CORONARY MICROVASCULAR DYSFUNCTION IN CHRONIC KIDNEY DISEASE – INSIGHTS FROM LIVING KIDNEY DONORS AND END-STAGE RENAL DISEASE

by

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ABSTRACT

Cardiovascular disease is the leading cause of morbidity and mortality among patients with chronic kidney disease (CKD). This risk is highest in patients with uraemic cardiomyopathy - a clinical syndrome characterised by left ventricular hypertrophy, myocardial fibrosis, systolic and diastolic dysfunction and an increased risk of sudden cardiac death. Uraemic cardiomyopathy shares similarities with other myocardial diseases such as heart failure with preserved ejection fraction, which are associated with a high burden of coronary microvascular dysfunction (CMD).

In this thesis I explore the prevalence and mechanisms of CMD in different populations with renal impairment. I have identified several novel findings. Firstly, I have shown that living kidney donors have reduced coronary flow velocity reserve (CFVR – a measure of coronary microvascular function) and increased inflammatory markers compared to healthy controls, suggesting that uni-nephrectomy and its associated loss of renal function may lead to subclinical inflammation and microvascular changes. Secondly, I have demonstrated a high prevalence of CMD among patients with CKD stage 5 that are on the kidney transplant waiting list, with lower CFVR among patients with more profound anaemia. Finally, I have shown a negative correlation between CFVR and T1 times (a marker of myocardial fibrosis) in patients with advanced CKD.

These findings suggest that CMD may be a key contributor to the cardiovascular risk associated with renal dysfunction. Larger studies are necessary to confirm the findings shown in these cross-sectional data.

DEDICATION

To my amazing wife, Anisha. She has been my rock and has supported me through all the highs and lows of this process. And to my wonderful son, Arihaan, who has brought such joy to our lives.

ACKNOWLEDGEMENTS

I would like to express my sincere thanks to everyone who has helped me with this project. I am particularly indebted to my lead supervisor, Prof Jonathan Townend, for his guidance, support and mentorship. I am also grateful to Prof Charles Ferro and Dr Rick Steeds for their scientific input, and to Prof Larissa Fabritz, who helped me to secure the funding necessary to complete my MD. A special thanks must go to all the staff in the Centre for Rare Diseases at the Queen Elizabeth Hospital, who provided assistance with my clinical studies and who were also willing participants as control subjects. I would also like to thank the cardiology and renal research nursing teams for their help with navigating the necessary regulatory and ethical approvals for this project. I am grateful to Dr Sara Svedlund and Prof Li Min Gan from Sahlgrenska University, Gothenburg, Sweden, and Prof Roxy Senior and Reinette Hampson from Northwick Park Hospital, London. These individuals devoted their time and expertise in teaching me the techniques that formed the basis of my thesis, and their generosity truly epitomises the spirit of collaborative research. Similarly, I would like to thank Dr Peter Nightingale at the Queen Elizabeth Hospital for his invaluable statistical advice. I would especially like to thank the other research fellows in the Birmingham Cardio-Renal Group (Dr Luke Pickup, Dr Anna Price and Dr Jonathan Law) for their camaraderie, support and friendship throughout my MD. I also wish to acknowledge the support from Birmingham Health Partners, the Institute of Cardiovascular Sciences, University Hospitals Birmingham Charity and the Metchley Park Medical Society, who all provided funding for my salary and this project. Finally, I wish to wholeheartedly thank all the patients who took part in my studies, without whom this project would not have been possible.

EXTENT OF PERSONAL CONTRIBUTION

The studies presented in this thesis were designed by Prof Jonathan Townend and I, with input from Prof Charles Ferro and Dr Rick Steeds. Applications for funding from University Hospital Birmingham Charity and the Metchley Park Medical Society were submitted and secured by Prof Townend and I. Application to the Research Ethics Committee for ethical approval for the CRIB-FLOW study was obtained by Prof Townend and I. Ethical approval for the RETRACT echocardiogram sub-study was obtained by Dr Luke Pickup and Prof Charles Ferro.

I recruited all the participants enrolled in the CRIB-FLOW study. I also recruited participants enrolled in the RETRACT echocardiogram sub-study, in conjunction with Dr Luke Pickup. I performed all the transthoracic echocardiograms, Doppler coronary flow velocity reserve measurements and myocardial contrast echocardiograms. Arterial stiffness measurements were performed by Dr Luke Pickup and I.

Serum multiplex immunoassay samples were performed at the University of Birmingham laboratories by Dr Anna Price, with my assistance. Cardiac magnetic resonance scans included in this thesis were performed and analysed by Dr Luke Pickup. The high sensitivity C reactive peptide assay was processed by Mohammed Shaikh at Birmingham Heartlands Hospital. Statistical advice was provided by Dr Peter Nightingale at the Queen Elizabeth Hospital, Birmingham. I analysed all the data and performed the statistical analyses presented in this thesis.

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LIST OF ABBREVIATIONS

ACE	Angiotensin converting enzyme
ACR	Albumin-creatinine ratio
ADH	Anti-diuretic hormone
ADMA	Asymmetric dimethylarginine
AIx	Augmentation index
ARB	Angiotensin receptor blocker
BMI	Body mass index
BP	Blood pressure
BSA	Body surface area
BSE	British Society of Echocardiography
CAD	Coronary artery disease
CBF	Coronary blood flow
CFR	Coronary flow reserve
CFV	Coronary flow velocity
CFVR	Coronary flow velocity reserve
CKD	Chronic kidney disease
CKD-EPI	Chronic kidney disease epidemiology collaboration
CMD	Coronary microvascular dysfunction
CMR	Cardiac magnetic resonance imaging
CRIB-Donor	Chronic Renal Impairment in Birmingham Donor study
CRIB-FLOW	Chronic Renal Impairment in Birmingham coronary FLOW reserve study
CRIC	Chronic Renal Insufficiency Cohort
CRP	C reactive peptide
CSF	Coronary sinus flow
CVD	Cardiovascular disease
DCM	Dilated cardiomyopathy

EARNEST	Effect of A Reduction in glomerular filtration rate after NEphrectomy on arterial STiffness and central hemodynamics
ECG	Electrocardiogram
EDV	End-diastolic volume
eGFR	Estimated glomerular filtration rate
EPO	Erythropoietin
ESC	European Society of Cardiology
ESRD	End-stage renal disease
ESV	End systolic volume
FFR	Fractional flow reserve
FGF-23	Fibroblast Growth Factor-23
GFR	Glomerular filtration rate
GLS	Global longitudinal strain
HCM	Hypertrophic cardiomyopathy
HD	Haemodialysis
HR	Heart rate
HRA	Health Research Authority
hsCRP	High sensitivity C reactive peptide
ICC	Intra-class correlation coefficient
ICD	Implantable cardioverter defibrillator
IL-18	Interleukin-18
IL-6	Interleukin-6
KDIGO	Kidney Disease Improving Global Outcomes
LAD	Left anterior descending artery
LDL	Low density lipoprotein
LKD	Living kidney donors
LOA	Limits of agreement
LV	Left ventricular
LVEF	Left ventricular ejection fraction

LVH	Left ventricular hypertrophy
LVIDd	Left ventricular internal diameter in diastole
LVIDs	Left ventricular internal diameter in systole
LVMI	Left ventricular mass index
MACE	Major adverse cardiovascular events
MAP	Mean arterial pressure
MBF	Myocardial blood flow
MCE	Myocardial contrast echocardiography
MDRD	Modification of diet in renal disease
MI	Mechanical index
MOLLI	Modified Look-Locker inversion recovery
NO	Nitric oxide
NTpro-BNP	N terminal pro-B type natriuretic peptide
PCI	Percutaneous coronary intervention
PD	Peritoneal dialysis
PET	Positron emission tomography
PTH	Parathyroid hormone
PW	Pulse wave
PWA	Pulse wave analysis
PWTd	Posterior wall thickness diastole
PWV	Pulse wave velocity
PWVadj	Adjusted pulse wave velocity
QEHB	Queen Elizabeth Hospital, Birmingham
RAAS	Renin-angiotensin-aldosterone system
RETRACT	Prospective Study of the Effects of Renal Transplantation on Uraemic Cardiomyopathy using Magnetic Resonance Imaging study
ROI	Region of interest
RU	Relative upslope

RWT	Relative wall thickness
SCD	Sudden cardiac death
SGLT-2	Sodium-glucose-cotransporter-2
SNS	Sympathetic nervous system
SPECT	Single photon emission computed tomography
SSFP	Steady state free precession
SWTd	Septal wall thickness diastole
TNFα	Tumour necrosis factor alpha
TTE	Transthoracic echocardiography
UK	United Kingdom

CHAPTER 1: INTRODUCTION

Section I: The kidneys and cardiovascular disease

I.1 The kidneys and their function

The kidneys are a pair of abdominal retroperitoneal organs, located between the vertebral levels of T12 to L3. Each kidney receives arterial blood supply from a renal artery arising directly from the aorta, drains venous blood to a renal vein, and is connected to the bladder by a ureter.(1) Despite their small size, blood flow to the two kidneys accounts for approximately 25% of cardiac output under normal resting conditions. Far beyond simply producing urine to excrete waste products, these complex organs are responsible for a variety of vital homeostatic functions including acid-base balance, drug metabolism and excretion, hormone secretion, regulation of salt and water, blood pressure (BP) control, and bone mineral metabolism - figure 1-1.(2)

