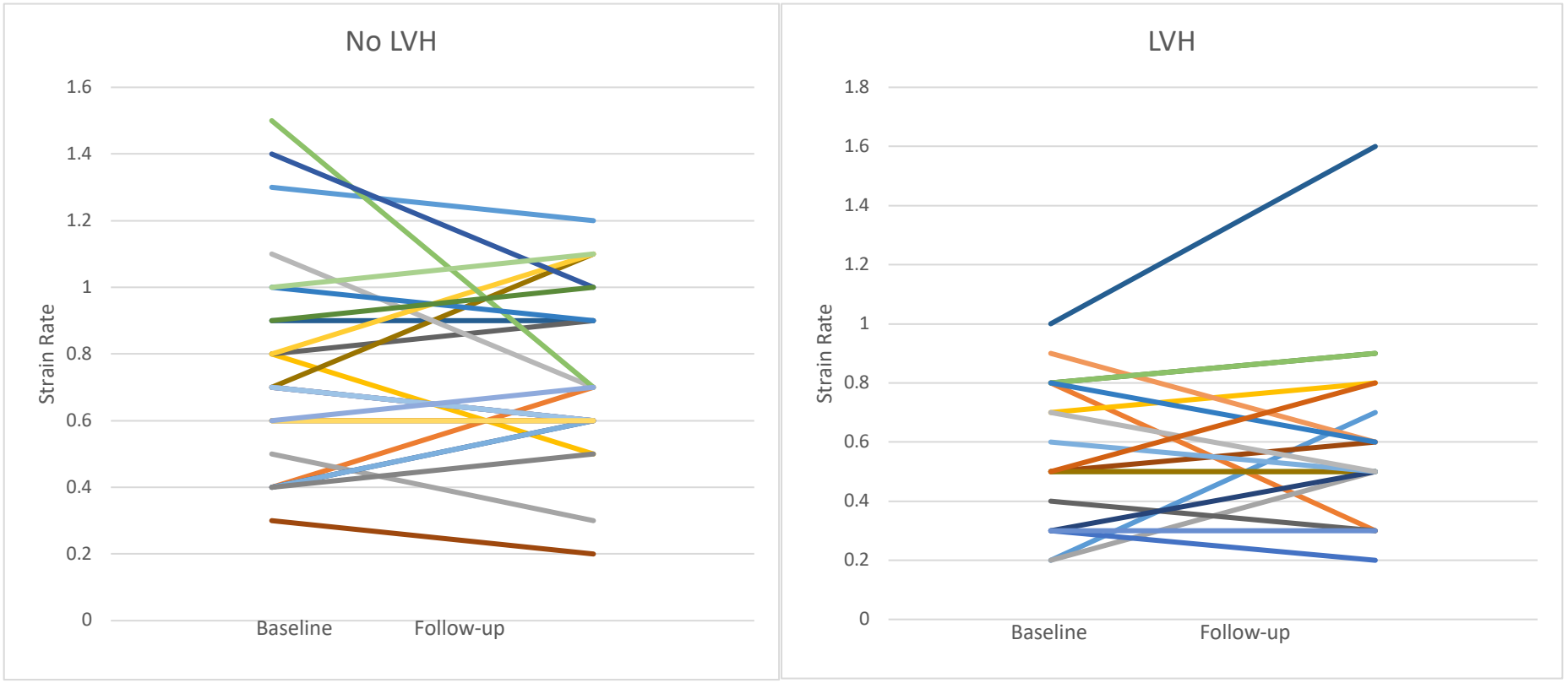
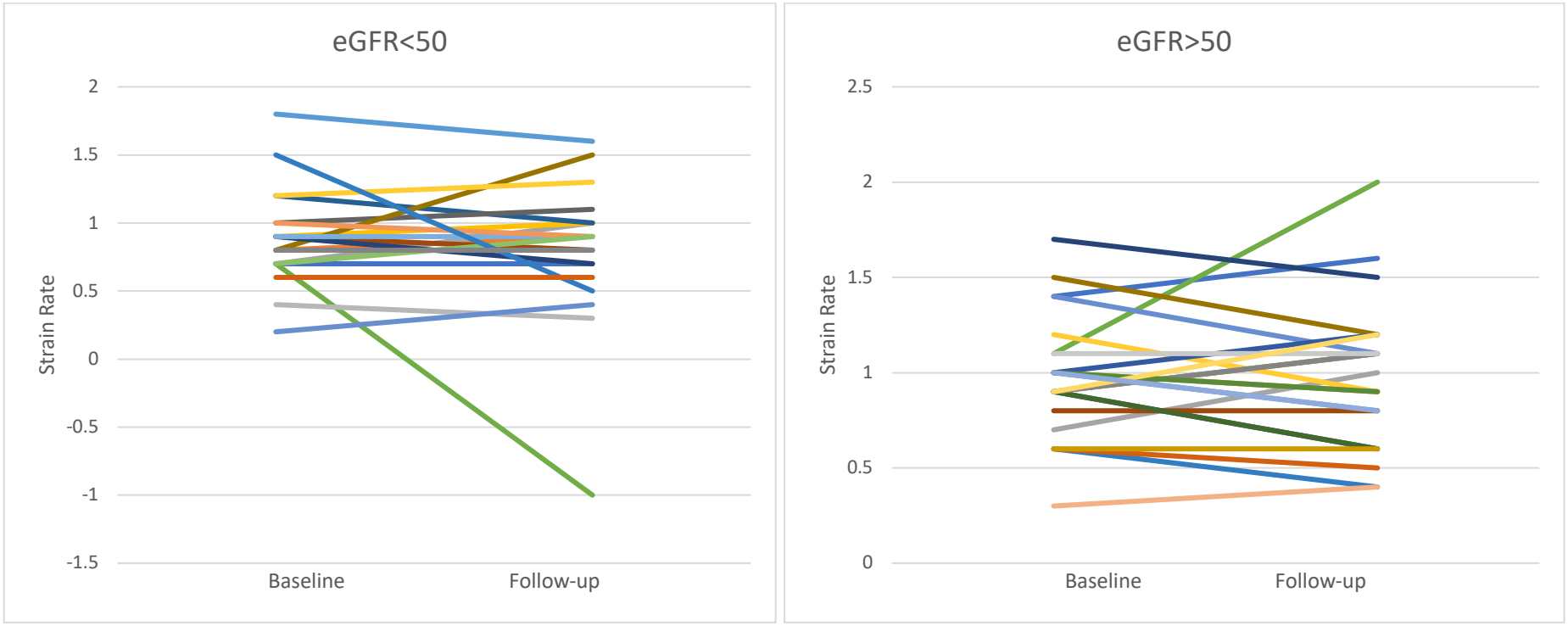


Figure 5.15 Diastolic strain rate analysis of transplant recipients stratified based on renal function at follow-up.

	N	Baseline	Follow-up	Within group change	P Value	Between group change	P Value
GLS e' eGFR<50 eGFR>50	 20 25	 0.55(0.40 – 0.80) 0.70(-0.50 – 0.90)	 0.159	 0.60(0.40 – 0.85) 0.60(0.50-0.90)	 0.380	 0.00(-0.10 – 0.20) 0.00(-0.15 – 0.10)	 0.446
GLS a' eGFR<50 eGFR>50	 20 25	 0.65(0.53 – 0.80) 0.60(0.60 – 0.80)	 0.515	 0.60(0.50 – 0.70) 0.60(0.50 -0.70)	 0.958	 -0.10(-20-0.10) 0.00(-0.10 – 0.10)	 0.255
GCS e' eGFR<50 eGFR>50	 20 25	 0.85(0.70 – 1.00) 0.90 (0.80 – 1.20)	 0.319	 0.90(0.65 – 0.85) 0.90(0.90 -1.10)	 0.191	 0.00(-0.17 – 0.10) 0.10(-0.20 – 0.20)	 0.541
GCS a' eGFR<50 eGFR>50	 20 25	 0.80 (0.50 – 1.00) 0.70(0.60 – 0.80)	 0.564	 0.70(0.60 – 0.85) 0.70 (0.60 – 0.80)	 0.681	 -0.10(-0.27 – 0.17) 0.00(-0.10 – 0.10)	 0.431
GRS e' eGFR<50 eGFR>50	 20 25	 -1.75(-2.18 - -1.10) -1.80 (-2.30 - -1.37)	 0.513	 -1.70(-2.35 - -1.45) -2.00(-2.50 - -1.50)	 0.460	 -0.30(-0.57 -0.40) -0.10(-0.75 – 0.45)	 0.859
GRS a' eGFR<50 eGFR>50	 20 25	 -0.70 (-1.10 - -0.43) -0.70 (-1.00 - -0.50)	 0.806	 -0.85(-1.60 - -0.60) -0.70(-0.90 - -0.50)	 0.058	 -0.20(-0.50 – 0.10) 0.00 (-0.40 – 0.48)	 0.064

Data are presented as median (IQR). Statistical significance was established using non-parametric Mann-Whitney U test. GLS- global longitudinal strain GRS- global radial strain GCS- global circumferential strain

Figure 5.16 Individual cases plotted from baseline to follow-up showing change in early diastolic circumfrential strain rate in trasnsplants startified by eGFR at follow-up.



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5.5 Discussion

5.5.1 Hypotheses

The current data has shown that following renal transplant.

i. There was no significant improvement in EF in transplant recipients compared to control participants.
ii. There was no significant difference observed in either contraction fraction or LVGFI.
iii. There were no significant changes observed in markers of left ventricular systolic strain.
iv. There were no significant changes observed in markers of left ventricular diastolic strain.

5.5.2 Systolic function

The data presented here and has shown that following renal transplantation there were no significant differences between transplant recipients and control participants when examining markers of left ventricular systolic function.

Recent work by Lim et al. (283) using echocardiography reported significant increases in ejection fraction following transplantation (60.0% vs 63.2% P = 0.02). Currently there are three studies which employed CMR to assess left ventricular function following renal transplant. These studies have been reviewed in detail as part of the meta-analysis presented in **Chapter 2.** Hayer et al. (85) reported significant improvements in ejection fraction early after renal transplant (EF 68 ± 9% to 73 ± 9%; $P < 0.05$). These findings however were not reproduced by Prasad et al. (220) or Patel et al. (221) where data indicated that there were no significant changes in EF when compared to patients on transplant waiting lists. The current work supports these findings.

While the current data has failed to show any improvements in systolic function it is noteworthy that EF was normal at baseline in both the transplant and control groups. The current work has therefore not addressed the effects of renal transplantation in those with LVSD. At present there is a lack of controlled data using either echocardiography or CMR to address this question. Longitudinal observational studies however have reported positive findings with EF improving significantly in those with LVSD (216, 284). The current data is therefore only indicative of those with normal EF prior to transplant and should not be extrapolated outside this group.

It is also important to recognise that while LVSD is common in uraemic cardiomyopathy, many patients with significant LVSD will remain unsuitable for surgery(105, 285). It is therefore important for future work to establishing the potential benefits of both transplantation and of medical therapy. While historically those with advanced CKD have been excluded form clinical trials aimed at the treatment of heart failure, more recently there is an acceptance that this is an important group where improved treatment options are needed. The use of beta-blockade has long been established as a treatment choice for heart failure in those with ESKD (286, 287) . Recently Niu et al.(288) have also reported that Sacubitril/Valsartan improved both systolic and diastolic function in those with ESKD. Martinez-Esteban et al.(289) also reported similar results. The role SGLT-2 inhibitors and mineralocorticoid receptor antagonists in ESKD are currently not well established and further work is therefore required.

When examining measures of stroke volume (LVSV, LVSVI) there were no significant differences between the two groups. Longitudinal analysis of transplant recipients did however show a significant within group change, which was not observed in control participants. This finding is in keeping with the early work of Himelman et al. (290) where LVSV reduced

significantly following transplantation in 41 recipients (68±27 Vs 51±16 P<0.001). It is also in keeping with previous analysis from **Chapter 4** which has shown that following transplantation there are significant reduction in left ventricular volumes. These findings are likely to represent restoration of euvolemia following transplant. This conclusion is supported by the data presented in **Chapter 6** which has shown significant reduction in NTproBNP in transplant recipients compared to controls.

Contraction fraction is a novel marker which quantifies the ratio between stroke volume and myocardial volume. This measure was considered of interest as previous work has indicated that contraction fraction can predict adverse events in several conditions including hypertrophic cardiomyopathy and amyloidosis (277, 291). At present it has not been investigated as a prognostic marker in either ESKD or following transplantation. The current data, indicated that contraction fraction was normal in both recipients and control participants at baseline and follow-up, in addition there were also no significant changes between the groups. At present comparisons with other data are not possible, but this represents an avenue for future study.

LVGFI is a second novel marker which has been shown to be an indicator of both incident heart failure and CVD (292). It is defined as, stroke volume divided by left ventricular total volume. At present the prognostic significance of LVGFI has not been established in either ESKD or following renal transplantation. The current study has shown that there were no between group or longitudinal changes noted for any of the analyses. This is similar to the findings noted for ejection fraction which is unsurprising given that Mewton et al.(279) have reported strong correlations between the two measures (R0.67 P=<0.0001).

Both contraction fraction and LVGFI combine measures of systolic function and left ventricular geometry. Individual analyses of these parameters in the previous and current chapter have indicated that in transplant recipients there are reductions in LVM, LVSV and left ventricular volumes. As such it is unsurprising that the ratio of LVSV to either myocardial volume or total volume is unchanged. In control participants however there was a significant reduction from baseline to follow-up noted in contraction fraction. This is explained by the fact that while left ventricular volumes and LVSV remained the same in this group the LVM increased significantly.

5.5.3 Systolic Strain

3D Feature tracking analysis was also performed. Strain analysis is now established as a method of detecting subtle changes in systolic function which may be present before overt reductions in ejection fraction (293). In addition, GLS has been shown to correlate with measures of arterial stiffness in CKD (294). Based on previous work by Liu et al.(254) all the currently reported values of strain at both baseline and follow-up were within normal range. Previous work by Barbosa et al.(281) reported that 6 months following renal transplantation there were no changes observed in either GRS or GCS which reflects the current data. This work however also suggested that here were significant improvements in GLS (-13.4 ± 3.0 baseline Vs -15.2 ± 2.7 follow-up $P<0.05$). This finding was not repeated here as such further work is required to fully understand the relationship between these parameters and renal transplantation.

5.5.4 Diastolic Strain

Diastolic function was also examined. This indicated that in both transplant and control groups diastolic strain values were normal at baseline and remained normal at follow-up. As has previously been discussed, all the participants in the study were on the renal transplant waiting list at baseline and had undergone rigorous cardiovascular screening to establish their

suitability for surgery. As such the current cohort does not include those patients with signs and symptoms of established diastolic dysfunction and heart failure. It is therefore not possible to comment on the impact transplantation may have in this group. The current findings, however, mirror the findings of both Hamidi et al.(217) and Hewing et al.(224) where measures of diastolic function failed to improve following transplant.

It should also be acknowledged, that as with LVSD, severe diastolic dysfunction may preclude patients from transplant listing. As such there is a need to focus research on medical therapy for advanced diastolic dysfunction associated with CKD. Recent research has suggested that SGLT-2 inhibitors represent a promising avenue for ongoing research. The EMPEROR-preserve trial has recently demonstrated that empagliflozin reduced hospitalisations and cardiovascular mortality in those with HFpEF (295). In addition, the EMPA-kidney trial has also demonstrated that empagliflozin can significantly reduce progression of CKD and cardiovascular mortality (296). There are presently no studies which have used imaging to directly establish the effects of this class of drug on diastolic dysfunction in CKD. Current results in those with type 2 diabetes, however, have shown significant reductions in measures of diastolic function (297).

It is also of note that the assessment of diastolic function using CMR is not a part of routine clinical practice. As such long-term clinical outcomes based on CMR derived measures of diastolic function have not been established (298). In addition, the best method of measuring diastolic function with CMR has not been established within the current literature. There is also currently only a small body of evidence which has sought to establish normal values for CMR derived parameters of diastolic function(254). The current data must, therefore, be interpreted within this context.

5.6 Limitations

The current data has several limitations. Several of the parameters reported in this chapter were subject to small changes. This may be explained by the fact that mean and median values for all parameters studied were within normal range at baseline. The RETRACT study was powered to detect clinically significant changes in LVM rather than marker of systolic function. As such it is possible that due to these small changes observed the current sample size may be inadequate.

During feature tracking analysis it was also necessary to exclude 5 transplant recipients and 1 control recipient from the analysis. When analysing 3D feature tracking it is necessary to maintain endocardial definition throughout the cardiac cycle. Due to the presence of left ventricular cavity obliteration during systole it was not possible to obtain accurate data for these participants.

5.7 Conclusion

The current work suggests none of the markers of systolic function including both novel indices and those in routine clinical practice changed significantly after renal transplant. It must therefore be concluded that left ventricular systolic function does not improve after renal transplantation. The current data however are not representative of those with LVSD as such further work is needed to establish the effects of transplantation in this group. This work has also indicated diastolic function does not change significantly after transplant. Again, this data is not representative of those with advanced diastolic dysfunction as such it is not possible to assess what changes renal transplant may have in this group. Finally it is acknowledged that both LVSD and advanced diastolic dysfunction will always remain a significant barrier to renal reverse the pathological changes associated with uraemic cardiomyopathy

Chapter 6: CHANGES IN METABOLIC AND BIOCHEMICAL PARAMETERS FOLLOWING SUCCESSFUL RENAL TRANSPLANTATION

Abstract

Background

End stage renal disease is associated with several metabolic and biochemical derangements. Following renal transplant there is at least partial normalisation of many haemostatic feedback mechanisms leading to improvements in electrolyte balance, markers of CKD-MBD and anaemia. Understanding these changes is important as a persistently abnormal metabolic profile after transplantation is associated with increased risk of both graft failure and all-cause mortality.

Objectives

To assess both metabolic and biochemical parameters in both renal transplants recipients and a similar group of control participants with end ESKD

Methods

Standard metabolic and biochemical parameters were assessed at baseline and follow-up at between 12 and 24 months. All analyses were performed within the clinical laboratories at the Queen Elizabeth Hospital, University Hospitals Birmingham Foundation Trust.

Results

There were indicated significant differences between transplanted and control participants in haemoglobin (18.39 g/l 95%CI(7.12-29.66)P<0.001), creatinine (x0.19 95%CI (0.15 - 0.22) P<0.001), NTproBNP (x0.10(0.05-0.22)P<0.001), PTH (x0.40 95%CI[0.17 – 0.93]p+0.035, phosphate (-0.28 mmol/L (-0.41 - -0.16)p<0.001) and Vitamin D (31.54 nmol/L 95%CI(16.03 -47.05)P<0.001)

Sub-group analysis based on the presence of LVH indicated that only NTproBNP was significantly different between the two groups (mean difference x0.23 95%CI [0.11 – 0.49] P<0.001).

Sub analysis based on renal function did not produce any significant difference between the groups

Conclusion

Following renal transplant anaemia, NTproBNP, and markers of CKD-MBD improve significantly compared to control participants.

6.1 Introduction

CKD is associated with increasing cardiovascular risk. This cardiovascular risk is not associated with the traditional risk factors of cardiovascular disease observed in the general population. In the United States (US) heart disease is the most common cause of death, the development of which overwhelmingly due to the presence of coronary artery disease (299). In those with ESKD, however, coronary artery disease and acute myocardial infarction accounts for only 3.4 % of recorded deaths. In this group arrythmia and sudden cardiac death account for 43% of recorded deaths. These observed differences are due to the development of uraemic cardiomyopathy(300). This cardiovascular phenotype is the result of a complex interplay between non-traditional risk factors which are considered unique to CKD(103) . Of these risk factors, the altered metabolic state produced by reduced renal function is considered one of the most pivotal .

Anaemia is common at all stages of CKD with a prevalence of 8.4% at stage rising to 53.4% at stage 5 (301). Anaemia is associated with structural changes within the cardiovascular system. Recent work has highlighted significant association between coronary microvascular dysfunction and haemoglobin levels, the development of which is associated with a 2.1 fold increase in cardiovascular mortality(302). Microvascular dysfunction also has a well-established associated with the development of LVH in other conditions including hypertrophic cardiomyopathy (105). The correction of anaemia in patients with CKD has been shown to produce positive cardiovascular remodelling and reduced LVMI(303) Following transplant anaemia remains a significant issue with rates as high as 50%. It is associated with both increased mortality and acute rejection and graft loss (304).

CKD-MBD is also a common finding in CKD. The classical picture of CKD-MBD includes hyperphosphatemia, reduced calcitriol, increased PTH, increased FGF23 levels and reduced αklotho levels.

Hyperphosphatemia is common CKD and almost universal in ESKD. It has a well-known association with vascular calcification, and correlates significantly with increased LVM in this group (127, 305, 306). 10-year outcome data from the Q-cohort study also demonstrated significant association between mortality and hyperphosphatemia in dialysis recipients (307). Following transplant Benaventa et al.(308) also demonstrated elevated levels at 6 and 12 months were associated with an increased risk of graft loss. Due to the physiological association between hyperphosphatemia and PTH it is unsurprising that increased levels of PTH show similar associations with both increased LVM and mortality in CKD. Elevated PTH is also similarly associated with both mortality and graft loss after transplant (309).

FGF23 plays a pivotal role in the development of fibrotic changes within the cardiovascular system (138). The physiological function of FGF23 is to enhance urinary phosphate excretion, achieved by binding to receptors in the presence of α-Klotho. FGF23 levels experience significant increases in response to only minor reductions in renal function and to rise as renal function declines. Concurrently, reductions in α-Klotho levels are also evident as renal function declines (264). This pattern of reduced α-Klotho and increased FGF23 is now believed to induce signalling cascades within the myocardium which can stimulate myocardial fibroblasts ultimately leading to the formation of diffuse interstitial myocardial fibrosis (104). This hypothesis is supported by the findings of Gutierrez et al.(310) which demonstrated an independent association between LVM and FGF23 levels.

Elevated levels of high sensitivity (Hs-Trop-T) are known to be associated with increased prevalence of LVH and risk of all-cause mortality in the general population without established cardiovascular disease (311). This relationship was also demonstrated in those with CKD by the KNOW-CKD cohort study (312) In addition to LVH Chesnaye et al. (313) demonstrated that in 927 participants with CKD stages 4 and 5, every standard deviation increase in Hs-Trop-T level was associated with 3.1 fold increase in mortality . As with the previous factors discussed elevated Hs-Trop-T is also significant predictor of mortality following transplant (314) .

In ESKD hypervolaemia is often present, this leads to increased ventricular end diastolic pressure, which is associated with increased BNP levels. In those with increased levels there is a positive correlation with both LVM and mortality. This relationship is however difficult to observe in dialysis cohorts as BNP is cleared by this method of renal replacement. Following transplantation levels of BNP have been reported to fall with associated reductions in both LVM and mortality(315).

Following renal transplantation there is partial normalisation of the impaired homeostatic mechanisms associated with CKD. The responses to these changes however are not uniform with many recipients continuing to exhibit abnormal metabolic profiles (316). The following chapter examines the metabolic and biochemical profiles of the participants enrolled in the RETRACT study at both baseline and follow-up.

6.2 Methods

Recruitment and sample size calculation were as previously described in **chapter 3**.

6.2.1 Sample acquisition and analysis

Venepuncture was undertaken at the time of CMR. All samples were analysed at the clinical laboratory at University Hospitals Birmingham Foundation Trust. Standard techniques in clinical practice were used for sample analysis (see **Appendix A** for sample processing strategy). All dialysis patients were studied the day after a dialysis session (intradialytic day).

6.2.2 Statistical analysis

Normality of variable distributions was assessed using the Kolmogorov–Smirnov test. Non-normally distributed data were log transformed for analysis and then anti-logged with results presented as multipliers. Baseline comparisons were performed using with paired students T-test or fishes exact test where frequency data was presented. Primary analysis of change between groups was performed using independent sample T-tests. A 2-tailed $P<0.05$ was considered statistically significant. Statistical analysis was overseen by Dr James Hodgson medical statistician at University Hospitals Birmingham Foundation Trust.

6.3 Hypothesis

i. Renal function will improve significantly after renal transplant
ii. Haemoglobin will increase following transplantation
iii. Indices of CKD-MBD will improve following transplantation
iv. NTproBNP and High sensitivity Hs -Trop-T will reduce following transplantation.
v. The above parameters will show the greatest change in those with more advanced features of uraemic cardiomyopathy (LVH) at baseline.
vi. The above parameters will show the greatest change in those who achieve higher levels of renal function at follow-up (eGFR>50. ml/min/1.73 m^2).

6.4 Results

6.4.1 Transplants and Control Participants

Demographic data for the cohort is presented in **Figure 4.2**.

The main findings of this analysis are presented in **Figure 6.1.** As expected, following renal transplant there were significant improvements in renal function compared to controls; creatinine x0.19([0.15-0.22] P<0.001. Attained values of eGFR in the transplant group were as follows, mean eGFR 53.86±15.70 ml/min/1.73 m^2, The lowest eGFR attained was 18 ml/min/1.73 m^2 and the maximum >90 ml/min/1.73 m^2. The median value was 52 ml/min/1.73 m^2 IQR 42-62 ml/min/1.73 m^2.

There were also significant improvements in several other parameters; NTproBNP (x0.10 95%CI [0.05-0.22] P<0.001), PTH (x0.40 95%CI [0.17 – 0.93] P=0.035), phosphate (x0.52 95%CI [0.39 - 0.69] p<0.001), vitamin D (31.54 nmol/L 95%CI [16.03 -47.05] P<0.001) and FGF23. -4038.27RU/mL 95%CI [-6144.41- -1932.12] P<0.001

Analysis of longitudinal changes within the transplanted cohort indicated that there were significant improvements observed in the following parameters; Hb (16.44g/L 95%CI[10.22 - 22.66]P<0.001), potassium (-0.34 mEq/L 95%CI[-0.54 - -0.15]P<0.001), urea (-14.02 mmol/L 95%CI[-22.25 - -5.79] P<0.001), creatinine (x0.23 95%CI[0.19 - 0.26]P<0.001), NTproBNP(x0.10 95%CI (0.05-0.22)), PTH (x0.54 95%CI[0.43 - 0.68]P<0.001), phosphate (x0.66 95%CI[0.60 - 0.72]P<0.001), Hs-Trop-T (x0.76 95%CI[0.62 - 0.91] P0.007) , vitamin D (10.65nmol/L 95%CI[1.26 – 20.03]P0.027) and FGF23 (-2017.93 RU/mL 95%CI[-3290.54 - -745.31]P=0.001).

Analysis of longitudinal changes within control participants significant indicated reductions in Triglycerides (-0.22mmol/l 95%CI [-0.76 – -0.33] P<0.001) vitamin D (-20.89 nmol/L 95%CI [-33.29- -8.50] P=0.003) and FGF23 (2020.34 RU/mL 95%CI (209.12 - 3831.56) P=0.03. There was also a significant increase in creatinine over time (x1.20 95%CI (1.07 - 1.35) P=0.005).

Figure 6.1 Biochemical parameters before and after renal transplantation in transplant and control recipients.