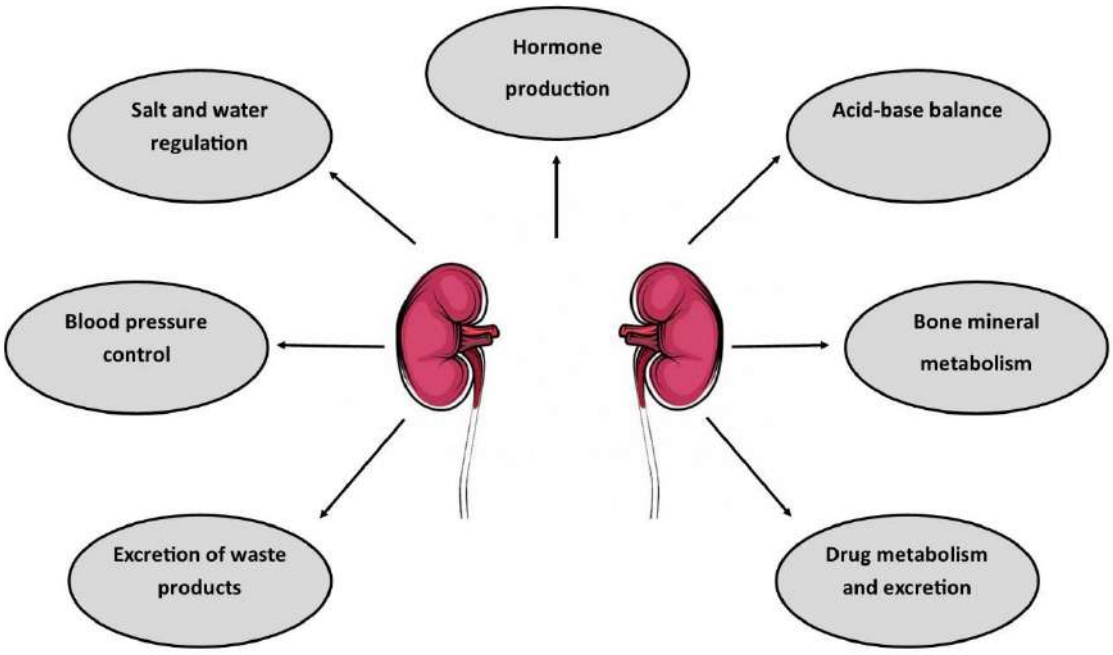


Figure 1-1: Functions of the kidneys.

The primary roles of the kidneys are salt and water regulation and the excretion of waste products by the production of urine. This is carried out by the functional unit of the kidney - the nephron. Each healthy kidney contains 400,000 to 1.2 million individual nephrons and each nephron comprises a number of constituent parts including the glomerulus, the proximal tubule, the loop of Henle and the collecting duct - figure 1-2.(3) The glomerulus receives its blood supply from the afferent arteriole. Pressure across the glomerulus leads to the passive movement of protein free fluid from the afferent arteriole into the Bowman's capsule, which surrounds the glomerulus. Only a proportion of plasma is filtered in this way, with the remaining volume leaving the glomerulus via the efferent arteriole.(2) The amount of plasma that is filtered over time is referred to as the glomerular filtration rate (GFR) and is an index of effective kidney function. Once plasma enters the glomerulus, it then undergoes a process of selective reabsorption of water and electrolytes in the proximal tubule, the loop of Henle and the collecting duct, ultimately leading to the production of urine – the main mechanism by which the kidneys excrete waste products from the body. This reabsorption process is under the control of hormones including anti-diuretic hormone (ADH) and aldosterone.(3)

In addition to salt and water regulation, the nephron also plays a key role in acid-base balance. Tubular cells secrete hydrogen ions into the tubular fluid through the Na^+/H^+ antiporter, which in turn leads to reabsorption of bicarbonate into the blood. This maintenance of acid-base homeostasis is vital for appropriate enzymatic function in cells around the body.(4)

The kidneys are also vital endocrine organs. Erythropoietin (EPO) is secreted by interstitial fibroblasts in the kidney and is an important stimulus for the bone marrow to produce red blood cells. The juxtaglomerular apparatus of the kidneys secretes renin which causes activation of the renin-angiotensin-aldosterone system (RAAS) and plays an important role in salt and water balance and BP control.(3)

Finally, the kidneys also play a vital role in bone mineral metabolism, primarily through their role in the activation of vitamin D. Calcitriol is the activated form of vitamin D and is produced in the proximal renal tubules by hydroxylation of 25-hydroxycholecalciferol by the enzyme 1α-hydroxylase. Calcitriol then regulates calcium and phosphate metabolism by binding to the vitamin D receptor (which is found in the intestine, kidney and bone), thereby promoting calcium and phosphate reabsorption in the kidney and gut, as well as having direct effects on osteoclast and osteoblast function.(5)

Thus, it is clear that the kidneys have a number of vital roles in normal homeostatic function and it is understandable why patients with chronic kidney disease (CKD) have significant metabolic and subsequently cardiovascular abnormalities.

I.2 Assessment of renal function

Glomerular filtration rate is a marker of glomerular, and thereby, renal function. An ideal exogenous marker has the following properties:

- It appears endogenously in the plasma at a constant rate
- It is freely filtered at the glomerulus
- It is neither reabsorbed nor secreted by the renal tubule
- It does not undergo elimination outside the kidney(6)

Historical assessment of GFR relied on 24-hour urine collections to assess creatinine clearance. However, this was a time consuming, inconvenient and slow method of assessing GFR. Accurate measures of GFR can be calculated by measuring the filtration of exogenous radioisotopes such as chromium-51 ethylene-diamine-tetra-acetic acid. In reality, measurement of isotopic GFR is restricted to specialised centres.

In everyday clinical practice, renal function is usually assessed through calculating estimated glomerular filtration rate (eGFR). This involves regression analysis in which the level of measured GFR is related to the serum concentration of an endogenous filtration marker.(7) Creatinine is the most widely used endogenous marker.(6) It is a breakdown product of creatine phosphate in muscle tissue. Creatinine is produced at a constant rate and is almost wholly excreted by the kidneys, with only minimal tubular reabsorption, thus making it a suitable marker of glomerular filtration.(8) It is important to note that eGFR is only an estimate. As it is mathematically derived based on variables such as creatinine, age, sex and race, significant error can occur from variations in any of these parameters. Multiple formulae including the Cockcroft-Gault, Modification of Diet in Renal Disease (MDRD) and Chronic Kidney Disease

Epidemiology Collaboration (CKD-EPI), exist for calculating eGFR. The Cockcroft-Gault formula is the oldest formula and was validated against 24-hour urinary creatinine clearance but is no longer used due to a tendency to overestimate eGFR.(9) Both the MDRD and the CKD-EPI formulae calculate eGFR using the same 4 variables (age, gender, race and serum creatinine). Current guidelines recommend use of the CKD-EPI formula, due to its more accurate estimation of eGFR, CKD prevalence estimates and better risk prediction.(7,10)

I.3 Chronic kidney disease

Chronic kidney disease is defined as the presence of abnormal kidney structure or function for >3 months.(10) It is extremely common, with an estimated worldwide prevalence of 13.4%.(11) In the Western world, diabetes and hypertension are the most common aetiologies.(12) – figure 1-2.

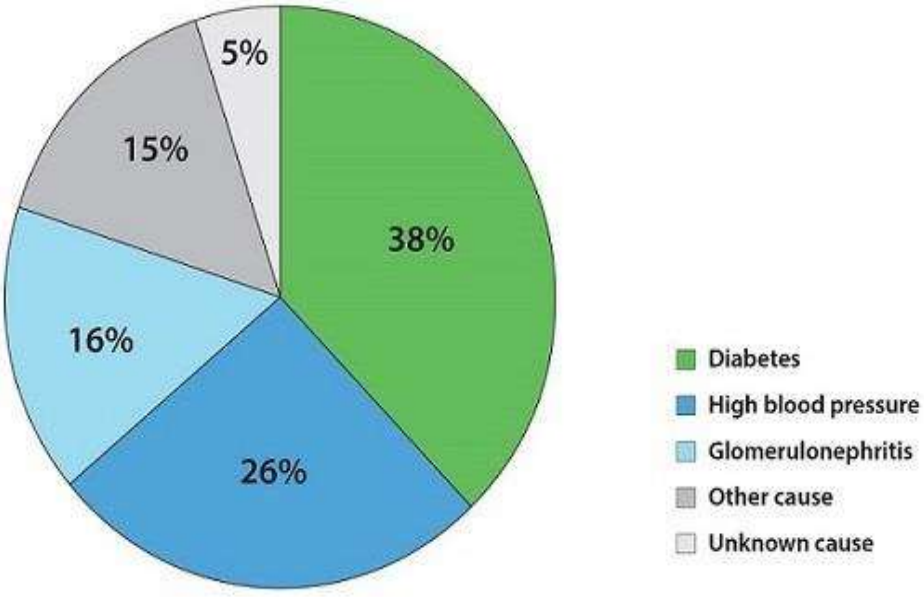


Figure 1-2: Aetiology of chronic kidney disease in the Western world. Reproduced from Chronic Kidney Disease in the United States, Centre for Disease Control, 2019.

The Kidney Disease Improving Global Outcomes (KDIGO) system classifies severity of CKD based on eGFR with higher stages reflecting more severe disease – table 1-1.(10) The degree of proteinuria is also important and can be further subdivided into 3 stages – A1 (<3mg/mmol), A2 (3-30mg/mmol) and A3 (>30mg/mmol). Proteinuria is an independent risk factor for both progression of CKD and the development of cardiac disease.(13)

Table 1-1: KDIGO staging of chronic kidney disease

Stage	***Description***	***eGFR (ml/min/1.73m^2)***
G1	Kidney damage* with normal or increased eGFR	≥90
G2	Kidney damage* with mild decrease in eGFR	60-89
G3a	Mild to moderate decrease in eGFR	45-59
G3b	Moderate to severe decrease in eGFR	30-44
G4	Severe decrease in eGFR	15-29
G5	Kidney failure	<15

*Evidence of functional or structural abnormalities for >3 months. In the absence of kidney damage, neither stage G1 or G2 fulfil the definition for chronic kidney disease. KDIGO: Kidney Disease Improving Global Outcomes, eGFR: Estimated glomerular filtration rate

Although the KDIGO classification suggests that CKD is a progressive condition, the reality is that the course of the disease in individual patients is very heterogenous, with the majority of patients showing no significant change in renal function on a yearly basis.(14)

I.4 The cardio-renal axis

The heart and the kidneys act in conjunction through a series of bi-directional physiological pathways – the so called “cardio-renal axis.” This axis plays a vital role in the regulation of systemic BP. There is a complex interplay between mechanisms favouring vasoconstriction and sodium retention and competing mechanisms favouring vasodilatation and natriuresis. In hypotensive states, with reduced systemic and renal perfusion pressures, there is a decline in vagal stimulation and increased sympathetic nervous system (SNS) mediated activation of the RAAS. This in turn leads to increased production of angiotensin II, a potent vasoconstrictor that preferentially vasoconstricts the efferent arteriole of the glomerulus, causing increased renal perfusion pressure and promoting glomerular filtration. Furthermore, activation of the RAAS increases secretion of aldosterone and ADH, which both act to promote salt and water retention.(15) These mechanisms lead to an expansion of the circulating blood volume, which causes increased venous return to the right heart, increased preload for the left ventricle and consequently an increase in stroke volume and systemic BP.(16) By contrast, in states of volume overload, activation of receptors in the atria and ventricles leads to the release of natriuretic peptides that promote vasodilatation, sodium and water excretion, and suppression of aldosterone and ADH, which all reduce circulating blood volume.(2)

As CKD progresses, there is dysregulation of these physiological pathways, leading to a state of chronic volume overload, particularly in patients with end-stage renal disease (ESRD).(17) A consequence of this chronic volume overload is the development of systemic hypertension, which is near universal in advanced CKD, and which itself is

implicated in further deterioration of renal function, as well as cardiovascular complications.(18,19) Additionally, chronic fluid overload itself is associated with adverse consequences including progression of CKD, development of ESRD, and cardiovascular mortality, independent of the effects of systemic hypertension.(20,21)

Abnormalities of the cardio-renal axis can be termed cardio-renal syndrome and are classified into 5 distinct phenotypes, depending on which is the primary organ affected – figure 1-3. The syndrome of uraemic cardiomyopathy, which represents cardiac disease in CKD, can be considered a form of type 4 cardio-renal syndrome, and is the focus of this thesis.

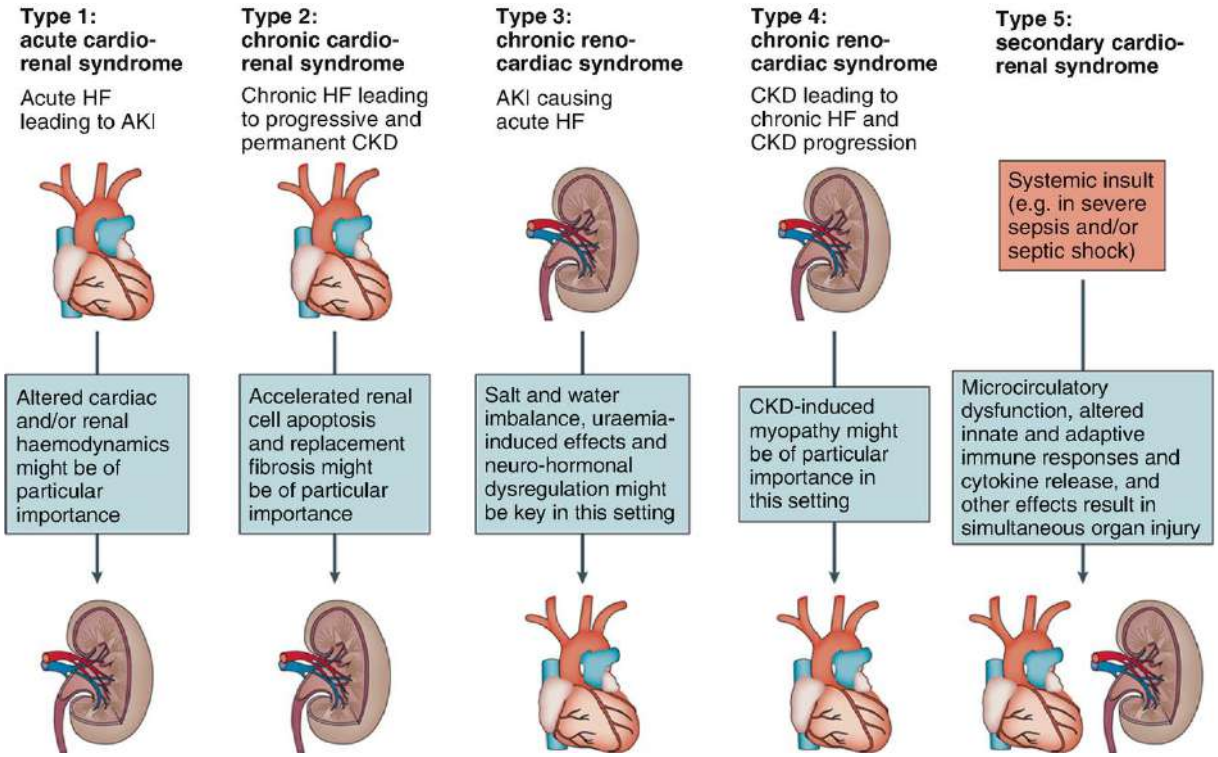


Figure 1-3: The five different phenotypes of cardio-renal syndrome. Reproduced with permission from Joki *et al.*(22) HF: heart failure, AKI: acute kidney injury, CKD: chronic kidney disease.

I.5 The burden of cardiovascular disease in chronic kidney disease

There is a wealth of epidemiological data showing that CKD is associated with increased cardiovascular morbidity and mortality. Cardiovascular disease (CVD) is the leading cause of mortality in CKD and was responsible for approximately 20% of deaths in patients with ESRD according to United Kingdom (UK) Renal Registry data from 2019. Higher cardiovascular mortality rates have been reported in other developed countries, with data from the United States reporting CVD as the cause of death in 42.5% of patients with ESRD.(23,24) The risk of cardiovascular death is highest among dialysis-dependent patients, who have a 10-fold increased risk of cardiovascular death compared to age- and gender-matched controls without CKD.(25) Proteinuria is an additional risk factor for cardiovascular death in CKD, with a 1.5-fold increase in cardiovascular mortality seen with stage 3 albuminuria (>30mg/mmol) compared to stage 1 (<3mg/mmol).(26) After adjustment for age, gender, cardiovascular risk factors, and degree of proteinuria, there appears to little increase in cardiovascular mortality until eGFR falls to below 75ml/min/1.73m^2, following which there is a linear increase in cardiovascular risk as eGFR falls, a pattern that accelerates rapidly once eGFR falls below 60 ml/min/1.73m^2.(26,27)

Although cardiovascular death is also common in the general population, this is heavily skewed towards the elderly, with low rates of cardiovascular death among younger age groups. By contrast, younger patients with ESRD have a disproportionately increased risk of cardiovascular mortality compared to the general population. Among older patients with advanced kidney disease, the relative risk of cardiovascular death is only

slightly increased compared to the general population, who also have high background levels of CVD – figure 1-4.