	N	Baseline	Follow-up	Mean Change	P Value	Between group difference	P Value
Hb (g/l)			**				
Transplant	50	117.40±16.93	133.84±16.14	16.44(10.22 - 22.66)	<0.001		
Control	20	112.15±14.17	110.20±17.06	-1.95(-11.26 - 7.36)	0.666	18.39(7.12-29.66)	0.002
Na+ (mEq/L)							
Transplant	50	139.16±5.55	139.80±2.66	0.64(-0.90 – 2.18)	0.408		
Control	20	139.25±3.34	139.00±3.18	0.25(-1.13 – 0.63)	0.561	-0.89(-3.38-1.60)	0.478
K+ (mEq/L)							
Transplant	49	4.63±0.63	4.28±0.42	-0.34(-0.54 - -0.15)	<0.001		
Control	20	4.67±0.69	4.49±0.61	-0.18(-0.46 – 0.11)	0.218	-0.017(-0.52-0.18)	0.343
Urea (mmol/L)			**				
Transplant	50	23.15±25.75	9.13±11.88	-14.02(-22.25 - -5.79)	<0.001		
Control	20	24.54±21.15	16.97±5.90	-7.57(-18.23 – 3.09)	0.539	-6.45(-20.90-8.00)	0.376
Creatinine (mg/dL)			**				
Transplant	50	520.60(445.14-608.70)	118.20(108.82-128.41)	x0.23(0.19 - 0.26)	<0.001		
Control	20	636.06(518.21-780.73)	761.73(610.38-950.83)	x1.20 (1.07 - 1.35)	0.005	x0.19(0.15 - 0.22)	<0.001
eGFR			**				
(ml/min/1.73m^2)	50	9.10±3.77	53.86±15.70	44.76(40.26 – 49.26)	<0.001		
Transplant	20	7.85±3.67	6.60±3.45	-1.25(-2.32 - -0.18)	<0.001	46.01(41.40-50.62)	<0.001
Control							

NTproBNP (ng/L) Transplant Control	 46 20	 852.90(551.19-1319.78) 775.18(349.87-1717.51)	** 147.50(114.34-190.28) 1333.83(664.51-2676.70)	 x0.17(0.11 - 0.26) x1.73(0.85-3.47)	 <0.001 0.12	 x0.10(0.05-0.22)	 <0.001
Cholesterol (mg/dl) Transplant Control	 49 20	 4.75±0.89 4.49±1.02	 4.79±0.77 4.46±1.03	 0.03(-0.25 – 0.32) -0.04(-0.49 – 0.41)	 0.820 0.854	 0.07(-0.45±0.60)	 0.783
Triglycerides(mmol/l) Transplant Control	 50 20	** 1.65±0.73 2.22±1.52	 1.87±0.92 2.00±1.46	 0.21(-0.04 – 0.47) -0.22(-0.76 – 0.33)	 0.098 <0.001	 0.43(-0.09±0.95)	 0.102
Calcium (mmol/l) Transplant Control	 50 20	 2.34(2.30-2.38) 2.34(2.29-2.40)	** 2.34(2.17-2.54) 2.06(1.80-2.37)	 x1.00(0.91 - 1.10) x0.99(-0.75 -1.02)	 0.98 0.079	 x1.15(0.97 - 1.32)	 0.10
Urate (µmol/L) Transplant Control	 40 13	 365.73±150.64 405.20±78.80	 380.38±83.85 378.69±105.59	 27.26(-21.99 – 76.51) -38.69(-96.05 - -18.66)	 0.270 0.057	 65.95(-26.18-158.08)	 0.157
PTH (pmol/L) Transplant Control	 46 20	 22.42(18.06-27.83) 24.81(14.74-41.76)	** 12.14(9.87-14.93) 33.90(18.88-60.87)	 x0.54(0.43 - 0.68) x1.38(0.60 - 3.09)	 <0.001 0.439	 x0.40(0.17 – 0.93)	 0.035
Phosphate (mmol/L) Transplant Control	 48 20	 1.47(1.35-1.60) 1.68(1.50-1.87)	 1.03(0.91-1.64) 2.13-1.43-3.18)	 x0.66(0.60 - 0.72) x0.10(0.83 - 1.95)	 <0.001 0.24	 x0.52(0.39 - 0.69)	 <0.001
Hs-Trop-T (ng/mL) Transplant Control	 42 17	 8.45(6.86-11.41) 10.98(7.47-14.15)	 6.64(5.27-8.35) 8.97(5.69-14.15)	 x0.76(0.62 - 0.91) x0.81(0.53 - 1.23)	 0.007 0.32	 x0.91(0.62 - 1.38)	 0.67

Mg (mEq/L) Transplant Control	 46 19	 1.10±1.28 0.94±0.12	** 0.73±0.08 0.94±0.11	 -0.38(-0.77 – 0.1) 0.01(-0.05 – 0.06)	 0.055 0.853	 -0.39(-0.99-0.22)	 0.206
Vitamin D (nmol/L) Transplant Control	 32 16	 41.45±28.88 54.56±29.29	 48.40±28.82 34.04±24.92	 10.65(1.26 – 20.03) -20.89 (-33.29- -8.50)	 0.027 0.003	 31.54(16.03 -47.05)	 <0.001
FGF23 RU/mL Transplant Control	31 18	 2109.04±3464.70 2025.06±3253.72	** 91.12±139.36 4045.40±4760.72	 -2017.93(-3290.54 - -745.31) 2020.34 (209.12 - 3831.56)	 0.003 0.03	 -4038.27(-6144.41 - -1932.12)	 <0.001
α-Klotho Pg/mL Transplant Control	 28 18	 783.31±672.44 889.57±640.14	 994.87±1050.48 1051.10±899.67	 211.56(-55.65 - 478.77) 161.527 (-303.76 - 626.81)	 0.12 0.47	 50.03 (-433.09 - 533.16)	 0.836

*** indicates significant difference between the two groups at either baseline or follow-up Data are displayed as mean ± SD or geometric mean (95%CI) at baseline and follow-up for the whole cohort. For normally distributed data within-group differences were determined by paired samples t tests. Between-group differences were determined using independent samples t tests. Results are displayed as the mean difference or mean change in values (95% CI). Where a variable was nonparametric data was log10 transformed before analysis. Values for within-group change and between-group differences are displayed as anti-logged multipliers (95% CI).*

Hb Haemoglobin, Na+ Sodium, K+ Potassium, PTH parathyroid hormone, Mg Magnesium

Figure 6.2 Box and whisker plot of haemoglobin levels at baseline and follow-up in transplants and controls.

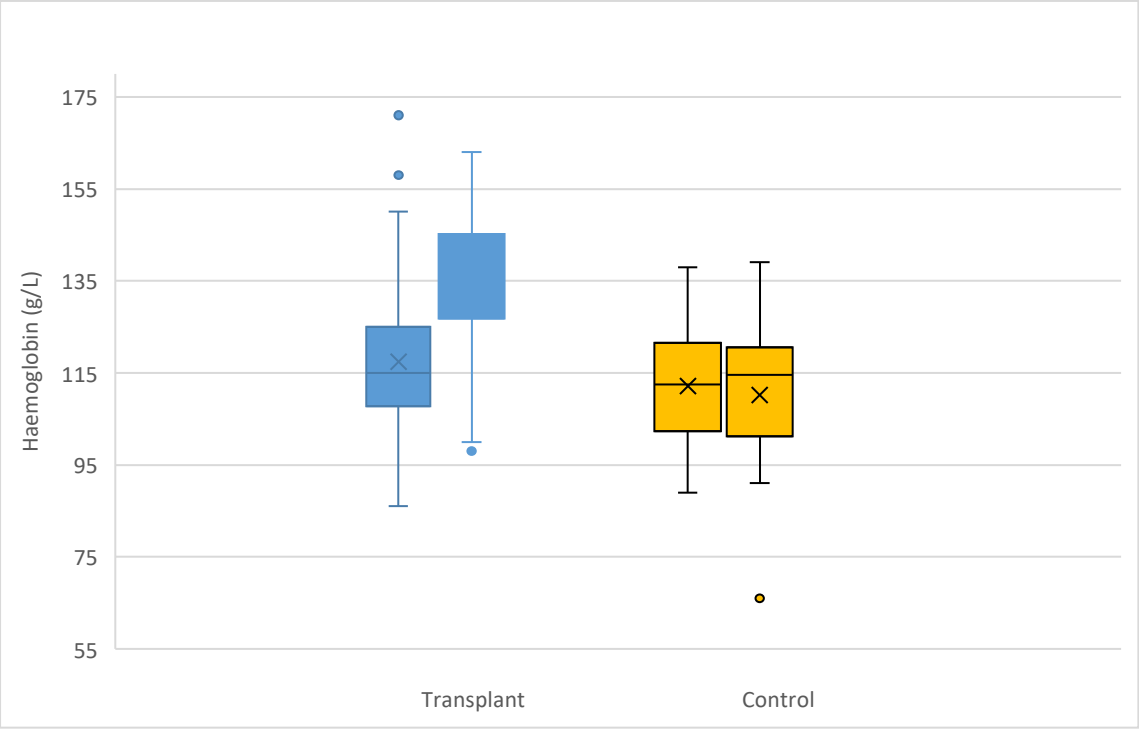


Figure 6.3 Individual cases plotted from baseline to follow-up showing change in NTproBNP in transplants and controls.

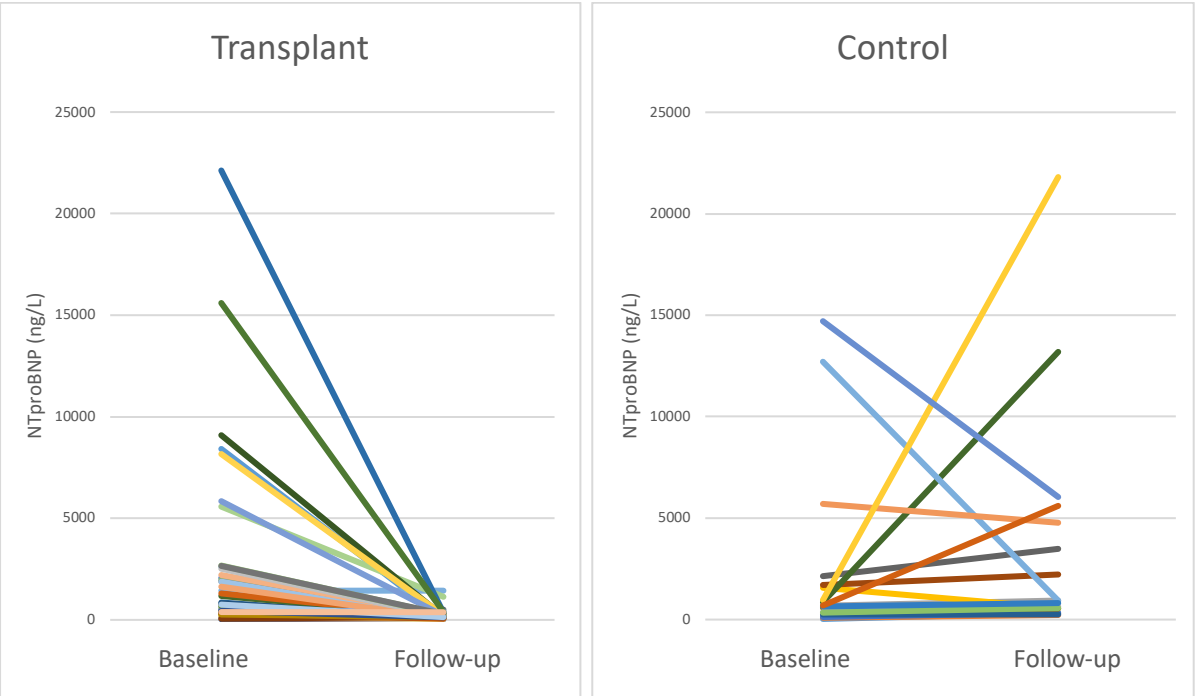
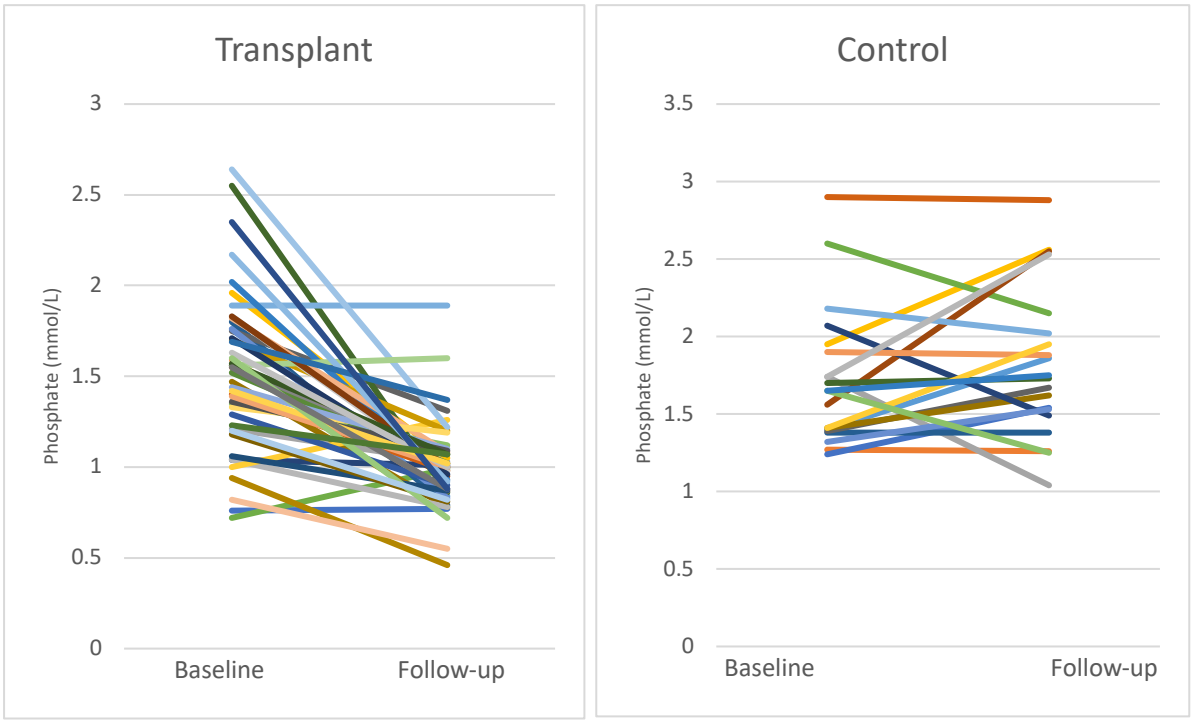


Figure 6.4 Individual cases plotted from baseline to follow-up showing change in phosphate in transplants and controls.



6.4.2 LVH subgroup analysis

Analysis of transplant recipients based on the presence of LVH at baseline was again performed. Demographic data for this analysis is presented in **Figure 4.7**.

There was only a significant difference observed between the two groups was in NTproBNP (x0.23 95%CI [0.11 – 0.49)] P<0.001). There were no significant differences in renal function, or the other parameters studied.

Analysis of the LVH cohort indicated significant longitudinal group change from baseline to follow-up in the following parameters; Hb (22.58g/L 95%CI[11.54 - 33.61]P=0.001), Na (1.79 mEq/L95%CI[0.15 - 3.43]P=0.03), potassium(-0.52 mEq/L 95%CI[-0.92 - -0.11]P=0.03), urea (-12.15 mmol/L 95%CI[-16.60 - -7.69]P=0.02), creatinine (-474.84 mg/dL 95%CI[-608.72 - -340.97] P<0.001), eGFR (48.21 ml/min/1.73m2 95%CI[40.40 - 56.02]P<0.001), NTproBNP (x0.07 95%CI [0.036 – 0.14]P<0.001), PTH (x0.46 95%CI[0.30 – 0.72]P=0.002), phosphate (-0.49mmol/L 95%CI[-0.73 - -0.24]P=0.001), Hs-Trop-T (0.6595 ng/mL %CI [0.46 – 1.09]P=0.018), and magnesium (x0.73 95%CI [0.64 – 0.83]P<0.001).

In those without LVH at baseline there were also significant longitudinal changes observed in the following parameters; Hb (mean change 12.68g/L 95%CI (5.08 - 20.27)P=0.001), K+ (-0.24 mEq/L 95%CI (-0.46 - -0.03)P=0.03), urea (-15.17 mmol/L 95%CI (-28.47 - -1.87)P=0.03), creatinine (-456.68 mg/dL 95%CI (-556.41 - -356.94) P<0.001), eGFR(42.65 ml/min/1.73m2 95%CI (36.96 - 48.33) P<0.001), NTproBNP (x0.30 95%CI (0.20 – 0.47)P=<0.001), PTH (x0.5995%CI (0.44 – 0.78)P=0.001), phosphate (-0.56 mmol/L95%CI (-0.71 - -0.41) P<0.001), magnesium (x 0.76 95%CI (0.64 – 0.91)P=0.03).

Figure 6.5 Biochemical parameters before and after renal transplantation stratified based on the presence of LVH at baseline.

	N	Baseline	Follow-up	Mean Change	P Value	Between group Difference	P Value
Hb(g/l)							
LVH	19	114.63±17.05	137.21± 17.53	22.58(11.54 - 33.61)	0.001		
NO LVH	31	119.10±16.91	131.77± 15.15	12.68 (5.08 - 20.27)	0.001	9.90 (-2.72 - 22.53)	0.121
Na+ (mEq/L)							
LVH	19	138.53±3.42	140.32± 2.36	1.79 (0.15 - 3.43)	0.03		
NO LVH	31	139.55±6.55	139.48± 2.83	-0.06 (-2.38 - 2.25)	0.095	-1.85(-5.02 - 1.31)	0.245
K+ (mEq/L)							
LVH	19	4.75±0.67	4.22±0.48	-0.52 (-0.92 - -0.11)	0.02		
NO LVH	31	4.55±0.61	4.31±0.39	-0.24 (-0.46 - -0.03)	0.03	-0.27 (-0.68 - 0.13)	0.176
Urea (mmol/L)							
LVH	19	18.86±9.80	6.72±1.45	-12.15 (-16.60 - -7.69)	<0.001		
NO LVH	31	25.77±31.73	10.60±14.95	-15.17 (-28.47 - -1.87)	0.03	3.02 (-14.09 - 20.13)	0.724
Creatinine (mg/dL)							
LVH	19	593.42±283.04	118.58±31.20	-474.84 (-608.72 - -340.97)	<0.001		
NO LVH	31	583.42±274.63	126.74±47.59	-456.68 (-556.41 - -356.94)	<0.001	-18.16 (-178.74 - 142.41)	0.821

eGFR(ml/min/1.73m^2)							
LVH							
NO LVH	19	9.05±3.24	57.26±16.60	48.21 (40.40 - 56.02)	<0.001		
	31	9.13±4.11	51.77±15.02	42.65 (36.96 - 48.33)	<0.001	5.57 (-3.67 - 14.80)	0.232
NTproBNP (ng/L)							
LVH	18	2142.90(1085.18 – 4231.56)	154.17(84.33 – 259.00)	x0.07(0.036 – 0.14)	<0.001		
NO LVH	30	471.63(293.63 – 757.70	143.38(107.87-190.55)	x0.30(0.20 – 0.47)	<0.001	x0.23(0.11 – 0.49)	<0.001
Cholesterol (mg/dl)		**					
LVH	18	4.39±1.00	4.58±0.86	0.18(-0.27 - 0.63)	0.42		
NO LVH	31	4.97±0.75	4.92±0.69	-0.05 (-0.44 - 0.33)	0.79	0.23 (-0.37 - 0.83)	0.44
Triglyceride							
(mmol/l)		xx					
LVH	18	1.300(1.10-1.72)	1.70(1.31 – 2.22)	x1.31(0.97 – 1.77)	0.77		0.216
NO LVH	31	1.57(1.31 – 1.88)	1.68(146 – 1.93)	x1.07(0.89 – 1.29)	0.47	x1.23(0.88 – 1.70)	
Calcium (mmol/l)		xx					
LVH	19	2.32(2.25 – 2.40)	2.43(2.38 – 2.49)	1.05(1.01 – 2.24)	0.12		
NO LVH	31	2.35(2.30 – 2.41)	2.92(2.02 – 2.60)	0.98 (0.85 – 1.11)	0.70	X1.07(0.90 – 1.27)	0.414
Urate (µmol/L)							

LVH NO LVH	12 28	351.91±147.87 374.19±154.12	368.67±86.71 385.39±83.70	45.97 (-81.76 - 173.71) 18.04 (-35.86 - 71.93)	0.445 0.498	27.94 (-83.44 - 139.31)	0.615
PTH (pmol/L) LVH NO LVH	 16 30	** 22.72(15.28 – 33.78) 22.26(16.94 – 29.25)	 10.49(7.07 – 15.55) 13.12(10.21 – 18.87)	 x0.46(0.30 – 0.72) x0.59(0.44 – 0.78)	 0.002 0.001	 x0.78(0.48 - 1.32)	 0.320
Phosphate (mmol/L) LVH NO LVH	 19 31	 1.57±0.41 1.49±0.42	 1.12±0.30 0.93±0.17	 -0.49 (-0.73 - -0.24) -0.56 (-0.71 - -0.41)	 0.001 <0.001	 0.07 (-0.20 - 0.34)	 0.586
Hs-Trop-T (ng/mL) LVH NO LVH	 17 25	 ** 11.46 (7.22 –18.19) 7.42(5.50 – 9.98)	 7.43(4.90 – 11.24) 6.15(4.62 – 8.18)	 0.65(0.46 – 1.09) 0.83 (0.64 – 1.07)	 0.018 0.150	 0.78(0.51 – 1.18)	 0.237
Mg (mEq/L) LVH NO LVH	 15 30	** 0.96(0.88 – 1.04) 0.97(0.82 – 1.15)	 0.700(0.66 – 0.75) 0.74(071 – 0.77)	 x0.73(0.64 – 0.83) x0.76(0.64 – 0.91)	 <0.001 0.03	 x0.96(0.77 – 1.18)	 0.755
Vitamin D (nmol/L)							

LVH	11	42.96±40.26	50.14±36.02	7.20 (-7.73 - 22.13)	0.31		
NO LVH	21	40.72±22.64	49.70±26.17	12.45 (-0.37 - 25.27)	0.06	-5.25(-25.27 -14.76)	0.60
FGF23 RU/mL							
LVH	9	3406.51±5950.23	61.36±50.43	-3345.15 (-7900.88 - 1210.59)	0.13		0.18
No LVH	22	1578.26±1626.19	3406.515950.23	-1474.97 (-2210.33 - -739.62)	<0.001	1870.18(-895.81 -4636.17)	
α-Klotho							
Pg/mL							
LVH	9	461.60±250.95	656.91±271.38	195.32(10.92 - 379.71)	0.04		
No LVH	19	935.70±757.63	1154.96±1240.31	219.25(-180.10 - 618.61)	0.26	23.94(-560.08-607.96)	0.93

*** indicates significant difference between the two groups at either baseline or follow-up Data are displayed as mean ± SD or geometric mean (95%CI) at baseline and follow-up for the whole cohort. For normally distributed data within-group differences were determined by paired samples t tests. Between-group differences were determined using independent samples t tests. Results are displayed as the mean difference or mean change in values (95% CI). Where a variable was nonparametric data was log10 transformed before analysis. Values for within-group change and between-group differences are displayed as anti-logged multipliers (95% CI).*

Hb Haemoglobin, Na+ Sodium, K+ Potassium, PTH parathyroid hormone, Mg Magnesium

Figure 6.6 Box and whisker plot of NTproBNP levels in LVH and No LVH groups at baseline and follow-up.

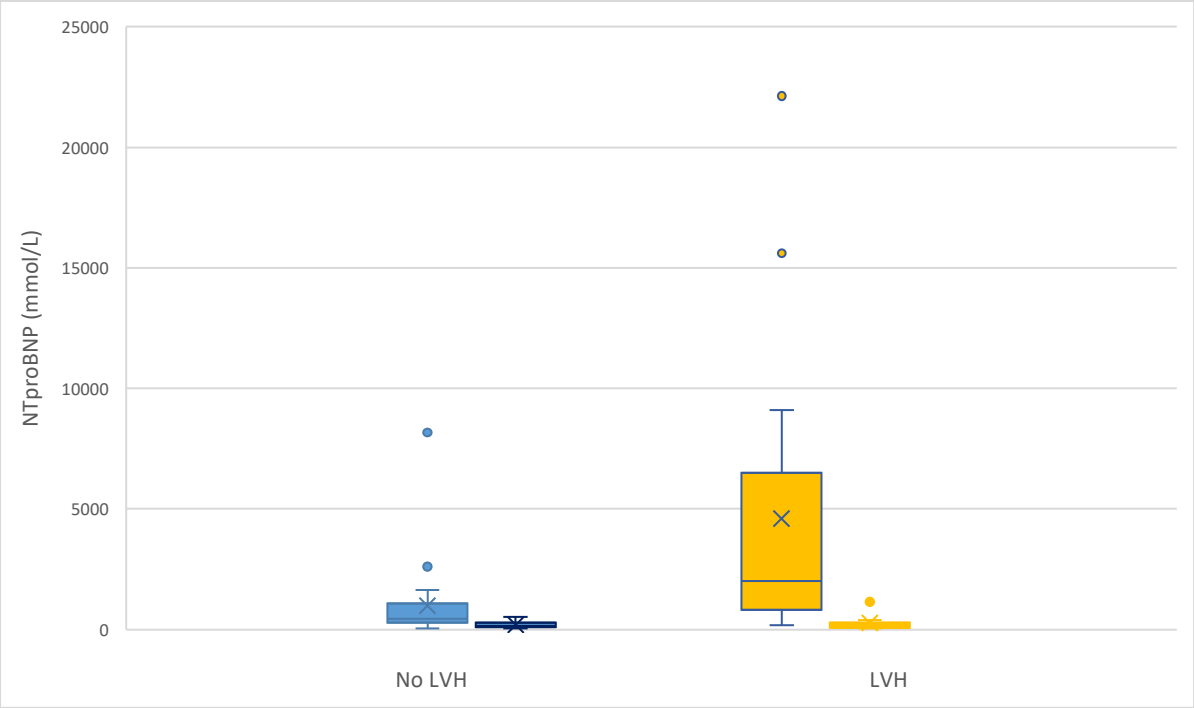
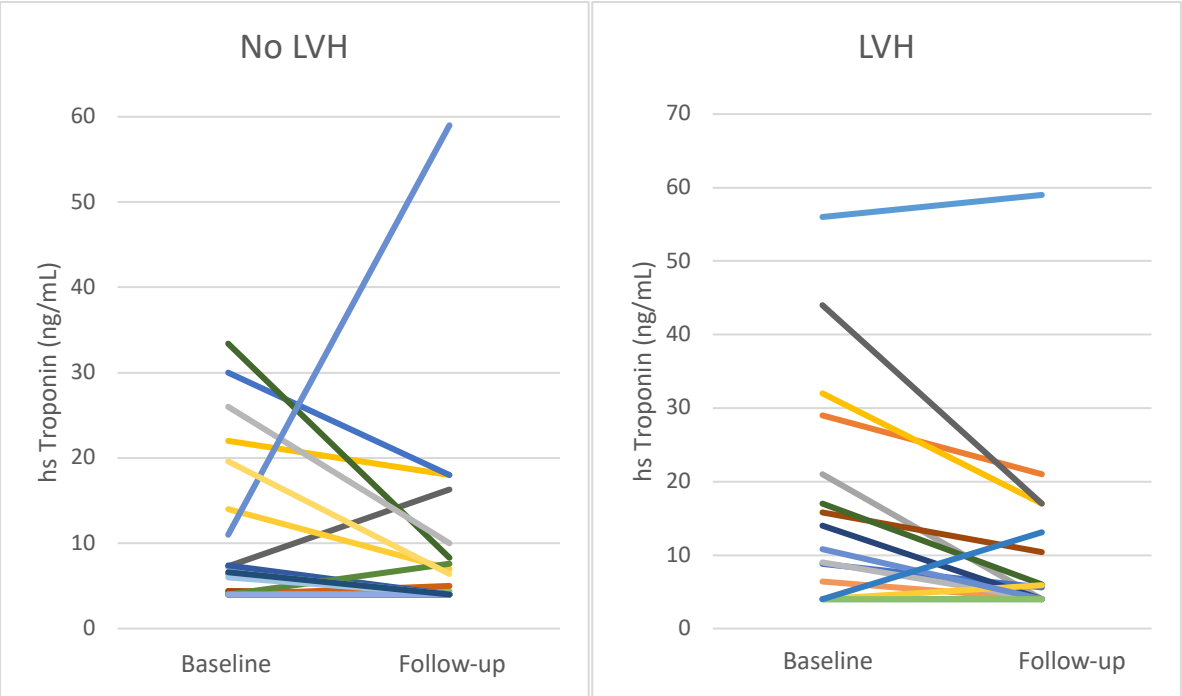


Figure 6.7 Individual cases plotted from baseline to follow-up showing change in Hs - Trop-T in transplant group in starified based on LVH at baseline.



6.4.3 eGFR subgroup analysis

Further analysis stratified based on eGFR was again performed as previously described in chapter 3. Demographic data for this analysis is presented in **Figure 4.13**.

As expected, there was a significant difference observed in eGFR between the two groups. This was the only parameter that differed between the groups.

Longitudinal analysis of those who achieved higher levels of renal function at follow-up revealed significant changes observed in haemoglobin (21.00g/L[11.46 – 30.54]P<0.001), NTproBNP (x0.15 95%CI[0.09 - 0.25]P<0.001), PTH (x0.56 95%CI[0.40 – 0.79]P<0.001), phosphate (-0.54 mmol/L 95%CI[-0.72 - -0.37]P<0.001), magnesium (x0.78 95%CI[0.71 - 0.85]P<0.001) and vitamin D (x1.35[1.04 – 1.74]P=0.03).

Longitudinal analysis of those who achieved lower levels of eGFR highlighted that there were significant changes in haemoglobin (10.14 g/l [3.39 – 16.89]P0.005), NTproBNP (x0.20 95%CI(0.09 – 0.45)P<0.001), calcium (x1.04 95%CI[1.01 - 1.07]P=0.01), urate (85.67 µmol/L 95%CI(8.43 – 162.91)P=0.03), PTH (x0.51 95%CI[0.35 – 0.72]P<0.001), Hs-Trop-T (x0.72 95%CI[0.53 – 0.98]P=0.03), magnesium (x0.72 95%CI[0.55 - 0.95]P=0.02) and FGF23 -1116.85 RU/mL 95%CI[-1959.38 - -274.32]P=0.01)

Figure 6.8 Biochemical parameters before and after renal transplantation stratified based on eGFR at follow-up.