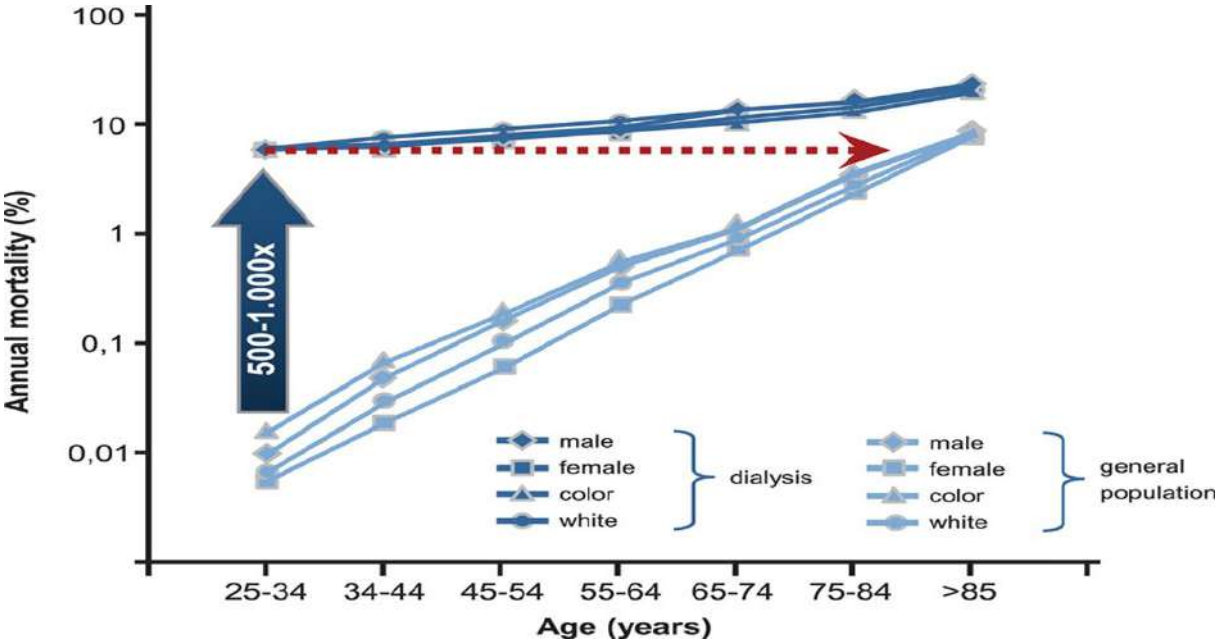


Figure 1-4: Cardiovascular mortality in the general population and in end-stage renal disease. Reproduced with permission from Jankowski *et al*.(28)

The reasons for this increased cardiovascular risk are probably multifactorial. Firstly, patients with CKD have high rates of epicardial coronary artery disease (CAD), reflecting cardiovascular risk factors such as diabetes and hypertension that are endemic in CKD. There is a graded relationship between eGFR and the risk of atherosclerotic CAD, with each 10ml/min reduction in eGFR associated with an adjusted hazard ratio for CAD of 1.05.(29) Patients with CKD are more likely to present with atypical symptoms, and present with myocardial infarction rather than stable angina.(30) Myocardial ischaemia is a frequent finding in patients undergoing

haemodialysis (HD), with studies demonstrating dialysis-induced asymptomatic ST-segment depression in 15-40% of cases.(31) However, this does not always correspond to epicardial CAD on coronary angiography and may represent myocardial oxygen-supply mismatch due to factors such as anaemia, left ventricular hypertrophy (LVH), abnormal loading conditions and microcirculatory changes.(31,32) In patients with proven CAD undergoing revascularisation with coronary angioplasty or coronary artery bypass grafting, there is also a higher risk of major complications including acute kidney injury and bleeding with the use of antiplatelet therapy.(33–36)

Secondly, heart failure is extremely prevalent in CKD. The Atherosclerosis Risk in Communities study demonstrated that over a 13-year follow-up period, a significantly higher proportion of patients with eGFR <60ml/min/1.73m^2 developed incident heart failure (defined as heart failure hospitalisation or death) compared to patients with normal renal function, and this risk was even higher in individuals who had CAD at baseline.(37) The relationship between heart failure and CKD appears to be bi-directional, with a large meta-analysis of studies including patients with both heart failure with reduced ejection fraction and heart failure with preserved ejection fraction (HFpEF) showing that 55% of patients had eGFR <60ml/min/1.73m^2.(38) Indeed, renal dysfunction is the second most common comorbidity (after systemic hypertension) among patients with heart failure.(39)

Thirdly, sudden cardiac death (SCD) is also extremely common in CKD and multiple studies have shown an association between reduced renal function and SCD. Retrospective analysis of the Multicenter Automatic Defibrillator Implantation Trial II

highlighted that baseline eGFR was the most powerful predictor of subsequent mortality in patients with previous myocardial infarction and left ventricular ejection fraction (LVEF) <30%. In this trial, the risk of SCD increased by 17% for each 10ml/min/1.73m^2 decrease in eGFR.(40) Deo *et al.* demonstrated that among postmenopausal women with a history of CAD, eGFR <40ml/min/1.73m^2 was associated with a two-fold increased risk of SCD, even after adjustment for baseline risk factors and incident myocardial infarction and congestive cardiac failure.(41) The risk of SCD is amplified in ESRD, with a 14-fold increased risk of sudden death in patients commencing HD.(42) Sudden cardiac death was responsible for 22-26% of all mortality in HD patients across multiple studies.(43) Similar findings were also seen in a prospective Chinese cohort of patients on peritoneal dialysis (PD).(44)

It was previously assumed that ventricular tachyarrhythmia was the main mechanism of SCD in CKD. However, data from implantable loop recorder studies in HD patients have demonstrated that although ventricular tachyarrhythmias commonly occur in patients on HD, terminal events were more frequently due to bradyarrhythmia or asystole.(45) Further evidence that the primary cause of SCD in CKD is not due to ventricular tachyarrhythmia comes from the ICD2 trial which randomised long term HD patients with LVEF >35% to implantable cardioverter defibrillator (ICD) therapy or standard therapy and found no reduction in rates of SCD [hazard ratio 1.02 (95% CI, 0.69–1.52; p=0.92)] and a high rate of adverse events from ICD implantation.(46)

Finally, CKD is also associated with a significantly increased risk of stroke, with a large meta-analysis showing a 43% increase in relative risk of incident stroke among

patients with eGFR <60ml/min/1.73m2 compared to those with eGFR >60ml/min/1.73m2. There was an increased risk of both ischaemic and haemorrhagic stroke.(47) Similar to other cardiovascular conditions, proteinuria increases the risk of stroke by 71% compared to CKD patients without proteinuria.(48) The high incidence of stroke in CKD is likely to be related to the high prevalence of hypertension, which is the major risk factor for both ischaemic and haemorrhagic stroke.(49) Patients with CKD also have a high burden of atrial fibrillation, with the Chronic Renal Insufficiency Cohort (CRIC) study identifying atrial fibrillation in 20.4% of patients with eGFR <45ml/min/1.73m2.(50) This is compounded by the increased bleeding risk in CKD from novel anticoagulant agents and limited data for their use among patients with renal impairment. Furthermore, CKD is associated with worse outcomes after both ischaemic (greater neurological deficit, poorer functional outcome, higher risk of haemorrhagic transformation and increased mortality) and haemorrhagic (increased haematoma volume) stroke.(51–53)

Thus, it is clear that patients with CKD are susceptible to a wide array of cardiovascular conditions that cause considerable morbidity and mortality. The pattern of CVD appears to change as renal function declines, with CAD playing a greater role in early stage CKD and late stage CKD characterised by a high burden of heart failure, valvular heart disease and SCD.(54)

I.6 Cardiovascular changes in chronic kidney disease

A number of direct adverse arterial and myocardial changes occur in CKD that contribute to the increased cardiovascular risk associated with the condition.

I.6.1 Arterial changes

Chronic kidney disease is characterised by pathological changes to the arterial system. This takes the form of both accelerated atherosclerosis (intimal disease), as well as arteriosclerosis (medial disease). Chronic kidney disease appears to be an independent risk factor for atheroma development,(55) and this process affects multiple vascular beds.(56) In contrast to atheroma in the general population, atheromatous plaques in CKD are characterised by increased calcification.(57) Coronary artery calcification in individuals with CKD is up to 8-fold higher than similar subjects without CKD.(58) However, this does not seem to equate to a corresponding increase in vaso-occlusive events.(18) A recent study by Jansz *et al.* demonstrated that, despite similar coronary artery calcification scores on computed tomography coronary angiography, dialysis patients had significantly lower odds ratio for obstructive coronary stenoses compared to a non-CKD cohort matched for cardiovascular risk factors, suggesting that some of the calcification detected in CKD patients may be medial, rather than intimal, calcification.(59)

Perhaps of greater significance is arteriosclerosis, which is a hallmark of arterial disease in CKD. Diffuse fibroelastic intimal thickening leads to an increase in collagen and ground substance in the medial layer of arteries, causing medial hypertrophy, calcification, secondary fibrosis and a reduction in the luminal area of the

vessel.(56,60) Arteriosclerosis impairs the cushioning effect of the large conduit arteries which during diastole store 50% of stroke volume and prevent excessive pressures in the distal circulation – the so called “Windkessel effect.” This impairment in turn increases arterial stiffness and has the harmful consequences of increased systolic pressures and downstream damage in distal organ beds exposed to pulsatile aortic flow such as the brain, heart and kidneys.(60,61) It is likely that there is a bi-directional relationship between arterial stiffness and hypertension, with some evidence that increased arterial stiffness predates and indeed accelerates the development of systemic hypertension.(62) Arterial stiffness increases as renal function declines,(56,63) and is an independent predictor of progression to ESRD and death.(64–66)

I.6.2 Left ventricular hypertrophy

Left ventricular hypertrophy is extremely common, with estimated rates of 16-30% in early stage CKD.(67,68) By ESRD, over 70% of patients develop LVH,(69) and worsening of LVH is the biggest risk factor for SCD in HD patients.(70) A variety of factors are thought to contribute to the development of LVH – figure 1-5. Pre-load related factors include intravascular volume expansion due to salt and water retention, anaemia and the presence of arteriovenous fistulae.(67,71,72) The primary afterload related factor is hypertension, which is near universal in CKD, and which promotes LVH through increased systemic arterial pressures and stiffness and reduced aortic compliance.(56,67) Neurohormonal factors such as activation of the RAAS also play a role, both indirectly through promotion of salt and water retention as well as directly

with small studies suggesting that aldosterone itself is responsible for the development of LVH.(73,74)

Disordered bone metabolism may also play a role in the development of LVH. There is an association between increasing serum phosphate and the development of LVH(75), although it is not clear whether this is a direct effect of phosphate or due to the influence of phosphate regulating hormones. Fibroblast growth factor-23 (FGF-23) is a hormone that has been particularly implicated in adverse cardiac remodelling in CKD. Its primary function is to augment phosphaturia by down-regulating expression of sodium-phosphate co-transporters in the renal proximal tubule.(76) Although effective in maintaining normal serum phosphate levels, significant elevations of FGF-23 occur in CKD, where it has a linear association with LVH and increased cardiac mortality.(76–78)

I.6.3 Myocardial fibrosis

Myocardial fibrosis is also extremely common in CKD. Histological data from myocardial biopsy studies in ESRD show that subjects have severe myocyte hypertrophy, myocyte disarray and extensive diffuse interstitial fibrosis – a pattern that resembles the dilated phase of hypertrophic cardiomyopathy (HCM).(79) Diffuse interstitial fibrosis is the consequence of activation of cardiac fibroblasts and myofibroblasts, leading to alterations in the turnover of fibrillary collagen, which results in an excessive synthesis and deposition of predominantly type 1 collagen within the myocardial interstitium.(80) Although most prevalent in ESRD, previous work has shown that the fibrotic process can be detected as early as CKD stage 2, with a

progressive increase in non-invasive markers of myocardial fibrosis as CKD stage progresses.(81,82) Myocardial fibrosis will be discussed further in Chapter 7.