	N	Baseline	Follow-up	Mean Change	P Value	Between group difference	P Value
Hb (g/l)							
eGFR<50	21	119.19±17.37	129.33±15.61	10.14(3.39 – 16.89)	0.005		
eGFR>50	29	116.10±16.78	137.10±15.99	21.00(11.46 – 30.54)	<0.001	-10.86(-23.19-1.48)	0.08
Na+ (mEq/L)							
eGFR<50	21	139.81±7.67	139.90±2.10	0.10(-3.19 – 3.38)	0.952		
eGFR>50	29	138.69±3.38	139.72±3.05	1.03(-0.39 – 2.46)	0.147	0.94(-2.21- 4.08)	0.55
K+ (mEq/L)							
eGFR<50	21	4.60±.57	4.32±0.48	-0.28(-0.53 - -0.03	0.030		
eGFR>50	29	4.64±.68	4.24±0.38	-0.39(-0.69 - -0.09))	0.013	0.11(0-.29- 0.51)	0.59
Urea (mmol/L)							
eGFR<50	21	18.30±11.15	9.32±3.43	-8.98 (-13.39 - -4.57)	<0.001		
eGFR>50	29	26.66±32.27	8.99±15.45	-17.67 (-31.74 – 3.61)	0.016	8.70(-7.97 -25.36)	0.30
Creatinine			**				
(mg/dL)	21	565.43±196.53	152.76±46.97	-412.67 -498.39 - -326.94)	<0.001		

eGFR<50 eGFR>50	29	603.00±322.72	102.55±20.18	-500.45(-620 - -380.01)	<0.001	87.78(-68.16-243.72)	0.26
eGFR(ml/min/1.73m^2) eGFR<50 eGFR>50	 21 29	 9.05±4.28 9.14±3.43	** 39.24±9.07 64.45±9.81	 30.19 (25.72 – 34.66) 55.31(51.58 – 59.04)	 <0.001 <0.001	 -25.12(-30.77 - -19.47)	 <.001
NTproBNP (ng/L) eGFR<50 eGFR>50	 19 27	 1275.56(637.36-2552.11) 642.539(361.539-1140.76)	 259.78(251.77-388 99.04(77.30-126.91)	 x0.20(0.09 – 0.45) x0.15(0.09 - 0.25)	 <0.001 <0.001	 x1.32(0.56 – 3.09)	 0.510
Cholesterol (mg/dl) eGFR<50 eGFR>50	 21 29	 4.51±0.70 4.92±0.98	 4.72±0.71 4.84±.82	 0.21(-0.16 – 0.58) -0.10(-0.54 – 0.33)	 0.24 0.63	 0.32(-0.26-0.90)	 0.27
Triglyceride (mmol/l) eGFR<50 eGFR>50	 21 29	 1.79±0.75 1.54±0.7	 2.02±1.06 1.75±0.80	 0.23(-0.23 – 0.68) 0.20(-0.11 -0.52)	 0.31 0.20	 0.03(-0.50-0.55)	 0.92

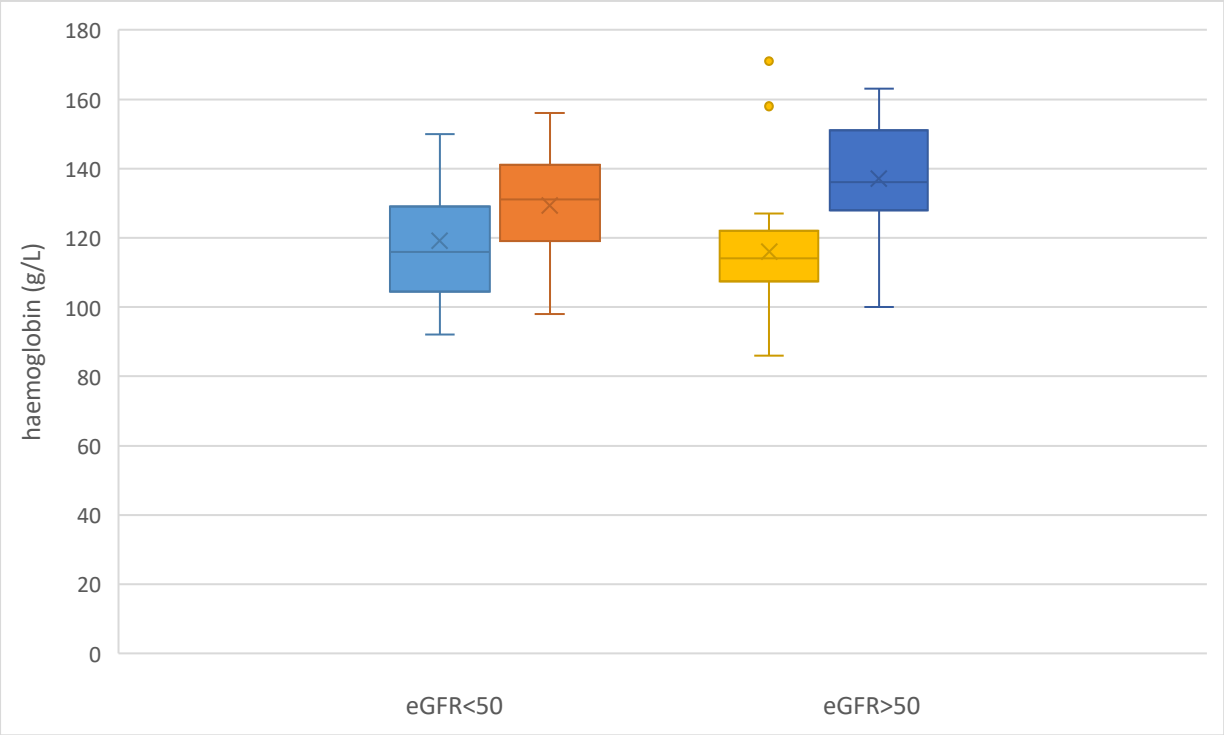
Calcium (mmol/l)							
eGFR<50	21	2.35(2.29-2.41)	2.44(2.39-2.49)	x1.04(1.01 - 1.07)	0.01		
eGFR>50	28	2.34(2.27-2.40)	2.28(1.9802.62)	x0.98(0.83 – 1.12)	0.72	x1.07(0.89-1.26)	0.451
Urate (µmol/L)			**				
eGFR<50	15	414.93±58.71	329.27±157.12	85.67(8.43 – 162.91)	0.03		
eGFR>50	26	361.96±89.62	368.40±157.74	-6.44(-70.08 -57.21)	0.84	94.80(-6.30-195.90)	0.07
PTH (pmol/L)							
eGFR<50	20	25.45(17.74-36.95)	13.10(9.01-18.56)	x0.51(0.35 – 0.72)	<0.001		
eGFR>50	26	20.34(15.52-26.65)	11.56(8.91-15.00)	x0.56(0.40 – 0.79)	<0.001	x0.89(0.55-1.44)	0.636
Phosphate (mmol/L)							
eGFR<50	20	1.56±0.37	1.07±0.30	-0.53(-0.71 - -0.34)	<0.001		
eGFR>50	28	1.49±0.45	0.95±0.17	-0.54(-0.72 - -0.37)	<0.001	0.02(-0.24-0.27)	0.90
Hs -Trop-T (ng/mL)							
eGFR<50	17	11.36(7.00-18.42)	8.21(5.33-12.66)	x0.72(0.53 – 0.98)	0.03		
eGFR>50	25	7.46(5.61-9.92)	5.74(4.42-7.45)	x0.77(-0.58 - 0.95)	0.08	x0.94(0.62-1.45)	0.679

Mg (mEq/L)			.				
eGFR<50	19	1.02(0.78-1.33)	0.74(0.70-0.78)	x0.72(0.55 - 0.95)	0.01		
eGFR>50	26	0.93(0.87-0.99)	0.72(0.69-0.75)	x0.78(0.71 - 0.85)	<0.001	x0.93(0.72-1.17)	0.552
Vitamin D (nmol/L)							
eGFR<50	12	33.16(21.52-51.11)	44.11(29.06-66.93)	x1.32(0.91 - 1.29)	0.13		
eGFR>50	20	31.68(23.47-42.76)	42.55(33.17-54.58)	x1.35(1.04 – 1.74)	0.03	x1.01(0.65 – 1.55)	0.962
FGF23 RU/mL							
eGFR<50	11	1193.17±1238.77	76.32±53.64	-1116.85(-1959.38 - -274.32)	0.01	1396.67(-1260.08-	0.291
eGFR>50	20	2612.78±4170.56	99.26±170.15	-2513.52(-4468.56 - -558.48)	0.01	4053.42)	
α-Klotho Pg/mL							
eGFR<50	10	727.63±842.79	1089.33±1532.48	361.70(-174.22-897.62)	0.161		
eGFR>50	18	814.25±582.48	942.39±707.92	128.15(-200.38-456.67)	0.422	233.55(-327.92-795.02)	0.40

*** indicates significant difference between the two groups at either baseline or follow-up Data are displayed as mean ± SD or geometric mean (95%CI) at baseline and follow-up for the whole cohort. For normally distributed data within-group differences were determined by paired samples t tests. Between-group differences were determined using independent samples t tests. Results are displayed as the mean difference or mean change in values (95% CI). Where a variable was nonparametric data was log10 transformed before analysis. Values for within-group change and between-group differences are displayed as anti-logged multipliers (95% CI).*

Hb Haemoglobin, Na+ Sodium, K+ Potassium, PTH parathyroid hormone, Mg Magnesium

Figure 6.9 Box and whisker plot of haemoglobin levels in eGFR<50 and eGFR>50 groups at baseline and follow-up.



6.5 Discussion

The current data indicates that following transplantation

i. Renal function improves significantly after transplant

ii. Hb levels increase significantly in transplant recipients compared to controls

iii. Several indices of CKD-MBD improve following transplant.

- . PTH
- . Phosphate
- . FGF23

iv. BNP but not Hs-Trop-T reduced in transplant recipients compared to control participants.

v. Only NTproBNP showed greater reductions in those with LVH at baseline. There was no difference between the groups for the other parameters studied.

vi. There were no significant differences observed when transplant recipients were stratified by eGFR

The finding that renal function improved following renal transplantation was expected. The current analysis was reassuring as it indicated the restoration of renal function in the current cohort is in line with previously published data (317).

Anaemia is common in ESKD due to multiple factors including reduced erythropoietin production and iron deficiency(318). Reduced Hb leads to tissue hypoxia as a consequence of reduced oxygen carrying capacity of blood. This leads to vasodilation, reduced peripheral vascular resistance and decreased afterload (319). Decreased blood viscosity also increases venous return leading, to an increased preload(256). There is also increased activation of the sympathetic nervous system which increases both heart rate and left ventricular contractility.

In combination, these changes lead to a hyperdynamic state which increases cardiac output in order to maintain adequate end organ perfusion. If anaemia is prolonged, however, this process can lead to the development of both LVH and heart failure. Foley et al.(320) reported that in 432 patients receiving renal replacement therapy each 1g/dl reduction in Hb was associated with the development of new onset cardiac failure (relative risk 1.28; P = 0.018). In addition, Levin et al. (321) conducted a multi-centre study of 246 patients with mild to moderate CKD which reported that every 0.5g/dl reduction in Hb was significantly associated with increasing LVM.

A number of studies have attempted to examine the effects of correcting anaemia on all cause and cardiovascular mortality in CKD. These studies have produced surprising results. The CHOIR study enrolled 1400 with CKD where epoetin alfa was used to correct anaemia to either >13.5 g/dL or >11.3 g/dL (322). This reported that improvements in quality of life were the same in each group. However, in those where a higher Hb was achieved, there was a significantly increased risk of a composite end point of death, myocardial infarction, and hospitalization for heart failure and stroke. The CREATE trial also demonstrated that administration of erythropoietin to achieve optimal Hb (13-15 g/dL) vs sub optimal Hb (10.5-11.5g/dL) did not produce any changes in LVM or incidence of adverse cardiac events between the groups (323). The TREAT trial, which was a placebo, controlled study also reported that the administration of darbepoetin alfa was not associated with reduced mortality but was associated with increased risk of stroke(324).

Following transplantation erythropoietin levels can increase to near normal levels within 3 months, however within the literature it is reported that between 20-50% of patients may remain anaemic (325). The presence of anaemia in transplant recipients is clinically

significant, results from the DIVAT cohort which comprised 4217 transplants recipients indicated that it is significantly associated with all-cause mortality and cardiovascular mortality (326). Rigatto et al. (29) also reported that the presence of anaemia in renal transplant recipients was an independent rick factor of the development of LVH at between and one and five years following transplantation. Ibernon et al.(327) also demonstrated that post-transplant anaemia was a significant independent predictor or of LVMI in this group. The current findings of significant improvements in both Hb and LVM/LVMI (data reported in **Chapter 4**) in transplant recipients, therefore, supports the hypothesis that there is an association between the two.

NTproBNP is released by cardiac myocytes in response to stretch which is produced during volume overladed states (328). In the general population elevated NTproBNP is recognised an indicator of cardiovascular mortality even when adjusted for the presence of traditional cardiovascular risk factors (329) In CKD, NTproBNP is known to increase with declining renal function as it is primarily excreted by the kidney (330). It, however, remains an important biomarker in addition to eGFR as it is a predictor of LVMI in CKD independently of renal function(106). Astor et al.(331) reported in the African American Study of Kidney Disease and Hypertension study that NT-proBNP levels predicted CVD events in a CKD cohort independently of kidney function. Elevated levels of NTproBNP must, therefore, be expected in CKD, however it remains as useful indicator of cardiovascular risk in this cohort

NTproBNP is known to reduce following transplantation due to increases in renal function, however, it remains a useful surrogate marker of cardiovascular risk in this group (332) Schwab et al (315) studied 176 ESKD patients with preserved ejection fraction who received renal transplantation. The data reported here indicated that 1- and 5-year MACE-free survival

rates were 78.82% and 74.68% in those with NTproBNP > 4350 pg/ml compared to 93.33% and 91.21% for patients with NTproBNP < 4350 pg/ml ($p < 0.01$). Massimetti et al.(333) also reported that in 81 participants BNP levels were significantly correlated with LVMI following transplantation. The current data therefore supports the current evidence base, with significant reductions observed in both LVM and NTproBNP compared to controls. As has previously discussed it is not possible to comment on the effects this change may have on mortality between the two groups due to the limited duration of follow-up

Hs-Trop-T is a surrogate marker for sub-clinical myocardial damage in the general population (311). As is the case with NTproBNP levels increased as eGFR declines, as it is a small peptide which is renally excreted (334). Regardless of this association Hs-Trop-T remains a significantly predictor of mortality in ESKD (313, 335). The EQUAL study, which included 176 participants with CKD stage 4-5, reported that both baseline and longitudinal measures of Hs-Trop-T remained significantly associated with mortality when corrected for eGFR (HR3.7 (2.7-5.1) P<0.001)(313). Hs-Trop-T is also known to be associated with increasing LVM in CKD. The KNOW-CKD study demonstrated that in CKD those in the highest quartile of Hs-Trop-T levels exhibited higher LVM levels(312). This finding was considered independent of eGFR in multivariable regression,

Connolly et al.(335) reported that in 372 asymptomatic renal transplant recipients followed up for over four years levels of Hs-Trop-T were a significant predictor of mortality when adjusted for traditional cardiovascular risk factors. The current analysis has shown that while there were significant reductions from baseline to follow-up in the transplant cohort, the overall change observed between the two groups was not significant. This, however, may reflect inadequate sample size given that the changes observed were small and baseline Hs-

Trop-T levels were only marginally elevated. Sub-group analysis also indicated that while the magnitude of change was not significantly different between the groups when stratified for the presence of LVH there was a significant longitudinal change observed in those with LVH, while no change was observed in those without LVH at baseline. This finding may suggest that following transplant those with LVH at baseline may benefit from the greatest reduction in cardiovascular risk (336).

Impaired homeostasis of phosphate, calcium, vitamin D, and PTH is commonly observed in CKD (337). This process begins with only minor reductions in eGFR, however in those with ESKD this process ultimately leads to the classical picture of CKD-MBD. This is characterised by hyperphosphatemia due to reduced renal excretion and reduced levels of calcitriol as a result of the kidneys inability to hydroxylate 25-hydroxyvitamin D. Reduced Calcitriol levels ultimately lead to hypocalcaemia as a result of reduced gastrointestinal calcium absorption, which in turn stimulates over production of PTH. This over production of PTH can then result in both tertiary and secondary hyperparathyroidism(338).

Following renal transplantation there is often partial correction of these homeostatic mechanism, however, levels of the indices of CKD-MBD following transplant are variable. Stavroulopoulos et al. (339) reported that up to 97% of transplant recipients remain vitamin D deficient at 12 months. Chevarria et al. (340) studied a cohort of 1525 reporting that 86.3% of recipients had hypophosphatemia and 36.1% exhibited hypercalcaemia. Crepeau et al.(341) also reported that increased levels of PTH persisted in 61% of recipients.

Understanding the behaviour of these parameters is clinically significant as they are associated with post-transplant prognosis. A systematic review conducted by Koimtzis et al.(342) has

indicated that Vitamin D deficiency post-transplant is associated with decreased renal function, acute rejection, and mortality. In addition analysis conducted from the ALERT trial indicated that increased levels of PTH were associated with a 4% increase in all-cause mortality and a 5% increase in allograft lost (309) Phosphate levels have also been shown to demonstrate a U- shaped relationship with both mortality and graft failure (343).

The current data has shown that levels of vitamin D increased significantly compared to controls. Whereas both phosphate and PTH reduced significantly. When considered in conjunction with the previous findings that LVM/LVMI reduced significantly after transplant these findings support previous work by Randon et al. (344) who reported that PTH and LVM were significantly positively correlated in ESKD. They are also in line with the work of Chue et al. (127) which demonstrated that in 208 patients with CKD phosphate was an independent predicator of LVM. When interpreting this type of data, it must be acknowledged that markers od CKD-MBD can be problematic. All transplant patients and those with CKD are regularly seen by renal physicians. Metabolic or biochemical abnormalities are therefore treated promptly, as a result the changes observed may not be directly related to the effects of transplantation.

Examination of biomarkers which have been associated with myocardial fibrosis was also conducted. The role of FGF23 in the development of myocardial fibrosis in CKD is discussed in detail in chapter 1. FGF23 is known to be a predicator of cardiovascular mortality in both the general population and CKD (345). The current data has shown that there were significant reductions in FGF23 following transplantation, which, reflects previous work by Prasad et al.(346) who reported that FG23 levels reduce dramatically following successful transplantation. The current findings are reassuring as previously, Wolf et al.(347),

demonstrated that increased levels of FGF23 are associated with increased mortality and graft loss. The current data however cannot confirm this finding, due to short length of follow-up.

FGF23 is also known to be associated with the development of cardiac diastolic dysfunction, which is often attributed to the development of myocardial fibrosis(348). At present, however, there is very little data which has sought to assess the association between biochemical and CMR derived markers of fibrosis. In the only published work to date Hayer et al. (349) failed to show an association between T1 mapping values and FGF23 in CKD. There no data attempting to correlate FGF23 with native T1 mapping following transplant. This will be addressed in **Chapter 8** where the relationship between these parameters will be examined.

In terms of α-Klotho previous meta-analysis data has indicated that following transplantation there are significant increases in this biomarker (350). The current data does not support this finding. At present, however, the RETRACT study is the first research that has compared α-klotho levels in transplants and controls, as the previous analysis was entirely based on cohort data without a control group for comparison. As such the current findings are a significant addition to the evidence base given the robust methodology employed to obtain this data.

The management of traditional cardiac risk in ESKD is complex due to limited efficacy of standard treatments approaches. Following renal transplantations dyslipidaemia is often reported due to effects of immunosuppression with 58.9% of recipients being reported to have hypercholesterolaemia and 86.6% to have elevated triglyceride levels(351). In addition, observation of the trends in lipid levels following transplantation has shown that both cholesterol and triglycerides may increase. The current data, however, has shown no significant changes between transplant and controls after transplant. In addition, sub-analysis

has also shown no differences between the groups. It is of note however the trends observed in transplant recipients was for an increase in both cholesterol and triglycerides, whereas in control participants this trend was reversed. This pattern of dyslipidaemia is well described and is attributed to the combination of calcineurin inhibitors which increase LDL cholesterol levels and corticosteroids which increase both triglycerides and cholesterol in a dose dependant fashion (207).

6.6 Limitations

The RETRACT study's primary outcome measure was LVM as such it was not powered to detect changes in biochemical or metabolic parameters. As such the data presented here may be subject to type 2 statistical error. This may be particularly true for parameters such as Hs-Trop-T where the changes observed were small and baseline values were only marginally outside of the normal range.

6.7 Conclusion

Following renal transplantation multiple biochemical parameters changed significantly. The current data has mirrored previous work indicating that anaemia, NTproBNP, FGF23 and indices of CKD-MBD improve following transplantation. These results in conjunction with the previous findings that LVM also reduces following transplantation support the notion that marks of CKD-MBD and anaemia are central to the development of uraemic cardiomyopathy.

Chapter 7: MARKERS OF LEFT VENTRICULAR FIBROSIS

Abstract

Background

ESKD is associated with the presence of diffuse interstitial myocardial fibrosis. Post-mortem studied have shown that the amount of fibrosis is strongly associated with mortality in this cohort. The improved cardiovascular risk profile following renal transplantation is hypothesised to be related to related to reductions in the burden of this diffuse fibrosis.

Objectives

To assess native T1 and T2 mapping times and biochemical markers of fibrosis to assess the fibrosis in both transplant recipients and control subjects.

Methods

Patients were recruited to the RETRACT study as previously described. Native T1 times and T2 times were assessed at baseline and follow-up at between 12 and 24 months.

Results

There were significant reductions in native T1 times between transplant and controls in the inferior-lateral segment of the basal left ventricular slice (between group difference -38.78ms 95%CI [-76.43 - -1.13] P=0.04) and the global average of all segments in the mid ventricular slice (between group difference -34.37ms 95%CI [-61.01 - -7.74] P=0.01. Stratification based on the presence of LVH at baseline and eGFR at follow-up did not show any clear pattern of results. There were no significant changes observed between the groups in T2 mapping times.

Conclusion

Significant reductions were observed in native T1 times independent of T2 times. This suggests that following transplantation there is a reduction in diffuse interstitial fibrosis rather than a reduction in myocardial oedema.

7.1 Introduction

Uraemic cardiomyopathy is characterised by increased LVM, diastolic and LVSD. Histologically this cardiovascular remodelling is associated with diffuse interstitial myocardial fibrosis, myocyte disarray and hypertrophy (30).

At present CMR serves as the gold standard imaging technique to assess left ventricular, volumes, mass, and systolic function in CKD. It is less affected by changes in volume status than other modalities, which is particularly important in those receiving regular HD where fluid shifts can be extreme (177). The use of gadolinium enhancement is also a well-established technique to detect focal aeras of fibroses within the myocardium. It is established in numerous clinical conditions where patterns of focal fibrotic change are well recognised. Examples of these conditions include myocardial infarction where transmural and subendocardial enhancement is seen, myocarditis where mid-wall enhancement is observed and sarcoidosis where typically epicardial fibrosis is seen (352).

The clinical application of this methodology is, problematic in uraemic cardiomyopathy. In those with ESKD the use of GBCA imaging has been associated with the development of NSF which is a progressive, potentially fatal multi organ fibrosing disease (353). While the risk is low with the current generation of gadolinium-based agents, it remains relatively contraindicated in this group. Gadolinium enhancement images are also not well able to detect the presence of diffuse fibrosis. This type of image acquisition detects fibrosis based on the differing image intensity between normal and pathological myocardium. In conditions such as uraemic cardiomyopathy where fibrosis is distributed throughout he myocardium there may be no discernible changes in signal intensity, leading to an under appreciation of any fibrosis present (354).

Due to these issues, native T1 and T2 CMR mapping techniques have been developed, and are now recognised as effective methods of establishing myocardial tissue composition (190). Native T1 times increase with the presence of fibrosis, oedema, and protein deposition. Systematic review by Diao et al.(355) demonstrated that there were favourable correlations between histologically proven fibrosis and native T1 mapping times with low levels of heterogeneity between studies included. Native T2 times, increase with myocardial free water content and oedema, with studies demonstrating that native T2 mapping times increase with biopsy proven inflammation (250, 356, 357). In terms of application to CKD cohorts, Rutherford et al.(83) demonstrated increased native T1 times in a HD cohort compared to healthy volunteers. Hayer et al. (191) also demonstrated that as renal function declines from early-stage CKD to ESKD, there is a corresponding incremental increase in native T1 time. Graham-Brown et al. (84) also demonstrated that in an HD cohort, increases in T1 times observed were independent of changes in T2 mapping times, indicating that alterations in native T1 values were primarily due to the presence fibrosis rather than increased myocardial water content related to fluid overload.

Presently, the impact of renal transplantation on myocardial fibrosis and T1 mapping times is not well established. Conntti et la. (192) reported significant changes in T1 times at 6 months; however, this study was based on only 30 patients and did not incorporate a control arm for comparison. Consequently, further evaluation of the effects of renal transplantation on native T1 times is required. There is presently no published data which has examined changes in native T2 mapping times following transplantation.

The current chapter will examine changes in both nativeT1 and T2 mapping times both before and after renal transplantation.

7.2 Methods

Recruitment and sample size calculation were as previously described in chapter 3.

7.2.1 Hypothesis

i. T1 times will reduce significantly in transplant recipients when compared to a group of similar controls.

ii. T2 times will not reduce significantly in transplant recipients when compared to a group of similar controls.

7.2.2 Sample acquisition and analysis

Native T1 and T2 mapping values were assessed according to the American Heart Association 17 segment model performed at the basal and mid ventricular slice.

7.2.3 Statistical analysis

Normality of variable distributions was assessed using the Kolmogorov–Smirnov test. Baseline comparisons were performed using with paired students T-test. Primary analysis of change between groups was performed using independent sample T-tests.. A 2-tailed $P<0.05$ was considered statistically significant. Statistical analysis was overseen by Dr James Hodgson medical statistician at University Hospitals Birmingham Foundation Trust.

7.3 Results

7.3.1 T1 Mapping: Transplants and controls

Demographic data for transplants and controls are presented in **Figure 4.2.**

When examining native T1 times in the basal left ventricular slice, the trend observed in all segments studied was for a decrease in transplant recipients and an increase in control participants. Significant changes, however, were only observed between control and transplant recipients in the inferior-lateral segment (mean difference -38.78 ms, 95% CI [-76.43 - -1.13], P=0.04). Within-group changes indicated that there were significant reductions from baseline to follow-up in transplant recipients in the septal average values (mean difference -15.61 ms, 95% CI [-30.36 - -0.86], P=0.04) and in the anterior septal segment (mean difference -17.10 ms, 95% CI [-33.12 - -1.09], P=0.04). There were no significant within-group changes observed in control participants (**Figure 7.1**).

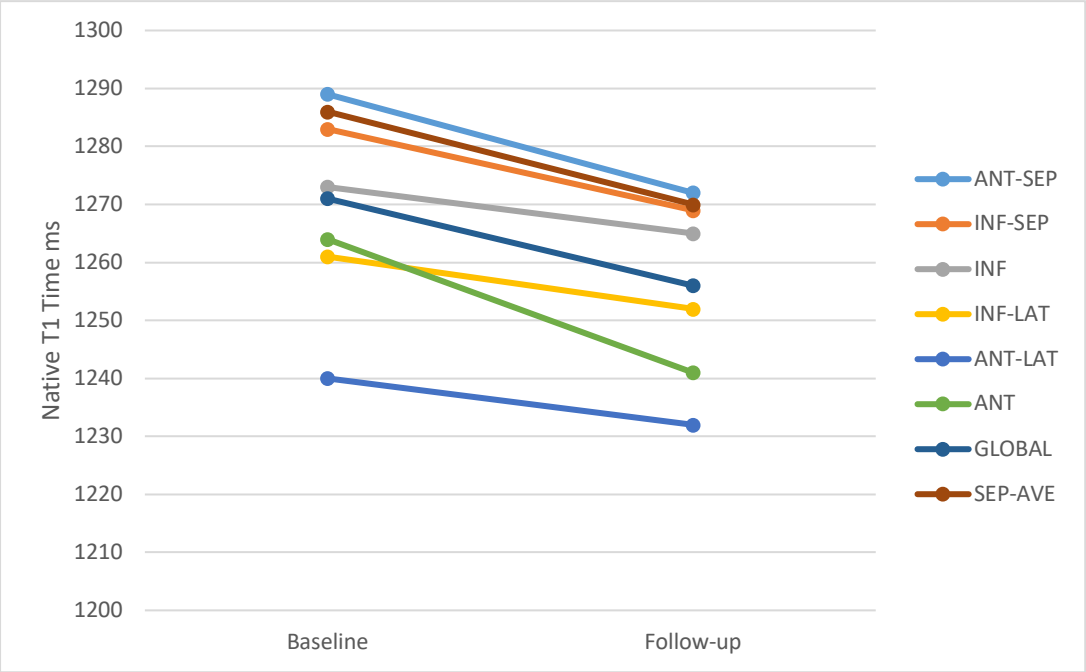
Data for T1 mapping times in the mid-ventricular slice for both transplant and controls showed a similar pattern to that seen in the basal slice. The trends observed showed reductions in all segments in the transplant recipients, with the reverse observed in control participants. There were significant between-group changes observed in the inferior (mean difference -46.55 ms, 95% CI [-80.04 - -13.06], P=0.01), inferior lateral segments (mean difference -51.28 ms, 95% CI [-81.87 - -20.70]), and the global average of all segments (mean difference -34.37 ms, 95% CI [-61.01 - -7.74], P=0.01)

Figure 7.1 Segmental analysis of basal slice nativeT1 times at baseline and follow-up.

Segment	N	Baseline	Follow-up	Within group change	P Value	Between group change	P Value
ANT-SEP							
Tx	32	1289.19±50.74	1272.09±42.68	-17.10 (-33.12 - -1.09)	0.04	-28.37 (-60.21- -3.47)	0.08
Cx	16	1269.4341.41	1280.70±40.33	11.27 (-22.889 - 45.418)	0.49		
INF-SEP							
Tx	32	1283.52±48.07	1269.40±46.26	-14.12 (-31.23 - 2.99)	0.10	-18.76 (-50.76 - 13.24)	0.24
Cx	16	1275.44±40.55	1280.08±45.81	4.65 (-27.40 - 36.68)	0.76		
INF			**				
Tx	32	1273.92±68.47	1265.51±43.12	-8.41 (-31.37 - 14.56)	0.461	-12.46 (-48.19 - 23.27)	0.49
Cx	16	1295.36±46.67	1299.41±48.02	4.05 (-19.33 - 27.44)	0.71		
INF-LAT			**				
Tx	31	1261.49±66.46	1242.42±53.28	-19.07 (-40.47 - 2.34)	0.79	-38.78 (-76.43 - -1.13)	0.04
Cx	16	1262.68±41.70	1282.39±60.18	19.71 (-15.04 - 54.47)	0.25		
ANT-LAT							
Tx	30	1240.31±52.08	1232.89±51.33	-5.69 (-30.94 -19.56)	0.65	-19.61 (-67.47 - 28.25)	0.41
Cx	15	1235.54±62.21	1249.46±55.27	13.92 (-35.07 -62.92)	0.55		
ANT							
Tx	31	1264.57±57.32	1241.76±43.82	-22.81 (-48.74 - 3.13)	0.83	-40.12 (-85.36 - 5.12)	0.08
Cx	15	1244.70±52.17	1262.01±44.67	17.32 (-22.98 - 57.61)	0.37		
GLOBAL							
Tx	32	1271.32±47.10	1256.58±41.50	-14.74 (-32.63 - 3.15)	0.10	-21.36 (-54.00 - 11.27)	0.19
Cx	16	1270.56±46.00	1276.68±44.81	6.623 (-24.87 - 38.11)	0.66		
SEP AVE							
Tx	32	1286.36±46.05	1270.74±40.43	-15.61 (-30.36 - -0.86)	0.04	-23.57 (-50.97- - 3.84)	0.09
Cx	16	1272.43±28.63	1280.39±41.65	7.955 (-19.24 - 35.14)	0.54		

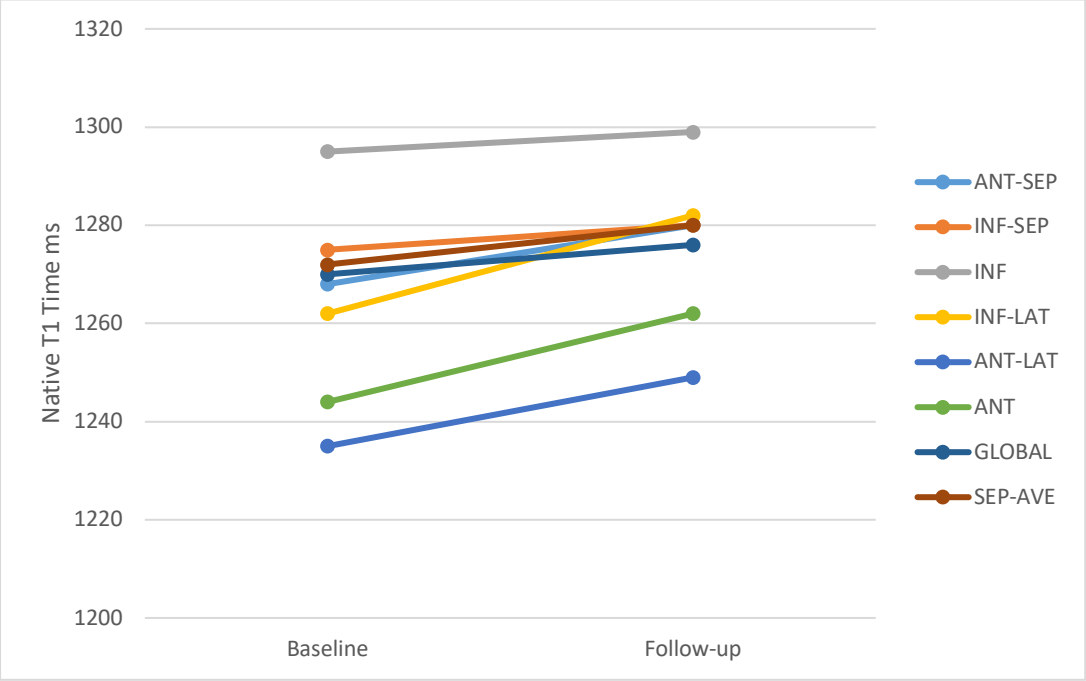
***indicates significant difference between the two groups at either baseline or follow-up. Data are displayed as mean±SD or geometric mean (95%CI) at baseline and follow-up for the whole cohort. Within-group differences were determined by paired samples t tests. Between-group differences were determined using independent samples t tests. Results are displayed as the mean difference in values (95% CI). (ANT- anterior, SEP - septum, INF-inferior, LAT – lateral Tx transplant Cx control)*

Figure 7.2 Individual cases plotted from baseline to follow-up showing change in native T1 times (ms) in each segment of the basal ventricular slice in transplant recipients.



(ANT- anterior, AVE- average, INF-inferior, LAT – lateral, SEP - septum)

Figure 7.3 Individual cases plotted from baseline to follow-up showing change in native T1 times (ms) in each segment of the basal ventricular slice in control participants.



(ANT- anterior, AVE- average, INF-inferior, LAT – lateral, SEP - septum)

Figure 7.4 Box and whisker plot of average global native T1 times at baseline and follow-up for transplant and control participants in the basal left ventricular segment

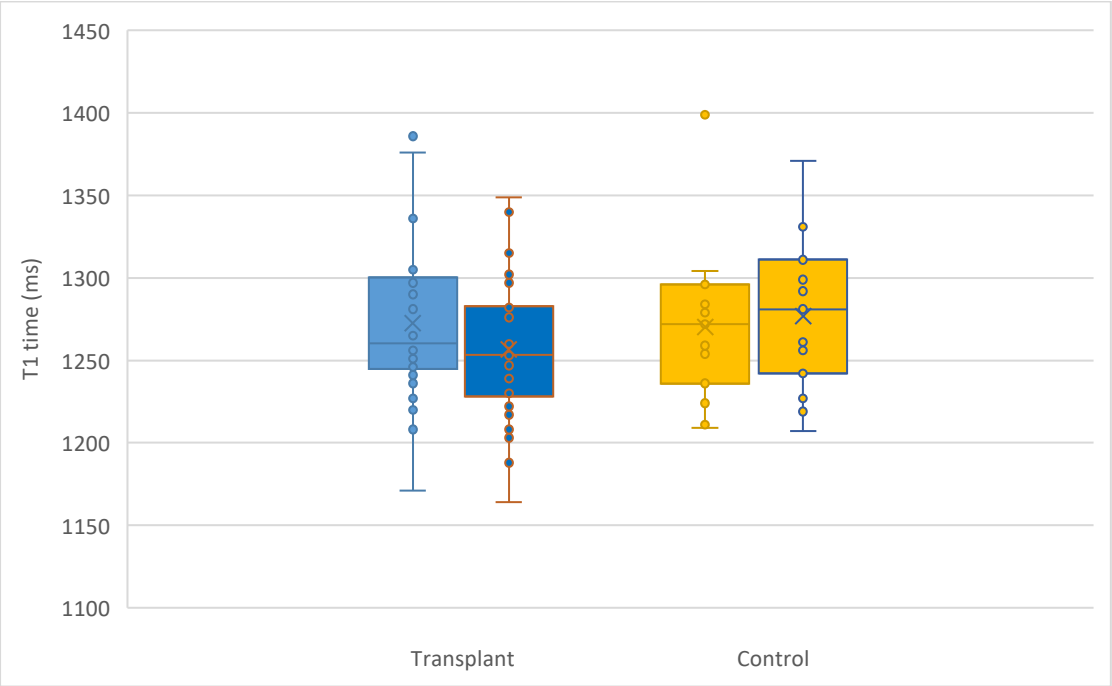


Figure 7.5 Box and whisker plot of average septal native T1 times at baseline and follow-up for transplant and control participants in the basal left ventricular segment

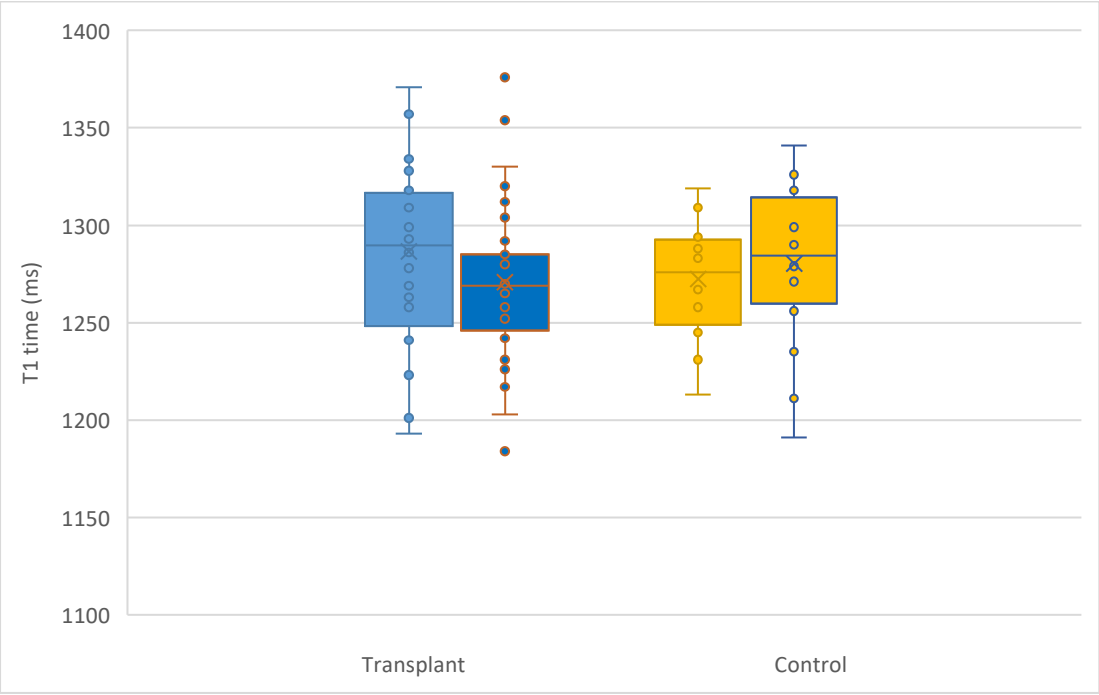


Figure 7.6 Segmental analysis of mid T1 times at baseline and follow-up.

Segment	N	Baseline	Follow-up	Within group change	P Value	Between group change	P Value
ANT-SEP							
Tx	31	1271.42±45.86	1263.59±43.73	-7.83 (-24.60 - 8.94)	0.35		0.10
Cx	17	1261.13±55.06	1280.57±50.57	19.44 (-15.06 - 53.94)	0.25	-27.27 (-60.15 - 5.61)	
INF-SEP							
Tx	31	1279.10±58.09	1263.94±43.08	-15.16 (-34.69 - 4.37)	0.12	-23.55 (-56.31 - 9.21)	0.16
Cx	17	1274.45±47.74	1282.84±50.89	8.39 (-20.00 - 36.78)	0.54		
INF			**				
Tx	31	1259.50±53.07	1227.14±46.31	-32.36 (-54.87 - -9.84)	0.01	-46.55(-80.04 - -13.06)	0.01
Cx	17	1261.56±64.13	1275.76±52.87	14.19 (-6.82 - 35.21)	0.17		
INF-LAT			**				
Tx	31	1249.05±52.34	1217.04±32.57	-32.01(-51.05--12.97)	<0.001	-51.28(-81.87 - -20.70)	0.002
Cx	17	1253.87±47.75	1273.14±54.27	19.27 (-5.04 - 43.58)	0.11		
ANT LAT			**				
Tx	31	1235.60±38.44	1225.11±47.68	-10.49 (-29.27 - 8.29)	0.26	-23.75(-58.52 - 11.02)	0.18
Cx	17	1244.24±49.00	1257.50±69.69	13.26 (-21.25 - 47.76)	0.43		
ANT							0.10
Tx	31	1238.15±40.85	1230.28±50.49	-7.88 (-28.11 - 12.36)	0.43	-30.30(-66.45 - 5.86)	
Cx	17	1238.79±61.92	1261.21±78.78	22.42 (-11.97 - 56.81)	0.19		
GLOBAL			**				
Tx	31	1255.82±41.22	1239.61±35.88	-16.21 (-31.97 - -0.45)	0.04	-34.37(-61.01 - -7.74)	0.01
Cx	17	1255.37±43.55	1273.54±55.00	18.16 (-5.21 - 41.54)	0.12		
SEP AVE							
Tx	31	1275.26±49.17	1263.76±39.40	-11.49 (-28.36 - 5.37)	0.17	-25.41(-55.64 -4.82)	0.10
Cx	17	1267.79±47.50	1281.71±47.19	13.92 (-14.96 - 42.79)	0.32		

***indicates significant difference between the two groups at either baseline or follow-up. Data are displayed as mean±SD or geometric mean (95%CI) at baseline and follow-up for the whole cohort. Within-group differences were determined by paired samples t tests. Between-group differences were determined using independent samples t tests. Results are displayed as the mean difference in values (95% CI). (ANT- anterior, SEP - septum, INF-inferior, LAT – lateral, Tx transplant Cx control))*

Figure 7.7 Individual cases plotted from baseline to follow-up showing change in native T1 times (ms) in each segment of the mid ventricular slice in transplant recipients.

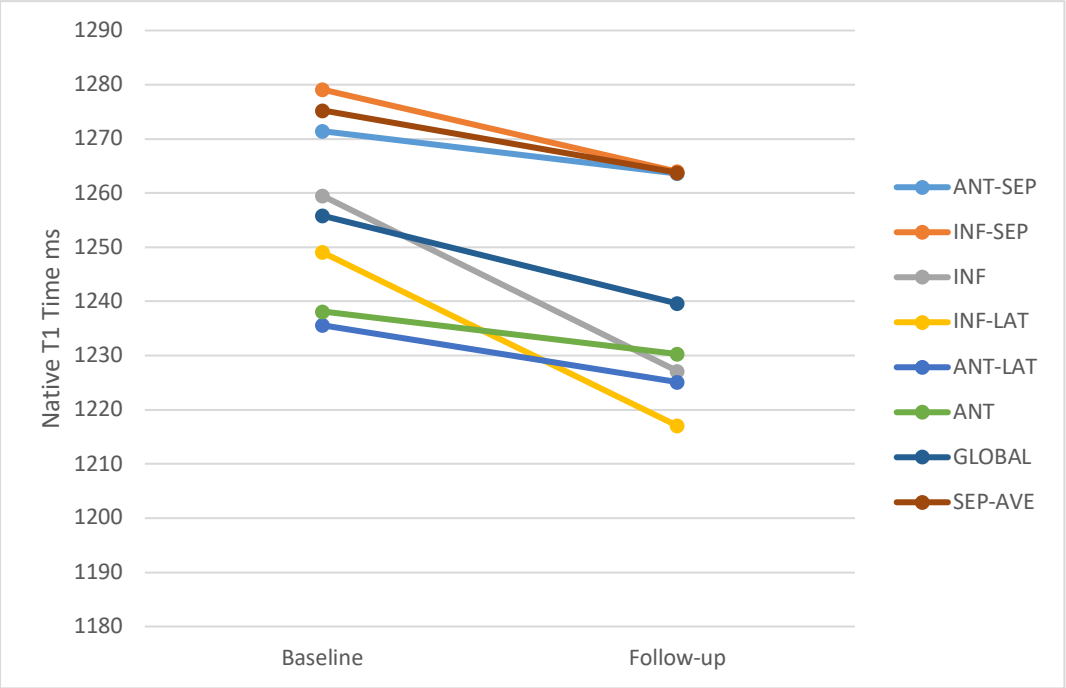


Figure 7.8 Individual cases plotted from baseline to follow-up showing change in native T1 times (ms) in each segment of the mid ventricular slice in control recipients.

(ANT- anterior, AVE- average, INF-inferior, LAT – lateral, SEP - septum)

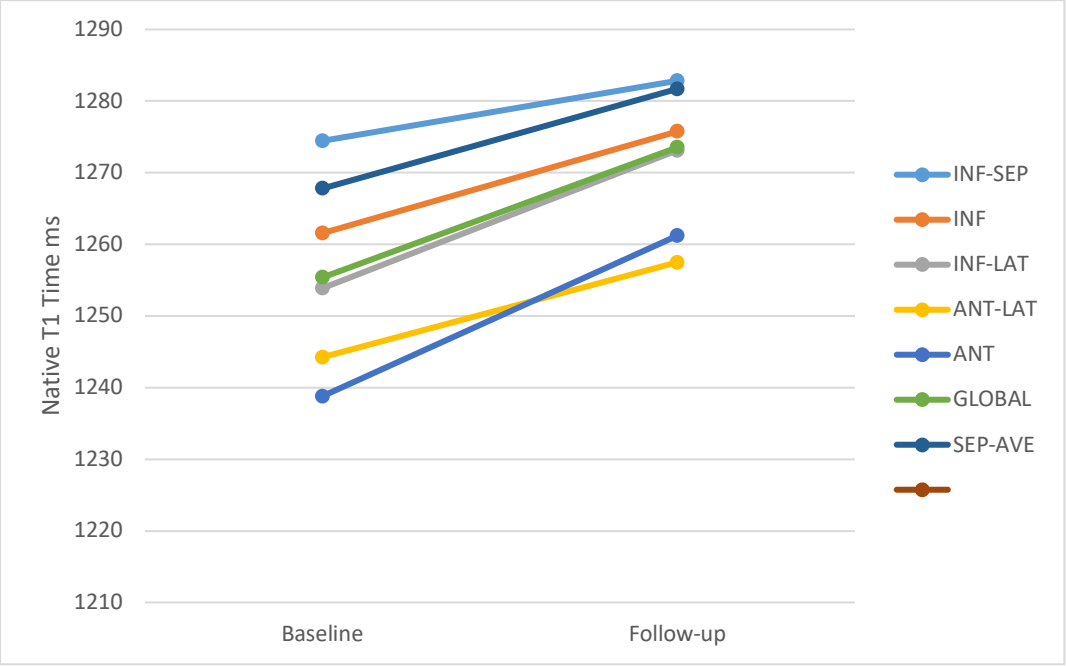


Figure 7.9 Box and whisker plot of global T1 times (ms) at baseline and follow-up for transplant and control participants in the mid left ventricular segment.

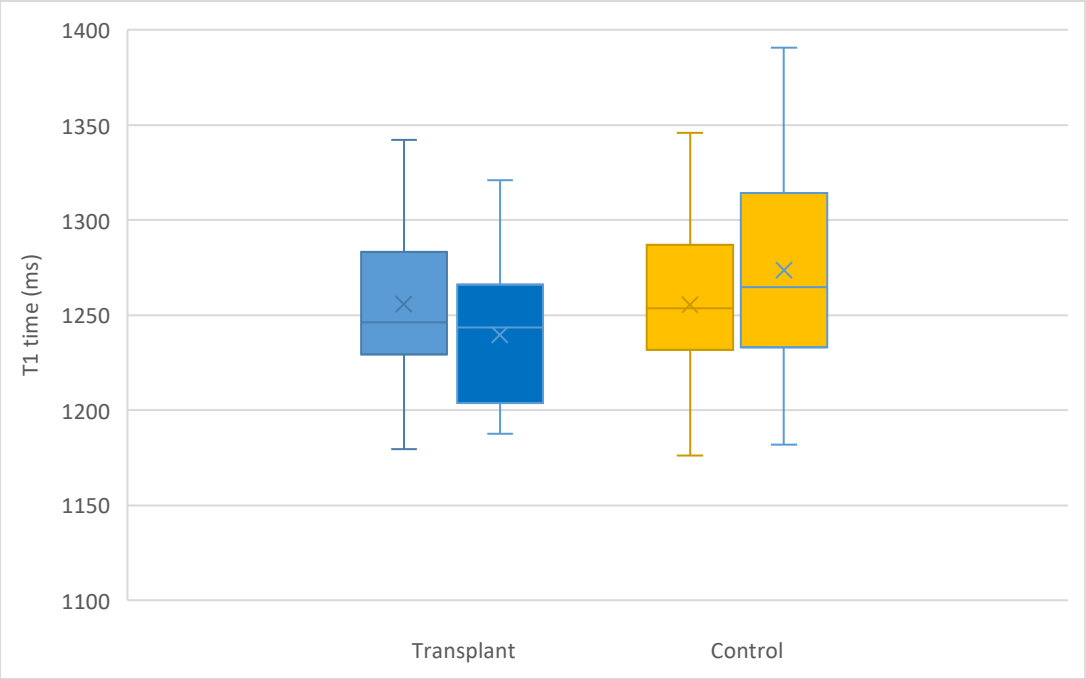
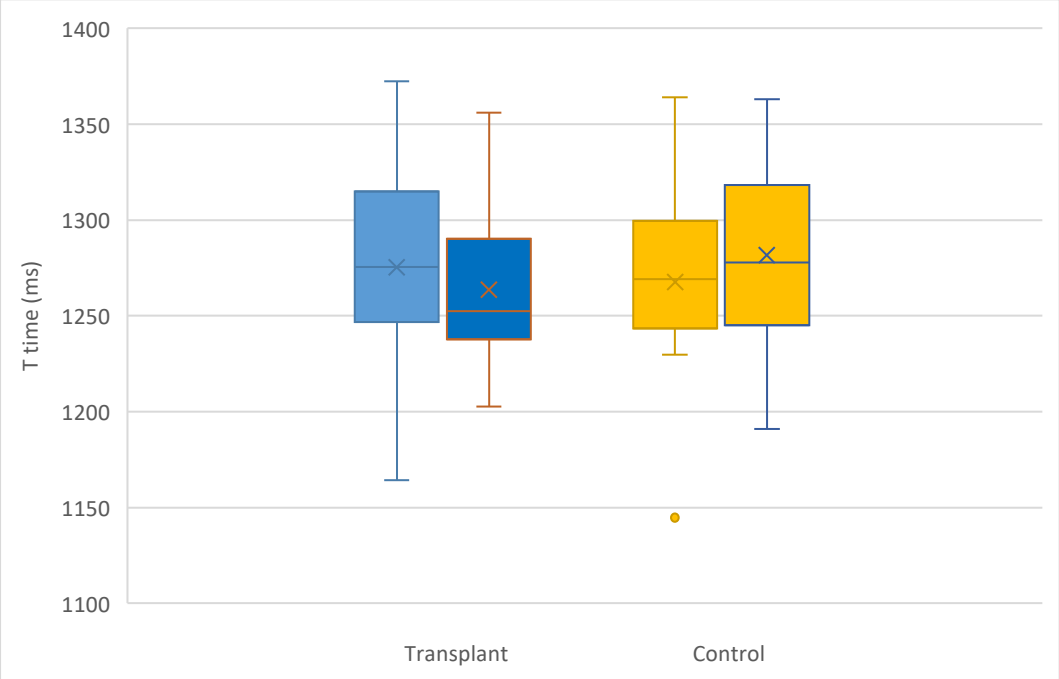


Figure 7.10 Box and whisker plot of average septal T1 times (ms) at baseline and follow-up for transplant and control participants in the mid left ventricular segment



7.3.2 LVH subgroup analysis

Demographic data for the two groups is presented in **Figure 4.7.** When stratified by the presence of LVH at baseline, analysis of T1 mapping data for the basal left ventricular slice failed to show any significant changes between the groups. There was a trend towards greater reductions in T1 times in those with LVH, with all parameters showing greater reductions in this group, with only the inferior septal segment showing the reverse. Significant reductions, however, were only observed in the inferior lateral segment (mean change -44.96ms 95%CI [-89.82 - -0.11] P=0.05) (**Figure 7.11)**

The same analysis conducted for the mid-ventricular slice indicated that there were significant reductions in those with LVH at baseline compared to those without.

Again, the data indicated a trend towards a greater reduction in those with LVH at baseline compared to those without. Significant reductions were observed within the LVH group from baseline to follow-up in the inferior (mean change -55.75ms 95%CI[-109.31 - -2.18]P=0.04) and inferior lateral segments (mean change -52.10ms 95%CI[-94.90 - -9.30]P=0.02), There was a significant reduction in the non LVH group from baseline to follow-up in the inferior later group(mean change -23.79ms 95%CI[-45.63 - -1.96]P=0.03). (**Figure 7.12).**

Figure 7.11 Segmental analysis of basal T1 times at baseline and follow-up stratified based on the presence of LVH at baseline.

Segment	N	Baseline	Follow-up	Within group change	P Value	Between group change	P Value
ANT SEP							
LVH	9	1293.12±61.34	1269.80±63.46	-23.32(-68.30 - 21.65)	0.27	-8.66(-44.77 - 27.45)	0.63
No LVH	23	1287.65±47.44	1272.98±33.15	-14.67(-31.49 - 2.15)	0.08		
INF SEP							
LVH	9	1281.53±61.03	1270.67±42.87	-10.85(-35.56 13.85	0.34	4.55(-34.14 - 43.24)	0.81
No LVH	23	1284.31±43.58	1268.91±48.44	-15.40(-38.24 7.44	0.18		
INF							
LVH	9	1268.00±47.70	1258.36±33.66	-9.65 (-45.69 - 26.40)	0.55	-1.73(-53.71 - 50.25)	0.95
No LVH	23	1276.23±75.89	1268.31±46.68	-7.92 (-38.24 - 22.40)	0.59		
INF LAT							
LVH	9	1285.84±47.55	1240.88±34.41	-44.96 (-89.82 - -0.11)	0.05	-34.90(-82.94 - 13.13)	0.15
No LVH	23	1253.02±70.80	1242.96±59.10	-10.06(-35.27 - 15.15)	0.42		
ANT LAT							
LVH	8	1265.57±22.38	1235.50±63.65	-26.24 (-84.03 - 31.55)	0.31	-31.17(-87.34 - 25.00)	0.27
No LVH	23	1231.53±56.80	1232.09±48.63	0.57 (-29.35 - 30.49)	0.97		
ANT							
LVH	7	1276.26±26.94	1240.78±59.72	-35.48(-84.27 - 13.32)	0.13	7.92(-66.81 - 82.65)	0.83
No LVH	23	1260.50±64.67	1242.10±38.51	-18.40(-50.92 - 14.12)	0.25		
GLOBAL							
LVH	8	1285.89±37.03	1258.85±49.16	-27.04(-59.49 - 5.42)	0.09	-16.58(-57.74 - 24.59)	0.42
No LVH	23	1266.25±49.84	1255.79±39.71	-10.46(-32.89 - 11.97)	0.34		
SEP AVE							
LVH	9	1287.32±59.26	1270.24±47.53	-17.09(-49.12 - 14.94)	0.25	-2.05(-35.43 - 31.33)	0.90
No LVH	23	1285.98±41.36	1270.94±38.50	-15.03(-32.99 - 2.93)	0.10		

*** indicates significant difference between the two groups at either baseline or follow-up. Data are displayed as mean±SD or geometric mean (95%CI) at baseline and follow-up for the whole cohort. Within-group differences were determined by paired samples t tests. Between-group differences were determined using independent samples t tests. Results are displayed as the mean difference in values (95% CI) ANT- anterior, SEP - septum, INF-inferior, LAT – lateral, LVH – Left ventricular hypertrophy*

Figure 7.12 Segmental analysis of mid T1 times (ms) at baseline and follow-up stratified based on the presence of LVH at baseline.

Segment	N	Baseline	Follow-up	Within group change	P Value	Between group change	P Value
ANT SEP							
LVH	9	1273.97±55.99	1259.90±69.56	-14.07(-46.86 - 18.73	0.35	-8.79(-46.27 - 28.70)	0.635
No LVH	22	1270.38±42.50	1265.10±29.67	-5.28(-26.40 - 15.84	061		
INF SEP							
LVH	9	1283.98±83.36	1260.32±57.55	-23.66(-73.07 - 25.74)	0.30	-11.98(-55.57 - 31.60)	0.58
No LVH	22	1277.10±46.47	1265.42±37.18	-11.68(-33.54 - 10.18)	0.28		
INF							
LVH	9	1263.48±71.58	1260.32±57.55	-55.75(-109.31 - -2.18)	0.04	-32.96(-81.92 - 15.99)	0.18
No LVH	22	1257.87±45.40	1265.42±37.18	-22.79(-47.88 - 2.31)	0.07		
INF LAT							
LVH	9	1246.36±50.51	1214.99±31.87	-52.10(-94.90 - -9.30)	0.02	-28.31(-69.66 - 13.05)	0.17
No LVH	22	1231.20±32.69	1217.87±33.55	-23.79(-45.63 - -1.96)	0.03		
ANT LAT							
LVH	9	1246.36±50.51	1218.22±41.60	-28.14(-67.74 - 11.45)	0.14	-24.87(-65.93 - 16.19)	0.23
No LVH	22	1231.20±32.69	1227.93±50.60	-3.27(-25.64 - 19.10)	0.35		
ANT							
LVH	9	1246.69±54.07	1232.86±46.41	-27.08(-80.42 - 26.26)	0.28	-23.16(-57.41 - 11.08)	0.22
No LVH	22	1234.66±35.02	1242.37±31.48	-0.02(-21.24 - 21.20)	0.76		
GLOBAL							
LVH	9	1278.98±65.26	1232.86±46.41	-32.65(-76.26 - 10.97)	0.12	-23.16(-57.41 - 11.08)	0.11
No LVH	22	1273.74±42.70	1242.37±31.48	-9.49(-25.14 - 6.17)	0.99		
SEP AVE							
LVH	9	1278.98±65.26	1232.86±46.41	-18.86(-54.29 - 16.56)	0.25	-10.38(-48.02 - 27.25)	0.58
No LVH	22	1273.74±42.70	1242.37±31.48	-8.48(-29.17 -12.21)	0.22		

*ANT- anterior, SEP - septum, INF-inferior, LAT – lateral, LVH – Left ventricular hypertrophy. ** indicates significant difference between the two groups at either baseline or follow-up. Data are displayed as mean±SD or geometric mean (95%CI) at baseline and follow-up for the whole cohort. Within-group differences were determined by paired samples t tests. Between-group differences were determined using independent samples t tests. Results are displayed as the mean difference in values (95% CI) (ANT- anterior, SEP - septum, INF-inferior, LAT – lateral, LVH – Left ventricular hypertrophy)*

7.3.3 eGFR Subgroup analysis

Demographic data for the two groups are presented in **Figure 4.13**.