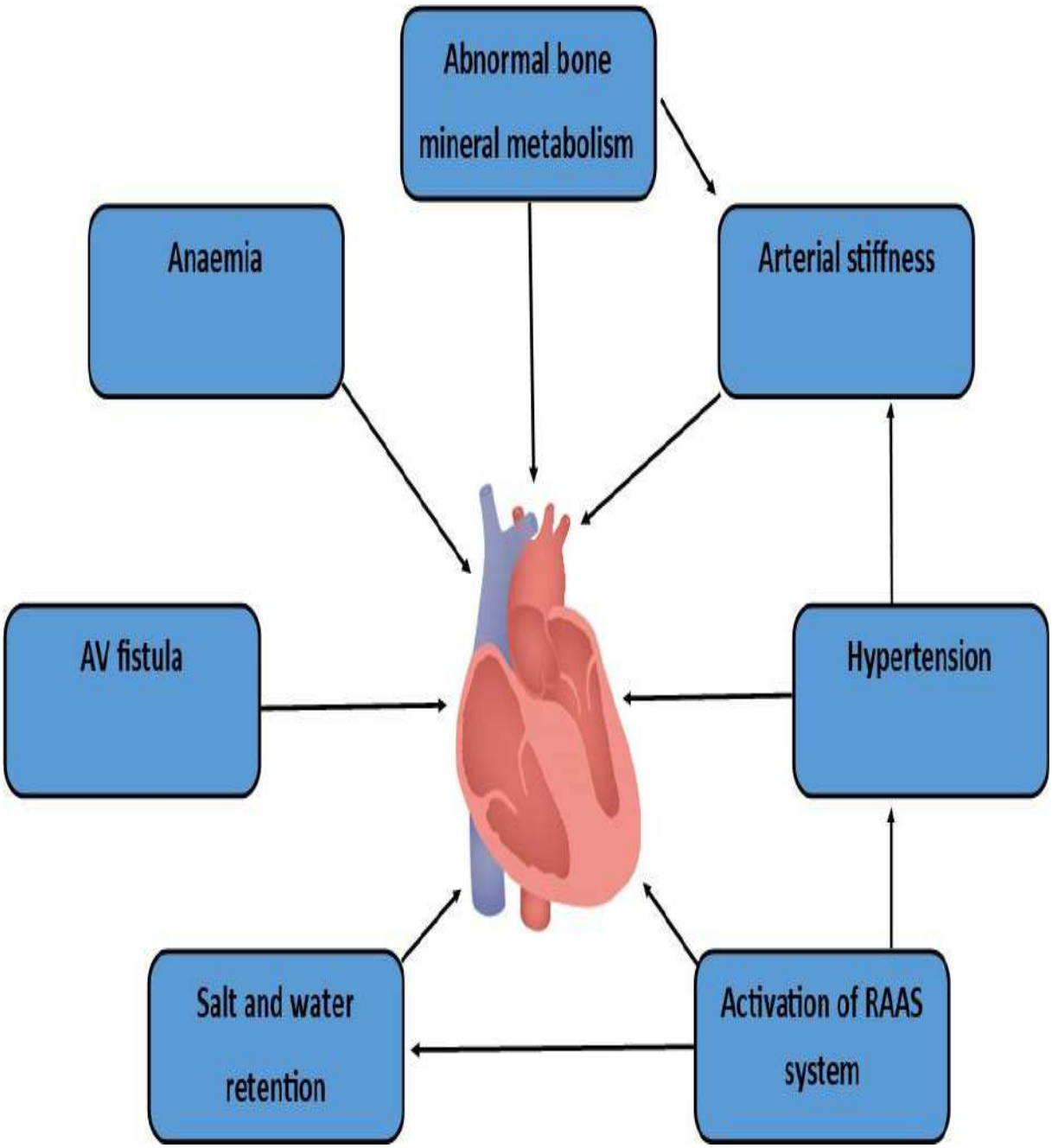


Figure 1-5: Schematic representation of factors causing left ventricular hypertrophy in chronic kidney disease. AV: arteriovenous, RAAS: renin-angiotensin-aldosterone system.

I.6.4 Systolic and diastolic dysfunction

While heart failure symptoms are common in CKD, severe left ventricular systolic dysfunction, as measured by reduced LVEF, is relatively rare. The CRIC study was a large multi-centre observational study of patients with pre-dialysis CKD (stage 2-4). Of the 3487 participants with echocardiographic data available and no prior history of clinical heart failure syndrome, systolic dysfunction, defined as LVEF <50%, was present in only 8% of the cohort.(83) Similar findings were found in a cardiac magnetic resonance imaging (CMR) based study by Mark *et al*.(69) However, it is recognised that LVEF is a relatively crude measure of assessing left ventricular systolic function.(84) Hensen *et al.* demonstrated that in a cohort of 304 patients with CKD stages 3b-5, including both pre-dialysis and dialysis patients, reduced left ventricular global longitudinal strain (GLS) was present in 32% of individuals despite preserved LVEF, and was associated with a significantly increased risk of subsequent heart failure hospitalisation.(85) Rakhit *et al.* demonstrated that in patients with late-stage CKD who had normal left ventricular (LV) mass and no overt myocardial ischaemia or dysfunction, subclinical abnormalities of myocardial deformation were present.(86) These abnormalities are evident even in early-stage CKD, with Edwards *et al.* showing that compared to healthy controls, patients with CKD stage 2-3 and no history of cardiovascular disease or diabetes, had reduced GLS.(87)

By contrast, diastolic dysfunction is extremely common in CKD, being present in 71% of the CRIC population.(83) Despite preserved LVEF, patients with CKD demonstrate increased markers of diastolic dysfunction including left atrial size and E/e'.(88) This is understandable given that CKD is associated with high levels of LVH and myocardial

fibrosis, both of which reduce LV compliance and impair myocardial relaxation.(89) Myocardial fibrosis in particular causes severe diastolic dysfunction as tissue collagen deposition affects viscoelasticity of the myocardium leading to impaired relaxation and diastolic recoil and increased passive stiffness.(90)

I.6.5 Uraemic cardiomyopathy

The term uraemic cardiomyopathy is used to describe cardiomyopathic features seen in patients with CKD. It is a clinical syndrome characterised by LVH, diffuse interstitial fibrosis, focal scarring and systolic and diastolic dysfunction. The diffuse fibrosis and focal scarring seen in uraemic cardiomyopathy is believed to be a major cause of the clinical syndrome of heart failure and the increased risk of arrhythmogenesis and SCD seen in CKD.(91) Its pathogenesis is poorly understood but is likely to be multifactorial. Both haemodynamic (hypertension, increased arterial stiffness, increased preload) and humoral (activation of the RAAS, hyperuricaemia, uraemic toxins such as asymmetric dimethylarginine [ADMA], abnormal bone mineral metabolism) factors are implicated.(91,92) A proposed mechanism for the development of uraemic cardiomyopathy is shown in figure 1-6.