When stratified for renal function at follow-up (**Figure 7.13**), analysis of basal native T1 times highlighted that while there were no significant between-group or within-group changes for any of the parameters measured. There was however a tendency for greater reductions in T1 times in those who achieved higher levels of renal function at follow-up in all slices (septal average, mean change eGFR>50 -19.98 (-39.67 - -0.30) vs eGFR<50 7.26 (-31.74 -17.22) P=0.412 global average, mean change value -20.82 (-46.65 - 5.02) vs -3.70 (-26.36 - 18.96) P=0.36)

The same analysis for T1 values at the mid-ventricular slice (**Figure 7.14**) indicated that there were again no significant between-group changes. There were also no significant longitudinal changes within the two groups Unlike the basal slice data, there was no obvious trend observed within the data.

Figure 7.13 Segmental analysis of basal T1 times (ms) at baseline and follow-up stratified based on eGFR levels at follow-up.

Segment	N	Baseline	Follow-up	Within group change	P Value	Between group change	P Value
ANT SEP			**				
<50	11	1311.85±45.236	1300.89±44.79	-10.96 (-35.43 - 13.51)	0.34		
>50	21	1277.32±50.364	1257.00±33.54	-20.321 (-42.45 - 1.81)	0.07	9.36 (- 24.78 – 43.50)	0.58
INF SEP			**				
<50	11	1303.03±45.64	1299.47±35.78	-3.56 (-31.62 - 24.49)	0.78		
>50	21	1273.30±47.13	1253.66±43.81	-19.65 (-42.52 - 3.22)	0.09	16.08 (- 20.08 – 52.25)	0.37
INF			**				
<50	11	1291.34±35.49	1286.84±41.17	-4.51 (-37.02 - 28.01)	0.76		
>50	21	1264.79±79.89	1254.34±40.67	-10.45 (-42.97 - 22.07)	0.51	5.94 (- 43.22 – 55.11)	0.81
INF LAT							
<50	11	1275.61±57.23	1263.1±66.13	-12.52(-55.82 - 30.78)	0.53		
>50	20	1253.72±71.22	1231.05±42.38	-22.67 (-48.95 - 3.61)	0.09	10.152 (- 35.25 – 55.56)	0.65
ANT LAT							
<50	11	1237.91±45.18	1248.18±43.71	10.27 (-15.23 - 35.77)	0.39		
>50	19	1238.96±56.83	1224.03±54.38	-14.92 (-53.24 - 23.40)	0.42	25.19 (- 27.32 – 77.70)	0.33
ANT			**				
<50	11	1267.50±35.45	1263.35±36.16	-4.15 (-26.73 - 18.44)	0.69		
>50	20	1262.96±67.22	1229.89±43.88	-33.07(-72.19 - 6.05)	0.09	28.923 (- 25.19 – 83.03)	0.28
GLOBAL			**				
<50	11	1281.29±38.29	1277.59±33.95	-3.70 (-26.36 - 18.96)	0.72		
>50	21	1265.84±51.39	1245.02±41.43	-20.82 (-46.65 - 5.02)	0.11	17.12 (-0 20.41 -54.65)	0.36
SEP AVERAGE			**				
<50	11	1307.44±44.00	1300.18±36.93	7.26 (-31.74 -17.22)	0.52		
>50	21	1275.31±44.12	1255.33±33.56	-19.98 (-39.67 - -0.30)	0.05	12.721 (- 18.53 - 43.97)	0.412

*** indicates significant difference between the two groups at either baseline or follow-up. Data are displayed as mean±SD or geometric mean (95%CI) at baseline and follow-up for the whole cohort. Within-group differences were determined by paired samples t tests. Between-group differences were determined using independent samples t tests. Results are displayed as the mean difference in values (95%CI) (ANT- anterior, SEP - septum, INF-inferior, LAT – lateral, LVH – Left ventricular hypertrophy)*

Figure 7.14 Segmental analysis of mid T1 times (ms) at baseline and follow-up stratified by eGFR levels at follow-up.

Segment	N	Baseline	Follow-up	Within group change	P Value	Between group change	P Value
ANT SEP							
<50	10	1283.21±46.78	1280.05±28.52	-3.16(-32.64 -26.33)	0.81		
>50	21	1265.81±45.46	1255.75±47.98	-10.06 (-32.15 - 12.04)	0.35	7.18(-27.49 - 41.86)	0.68
INF SEP			**				
<50	10	1300.23±55.20	1285.71±50.94	-14.51 (-48.24 -19.23)	0.35		
>50	21	1269.03±57.98	1253.56±35.59	-15.47 (-41.42 -10.48)	0.23	2.28(-38.23 - 42.79)	0.91
INF							
<50	10	1284.74±53.84	1241.75±35.03	-14.51 (-48.24 - 19.23)	0.07		
>50	21	1247.47±49.49	1220.18±50.07	-27.29 (-59.06 - 4.47)	0.09	-11.79 (-58.46 -34.87)	0.61
INF LAT							
<50	10	1266.09±54,08	1221.56±38.98	-44.53(-75.54 - -13.52)	0.01		
>50	21	1240.93±50.78	1214.88±29.87	-26.05 (-51.31 - -0.78)	0.04	-14.44 (-53.77 - 24.90)	0.46
ANT LAT							
<50	10	1239.66±42.23	1238.74±33.01	-0.92(-33.42 - 31.58)	0.95		
>50	21	1233.67±37.44	1218.62±52.76	-15.05 (-39.71 - 9.61)	0.22	14.21 (-24.36 - 52.79)	0.46
ANT							
<50	10	1240.65±41.65	1251.09±53.87	10.45(-29.42 - 50.31)	0.57		
>50	21	1236.97±41.45	1215.60±47.85	-21.36 (-48.38 - 5.66)	0.11	30.86 (-12.70 - 74.41)	0.16
GLOBAL							
<50	10	1270.69±43.58	1254.51±33.29	-15.57(-41.27 - 10.13)	0.20		
>50	21	1249.03±39.29	1232.51±35.61	-16.51 (-37.80 - 4.77)	0.12	2.36 (-30.36 - 35.07)	0.88
SEP AVE							
<50	10	1291.72±49.70	1282.89±37.75	-8.83 (-38.94 - 21.27)	0.52		
>50	21	1267.42±48.12	1254.66±37.65	-12.76 (-34.92 - 9.39)	0.24	4.73 (-30.21 - 39.67)	0.78

*** indicates significant difference between the two groups at either baseline or follow-up. Data are displayed as mean±SD or geometric mean (95%CI) at baseline and follow-up for the whole cohort. Within-group differences were determined by paired samples t tests. Between-group differences were determined using independent samples t tests. Results are displayed as the mean difference in values (95%CI) (ANT- anterior, SEP - septum, INF-inferior, LAT – lateral, LVH – Left ventricular hypertrophy.)*

7.4 T2 Mapping

7.4.1 Transplant and controls

Analysis of T2 mapping data indicated that at the basal slice, there were no significant differences observed between recipients and controls from baseline to follow-up (**Figure 7.15**).

Examination of longitudinal changes in transplant recipients revealed no obvious trends across the data as a whole. A significant reduction was observed in the anterior septal slice from baseline to follow-up; however, this trend was not replicated in other segments (mean change: (-2.33 ms, 95% CI [-4.45 - -0.21], P=0.03).

No significant longitudinal changes were observed in the control group.

Analysis of T2 mapping times in the mid-ventricular slice showed no significant differences between the two groups in any of the measured segments (See **Figure 7.20**).

Longitudinal within-group analysis indicated that in transplant recipients there was a trend towards reduced native T2 times in all segments. Three segments demonstrated significant reductions from baseline to follow-up: anterior septum (mean change: -1.24 ms, 95% CI [-2.43 - -0.05], P=0.04), inferior septum (mean change: -1.64 ms, 95% CI [-2.84 - -0.44], P=0.01), and septal average (mean change: -1.44 ms, 95% CI [-2.47 - -0.41], P=0.01) . In the control group there were no significant longitudinal changes observed in any of the segments. Additionally, there was no clear trend observed in control participants , with conflicting results seen across different segments.

Figure 7.15 Segmental analysis of basal T2 times (ms) at baseline and follow-up.

Segment	N	Baseline	Follow-up	Within group change	P Value	Between group change	P Value
ANT SEP							
Tx	30	41.95±3.46	39.62±4.78	-2.33(-4.45 - -0.21)	0.03		
Cx	17	40.62±2.46	40.61±1.98	-0.02 (-1.56 - 1.53)	0.98	-2.31 (-5.31 - 0.68)	0.13
INF SEP							
Tx	30	41.38±3.23	41.00±3.26	-0.38(-2.03 - 1.27)	0.64		
Cx	17	40.01±9.80	41.14±2.48	1.12(-3.77 - 6.02	0.63	-1.51 (-5.60 - 2.59)	0.46
INF							
Tx	30	41.40±3.39	41.84±4.01	0.44(-1.02 - 1.89)	0.54		
Cx	17	43.46±3.48	42.30±4.10	-1.16(-3.84 - 1.53	0.37	4.08 (-0.48 - 8.64)	0.08
INF LAT		**					
Tx	30	41.47±3.42	41.77±3.59	0.31(-1.05 - 1.67)	0.65		0.39
Cx	17	44.35±4.45	43.65±2.89	-0.70(-2.85 - 1.44)	0.50	1.01 (-1.34 - 3.36)	
ANT LAT		**	**				
Tx	30	40.61±3.01	40.08±2.99	-0.54(-1.72 - 0.64)	0.36		
Cx	17	42.76±2.80	42.10±2.81	-0.65(-2.48 - 1.18)	0.46	0.12 (-1.91 - 2.14)	0.91
ANT							
Tx	30	40.63±3.49	40.62±3.80	-0.01 (-1.36 - 1.34)	0.99		
Cx	17	42.29±3.23	41.16±5.85	-1.13 (-4.32 - 2.05)	0.46	1.12 (-1.75 -4.00)	0.43
GLOBAL							
Tx	30	41.07±3.92	41.09±2.74	0.03 (-1.36 - 1.42)	0.97		
Cx	17	42.51±2.23	41.91±2.16	-0.60 (-1.92 - 0.72	0.35	0.63 (-1.43 - 2.68)	0.54
SEP AVE							
Tx	30	41.67±3.17	40.31±3.47	-1.36 (-3.00 - 0.29)	0.10		
Cx	17	40.32±5.31	40.87±1.85	0.55 (-2.09 - 3.20)	0.66	-1.91 (-4.78 -0.96)	0.19

*** indicates significant difference between the two groups at either baseline or follow-up. Data are displayed as mean±SD or geometric mean (95%CI) at baseline and follow-up for the whole cohort. Within-group differences were determined by paired samples t tests. Between-group differences were determined using independent samples t tests. Results are displayed as the mean difference in values (95% CI) ANT- anterior, SEP - septum, INF-inferior, LAT – latera Tx transplant Cx control.)*

Figure 7.16 Individual cases plotted from baseline to follow-up showing change in native T2 times (ms) in each segment of the basal ventricular slice in transplant recipients.

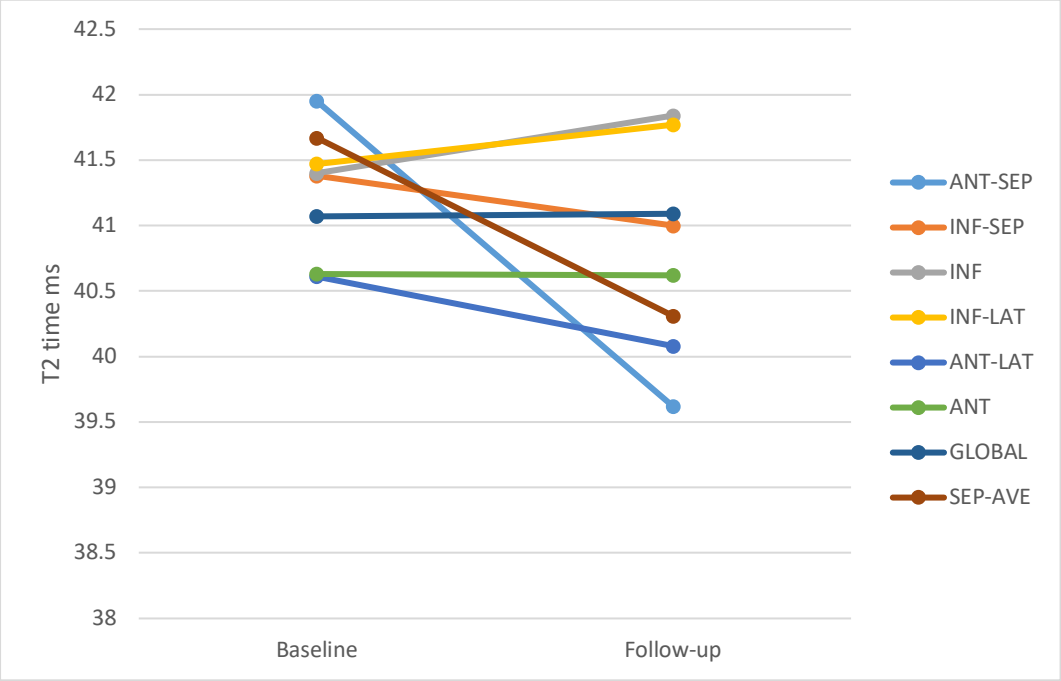
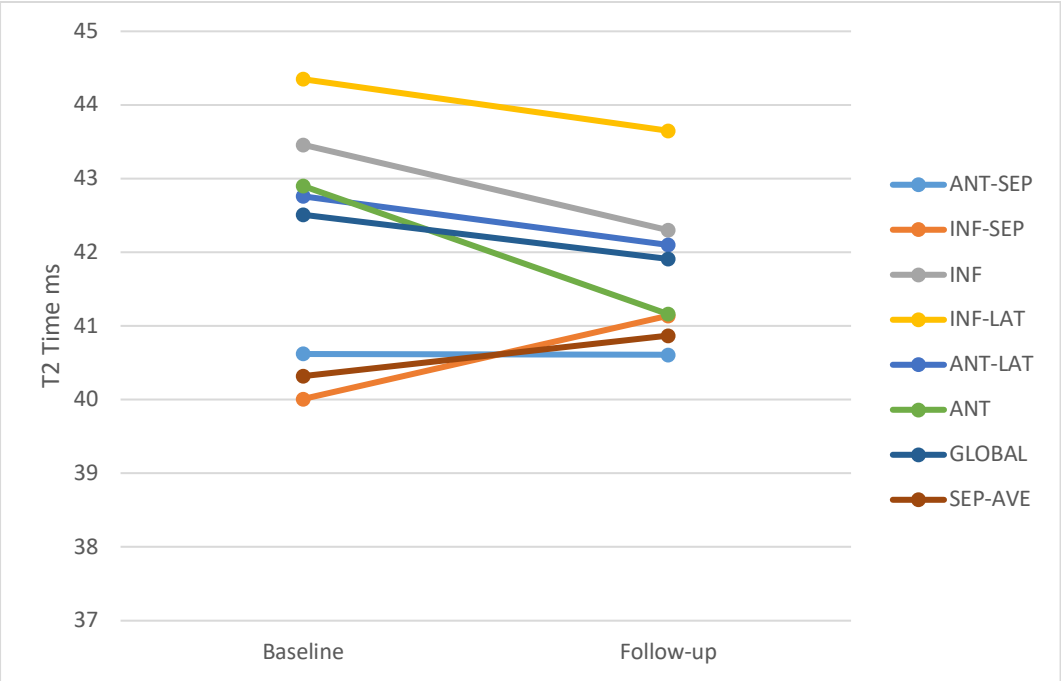


Figure 7.17 Individual cases plotted from baseline to follow-up showing change in native T2 times (ms) in each segment of the basal ventricular slice in control participants



(ANT- anterior, AVE- average, INF-inferior, LAT – lateral, SEP - septum)

Figure 7.18 Box and whisker plot of global T2 times (ms) at baseline and follow-up for transplant and control participants in the basal left ventricular segment.

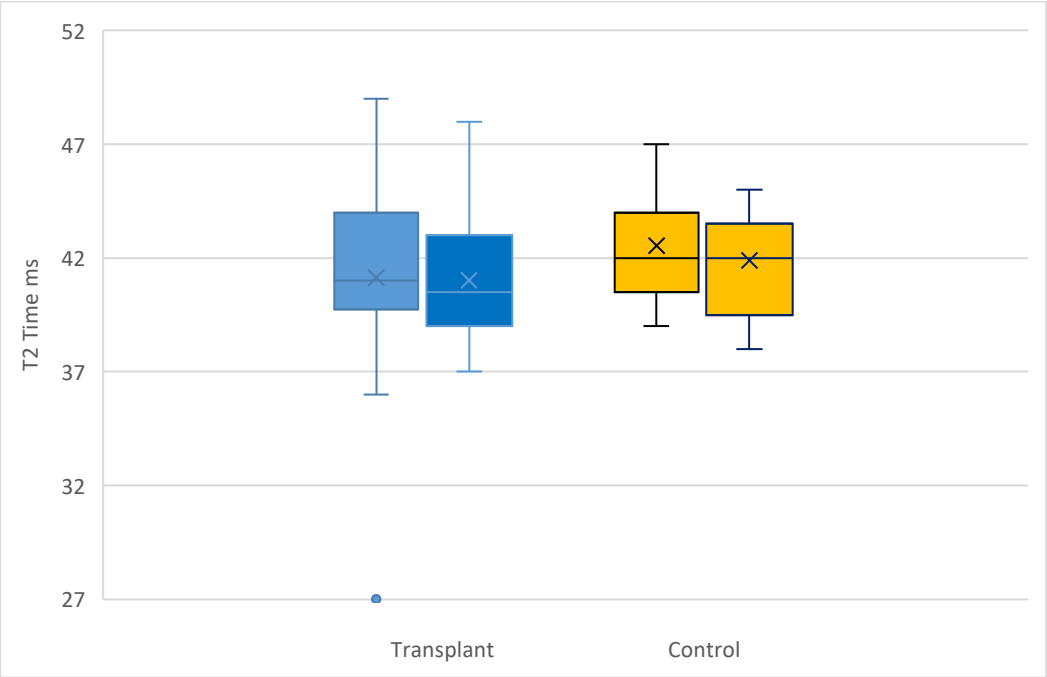


Figure 7.19 Box and whisker plot of septal average T2 times (ms) at baseline and follow-up for transplant and control participants in the basal left ventricular segment.

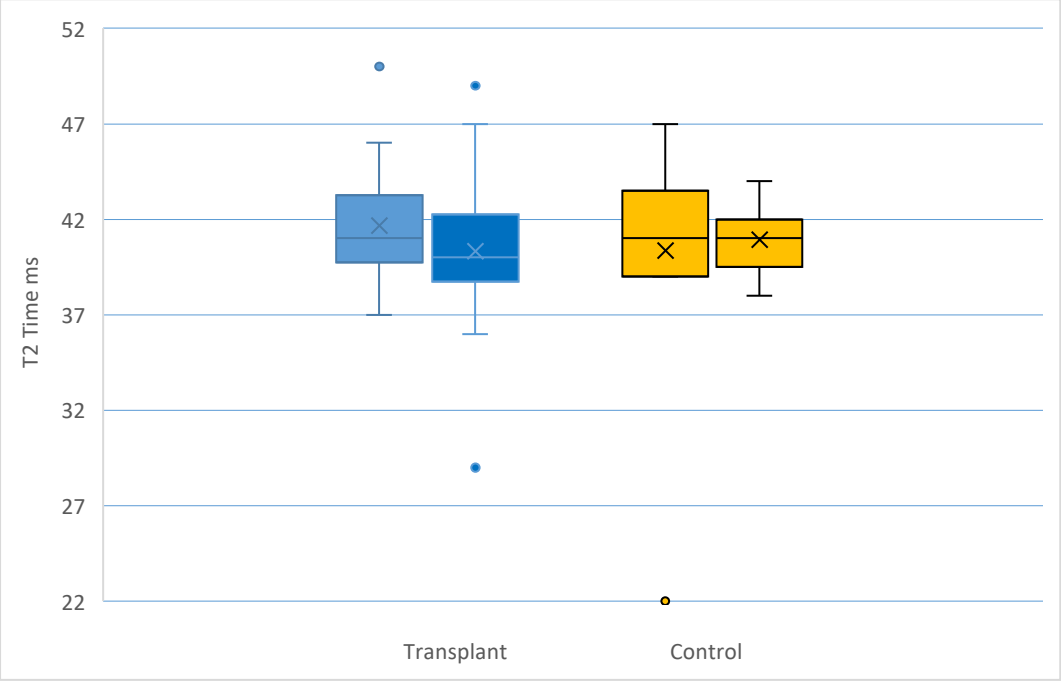


Figure 7.20 Segmental analysis of mid T2 times (ms) at baseline and follow-up for transplants and controls.

Segment	N	Baseline	Follow-up	Within group change	P Value	Between group change	P Value
ANT SEP							
Tx	29	43.46±3.32	42.22±3.57	-1.24 (-2.43 -0.05)	0.04		
CX	18	40.37±9.90	42.28±3.07	1.90(-3.23 - 7.03)	0.43	-3.14 (-7.11 - 0.82)	0.12
INF SEP							
Tx	30	42.19±3.59	40.55±2.22	-1.64 (-2.84 - -0.44)	0.01		
Cx	17	42.32±3.08	41.68±2.90	-.64(-2.83 - 1.54)	0.54	-1.00 (-3.21 - 1.21)	0.37
INF			**				
Tx	30	41.50±3.69	40.12±3.54	-1.38 (-2.97 - 0.22)	0.09		
Cx	17	43.90±5.58	42.60±4.51	-1.31 (-4.94 - 2.33)	0.46	-0.07(-3.40 - 3.25)	0.97
INF LAT			**				
Tx	30	42.24±4.42	41.27±3.25	-0.98 (-2.51 - 0.56)	0.20		
Cx	17	42.67±4.03	43.81±3.68	1.15 (-1.36 - 3.65)	0.35	-2.12(-4.81 - 0.57)	0.12
ANT LAT							
Tx	30	41.03±3.56	39.28±7.15	-1.75 (-4.65 - 1.15)	0.23		
Cx	17	42.56±2.83	42.91±3.39	0.35 (-1.92 - 2.62)	0.75	-2.10(-5.68 - 1.48)	0.31
ANT							
Tx	30	42.91±4.01	41.58±4.41	-1.33(-3.18 - 0.52)	0.15		
Cx	17	42.49±2.97	43.65±3.48	1.16 (-0.25 - 2.58)	0.10	-0.16(-4.15 - 4.46\)	0.94
GLOBAL			**				
Tx	30	42.17±3.13	41.22±2.55	-0.95 (-1.96 - 0.06)	0.06		
Cx	18	42.84±2.63	42.88±2.77	0.09 (-1.72 - 1.91)	0.91	-1.04(-2.83 - 0.75)	0.25
SEP AVE							
Tx	30	42.82±3.22	41.38±2.64	-1.44 (-2.47 - -0.41)	0.01		
Cx	17	41.35±5.54	41.98±2.80	0.63 (-2.60 - 3.86)	0.68	-2.07(-4.73 - 0.59)	0.12

*** indicates significant difference between the two groups at either baseline or follow-up. Data are displayed as mean±SD or geometric mean (95%CI) at baseline and follow-up for the whole cohort. Within-group differences were determined by paired samples t tests. Between-group differences were determined using independent samples t tests. Results are displayed as the mean difference in values (95% CI) (ANT- anterior, SEP - septum, INF-inferior, LAT – lateral)*

Figure 7.21 Individual cases plotted from baseline to follow-up showing change in native T2 times (ms) in each segment of the mid ventricular slice in transplant recipeints.

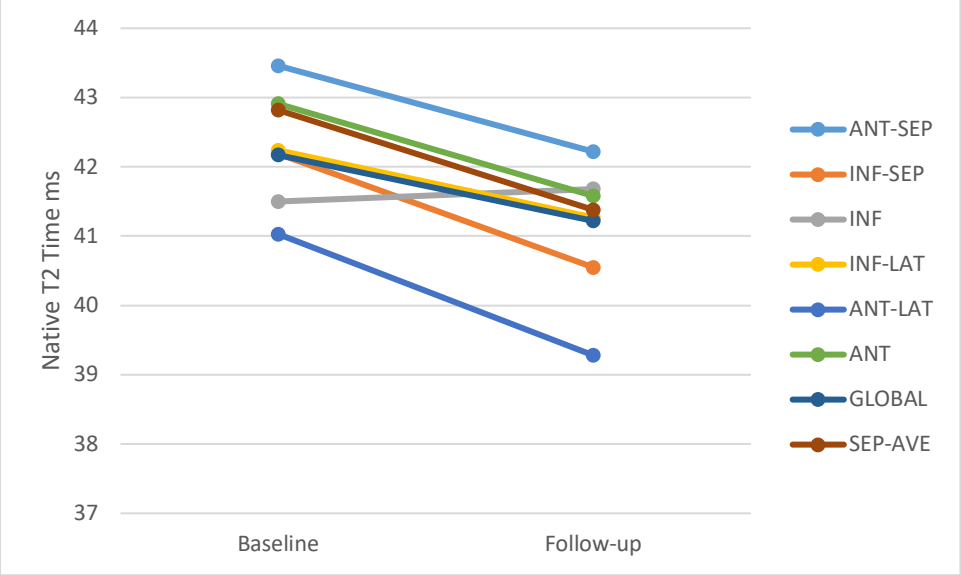
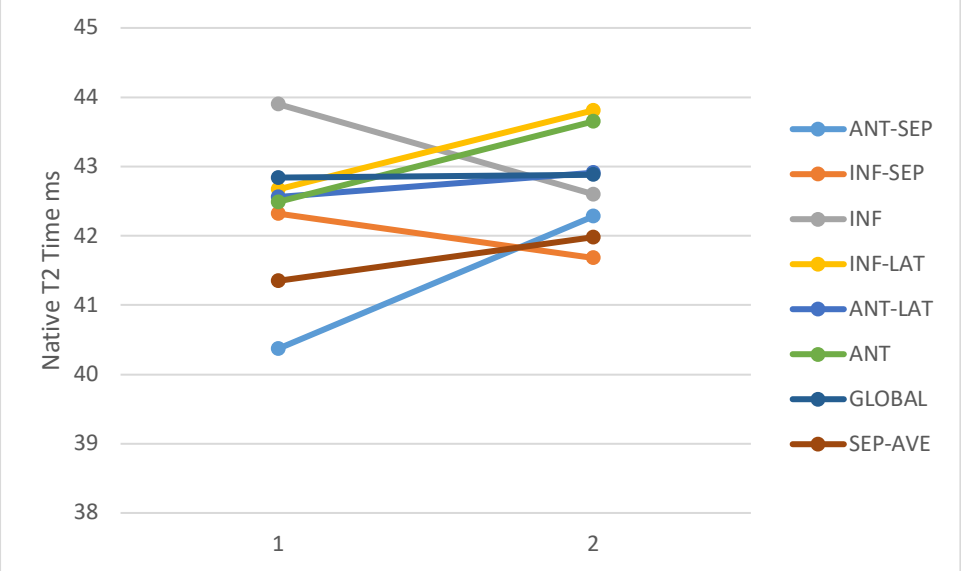


Figure 7.22 Individual cases plotted from baseline to follow-up showing change in native T2 times (ms) in each segment of the mid ventricular slice in control participants.



(ANT- anterior, AVE- average, INF-inferior, LAT – lateral, SEP - septum)

Figure 7.23 Box and whisker plot of global T2 times (ms) at baseline and follow-up for transplant and control participants in the mid left ventricular segment.