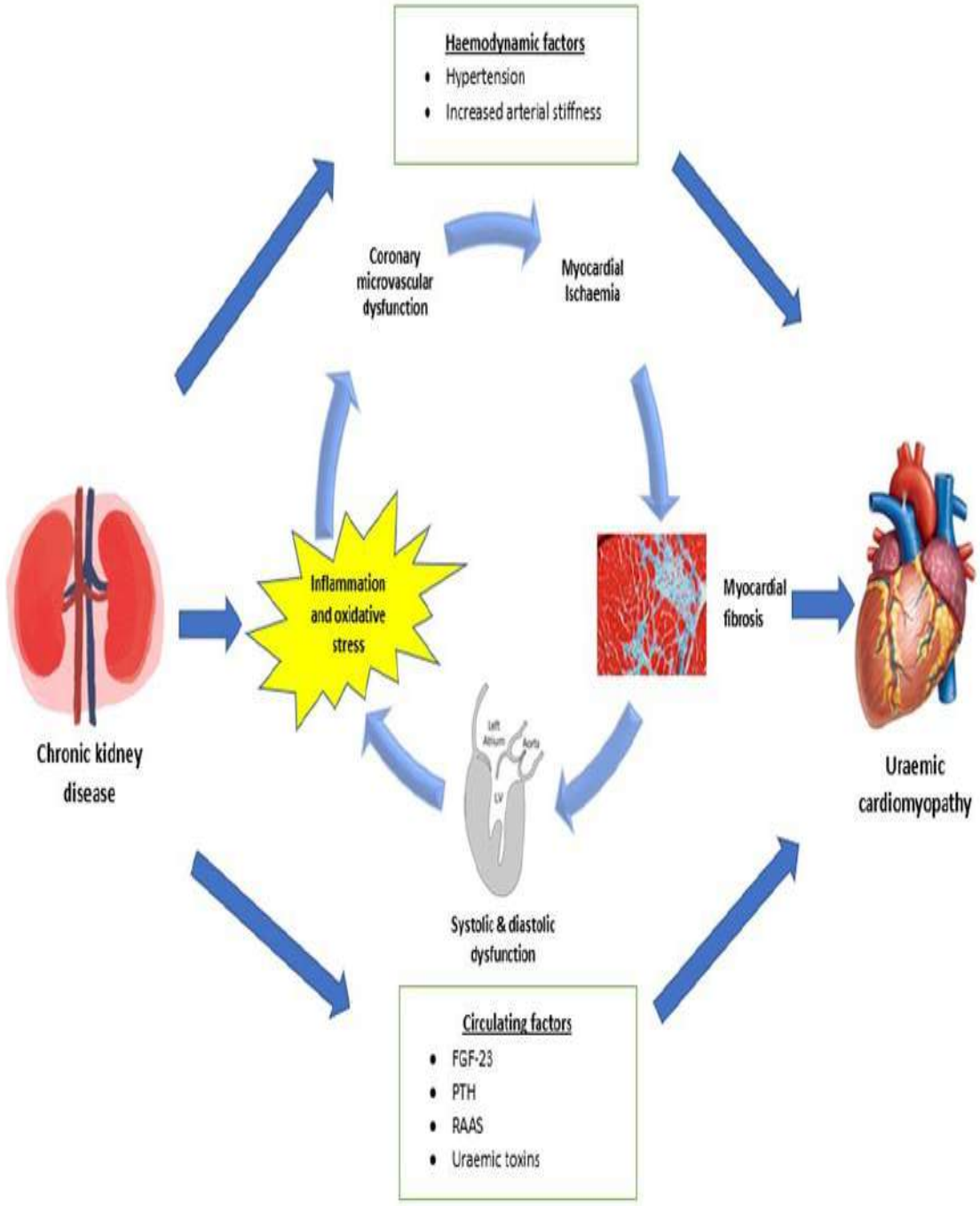


Figure 1-6: Proposed mechanism of uraemic cardiomyopathy. Reproduced with permission from Radhakrishnan *et al*.(93) FGF-23: fibroblast growth factor-23, PTH: parathyroid hormone, RAAS: renin-angiotensin-aldosterone system.

I.7 Cardiovascular risk in living kidney donors

Kidney transplantation from a live donor has been established as a treatment for ESRD since the 1950s. A kidney from a live donor provides the best outcome for recipients, with higher rates of graft and patient survival compared to deceased donor transplantation.(94) This benefit is especially seen if kidney transplantation is carried out pre-emptively before the onset of dialysis.(95,96) As a result of the improved recipient outcomes associated with live donor transplants, and the chronic shortage of deceased donor organs, there is increasing emphasis on the use of living kidney donors (LKD) in transplant programmes around the world. In the UK, LKD must be aged 18-80, have adequate renal function, and be free from any significant systemic disease.(97) Thus, they represent a unique and growing population of individuals with reduced eGFR but without the usual confounding cardiovascular comorbidities associated with CKD, therefore providing a useful model of early-stage CKD. After unilateral nephrectomy, the majority of LKD will have an eGFR consistent with CKD stage 2, and in over a third of LKD the final eGFR is <60 ml/min/1.73m^2, putting them into the category of CKD stage 3.(98)

Several studies have examined mortality in LKD compared to population matched controls. Segev *et al.* did not find any increased mortality in LKD compared to a control population drawn from participants in the third National Health and Nutrition Examination Survey, with no significant increased mortality in LKD at median follow-up of 6.3 years.(99) Garg *et al.* showed reduced cardiovascular events in LKD compared to controls and no increase in mortality at up to 10-year follow-up.(100) Similar findings were found by Reese *et al.* among a cohort of donors over the age of

55.(101) However, these studies can be criticised for the lack of an appropriate control group as LKD are often healthier than the general population from which control subjects are drawn. Furthermore, these studies have insufficient duration of follow-up. A Norwegian study with median follow-up of 24 years raised concerns about long term mortality in LKD when compared to a highly select control group who met the eligibility criteria for living kidney donation. All-cause death was significantly increased for LKD compared with controls [hazard ratio 1.30 95% confidence interval (CI) 1.11-1.52]. There were also corresponding significant increases in cardiovascular death (hazard ratio 1.40 95% CI 1.03-1.91) and the risk of ESRD (hazard ratio 11.38 95% CI 4.37-29.6).No difference in all-cause mortality between LKD and controls was seen in the first decade of follow-up, with the survival curves separating after 10 years.(102) This important study has emphasised the necessity of long-term follow-up and research in LKD.

I.8 Cardiovascular changes in living kidney donors

The cardiovascular effects of kidney donation are not fully understood. The current literature is hampered by poor quality studies that are often retrospective and lack appropriate control groups. However, there are possible structural and functional changes to the cardiovascular system in LKD that may lead to increased cardiac risk in this important population.

I.8.1 Hypertension

Data on the subsequent risk of systemic hypertension among LKD are conflicting.(92) Kasiske *et al.* showed no significant difference between LKD and controls in

ambulatory BP at 3-year follow up. However, there were no pre-donation ambulatory BP data available, so change from baseline in each group could not be assessed.(103) Studies by Eberhard *et al.* and Thiel *et al.* suggested that 29-43% of LKD develop hypertension within 10 years of donation. A meta-analysis also showed that on average, donors had a 5mmHg increase in blood pressure compared to controls.(104)

I.8.2 Arterial stiffness

The effect of living kidney donation on arterial stiffness is also unclear. Studies using pulse wave analysis (PWA) and pulse wave velocity (PWV) are conflicting. Fesler *et al.* showed no significant change in either PWA or PWV at 1-year post-nephrectomy. However, this study lacked a control group.(105) By contrast, a cross-sectional study of 101 Lebanese kidney donors demonstrated that PWV was 10% higher than healthy controls with a similar age and sex distribution.(106) An alternative method of assessing arterial stiffness is by measuring aortic distensibility on CMR. Moody *et al.* showed that at 12 months post-nephrectomy, there was a significant reduction in aortic distensibility (i.e. an increase in arterial stiffness) compared to controls.(107) The Effect of A Reduction in glomerular filtration rate after Nephrectomy on arterial Stiffness and central hemodynamics (EARNEST) study was the largest prospective controlled study of arterial stiffness in LKD. It compared carotid-femoral PWV at baseline and at 12 months post-nephrectomy in 168 LKD and compared this to a population of 138 controls who were eligible for kidney donation but did not proceed to donate. There was no significant difference in PWV at 12-months between LKD and controls. There was also no significant difference in the change from baseline PWV between the groups.(108)

1.8.3 Left ventricular structure

Very few studies have examined the effects of living kidney donation on cardiovascular structure and function. Bellavia *et al.* examined LV structure and function using CMR and transthoracic echocardiography (TTE) in a pilot study of 15 LKD and 15 controls and showed no difference in LV mass but a reduction in apical rotation and LV torsion on speckle echocardiography.(109) Hewing *et al.* studied 30 LKD pre- and post-nephrectomy and found no significant differences in left or right ventricular function after uni-nephrectomy.(110) The Chronic Renal Impairment in Birmingham Donor (CRIB Donor) study showed that unilateral nephrectomy is accompanied by a small but significant increase in left ventricular mass index (LVMI) at 12 months in LKD compared to controls, and this was independent of BP.(107) Similar findings were shown by Altmann *et al.* although their study lacked a control group.(111) However, a longer follow-up study of the CRIB Donor cohort showed that the difference in LVMI between LKD and controls seen at 12 months did not persist at 5 years, possibly suggesting that these early cardiovascular changes may be reversible over time.(112)

Although not definitive, the evidence suggests that LKD may have subtle structural and functional changes to the cardiovascular system after uni-nephrectomy. Therefore, there are plausible reasons why these individuals may have increased cardiovascular risk. Whether these cardiovascular changes represent the early stages of uraemic cardiomyopathy remains unknown. Given the increasing number of LKD worldwide, cardiovascular changes and their consequences in LKD require further careful investigation.

Section II: Coronary microvascular dysfunction

II.1 Coronary microvascular dysfunction – a potential contributor to cardiovascular risk in chronic kidney disease?

Although it is clear that the clinical syndrome of uraemic cardiomyopathy is associated with an adverse prognosis, the underlying pathophysiological mechanisms leading to this condition are not well understood. Some insights may be drawn from HFpEF, a condition that shares many phenotypical similarities with uraemic cardiomyopathy. Both HFpEF and uraemic cardiomyopathy are characterised by LVH, fibrosis, diastolic dysfunction, reduced systolic strain, and a predisposition to SCD.(113,114) They also share similar altered ventricular mechanics - patients with CKD have elevated ventricular and arterial elastances with preservation of the arterio-ventricular coupling ratio, a pattern characteristic of HFpEF.(115) Although initially considered a benign condition, there is now a weight of evidence that HFpEF is associated with significant morbidity and mortality.(116)

A developing body of literature suggests that coronary microvascular dysfunction (CMD) may play a key role in HFpEF. In the multicentre PROMIS HFpEF trial, 75% of patients with HFpEF had evidence of CMD and this was associated with kidney damage, as measured by albuminuria, as well as systemic arterial dysfunction.(117) The presence of CMD in HFpEF shows a linear association with markers of diastolic dysfunction (decreasing e' and increasing E/e'), with a significant reduction in diastolic function with worsening CMD.(118) This correlates with autopsy studies showing the presence of increased fibrosis and reduced capillary microvascular density in the hearts of patients with HFpEF compared to age-appropriate controls.(119)

Given the similarities between the two conditions, HFpEF may be a useful paradigm for uraemic cardiomyopathy. It raises the possibility that CMD also plays a role in the development of uraemic cardiomyopathy. A common consequence of the various disparate mediators implicated in the pathogenesis of uraemic cardiomyopathy may be the development of pathological changes in the coronary microcirculation. The coronary microcirculation and the potential role of CMD in the pathogenesis of cardiovascular disease in CKD forms the basis of this thesis.

II.2 The structure and function of the coronary circulation

The coronary circulation consists of both the epicardial coronary arteries and the vast network of small vessels that make up the coronary microcirculation – figure 1-7. The human heart functions primarily as an aerobic organ and the myocardium extracts 60-80% of oxygenated blood from the coronary circulation even during resting conditions. Thus, the main mechanism to increase myocardial oxygen delivery during periods of increased oxygen demand is to increase coronary blood flow (CBF).(120)

Coronary blood flow is characterised by marked phasic variations throughout the cardiac cycle. During systole, cardiac contractions lead to an increase in myocardial pressure to values equal to LV systolic pressure. As a result, there is impaired coronary arterial inflow but an increase in coronary venous outflow. By contrast, in diastole, coronary arterial inflow increases with a transmural gradient that favours perfusion to the subendocardial layers, and a reduction in coronary venous outflow.(121)

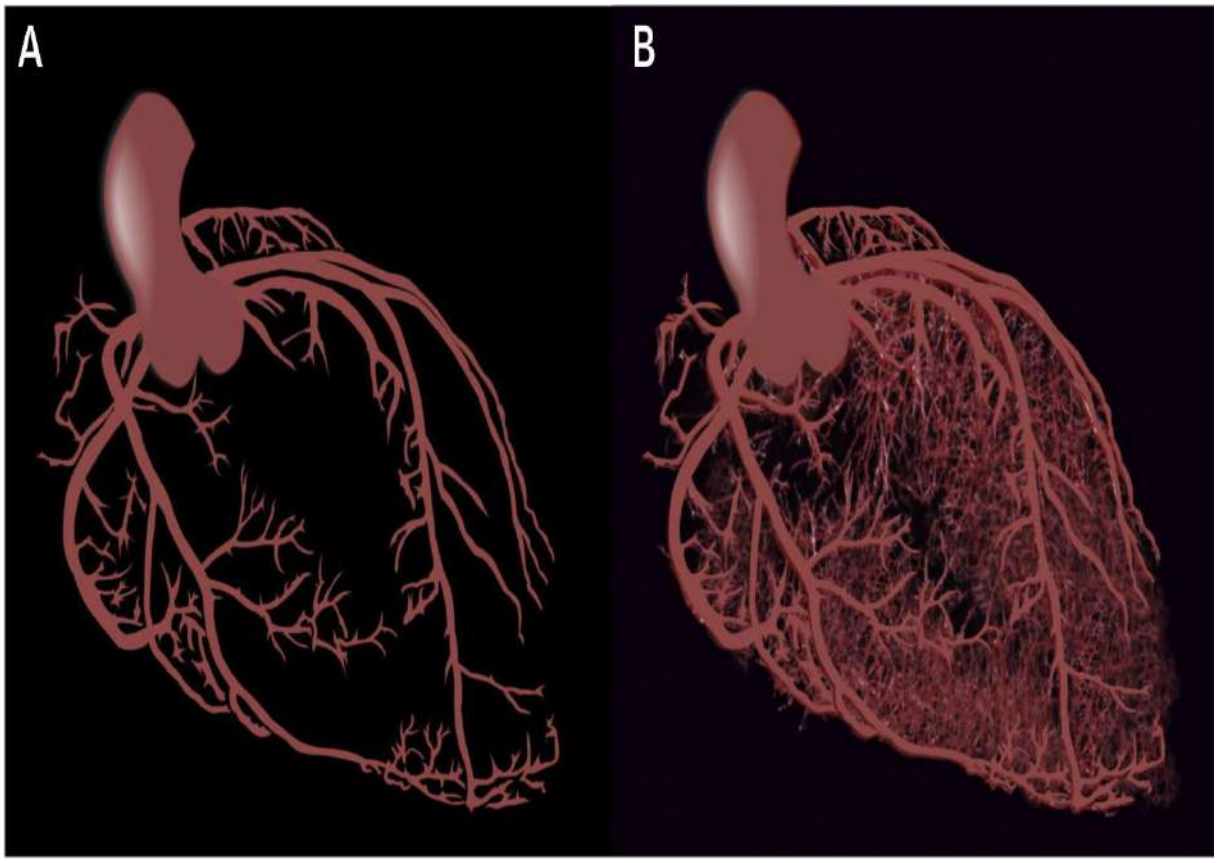


Figure 1-7: Schematic representation of the coronary circulation. Panel A shows the epicardial coronary arteries. Panel B shows the entire coronary circulation including the microcirculation. Reproduced with permission from Taqueti *et al.*(122)

Coronary blood flow is determined by the interplay between coronary perfusion pressure and diastolic filling time – the so-called diastolic pressure-time integral.(123) Coronary perfusion pressure relies on the pressure gradient between mean diastolic aortic pressure and LV diastolic pressure, and is maintained over a wide range of pressures through autoregulatory mechanisms in the coronary circulation. Heart rate (HR) is also an important consideration, as coronary filling occurs solely during diastole. In periods of tachycardia, with shortened diastolic filling time, CBF will be reduced. Any factor that reduces coronary perfusion pressure or increases HR will influence CBF, and by extension myocardial oxygen supply – figure 1-8.(121,123)

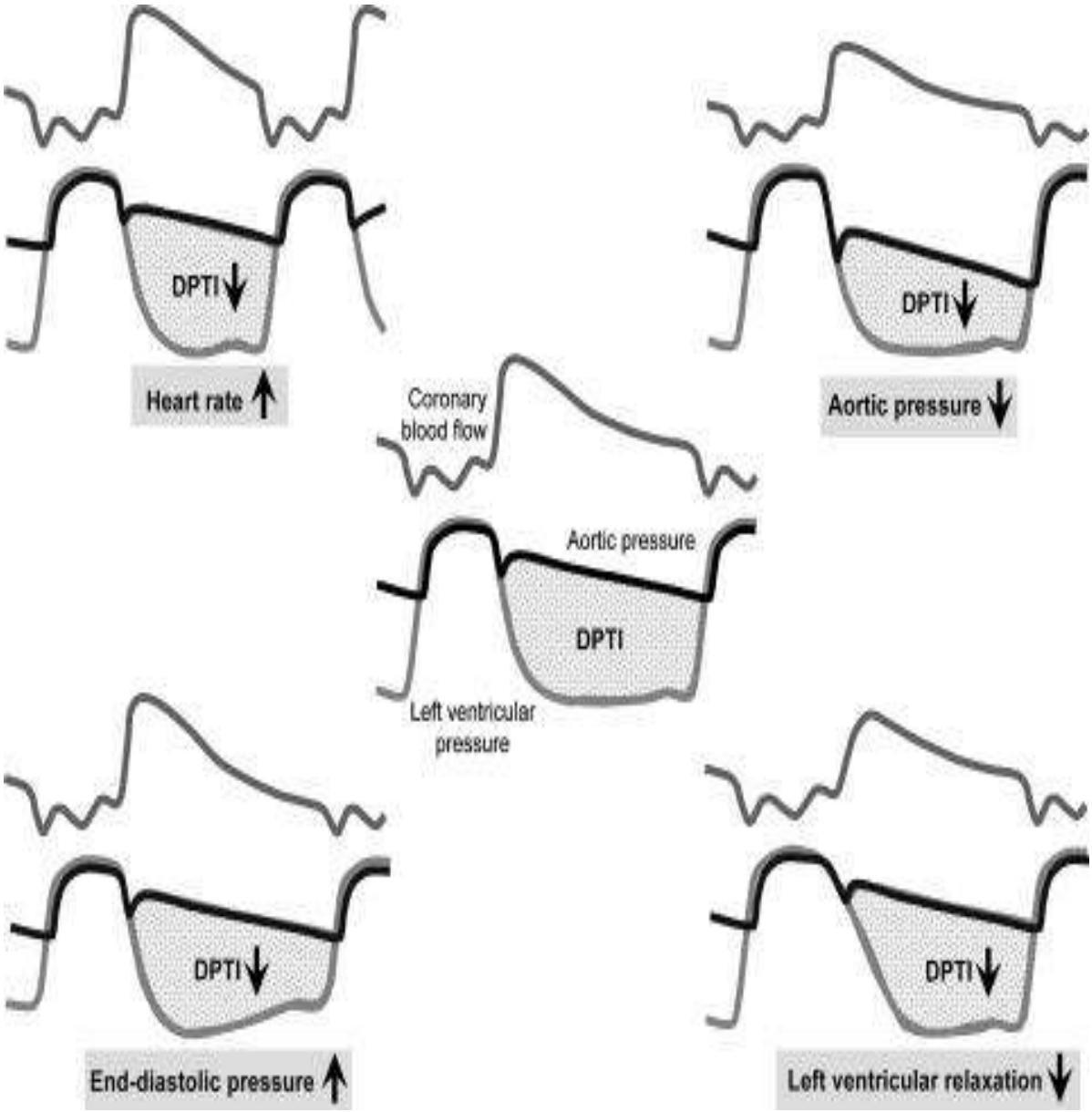


Figure 1-8: Schematic diagram of the diastolic pressure-time integral. Any decrease in diastolic aortic pressure (upper right), increase in diastolic ventricular pressure (lower left), delay in isovolumic ventricular relaxation (lower right) and decrease in diastolic duration (upper left) impedes coronary blood flow. DPTI – diastolic pressure-time integral. Reproduced with permission from Heusch *et al*.(123)