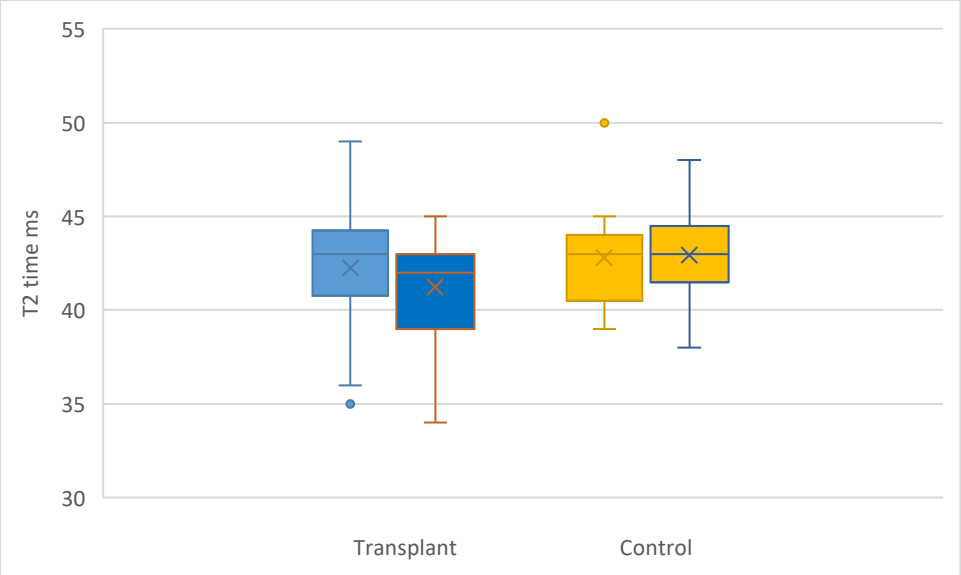
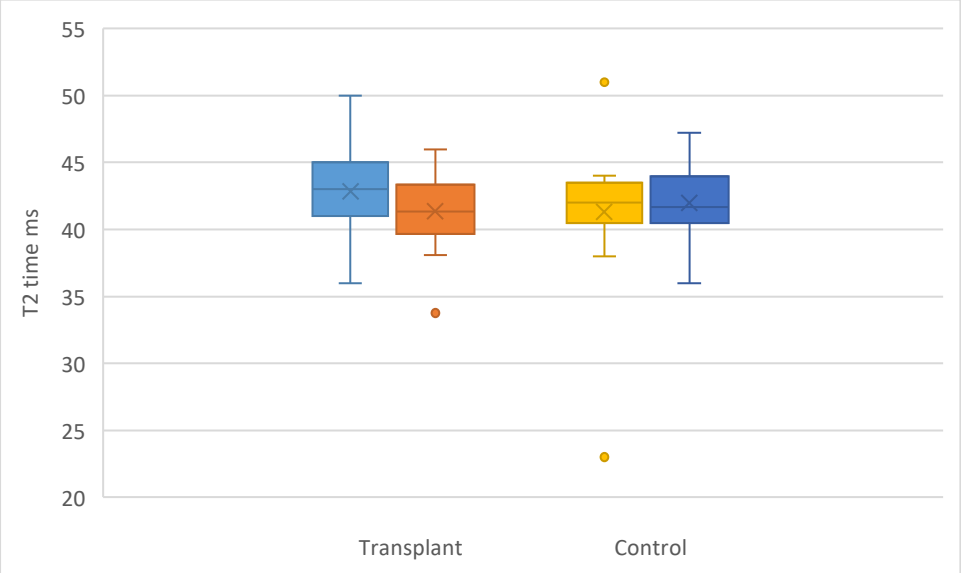


Figure 7.24 Box and whisker plot of septal average T2 times (ms) at baseline and follow-up for transplant and control participants in the mid left ventricular segment.



7.4.2 LVH subgroup analysis

Analysis of the T2 times, stratified by the presence of LVH at baseline showed significant reductions in the LVH group compared to those without. Inferior septum (between group difference-3.89ms 95%CI [-7.23 - -0.54] P=0.02), inferior (between group difference -3.34ms 95CI% [-6.30 - -0.38] P=0.03) and anterior lateral (between group difference -2.44ms 95%CI [-4.89-0.02] P=0.05) segments (**Figure 7.25**). There was no difference between the groups at baseline.

Longitudinal within group analysis for the basal slice indicated that there were significant reductions in T2 times observed within the LVH group in the anterior septal (mean difference -2.94ms 95%CI [-5.54 - -0.34] P=0.03) anterior lateral (mean difference -2.24ms 95%CI [-3.49 - -0.99] P<0.001) septal average (mean difference -3.02ms 95%CI [-6.02 - -0.02] P=0.05). There was also a tendency for a reduction in T2 times in the other segments studied, even where the difference was not statistically significant. There were no significant reductions in the non LVH group

The same analysis conducted for the mid-ventricular slice revealed that there were no significant differences between the two groups for any of the parameters studied.

Longitudinal analysis of the mid ventricular slice data for those with LVH demonstrated significant reductions in the anterior septum (mean change -2.48ms 95%CI [-4.44 - -0.51] P=0.02), inferior septum (-3.12ms 95%CI [-5.43 - -0.81] P=0.01), and global average (mean change -1.74ms 95%CI [-3.29 - -0.19] P=0.03) and septal average values (mean change -3.02ms 95%CI [-6.02 - -0.02] P=0.05). In those without LVH no significant longitudinal changes were observed (**Figure 7.26**).

Figure 7.25 Segmental analysis of basal T2 times (ms) at baseline and follow-up stratified based on the presence of LVH at baseline.

Segment	N	Baseline	Follow-up	Within group change	P Value	Between group change	P Value
ANT SEP							
LVH	9	43.94±3.54	41.00±1.09	-2.94(-5.54 - -0.34)	0.03		
No LVH	21	41.11±3.14	39.04±5.61	-2.07(-5.02 - 0.88)	0.16	-0.87(-5.57-3.84)	0.71
INF SEP	9						
LVH	21	43.95±4.23	40.85±1.79	-3.10(-6.64 - 0.44)	0.08		
No LVH		40.29±1.94	41.07±3.76	0.79(-0.98 - 2.55)	0.36	-3.89(-7.23 - -0.54)	0.02
INF	9						
LVH	21	42.87±2.27	40.97±4.00	-1.90\ (-4.31 - 0.50)	0.10		
No LVH		40.77±3.63	42.21±4.05	1.44(-0.29 - 3.17)	0.10	-3.34(-6.30 - -0.38	0.03
INF LAT	9						
LVH	21	41.68±1.76	41.37±2.58	-0.31(-1.91 - 1.28)	0.66		
No LVH		41.37±3.96	41.95±3.99	0.57(-1.32 - 2.47)	0.54	-0.89(-3.89-2.12)	0.55
ANT LAT	9						
LVH	21	41.53±1.50	39.29±1.34	-2.24(-3.49 - -0.99)	<.001		
No LVH		40.22±3.42	40.42±3.44	0.19(-1.36 - 1.74	0.80	-2.44(-4.89-0.02)	0.05
ANT	9						
LVH	21	41.84±2.36	40.00±2.25	-1.84 (-3.92 - 0.24)	0.08		
No LVH		40.11±3.80	40.89±4.32	0.78(-0.92 - 2.47)	0.35	-2.61(-5.44-0.21)	0.07
GLOBAL	9						
LVH	21	42.74±1.61	41.37±2.36	-1.37 (-3.97 - 1.23)	0.26		
No LVH		40.35±4.41	40.98±2.94	0.63 (-1.10 - 2.35)	0.46	-2.38(-5.92-1.17)	0.18
SEP AVERAGE	9						
LVH	21	43.94±3.87	40.92±1.27	-3.02 (-6.02 - -0.02)	0.05		
No LVH		40.70±2.29	40.05±4.07	-0.64(-2.70 - 1.41)	0.52	-2.38(-5.92 - 1.17)	0.18

*** indicates significant difference between the two groups at either baseline or follow-up. Data are displayed as mean±SD or geometric mean (95%CI) at baseline and follow-up for the whole cohort. Within-group differences were determined by paired samples t tests. Between-group differences were determined using independent samples t tests. Results are displayed as the mean difference in values (95% CI) (ANT- anterior, SEP - septum, INF-inferior, LAT - lateral.)*

Figure 7.26 egmental analysis of mid T2 times (ms) at baseline and follow-up stratified based on the presence of LVH at baseline.

Segment	N	Baseline	Follow-up	Within group change	P Value	Between group change	P Value
Ant Sep							
LVH	9	45.36±2.77	42.88±2.67	-2.48(-4.44 - -0.51)	0.02		
NoLVH	21	42.65±3.25	41.94±3.91	-0.71(-2.23 - 0.81)	0.34	-1.77(-4.33 - 0.80)	0.17
INF SEP							
LVH	9	44.03±2.94	40.91±1.12	-3.12(-5.43 - -0.81)	0.01		
NoLVH	21	41.40±3.61	40.39±2.56	-1.01(-2.44 - 0.43)	0.16	-2.11 (-4.65 - 0.43)	0.10
INF							
LVH	9	42.84±2.50	40.97±2.75	-1.87(-4.35 - 0.62)	0.12		
NoLVH	21	40.92±4.01	39.75±3.83	-1.17(-3.31 - 0.97)	0.27	-0.70(-4.24 - 2.84)	0.69
INF LAT							
LVH	9	42.27±2.36	41.37±2.04	-0.91(-3.49 - 1.68)	0.44		
NoLVH	21	42.23±5.11	41.22±3.70	-1.01(-3.04 - 1.03)	0.31	0.10(-3.32 - 3.52)	0.95
ANT LAT							
LVH	9	40.69±2.20	39.94±1.53	-0.75(-3.01 - 1.50)	0.46		
NoLVH	21	41.18±4.04	39.00±8.54	-2.18(-6.34 - 1.98)	0.29	1.43 (-5.00 - 7.86)	0.65
ANT							
LVH	9	43.41±2.29	40.96±4.87	-2.45(-7.05 - 2.15)	0.25		
NoLVH	21	42.70±4.59	41.85±4.29	-0.85(-2.91 - 1.22)	0.40	-1.61 (-5.68 - 2.47)	0.43
GLOBAL							
LVH	9	43.14±1.93	41.40±1.45	-1.74(-3.29 - -0.19)	0.03		
No LVH	21	41.76±3.48	41.14±2.93	-0.61(-1.95 - 0.72)	0.35	-1.13(-3.34 - 1.08)	0.30
SEP AVE							
LVH	9	44.70±2.74	41.90±1.51	-2.80(-4.59 - -1.01)	0.01		
NoLVH	21	42.02±3.13	41.16±3.00	-0.86(-2.12 - 0.40)	0.17	-1.94(-4.10 - 0.22)	0.77

*** indicates significant difference between the two groups at either baseline or follow-up. Data are displayed as mean±SD or geometric mean (95%CI) at baseline and follow-up for the whole cohort. Within-group differences were determined by paired samples t tests. Between-group differences were determined using independent samples t tests. Results are displayed as the mean difference in values (95%CI) (ANT- anterior, SEP - septum, INF-inferior, LAT - lateral.)*

7.4.3 eGFR subgroup analysis

Analysis of T2 mapping data, stratified based on renal function at follow-up, indicated that for the basal ventricular slice, there was no difference between the groups from baseline to follow-up. Further analysis of longitudinal changes for each group failed to demonstrate any meaningful trends within the data (**Figure 7.27).**

The same analysis conducted for the mid-ventricular slice again showed that there were no significant changes observed between the two groups for any of the segments analysed. Further assessment of within-group changes for both groups again failed to show any evidence of a trend in the two groups (**Figure7.28**)

Figure 7.27 Segmental analysis of basal T2 times (ms) at baseline and follow-up stratified based on eGFR at follow-up.

Segment	N	Baseline	Follow-up	Within group change	P Value	Between group change	P Value
ANT SEP							
<50	11	42.67±2.77	39.81±7.59	-2.86 (-8.75 -3.02)	0.30		
>50	19	41.42±3.78	39.58±2.26	-1.85 (-3.34 - -0.36)	0.02	1.02(-3.48 - 5.51)	0.65
INF SEP			**				
<50	11	41.93±2.17	43.13±3.54	2.12 (-1.42 - 5.66)	0.21		
>50	19	41.16±3.69	39.69±2.25	-0.66 (-1.78 - 0.47)	0.24	-2.66 (-5.88 - 0.55)	0.10
INF							
<50	11	42.64±3.57	43.64±4.21	1.00 (-1.89 - 3.89)	0.46		
>50	19	40.69±3.15	40.62±3.45	-0.07 (-1.78 - 1.65)	0.93	-1.07 (-4.05 - 1.91)	0.47
INF LAT							
<50	11	43.15±2.33	42.45±3.82	-0.70 (-2.67 - 1.26	0.44		
>50	19	40.54±3.65	41.33±3.45	0.79 (-1.12 - 2.70)	0.40	1.49 (-1.33 - 4.30)	0.29
ANT LAT							
<50	11	41.01±3.52	41.62±3.07	0.61 (-1.39 - 2.61)	0.51		
>50	19	40.35±2.71	39.04±2.37	-1.31 (-2.80 - 0.18)	0.08	-1.92 (-4.28 - 0.45)	0.11
ANT							
<50	11	41.88±3.56	43.04±4.59	1.16 (-1.92 - 4.25)	0.42		
>50	19	40.05±3.25	39.27±2.48	-0.78 (-2.09 - 0.52)	0.22	-1.95 (-4.66 - 0.77	0.15
GLOBAL							
<50	11	42.61±3.29	42.49±2.66	-0.13 (-2.39 - 2.14)	0.90		
>50	19	40.19±4.06	40.24±2.47	0.05 (-1.87 - 1.97)	0.96	0.18 (-2.75 - 3.10)	0.90
SEP AVE							
<50	11	42.30±2.24	41.47±5.00	-0.83 (-4.90 - 3.23)	0.66		
>50	19	41.29±3.60	39.63±2.03	-1.66 (-3.24 - -0.08)	0.04	-0.82 (-4.29 - 2.64)	0.63

**

***indicates significant difference between the two groups at either baseline or follow-up. Data are displayed as mean±SD or geometric mean (95%CI) at baseline and follow-up for the whole cohort. Within-group differences were determined by paired samples t tests. Between-group differences were determined using independent samples t tests. Results are displayed as the mean difference in values (95% CI) ANT- anterior, SEP - septum, INF-inferior, LAT - lateral.)*

Figure 7.28 Segmental analysis of basal T2 times (ms) at baseline and follow-up stratified based on eGFR at follow-up.

Segment	N	Baseline	Follow-up	Within group change	P Value	Between group change	P Value
ANT							
<50	11	44.03±2.20	43.38±3.21	-0.65 (-2.37 - 1.07)	0.42		
>50	19	43.13±3.84	41.55±3.67	-1.58 (-3.28 - 0.12)	0.07	0.93 (-1.57 - 3.43)	0.45
INF SEP							
<50	11	42.59±2.44	41.55±1.99	-1.04 (-2.73 -0.65)	0.20		
>50	19	41.96±4.16	39.97±2.19	-1.99 (-3.71 - -0.27)	0.03	0.95 (-1.56 - 3.46)	0.45
INF							
<50	11	42.88±2.33	40.91±2.14	-1.97 (-3.68 - -0.27)	0.03		
>50	19	40.70±4.13	39.66±4.13	-1.04 (-3.47 - 1.40)	0.38	-0.94 (-4.29 - 2.42)	0.57
INF LAT							
<50	11	43.33±3.05	42.00±2.89	-1.33 (-3.47- 0.82)	0.20		
>50	19	41.62±5.01	40.84±3.45	-0.77 (-3.01 - 1.46)	0.48	-0.55 (-3.80 - 2.69)	0.73
ANT LAT							
<50	11	42.48±2.86	38.95±11.85	-3.53 (-11.56 - 4.49)	0.35		
>50	19	40.19±3.72	39.47±2.06	-0.72 (-2.58 - 1.13)	0.42	-2.81(-8.85 - 3.23)	0.35
ANT							
<50	11	44.43±3.82	43.41±4.10	-1.02 (-3.10 - 1.05	0.30		
>50	19	42.03±3.94	40.52±4.33	-1.50 (-4.32 - 1.32	0.28	0.48 (-3.43 - 4.39)	0.80
GLOBAL							
<50	11	43.12±1.71	42.38±1.99	-0.74 (-1.99 - 0.50)	0.21		
>50	19	41.62±3.64	40.55±2.65	-1.07 (-2.59 - 0.44)	0.15	0.33 (-1.80 - 2.47)	0.75
SEP AVE							
<50	11	43.31±1.66	42.46±2.14	-0.85 (-2.21 - 0.52)	0.20		
>50	19	42.54±3.87	40.76±2.74	-1.79 (-3.28 - -0.29)	0.02	0.94 (-1.20 - 3.08)	0.34

*** indicates significant difference between the two groups at either baseline or follow-up. Data are displayed as mean±SD or geometric mean (95%CI) at baseline and follow-up for the whole cohort. Within-group differences were determined by paired samples t tests. Between-group differences were determined using independent samples t tests. Results are displayed as the mean difference in values (95% CI) (ANT- anterior, SEP - septum, INF-inferior, LAT - lateral.)*

7.5 T1 Stability

To assure quality of the mapping sequences used in this study, a QA protocol had been set up using EUROSPIN phantom (ELSE Solutions s.r.l.). The sequences used in the QA protocol have the same imaging parameters, whilst physiological parameters have been set constant to exclude potential small variations between the acquisitions and related map-fitting algorithm. In addition to all the mapping sequences, a set of basic inversion recovery (IR) turbo spin echo (TSE) sequences with 10 different inversion times (TI) were added to monitor potential phantom degradation. A total of 19 QA protocols were acquired during the time of this study, averaging 1 every 2-3 months. 12 independent tubes containing various gel solutions covering were used, which allowed for a large range of physiological (myocardial) T1 values from T1≈200 to 1665 ms to be assessed. The analysis was performed using the maps calculated and reconstructed on the scanner as well as using in-house software developed by the MRI Physics team to produce goodness-of-fit maps (R^2). Individual T1 values were extracted using the same size ROIs and their temporal stability was then assessed. The variation in T1 values for each tube across the entire period of this study was less than 1% at any tested timepoint. This QA was also necessary to assure that the mapping sequences remained the same in case of any potential software upgrades of the scanner used in that period

7.6 Discussion

i. There was a trend towards reduced native T1 times in transplant recipients in all myocardial segment's studies compared to a group of similar controls.

ii. There was no clear pattern of change in native T2 times at the basal level. There was a trend towards reduced T2 values at the mid-ventricular level in transplant recipients when compared to a group of similar controls.

7.6.1 Transplant and Control participants

Native T1 mapping is advocated as a way of characterising changes in the myocardium particularly where reduced renal function contraindicates the use of gadolinium. It is influenced by a number of factors including myocardial water content , lipid and iron deposition and the presence of diffuse interstitial fibrosis (358). It has been validated against invasively derived measures of fibrosis, with Nakamori et al.(189) confirming that histological derived collagen fraction is correlated with native T1 times=0.673, P=0.001). It is therefore an important modality when assessing uraemic cardiomyopathy, with multiple studies confirming elevated native T1 times in CKD (83, 191, 358).

Native T2 mapping is also used to assess the myocardium in uraemic cardiomyopathy, as it is considered as a proxy marker of free myocardial water content and oedema. This notion is supported by the work of Wolter et al. (359) who demonstrated that in myocarditis native T2 mapping was correlated with both plasma volume status, while also being independently correlated with the presence of inflammation. Further work by Arcari et al.(194) also reported that in uraemic cardiomyopathy native T2 times were elevated compared to hypertrophic cardiomyopathy. This difference was attributed to the presence of intramyocardial fluid.

The current data has shown that there were significant reductions observed in T1 mapping times in both the basal and mid ventricular slice. While not all segments analysed produced significant differences there was a trend in all segments toward a reduction in T1 mapping times compared to controls. Conversely assessment of T2 mapping times highlighted there no significant changes. There was also no consistent trend within the data across the two levels of the ventricle to suggest a clear effect of transplantation on T2 mapping times. The current data are in agreement with previous work by Contti et al (192) which indicated that following transplantation there were significant reductions in native T1 with values decreasing from 1331 ± 52 ms to 1298 ± 42 ms ($p < 0.001$) at 6 months. This is currently the only published work which has examined native T1 times following renal transplantation. There are currently no published studies examining changes in native T2 mapping changes after transplantation, comparisons with the current data are therefore not possible.

While native T1 times are regarded as a proxy measure of diffuse fibrosis, there is debate regarding the influence of myocardial water on native T1 values. Kotecha et al. (360) demonstrated that in 25 stable HD patients both native T1 and T2 times reduced significantly following HD supporting the notion that myocardial water content due to fluid overload has a significant influence on T1 times in ESKD. Further work by Graham-Brown et al.(361), however, has suggested that while myocardial water content may influence T1 times it is not the only factor. Comparing 127 subjects receiving HD and 137 health controls indicated that while native T1 times were significantly elevated in ESKD compared to controls, there was no corresponding difference in native T2 times. Due to this pattern of results, it was concluded that elevated T1 mapping times in ESKD were likely to represent the presence of diffuse intestinal myocardial fibrosis rather than myocardial oedema. The current findings of

reductions in native T1 values that were independent of native T2 values suggests that following transplant there was a reduction in the burden of myocardial fibrosis observed.

The current data confirms that there is regression of myocardial fibrosis following transplantation. This is clinically important as the presence of fibrosis, is associated with development of both diastolic and LVSD in a number of conditions. It is also associated with the development of heart failure and sudden cardiac death (84) . This data therefore adds to the current understanding of how renal transplantation improves mortality (362). These findings are also significant for those where transplant is not an option, as they confirm that the cardiovascular remodelling seen in uraemic cardiomyopathy is reversible. This data ,therefore, supports that development of therapies aimed at reversing uraemic cardiomyopathy in order to ameliorate cardiovascular risk.

It was also noteworthy that in control participants there was a tendency for an increase in T1 times in all segments studied in both the basal and mid ventricular levels. This finding is in stark contrasts to recipients and suggests that myocardial fibrosis is an ongoing process that can worsen over a short period of between12-24 months. This further demonstrates the requirement to developing strategies aimed at both preventing and reversing the cardiovascular remodelling seen in CKD.

7.6.2 LVH subgroup analysis

When the data was stratified based on the presence of LVH at baseline there were no significant differences between the groups when examining both native T1 and T2 values. There was a trend towards greater reduction in native T1 times in those with LVH, however this did not consistently reach the level of statistical significance. The same pattern of data was observed in T2 times. This data indicates that while there may be greater reductions in

myocardial fibrosis in those with LVH some of the changes observed in T1 times may be due to reductions in myocardial water content. This finding is in keeping with the previous data which has shown that there were greater reductions in ventricular volumes and levels of NTproBNP in those with LVH at baseline.

7.6.3 eGFR subgroup analysis

There were no differences between the groups in this analysis. There was a consistent trend across all segments for a reduction in native T1 times although the majority of this analysis did not reach the level of change required for statistical significance. There were no clear trends observed in the T2 mapping data. This pattern of data when viewed in conjunction in with the previous findings presented in **chapter 4**, where higher levels of achieved eGFR were associated with greater reductions in LVM suggest that achieved renal function has a direct impact on the presence of fibrosis. This is in keeping with previous data from Hayer et al. (349) who demonstrated that native T1 mapping times increase incrementally from CKD stages 2 – 5. The current findings also suggest that this relationship between eGFR and T1 mapping is bi-directional.

7.7 Limitations

There are several limitations to the data. Due to the nature of data acquisition, only patients who were studied on a 3T CMR were included for analysis. This was necessary, as native T1 times differ substantially between 1.5T and 3T scanners; therefore, direct comparisons between the two are not possible. Additionally, due to the nature of native T1 and T2 mapping, which means that images can be subject to artefacts, particularly breathing artefacts, it was necessary to discard some images due to inaccurate data which could not be reliably analysed. As a result, eighteen transplant recipients and three control participants were excluded from this analysis.

A further limitation was that both T1 and T2 mapping techniques are proxy rather than direct measures of the myocardium. Both measures are influenced by numerous factors, as such, it is possible that the changes observed may not reflect true changes in fibrosis. In order to address this, invasive endomyocardial biopsy would have been required. This is an invasive procedure with potentially life-threatening complications including ventricular perforation. It was therefore not considered an appropriate research methodology.

7.8 Conclusion

The RETRACT study has demonstrated that following renal transplantation, there are significant reductions in native T1 values in the absence of any change in native T2 times. These findings suggest that diffuse interstitial fibrosis is reversible following renal transplantation. These observed changes provide a greater understanding of the improved cardiovascular risk profile observed following renal transplant.

Chapter 8: PREDICTORS OF CARDIOVASCUALR REMODELLING AFTER SUCCESSFUL KIDNEY TRANSPLANT

Abstract

Background

The RETRACT study has demonstrated that several of the features of uraemic cardiomyopathy are reversed following renal transplantation. This is the first study with robust research methodology to demonstrate this using cardiac magnetic resonance imaging (CMR). Understanding the factors that influences these changes is critical to understanding the aetiology of uraemic cardiomyopathy. This understanding is essential to direct ongoing treatment strategies for this condition.

Aim

To understand the main drivers behind changes in the features of uraemic cardiomyopathy observed following renal transplantation

Methods

Recruitment and follow-up were as previously described in **Chapter 3**. Statistical analysis was performed using both univariate and multivariate regression.

Results

When the entire cohort were analysed both transplant group status and changes in systolic blood pressure were significantly correlated with both changes in LVM ($r^2$0.131 P=0.002 and r^2 P=<0.001). Analysis of transplant recipients alone highlighted that achieved eGFR ($r^2$0.145 P=0.005) the presence of baseline LVH ($r^2$0.140 P=0.007) and changes systolic blood pressure ($r^2$0.421 P=<0.001) all correlated significantly with changes in LVM. Changes in native T1 mapping times were only correlated with changes in magnesium ($r^2$0.174 P=0.027). Analysis of the correlation between native T1 and T2 mapping changes indicated that there was no relationship between these parameters.

Conclusion

Transplant status was significantly correlated with changes in LVM which supports the previous data reported in **chapter 3**. In addition, the current data suggests that improvements

in renal function are positively correlated with cardiovascular remodelling following transplantation. These findings supports the argument that renal function directly affects the structure and function of the cardiovascular system.

8.1 Introduction

The features of uraemic cardiomyopathy, and in particular left LVH following renal transplantation are a clinically significant, as they are strong predictors of both adverse cardiovascular events and all-cause mortality (363). As has previously been discussed in, **Chapter 1**, the development of uraemic cardiomyopathy is a multifactorial process (103).

Numerous biochemical and metabolic parameters are corrected following renal transplantation and are associated with cardiovascular remodelling. Anaemia is associated with the development of both coronary microvascular dysfunction and LVH in CKD (105) . It is a strong predicator of mortality in both dialysis and post-transplant cohorts, improvements in haemoglobin have also been shown to significantly correlate with regression of LVH following transplantation (364). Increased PTH levels are associated with the development of CKD mineral bone disease and cardiovascular calcification, also have an established association with the development of LVH and mortality in CKD (137). Following transplantation reduced levels of PTH are also associated with regression of LVH (364). The hormone FGF-23 is also positively correlated with increased LVM and negatively correlates with ejection fraction in those receiving HD (365). In transplant recipients higher levels of FG23 are associated with markers of increased arterial stiffness, however at present the relationship between FGF23 and changes in LVM have not been adequately studied within the literature (366).

Following transplantation reductions in LVM are potentially due to reductions in the burden of diffuse interstitial fibrosis (81). Currently native T1 and T2 mapping are regarded as the optimal imaging strategy for assessing this fibrosis in those where gadolinium administration is contraindicated (177). At present only one small study has examined changes in native T1

mapping after transplantation (192) At present however there is no published data which has sought to examine the relationship between changes in LVM and native T1 mapping values. In addition, there is also currently no published data examining changes in native T2 mapping values. As such the relationship between LVM and native T2 mapping and the relationship between native T1 and T2 values is currently unknown.

It was the aim of the RETRACT study to identify factors which were associated with changes in the main features of uraemic cardiomyopathy.

8.2 Methods

Recruitment and data acquisition was as set out in the chapter 3.

8.3 Aim

The aim of chapter 8 of the Retract study was to examine the drivers of change in the three main features of uraemic cardiomyopathy

i. LVM
ii. Systolic and diastolic function
iii. Measures of Fibrosis

8.4 Statistical analysis

Full detailed description of the RETRACT study is set out in chapter 3.

In this chapter univariate regression was conducted to establish which independent variables were significantly correlated with the measures of the three main features of uraemic cardiomyopathy: LVM, systolic function and markers of fibrosis. In addition, further analysis was conducted which sought to examine the relationship between changes in LVM and markers of fibrosis. The relationship between native T1 and native T2 mapping was also examined. Detailed description of statistical methods is described in **chapter 3**.

8.5 Results

8.5.1 Transplant and control participants

Initial univariate analysis for the entire cohort indicted that, age at baseline, gender, length of follow-up and body surface area at baseline was not significantly correlated with either change LVM or LVMI (P>0.05). Having received a transplant (r^2 0.136 P=0.002) and change in systolic blood pressure (defined as difference between baseline and follow-up value of Bp-tru values) (r^2 0.264 P=<0.001) did however have a significant impact on both LVM and LVMI. (**Figure 8.1** and **8.2**)

The same variables were then used to conduct a multivariable regression model (**Figure 8.3 and 8.4**). This indicated that transplant status continued to have a significant relationship with changes in LVM and LVMI. The remaining variables were not statistically associated with changes in LVM and LVMI.

Figure 8.1 Univariate analysis of LVM whole cohort.

	R	R^2	B coefficient	P-value
Transplant status	0.361	0.13	18.76	0.002
Age at baseline	0.41	0.002	0.74	0.74
Gender	0.01	0	-0.25	0.97
Length of F/U	0.05	0.003	0.01	0.66
BSA at baseline	0.19	0.04	18.75	0.12
Change in systolic BP	0.51	0.26	0.57	<0.001

Figure 8.2 Univariate analysis of LVMI whole cohort.

	R	R^2	B coefficient	P-value
Transplant status	0.36	0.13	11.37	0.002
Age	0.07	0.004	0.72	0.59
Gender	0.004	<0.001	0.11	0.97
Length of F/U	0.61	0.004	0.01	0.62
BSA at baseline	0.25	0.61	14.96	0.04
Change in systolic BP	0.47	0.23	0.32	<0.001

Figure 8.3 Multivariate analysis of the whole cohort for LVM.

	Beta	95% CI	P-value
Tx status	19.20	8.77 – 29.64	<0.001
Age	-0.05	-0.41 – 0.31	0.78
Gender	0.77	-8.98 – 10.53	0.88
F/U time	-0.01	-0.05 – 0.031	0.72
BSA	11.55	-8.70 – 31.80	0.26
Change in systolic BP	0.58	0.37 – 0.80	<0.001

R 0.648 R2 0.420

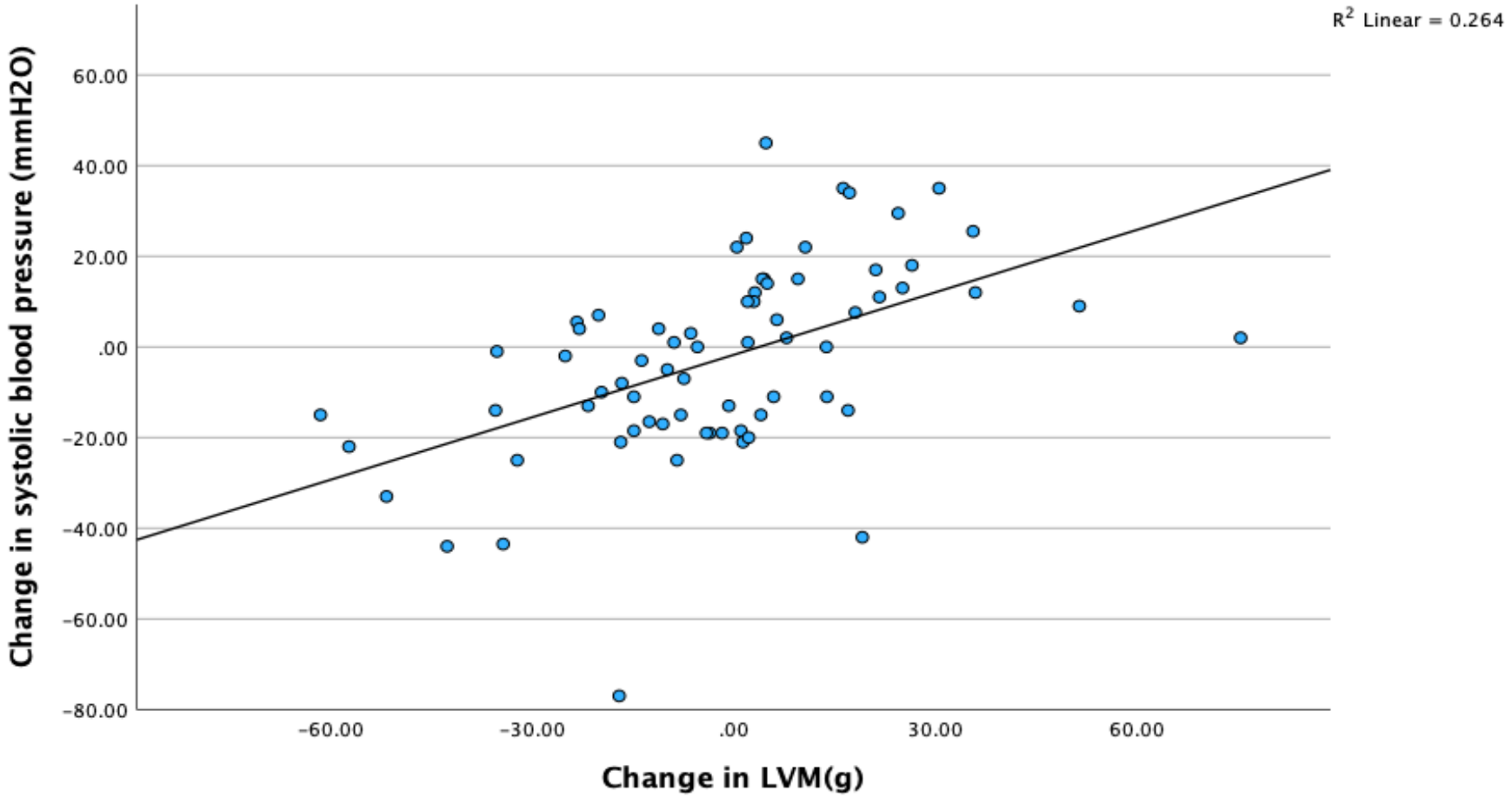
Figure 8.4 Multivariate analysis of the whole cohort for LVMI

	Beta	95% CI	P-value
Tx status	11.13	4.66 – 17.60	0.001
Age	-0.01	-0.23 – 0.22	0.97
Gender	-0.03	-6.07 – 6.02	0.99
F/U time	-0.003	-0.03 – 0.021	-0.03
BSA	11.11	-1.44 – 23.66	-1.44
Change in systolic BP	0.32	0.19 – 0.46	0.19

R 0.630 R2 0.397

(BP blood pressure, BSA body surface area, F/U follow-up, Tx transplant)

Figure 8.5 Correlation between change in LVM and systolic blood pressure.



(LVM, left ventricular mass)

8.6 Transplant recipients

Following assessment of the entire cohort, it was then necessary to establish the relationship between the main primary features of uraemic cardiomyopathy and changes in parameters that were observed with successful renal transplantation. These analyses were therefore performed in transplant recipients only.

8.6.1 Left ventricular mass

LVM and LVMI were the primary outcome measures of the RETRACT study. Univariate analysis was therefore performed to establish the relationship between changes in LVM and LVMI and the other parameters that were studied. This indicated that changes in systolic, diastolic blood pressure, eGFR, BNP, presence of LVH at baseline and creatinine were all significantly correlated with changes in LVM. The same relationships were also observed when the dependent variable for analysis was LVMI (**Figure 8.6 and 8.9**). The relationship between change in systolic blood pressure and LVM is presented in **Figure 8.8.**

Multivariable analysis was also conducted. In addition to those factors which were found to have a significant association with changes in LVM in univariate analysis, age, gender, and length of follow-up were also included in the analysis (**Figure 8.7**). This highlighted, that changes in systolic blood pressure, attained eGFR, BNP, presence of LVH at baseline and length of follow-up were all significant predictors of change in LVM. This same analysis conducted with LVMI as the dependent variable produced the same relationships except here length of follow-up was not a significant predictor of change in LVMI **(Figure 8.10**)

Figure 8.6 Univariate analysis of LVM for the transplant group only.

Variable	R	R^2	B coefficient	P Value
Age	0.03	0.00	-0.06	0.82
Gender	0.15	0.02	-7.03	0.29
Length of F/U	0.16	0.03	-0.03	0.27
BSA	0.17	0.03	15.85	0.23
eGFR> 50 ml/min/1.73 m^2 at F/U	0.18	0.03	-8.41	0.21
Presence of LVH at baseline	**0.37**	**0.14**	**-19.54**	**0.007**
Δ Systolic BP	**0.65**	**0.42**	**0.73**	**<0.001**
Δ Diastolic BP	**0.40**	**0.16**	**0.65**	**0.00**
Δ Hb	0.24	0.06	-0.26	0.89
Δ eGFR	**0.41**	**0.16**	**-0.59**	**0.00**
Δ α-Klotho	0.15	0.02	-0.01	0.46
Δ FGF23	0.25	0.06	0.00	0.18
Δ HS TROP	0.06	0.00	-0.13	0.69
Δ BNP	**0.57**	**0.32**	**0.003**	**<0.001**
Δ PTH	0.08	0.01	0.07	0.60
Δ Calcium	0.15	0.02	-9.60	0.31
Δ Creatinine	0.30	0.09	0.03	0.03
Δ Phosphate	0.09	0.01	4.66	0.57
Δ Magnesium	0.05	0.00	-0.92	0.73
Δ Vitamin D	0.03	0.00	-0.03	0.87
Δ Cholesterol	0.20	0.04	-4.41	0.17
Δ Urea	0.04	0.00	-0.03	0.80
Δ Sodium	0.07	0.01	-0.29	0.64
Δ Potassium	0.03	0.00	1.10	0.83
Attained values at F/U				
Systolic BP	0.14	0.02	0.20	0.33
Diastolic BP	0.09	0.01	0.19	0.56
Hb	0.02	0.00	-0.02	0.91
eGFR	**0.38**	**0.14**	**-0.55**	**0.01**
α-Klotho	0.05	0.00	0.00	0.80
FGF23	0.15	0.02	0.02	0.41
HS TROP	0.06	0.00	-0.11	0.70
NTproBNP	0.04	0.00	0.03	0.79
Parathyroid Hormone	0.10	0.01	0.10	0.50
Calcium	0.13	0.16	-9.50	0.38
Creatinine	**0.44**	**0.20**	**0.24**	**0.00**
Phosphate	0.09	0.01	-9.25	0.53
Magnesium	0.03	0.00	9.78	0.82
Vitamin D	0.16	0.03	-0.13	0.35
Cholesterol	0.00	0.02	0.64	0.88
Urea	0.14	0.02	0.27	0.33
Sodium	0.12	0.02	1.08	0.39
Potassium	0.15	0.02	-8.46	0.29

Figure 8.7 Multivariate analysis showing relationship between LVM and parameters found to have a significant correlation with LVM in univariate analysis for the transplant group only.

	Beta	95% CI	P-value
Gender	-2.89	-11.55 – 5.76	0.50
Age	0.06	-0.024 – 0.35	0.69
Length of Follow-up	-0.04	-0.07 - -0.01	0.02
Δ Systolic BP	0.45	0.25 – 0.66	<0.001
Δ NTproBNP	0.002	0.001 – 0.003	<0.001
Attained eGFR	-0.37	-0.63 - -0.11	0.01
LVH at baseline	-10.41	-20.81 - -0.01	0.05

*** R 0.862 R square 0.743**
(BSA body surface area, F/U follow-up)

Figure 8.8 Correlation between change in LVM and systolic blood pressure.

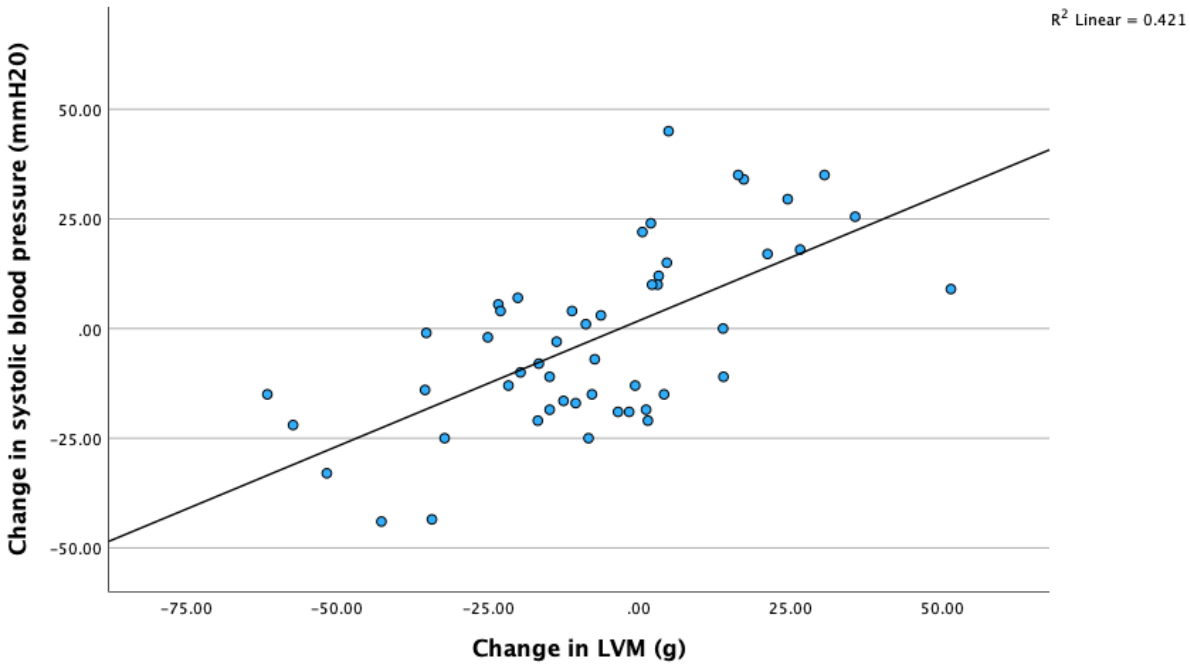


Figure 8.9 Univariate analysis of LVMI for the transplant group.

	R	R2	B coefficient	P-value
Age	0.000	0.003	-0.003	0.986
Gender	0.137	0.019	-3.790	0.344
Length of F/U	0.120	0.015	-0.015	0.405
LVH at baseline	**0.374**	**0.140**	**-19.504**	**0.007**
eGFR> 50 ml/min/1.73 m2 at F/U	0.219	0.048	-0.064	0.127
Δ Systolic BP	**0.619**	**0.383**	**0.419**	**<0.001**
ΔDiastolic BP	**0.372**	**0.139**	**0.360**	**0.008**
ΔHb	0.232	0.054	-0.147	0.105
Δ eGFR	**0.418**	**0.175**	**-0.0365**	**0.003**
Δ α-Klotho	0.028	0.001	0.000	0.886
Δ FGF23	0.317	0.101	0.001	0.082
Δ HS TROP	0.061	0.004	-0.073	0.0703
ΔBNP	**0.595**	**0.354**	**0.002**	**<0.001**
ΔPTH	0.089	0.008	0.046	0.554
Δ Calcium	0.139	0.019	-5.442	0.337
Δ Creatinine	**0.277**	**0.077**	**0.014**	**0.050**
ΔPhosphate	0.099	0.010	3.254	0.503
Δ Magnesium	0.054	0.003	-0.571	0.721
ΔVitamin D	0.068	0.005	-0.036	0.710
Δ Cholesterol	0.222	0.049	-2.958	0.125
ΔUrea	0.042	0.002	0.020	0.773
Δ Sodium	0.90	0.008	-0.230	0.534
Δ Potassium	0.038	0.001	0.774	0.797
Attained values at F/U				
Systolic BP	0.098	0.010	0.082	0.500
Diastolic BP	0.038	0.001	0.051	0.792
Hb	0012	0.000	-0.011	0.932
FGF23	0.160	0.026	0.015	0.389
α-Klotho	0.040	0.002	0.001-	0.841
eGFR	**0.392**	**0.145**	**-0.345**	**0.005**
HS TROP	0.073	0.005	-0.080	0.644
BNP	0.095	0.009	0.005	0.532
PTH	0.130	0.017	0.078	0.389
Calcium	0.139	0.019	-6.169	0.337
Creatinine	**0.455**	**0.207**	**0.150**	**<0.001**
Phosphate	0.083	0.007	-4.904	0.575
Magnesium	0.136	0.019	23.668	0.361
Vitamin D	0.194	0.038	-0.094	0.264
Cholesterol	0.03	0.000	-0.51	0.984
Urea	0.209	0.044	0.244	0.145
NA	0.110	0.012	0.569	0.449
K	0.184	0.034	-6.073	0.206

Figure 8.10 Multivariate analysis showing relationship between LVMI, and parameters found to have a significant correlation with LVMI in univariate analysis for the transplant group only.

	Beta	95% CI	P-value
Age	0.07	-0.11 – 0.25	0.42
Gender	-1.18	-6.46 – 4.10	0.065
Length of Follow-up	-0.02	-0.04 – 0.001	0.061
Δ in Systolic BP	0.23	0.11 – 0.37	<0.001
Δ NTproBNP	0.001	0.001 – 0.002	<0.001
Attained eGFR	-0.24	-0.40 - -0.083	0.004
LVH at baseline	-5613	-11.96 – 0.73	0.08

R 0.857 R square 0.735

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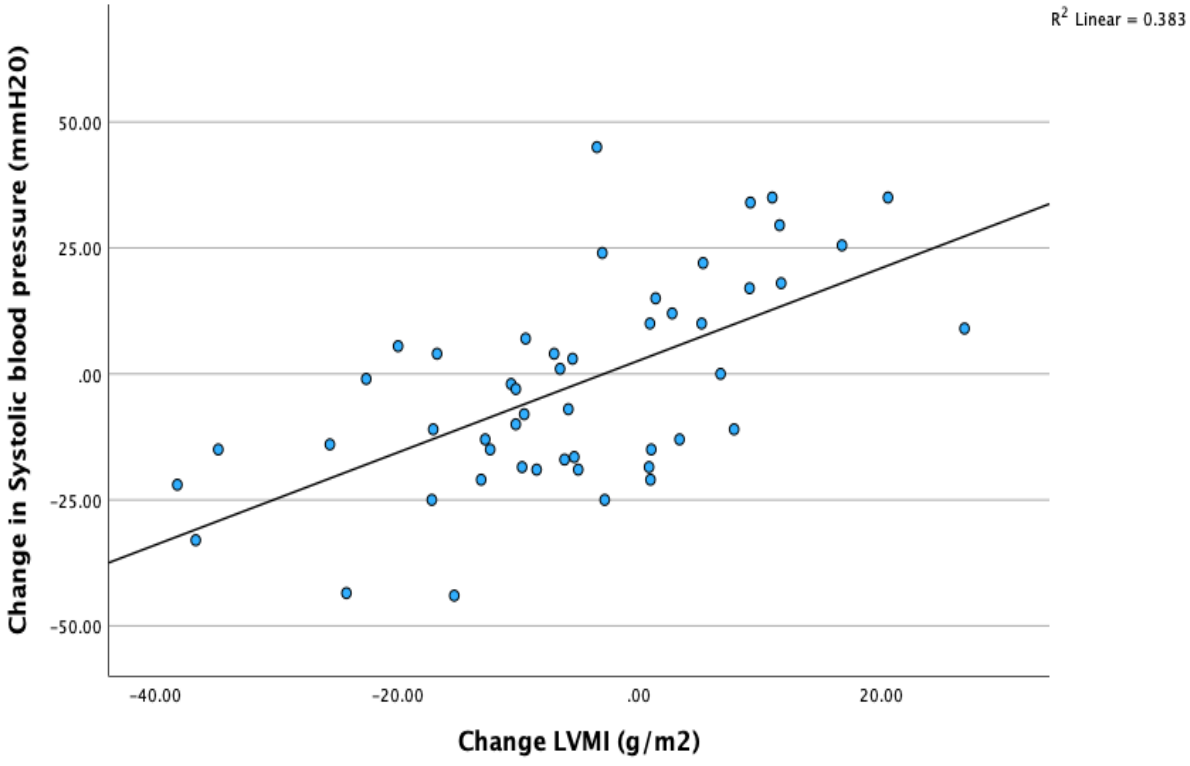
Figure 8.11 Correlation between change in LVMI and systolic blood pressure.



8.6.2 Systolic and diastolic function

The RETRACT study also examined both systolic and diastolic function. Analysis presented in, **chapter 5**, indicated that following transplantation none of the parameters studied changed significantly. Regression analysis was therefore not conducted.

8.6.3 Myocardial fibrosis

When examining the changes observed in native T1 mapping values which are presented in **Chapter 7**, there were clear trends in all the data towards reductions in native T1 values. Significant differences between transplant and controls were observed in the global native T1 times for the mid-ventricular level and this was considered the most significant finding. This parameter was therefore used to examine the relationship of native T1 mapping changes with other parameters studied.

Initial univariate analysis for the entire cohort indicted that, age at baseline, gender, length of follow-up and body surface area at baseline was not significantly correlated with T1 changes ($P>0.05$). Having received a transplant (r^2 0.128 $P=0.013$) and change systolic blood pressure did however have a significant impact on changes T1 times (**Figure 8.12**)

The same variables were then used to conduct a multivariable regression model (**Figure 8.12 and 8.13**). This indicated that transplant status continued to have a significant relationship with changes in T1 times. The remaining variables were not statistically associated with native T1 time changes.

Figure 8.12Univariate analysis of native T1 mapping for the whole cohort.

	R	R^2	B coefficient	P-value
Transplant status	0.36	0.13	34.37	0.01
Age at baseline	0.09	0.007	-0.29	0.56
Gender	0.05	0.002	4.52	0.75
Length of F/U	0.08	0.006	0.03	0.59
BSA at baseline	0.12	0.01	23.35	0.42
Δ Systolic BP	0.19	0.03	0.40	0.21

Figure 8.13 Multivariate analysis of the whole cohort for LVM.

	Beta	95% CI	P-value
Transplant status	37.83	9.20 – 66.46	0.01
Age	-0.60	-1.61 – 0.41	0.24
Gender	7.49	-21.37 – 36.35	0.60
F/U time	0.02	-0.09 – 0.12	0.77
BSA	3.52	-55.04 – 62.09	0.90
Δ Systolic BP	0.42	-1.61 – 0.41	0.18

R0.448 r0.201 P=0.142

(BP blood pressure, BSA body surface area, F/U follow-up, Tx transplant)

8.7 Native T1 mapping in transplant recipients

The relationship between native T1 mapping changes with the other parameters studied was then examined in transplant recipients only. Univariate analysis indicated that only changes in magnesium and phosphate levels were correlated with changes in global average native T1 time in the mid ventricular slice.

Due to the small numbers of variables which were significantly correlated with native T1 mapping in univariate analysis, a multivariable regression model was designed based on both variables positively correlated in univariate analysis and those which were considered clinically significant. Analysis is presented in **Figure 8.15** and indicates that only change in magnesium levels post transplantation was found to be a significant within the model.

Figure 8.14 Univariate analysis of global native T1 times for the mid-ventricular level

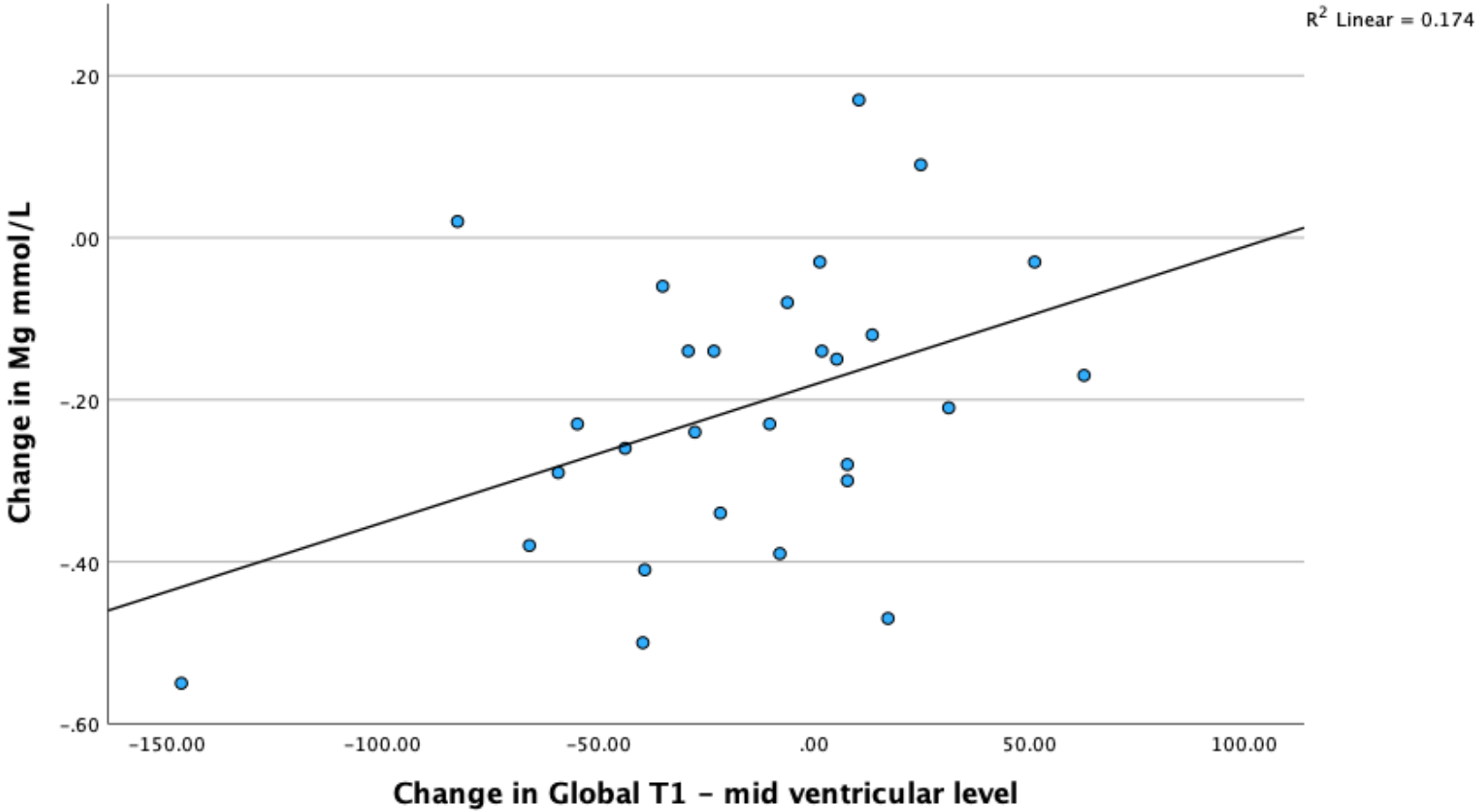
	R	R2	B coefficient	P-value
Age	0.21	0.04	-0.65	0.26
Gender	0.11	0.01	-9.18	0.57
Length of F/U	0.17	0.03	0..060	0.37
LVH at baseline	0.03	<0.001	-0.21	0.87
eGFR> 50 ml/min/1.73 m2 at F/U	0.01	<0.001	-0.94	0.96
Δ Systolic BP	0.29	0.09	0.64	0.11
Δ Diastolic BP	0.22	0.05	0.75	0.24
Δ Hb	0.15	0.02	-0.28	0.42
Δ eGFR	0.19	0.36	-0.19	0.31
Δ α-Klotho	0.20	0.04	-0.01	0.25
Δ FGF23	0.14	0.02	0.00	0.47
Δ HS TROP	0.18	0.03	1.25	0.37
Δ BNP	0.22	0.05	0.00	0.24
Δ PTH	0.03	0.00	0.40	0.90
Δ Calcium	0.16	0.03	-37.55	0.39
Δ Creatinine	0.25	0.06	0.04	0.18
Δ Phosphate	0.06	0.00	7.21	0.75
Δ Magnesium	**0.42**	**0.17**	**102.32**	**0.03**
Δ Vitamin D	0.07	0.01	-0.11	0.73
Δ Cholesterol	0.10	0.01	4.03	0.59
Δ Urea	0.06	0.00	-0.72	0.75
Δ Sodium	0.03	0.00	0.40	0.86
Δ Potassium	**0.50**	**0.25**	**29.11**	**0.004**
Attained values at F/U				
Systolic BP	0.09	0.01	-0.27	0.62
Diastolic BP	0.22	0.05	0.75	0.24
Hb	0.18	0.03	-0.51	0.34
FGF23	0.15	0.02	-0.41	0.41
α-Klotho	0.13	0.02	0.04	0.51
eGFR	0.07	0.01	0.00	0.73
HS TROP	0.01	0.00	0.13	0.97
BNP	0.01	0.00	0.00	0.96
PTH	0.03	0.00	-0.05	0.87
Calcium	0.07	0.01	-31.76	0.71
Creatinine	0.24	0.06	0.23	0.19
Phosphate	0.01	0.00	3.07	0.95
Magnesium	0.22	0.05	131.00	0.26
Vitamin D	0.15	0.02	0.24	0.45
Cholesterol	0.29	0.08	16.69	0.12
Urea	0.22	0.05	0.63	0.24
NA	0.07	0.01	1.06	0.71
K	0.19	0.03	17.50	0.32

Figure 8.15 Multivariate analysis showing relationship between global native T1 times for the mid-ventricular level as the dependent variable.

	Beta	95%CI	P value
Gender	-12.23	-54.40-29.93	0.55
Age	-0.37	-1.66 -0.92	0.55
length of follow-up	0.05	-0.09 - 0.18	0.47
Δ BNP	<0.001	-0.004 - 0.004	0.93
Δ eGFR	-0.15	-1.35 - 1.05	0.80
Change systolic BP	0.77	-0.27 - 1.82	0.14
LVH at baseline	-9.80	-65.50 - 45.89	0.72
Δ Magnesium	118.42	1.46 - 235.38	0.05

R0.637 R2 0.406

Figure 8.16 Correlation between change in serum magnesium and change in global native T1 time for the mid ventricular slice.



8.7.1 Correlation between changes in Left ventricular mass and native T1 and T2 mapping

Univariate analysis was also conducted to examine the relationship between changes in LVM and LVMI and markers of diffuse myocardial fibrosis in transplant recipients (native T1 mapping time). Here LVM/LVMI were considered the dependent variable and native T1/T2 mapping times were considered the independent variable.

This indicated that there were significant correlations observed between LVM and global average native T1 values at the basal and mid ventricular levels. There was also a significant correlation observed between LVM and septal average native T2 values at the basal level (**Figure 8.17**). This relationship remained significant in multivariant analysis (**Figure 8.18**)

Analysis of the same parameters where LVMI was the dependent variable indicated that global native T1 times at the basal level, was significantly correlated with LVMI. In addition, septal average T2 times at both the basal and mid-levels were significantly associated with changes in LVMI (**Figure 8.19**) This relationship remained significant in multivariant analysis (**Figure 8.18**)

Figure 8.17 Univariate analysis of native T1 and T2 times with change in LVM s the dependant variable in transplant recipients.

	R	R2	B coefficient	P Value
Δ Basal T1 Global value	0.46	0.21	0.22	0.01
Δ Basal T1 Septal average	0.35	0.12	0.20	0.05
Δ Mid T1 Global value	0.41	0.17	0.09	0.02
Δ Mid T1 Septal average	0.30	0.09	0.15	0.10
Δ Basal T2 Global value	0.22	0.05	1.36	0.25
Δ Basal T2 Septal average	0.15	0.02	-0.59	0.56
Δ Mid T2 Global value	0.21	0.04	1.77	0.09
Δ Mid T2 Septal average	0.56	0.32	5.17	0.001

Figure 8.18 Multivariate analysis showing relationship between LVM and change in global T1 value in mid ventricular slice.

	Beta	CI	P Value
Age	-0.02	-0.64 – 0.61	0.96
Δ Mid T1 Global value	0.23	0.03 – 0.43	0.03
Follow-up	-0.03	-0.10 – 0.04	0.10
Gender	-3.34	-20.61 – 13.93	0.69

R 447 R2 0.200

Figure 8.19 Univariate analysis of native T1 and T2 times with change in LVMI as the dependant variable.

	R	R2	B coefficient	P value
Δ Basal T1 Global value	0.40	0.16	0.12	0.03
Δ Basal T1 Septal average	0.29	0.08	0.06	0.11
Δ Mid T1 Global value	0.30	0.09	0.10	0.10
Δ Mid T1 Septal average	0.21	0.04	0.06	0.27
Δ Basal T2 Global value	0.17	0.03	0.64	0.37
Δ Basal T2 Septal average	0.36	0.13	1.17	0.05
Δ Mid T2 Global value	0.19	0.04	1.01	0.31
Δ Mid T2 Septal average	0.36	0.13	1.87	0.05

Figure 8.20 Multivariate analysis showing relationship between LVM and change in global T1 value in mid ventricular slice.

	Beta	95% CI	P value
Age	-0.04	-0.43 – 0.35	0.84
ΔMid T1 Global value	0.10	-0.03 – 0.22	0.14
Follow-up	-0.01	-0.06 – 0.04	0.66
Gender	-0.95	-11.82 – 9.92	0.86

R0.316 R20.100

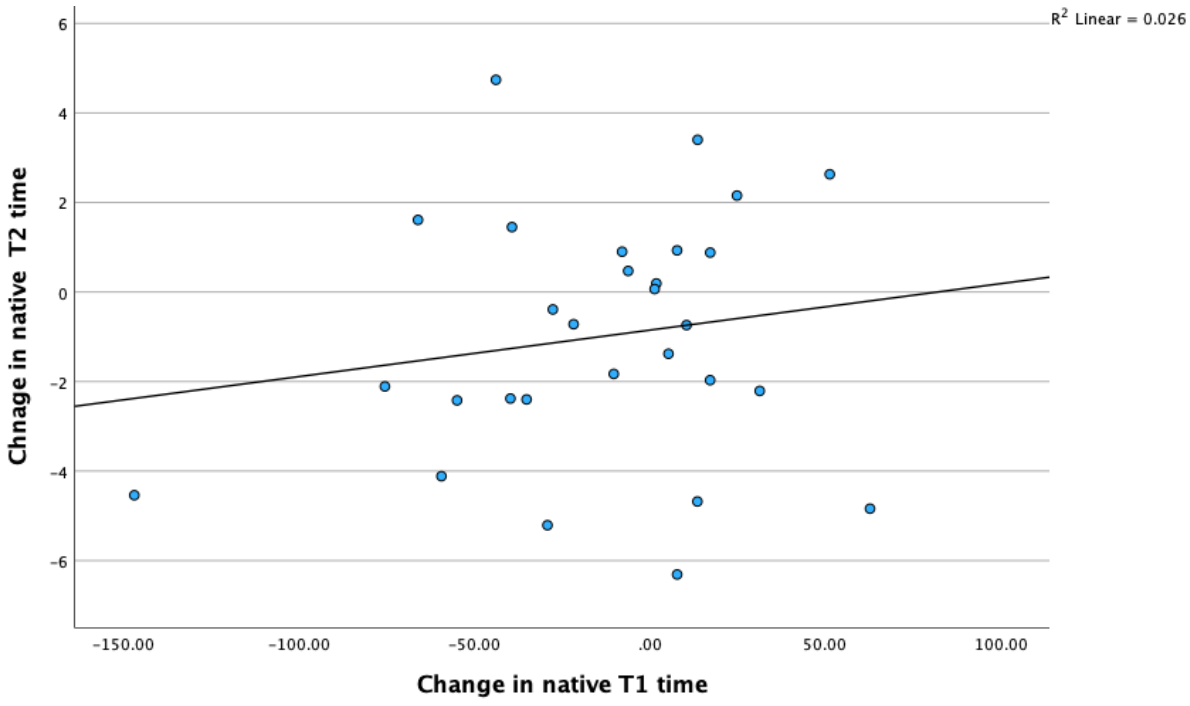
8.7.2 Correlations between changes in native T1 and T2 in transplant recipients

Univariate analysis was also conducted in order to examine the correlation between and native T1 and T2 times (**Figure 8.22)**. Here T1 times were adopted as the independent variable and the corresponding native T2 data was used as the dependent variable This analysis indicated that there was no correlation between the changes observed in T1 and T2 mapping times. As a result, no further, multivariate analysis was conducted.

Figure 8.21 Univariate analysis of correlations between native T1 and T2 mapping times.

	R	R2	B coefficient	P Value
Δ Basal-Septal average	0.16	0.03	1.27	0.41
Δ Basal Global average	0.18	0.03	-2.03	0.35
Δ Mid Septal average	0.10	0.00	-0.19	0.96
Δ Mid Global average	0.16	0.03	3.55	0.40

Figure 8.22 Correlation between native T1 and T2 mapping times for global average values at the mid ventricular level.



8.8 Discussion

8.8.1 Transplant and Control participants

This analysis of the RETRACT study has sought to identify potential drivers behind the changes observed in the cardiovascular system following renal transplantation. Initial analysis which involved the entire cohort has indicated that of the variables studied only transplant status and change in arterial systolic blood pressure were significantly correlated with changes in LVM and LVMI. These relationships remained clinically significant for both univariate and multivariate analysis. This finding reflects the previous data presented in **Chapter 3** which has shown significant reductions in both LVMI and LVM in transplant recipients compared to control participants. The current data supports the assertion that following renal transplantation there is cardiovascular remodelling which remains significant even when corrected for demographic factors. It was also a significant finding that in multivariate analysis the relationship between LVM and transplant status remained significant even when systolic blood pressure was included in the model. Such findings suggest that the changes produced by improved renal function following transplantation are not solely produced by change in the loading conditions of the ventricle. The notion that uraemic cardiomyopathy is not an entirely blood pressure dependant phenomena are innkeeping with previous work by Moody et al.(93) which demonstrated that in an early model of CKD reductions in renal function produced adverse cardiovascular remodelling that was independent of changes in blood pressure.

As previously stated, multivariable analysis indicated that there was a correlation between change in blood pressure and LVM. This data has previously been discussed in **Chapter 4**. It must also be acknowledged that the relationship between arterial blood pressure and cardiovascular remodelling in uraemic cardiomyopathy is difficult. In CKD stage 5, 85% of patients are reported as having clinically defined arterial hypertension (367). Following transplantation blood pressure is inversely related to achieved renal function as such a large

proportion of transplant recipients benefit from improved blood pressure control. Due to this relationship establishing the relative contribution of arterial blood pressure versus changes in the metabolic state is fraught with difficulty. The relationship between blood pressure and the presence of LVH in other conditions, however, indicates that undoubtably this factor partially contributes to the changes observed. Yun et al. studied 984 participants over a period of 10-year follow-up demonstrated that the presence of hypertension was significantly associated with the development of LVH. Furthermore Ahmed et al.(368) have demonstrated that this relationship is bidirectional with improved blood pressure control being related to regression of LVH.

Reassuringly the data presented for the entire cohort also indicated that the length of follow-up time did not have a significant effect on LVM or LVMI. The changes in follow-up which are discussed are in **Chapter 3**, were necessary due to the restrictions placed on clinical research by the COVID-19 pandemic. As such it can be concluded that while there were differences in follow-up times observed the data collected remains robust.

8.8.2 Left ventricular mass

Further analysis was then conducted in which only transplant recipients were studied to establish the drivers of changes in the three main features of uraemic cardiomyopathy in this group. In terms of LVM and LVMI, achieved eGFR, changes in systolic and diastolic blood pressure, and changes in BNP levels were all significantly correlated under univariate analysis and remained so when multivariable analysis was conducted.

The finding that higher levels of eGFR after transplant were negatively correlated with changes in LVM and LVMI, suggests that restoration of renal function and the magnitude of this improvement is contributing to post-transplant cardiovascular remodelling. The notion that

LVM declines as stage of CKD worsens has previously been suggested by our research group (349). At present however this relationship has not been demonstrated following renal transplant within the literature. As such the RETRACT study is the first to confirm that improved renal function significantly correlates with cardiovascular remodelling even when corrected for blood pressure changes.

The presence of LVH at baseline was also shown to significantly correlate with change in LVM. This finding reflects the results previously presented in **Chapter 4.** This data revealed greater reductions in LVM/LVMI in those with LVH at baseline compared to those without. It is acknowledged that this type of observation may be related to the statistical phenomena of regression to the mean. The current data, however, does indicate renal transplantation confers the most benefit in those participants the with most severe uraemic cardiomyopathy phenotype. The association between pre-transplant LVM and subsequent changes following transplant has also been demonstrated previously by Prasad et al.(220). In this work however, the reductions in LVM that were observed these were not statically significant due to inadequate statistical power. The current data which is more methodologically more robust and confirms this association.

BNP levels were also shown to be significantly correlated with changes in LVM. Slubowska et al. (369) previously demonstrated that BNP levels are significantly associated with LVM. Wei et al. (370) have also reported that following successful renal transplant BNP levels reduce significantly. The current results therefore reflect previously published data. This finding was considered unsurprising as BNP is recognised as a strong surrogate marker of fluid status and ventricular pre-load both of which are involved in the development of LVH in ESKD(103).

This finding is consistent with the findings presented in, **Chapter 5**, which demonstrated significant reductions in ventricular volumes after transplant.

The relationship between blood pressure and changes in LVM continued to be statistically significant when examined in transplant recipients only as discussed in the previous section. It is, however, noteworthy that the significant correlation between eGFR and LVM remained even when corrected for systolic blood pressure.

In addition to those parameters which demonstrated significant correlations, it was also noteworthy that several parameters which have been strongly implicated in the development of uraemic cardiomyopathy did not demonstrate a relationship with changes in LVM. There was no association observed between makers of CKD-MBD including FgF23 and α-Klotho and changes in LVM or LVMI. This was an unexpected finding given that makers of CKD-MBD were shown in to change significantly in **Chapters 7** and **8**. It should, however, be noted that the study was not powered to detect these types of association as such it is possible that the lack of statical significance was due to a type 2 error.

8.8.3 Systolic and Diastolic Function.

As was previously discussed there were no significant reductions in parameters of either systolic or diastolic function as such regression analysis was not performed.

8.8.4 Myocardial Fibrosis

As has been previously stated in **section 8.4.5** the global average values of the mid ventricular slice were used as the dependent variables for this analysis. Analysis indicated that there weas a significant association between this dependent variable and changes in magnesium levels only. This relationship remained significant during multivariate analysis.

The clinical significance of this correlation is uncertain as the r^2 obtained was low suggesting that only a small amount of the variation in LVM was explained by changes in magnesium. It is also surprising that increasing levels of magnesium were associated with increasing native T1 times. In the literature hypomagnesaemia is associated with several cardiac pathologies including endomyocardial fibrosis however, the reverse is not true of increased magnesium levels (371).In addition it is also noteworthy that magnesium levels are a part of the axis of CKD-MBD which is common in ESKD. However, in the current analysis other parameters such as PTH and FGF23 were not found to be significantly correlated with changes in T1 mapping values. This lack of a consistent relationship between markers of CKD-MBD and native T1 changes further calls into question the clinical significance of this observed relationship. It is also important to appreciate that that following renal transplantation patients are intensively followed-up by renal physicians. Electrolyte imbalances such as hypomagnesaemia which are common following transplant related to the use of both tacrolimus and proton pump inhibitors. Such imbalances are treated promptly which can obviously make the interpretation of this data difficult.

8.8.5 Left ventricular mass and native T1 and T2 mapping

A major component of the LVH observed in uraemic cardiomyopathy is often attributed to the development of diffuse interstitial myocardial fibrosis (177)**.** Due to this the relationship between LVM and LVMI and native T1 and T2 mappings was assessed. The current data indicated that there were statically significant positive correlations between change in LVM and LVMI and global T1 values. While the correlations were relatively weak these findings do support the notion that myocardial fibrosis following renal transplantation is reversible. This finding is in keeping with previous work from Mall et al. (81) who suggested that following transplantation the development of diffuse fibrosis is partially reversible. It is however important to recognise that these relationships were not consistent across all the

parameters examined, with no correlation between septal average values and native T1 mapping. The significance of this is unclear. It may however represent the lack of statistical power in this analysis, as the RETRACT study was not powered for changes in native mapping values.

The relationship between T2 values and changes in LVM and LVMI was again not straightforward. In the current analysis both basal and mid septal average values for Native T2 mapping were significantly correlated with changes in both LVM and LVMI. There was also a significant correlation between LVMI and basal septal average values. The other measures of T2 however did not show significant corelations. It is not possible to compare these findings with previous literature as at present there have been no studies which have published on native T2 mapping following renal transplantation.

8.8.6 Native T1 and T2 mapping.

The relationship between native T1 and T2 times was also assessed. (84). The data here has indicated that there was no correlation between changes in native T1 and T2 mapping times. This relationship is important as it suggests that the changes observed in these parameters are independent of each other.

The data presented in **Chapter 7** which suggested that while here were no significant changes in T2 mapping values, there were significant changes in T1 mapping for global average values at the mid ventricular levels. The findings, therefore, suggest that these changes in T1 values are independent of changes in T2 values. This conclusion is also supported by the previous section which has shown that there were significant correlations between LVM and global T1 values at the mid ventricular slice, however no correlation for T2 mapping was observed. Findings such as these, where changes in native T1 mapping are independent of native T2

times are indicative of a reduction in levels of myocardial fibrosis rather than myocardial water content (84).

8.9 Limitations

The current work suffers from several limitations. First as has previously been discussed not all participants underwent native T1 mapping conducted on a 3T CMR and as such were not included in this analysis. Reductions in sample size therefore leave the data more susceptible to type 2 statistical errors. Further to this it must also be noted that the current data was based on power calculations which were designed to detect changes in LVM which again raises the possibility of type 2 statical error.

Several of the parameters here are subject to changes other than renal transplant. Following the restoration of renal function there are numerous metabolic and biochemical abnormalities that are commonly described within the literature. Due to the regular medical follow-up this group of patients receive patients any defragments in biochemical properties are corrected pharmacologically. As such some of the relationships observed here may not reflect a physiological response to transplantation.

8.10 Conclusion

This current data has shown that renal transplantation is significantly correlated with changes in LVM and LVMI for the whole cohort. This data has also indicated that in transplant recipients, eGFR. and changes in systolic in blood pressure are independently correlated with changes in LVM. The current data has also indicated that there were significant correlations between LVM and changes in T1 mapping times. These findings support the conclusions made in the previous chapters that both LVM and associated myocardial fibrosis are reversible following renal transplant.

Chapter 9: CONCLUSIONS AND FUTURE DIRECTIONS

9.1 Summary of Key findings

The RETRACT study sought to establish if the cardiovascular remodelling associated with uraemic cardiomyopathy is reversed following renal transplant.

Chapter 2, established via systematic review and meta-analysis that the current evidence base examining cardiovascular changes after renal transplant is inadequate. This review indicated that at present the published literature mainly consists of uncontrolled echocardiographic studies, with only two controlled studies reporting CMR data. Overall, the methodological quality of studies available was poor, as such, it was concluded that there is currently insufficient evidence to fully understand how LVM, and indeed other features of cardiomyopathy change after transplant. This lack of evidence was the rationale for undertaking the RETRACT study which aimed to address these issues.

Chapter 4 presented data for volumetric and mass analysis. This reported that the restoration of renal function following successful renal transplant was associated with decreased LVM and LVMI. Reduction in left ventricular volumes were also observed in the transplant group. It also indicated that reductions in LVM/LVMI were greatest in those where LVH was present at baseline. Higher levels of renal function (eGFR>50 ml/min/1.73 m^2) following successful transplantation were also associated with greater reductions in LVM and LVMI. There were no significant differences in blood pressure changes between the two groups. These data indicate that uraemic cardiomyopathy is reversible following renal transplant, and that the changes observed are not totally dependent on changes in blood pressure. In addition, they also suggest that those with the most advanced features of this uraemic cardiomyopathy, and those who achieve higher levels of renal function at follow-up benefit the most from transplantation.

Chapter 5 presented data for systolic and diastolic function. This indicated that there were no changes observed in LVEF for transplant recipients compared to control participants. There were also no significant differences noted in novel markers of left ventricular systolic function (contraction fraction or LVGFI). Both diastolic and systolic strain analysis also failed to show any significant differences between the groups. While this analysis failed to show any significant change in systolic or diastolic function it was acknowledged that the cohort studied had normal systolic and diastolic function at baseline, as those with advanced features of cardiac failure would not be suitable for transplantation. The current data can therefore not be used to support transplant as an intervention which will improve either systolic or diastolic function. These findings support the need for further medical interventions which can prevent and treat systolic and diastolic dysfunction in those in whom transplantation is not a viable treatment option,

Chapter 6 examined changes in both biochemical and metabolic parameters. The confirmed that there were significant increases in both renal function and Hb following transplant. Markers of CKD-MBD (Vitamin D, PTH, phosphate and FGF23) also improved following transplant, which mirrored previous findings within the data. NTproBNP also declined significantly following transplant, this was likely due to correction of volume status associated with improved renal function. HS troponin did not change significantly following transplantation, levels of HS troponin prior to transplant, however, were normal. As such it was unsurprising that significant changes were not observed.

Chapter 7 reported changes in in native T1 and T2 mapping. This indicated that there were significant reductions in observed native T1 times following transplantation. It was also noted that while not all segmental analysis was statically significant all analysis suggested a trend

towards reduction in native T1 following transplant. There was no clear pattern of change observed in native T2 mapping times between transplants recipients and controls. Subgroup analysis indicated trends towards greater reduction in Native T1 values in those with LVH at baseline and those who achieved higher renal function at follow-up. These data suggested that following transplantation there was a reduction in the burden of myocardial fibrosis in transplant recipients rather than reductions in myocardial water content. These findings are clinically important and further research powered to detect changes in myocardial fibrosis are required.

Chapter 8 examined the relationships between the different parameters under study. This indicated that both transplant status and change in systolic blood pressure were independently correlated with changes in LVM. Further analysis conducted in transplant recipients only indicated that changes in systolic blood pressure and attained eGFR at follow-up were both predictors of subsequent changes in LVM. This analysis also highlighted that there were significant correlations observed between the changes in LVM and native T1 mapping times suggesting that changes in LVM observed were due to reductions in levels of myocardial fibrosis

9.2 Further Research Consideration

While the current data adds significantly to the evidence base there are several further research considerations which will lead to a greater understanding of cardiovascular remodelling after renal transplant.

9.2.1 Extended length of follow-up

The current data is limited by the short length of follow-up. Establishing the effects of renal transplantation in the longer-term would provide valuable information. Initially this would be achieved by following-up the current cohort at five years following transplantation. This approach is necessary, as it is currently unclear if cardiovascular remodelling takes place rapidly following transplant, or if cardiovascular remodelling is an ongoing process. If this is the case it is possible that the current data may underestimate the positive effects of renal transplantation.

A longer follow-up period would also allow a greater understanding of the natural history of uraemic cardiomyopathy. The current data has suggested that in the controls group there were increases in both LVM and native T1 mapping times from baseline to follow-up. This finding over a short follow-up period suggests that uraemic cardiomyopathy may be a rapidly progressive condition. At present there are several cross-sectional studies examining the prevalence of LVH in ESKD, there is however a paucity of longitudinal data using CMR data. This is therefore a research gap that should be addressed.

9.2.2 Mortality and cardiovascular remodelling

Within the previous chapters it has been acknowledged that it was not possible to collect data related to mortality. A longer follow-up would enable a greater understanding of how changes in cardiovascular structure are associated with subsequent mortality. This would allow the validity of LVM as a proxy maker of mortality to be better understood. This is required as at present there is conflicting evidence, with meta-analysis produced by Badve et al. (70, 262) suggesting that there is no consistent relationship between interventions that change LVM and mortality. Maki et al. (70) however in their meta-analysis concluded that changes in LVM were a useful surrogate of mortality risk. At least part of this discrepancy can be explained by Maki

et al. excluding studies of short duration (<6 months) and excluding studies targeting higher haemoglobin levels which have been repeatedly shown to be associated with a higher mortality despite regressing LVH. Further clarification of this, is therefore, required using robust research methodology.

9.2.3 Arterial Stiffness

As was discussed in **chapter 3** due to the limitations on research activity during COVID19 it was not possible to study parameters of arterial stiffness, in conjunction with changes in left ventricular volumetric and mass analysis. Performing this analysis would allow further understanding of the effects of renal transplant on the cardiovascular system.

9.2.4 Diastolic function

In the current study parameters of diastolic function were assessed using CMR parameters of early and late diastolic strain. At present echocardiography rather than CMR is considered the gold standard for assessing diastolic function(372). It would therefore be a future consideration to perform analysis combining both imaging techniques.

9.3 Targeted therapy for Uraemic cardiomyopathy, is this the future?

It has been noted previously that renal transplantation is the gold standard treatment for ESKD. Transplant, however, is not available to all persons with ESKD due to either high operative risk or the inability to find a suitable donor. In this group cardiovascular morbidity and mortality remain unacceptably high with current treatment strategies.

The data presented in this thesis indicate that the adverse cardiovascular remodelling associated with uraemic cardiomyopathy is reversible following renal transplant. This finding

supports the hypothesis that targeted therapies may be able to induce similar changes which could ameliorate cardiovascular risk where transplantation is not an option. Such therapies would have the advantage that they may be suitable in earlier stages of CKD, which could prevent the development of uraemic cardiomyopathy which is known to begin with only minor reductions in renal function (43).

At present there are multiple research avenues which have shown promise. These include vitamin D therapy where observation studies have demonstrated improved mortality in CKD In addition, modulators of calcium sensing receptors such as cinacalcet have been associated with significant reductions in cardiovascular mortality (148). A further promising avenue of research which is currently in early stages has been directly targeting FGF23. While blocking the activation of all FGF23 receptors is associated with hyperphosphatemia and vascular calcification, the potential for blocking FGFR4 alone represents a promising target for future therapies(264). Blocking FGFR4 has already been shown to reverse LVH in animal models of CKD(373). At present there is no active development of agents to target FGF23 receptors for the treatment of uraemic cardiomyopathy, however, several agents for use in oncology are currently being studied. Such agents may prove pivotal in the treatment of uraemia cardiomyopathy in the future.

9.4 Conclusion

The RETRACT study provides robust evidence that the adverse cardiovascular remodelling associated with uraemic cardiomyopathy is a reversible process. These findings allow greater understanding of the improvements in cardiovascular mortality seen following transplant. These findings also provide a strong mandate for ongoing research aimed at developing agents to both reverse and prevent the development of uraemic cardiomyopathy in those in whom a transplant is not a viable treatment option.

Chapter 10: Appendices

10.1 APENDIX A

Figure 10.1 Serum and Plasma laboratory processing methodology.

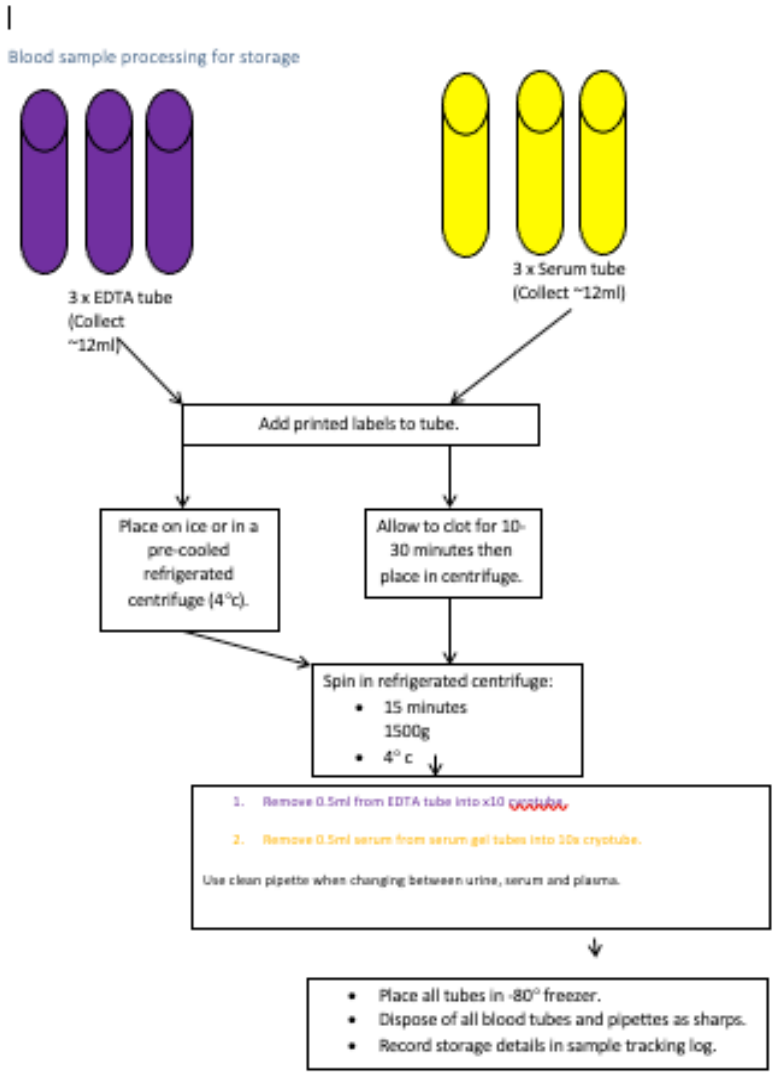


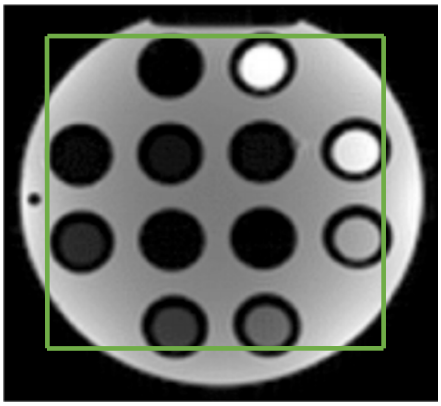
Figure 10.2 Urine processing methodology.

10.2 APPENDIX B

RETRACT EUROSPIN TO5 PHANTOM SCANNING PROTOCOL

1. Set up the simulated electrocardiographic signal with R-R interval of 900ms
2. Before starting the scans make sure all relevant coils are switched on.
3. Short Cable Body Coil should be used in all phantom and participant scanning.
4. Start with a localizer with no image-plane or field of view (FOV) offsets.
5. The phantom should be positioned at the isocentre of the scanner. Check that the physical positioning of the Eurospin TO5 phantom is correct (along all three directions) so that the phantom is not tilted or twisted beyond 10 degrees.
6. FOV should be set at 360 with FOV Phase at 85.2
7. Shimming volume should be set at A-P170mm, H-F20mm, R-L 170mm.

Figure 10.3 Example of Shim box position.



8. Slice thickness 8mm.
9. Shim Volumes and FOV parameters are to remain constant throughout the period of the RETRACT and CRIB Donor II studies.
10. Phantom to be scanned on monthly basis.
11. T1 Sequences – single transverse 8mm slice A-P orientation to follow study protocols as previously set-out.
    a. MOLLI - Pre-contrast sequence.
    b. ShMOLLI - Pre-contrast sequence.

c. Post Contrast -MOLLI sequence.

d. Turbo Spin Echo Sequence – Magnitude images. (Base resolution 192mm A>>p orientation)

12. Eurospin Tubes to be used see Figure 1.

Figure 10.4 Orientation of tubes to be used when scanning Eurospin TO5Phanton

Figure 10.5 Values of Tubes in the EurospinTO5 phantom

		Temperature	(k)			
	292	296	300	292	296	300
Tube No		T1ms			T2ms	
1	200	223	249	52	50	48
2	299	334	372	73	70	67
3	296	331	368	113	111	110
4	463	516	574	53	49	46
5	450	502	559	94	89	85
6	444	496	552	154	151	148
7	604	674	750	95	89	84
8	596	666	741	136	129	124
9	754	841	935	116	109	103
10	745	831	924	157	149	142
11	903	1007	1120	137	128	121
12	1448	1615	1796	390	373	359
13	966	1078	1199	224	212	203
14	1034	1153	1282	167	156	148
15	1160	1293	1437	214	201	191
16	1276	1422	1581	204	190	180
17	1262	1407	1563	184	171	161
18	1415	1576	1750	174	161	151

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