To further understand the coronary circulation, Chilian proposed an elegant model dividing the coronary circulation into three anatomically distinct but functionally interlinked compartments – figure 1-9.(124) The proximal compartment consists of the most well-known element of the coronary circulation – the epicardial coronary arteries. Their primary role is the delivery of oxygenated blood to the coronary circulation. They function as capacitance vessels and respond to changes in coronary blood flow by endothelial mediated dilatation with the aim of maintaining a constant level of shear stress.(125,126) During normal conditions, there is no measurable pressure drop in the epicardial arteries, and thus they contribute very little to coronary vascular resistance. However, once a stenosis >50% develops, then epicardial coronary artery resistance starts to increase and when the vessel is severely narrowed (>90% stenosis), basal CBF may fall.(121)

The middle compartment consists of pre-arterioles that are characterised by a measurable pressure drop along their length.(124) Their role is to maintain pressure at the origin of the arterioles within a narrow range, despite changes in coronary perfusion pressure or flow.(125)

The distal compartment consists of the intramural arterioles that have diameters <100μm, and are responsible for the majority of coronary vascular resistance and autoregulation of coronary perfusion pressure.(127) These vessels have high resting tone and constrict and dilate in response to myogenic stimuli.(125,128) The mechanisms behind this myogenic response are not fully understood but are likely to involve vascular smooth muscle calcium entry, leading to an increase in intracellular

calcium concentration that activates several downstream signalling pathways.(129) *In vitro* experiments show that this myogenic response does not depend on an intact endothelium.(130,131) In addition to myogenic stimuli, the intramural arterioles are also sensitive to local metabolic stimuli. A number of vasoactive mediators, including adenosine, hydrogen peroxide and endothelium derived relaxing factors act directly on the endothelium of these arterioles to produce vasodilatation.(128) Nitric oxide (NO) appears to be a key mediator with animal studies showing attenuated vasodilatation of the coronary microvasculature when NO synthesis is inhibited.(128)

Finally, the capillary bed delivers oxygen and substrates to the myocytes. The capillary density of the normal myocardium averages 3500/mm^2, which is far greater than the capillary density in other organs (skeletal muscle 400/mm^2) and is a physiological adaptation to the high oxygen demand of the myocardium.(132)

Thus, the coronary circulation is a complex homeostatic system that matches myocardial oxygen demand with supply through a series of interlinked components including myogenic tone, metabolic signals, circulating hormones and the intrinsic properties of the endothelium.(124) Despite the attention given to diseases affecting the proximal epicardial coronary arteries, it is in fact the coronary microcirculation that plays the major role in substrate delivery to the myocardium. Therefore, impaired microvascular function is likely to play a key role in the pathogenesis of many cardiovascular conditions.

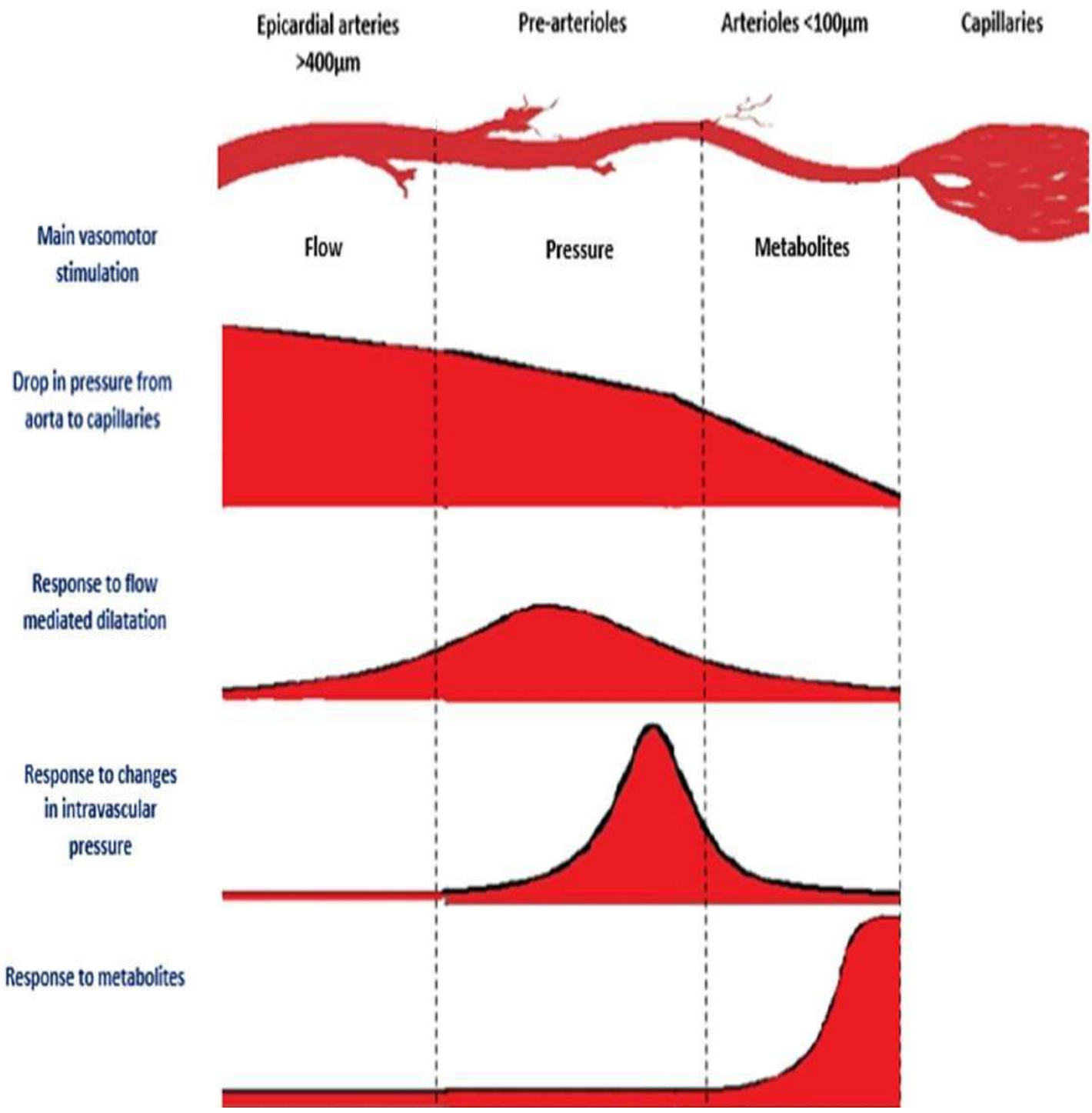


Figure 1-9: Functional anatomy of the coronary circulation. Reproduced with permission from Radhakrishnan *et al*.(93)

II.3 Assessing coronary microvascular function

Coronary flow reserve (CFR) is the most widely used parameter to measure coronary microvascular function. It is a measure of the magnitude of increase in CBF from a resting state compared to maximal hyperaemia. Since the majority of coronary flow resistance is in the microcirculation, CFR is primarily a measure of the ability of small vessels to respond to a vasodilatory stimulus and thus provides a surrogate measure of their function.(125)

To calculate CFR, hyperaemia is induced with a pharmacological vasodilator, and CFR is measured as the ratio of maximal hyperaemic to resting flow.(93) In modalities where flow velocity is measured (Doppler TTE, Doppler angiography), it is more accurate to refer to the surrogate parameter - coronary flow velocity reserve (CFVR). Unlike CFR based techniques that directly estimate CBF, CFVR based techniques rely on the assumption that the diameter of the coronary artery at hyperaemia remains constant.(133) Thus, at a constant vessel diameter, an increase in flow velocity through the vessel (which can be measured with Doppler) equates to an increase in flow through it. The two parameters are both widely described and used in the literature.

Both CFR and CFVR do not discriminate between epicardial coronary artery function or microvascular dysfunction. Haemodynamically significant epicardial coronary stenoses reduce both CFR and CFVR.(134,135) Therefore, exclusion of significant obstructive CAD is necessary before reduced CFR or CFVR can be attributed to CMD.(125)

In normal subjects, CBF should at least double at hyperaemia. However, animal and human cardiac catheterisation studies have shown that during hyperaemic states, such as exercise, CBF can increase up to 5-fold to meet myocardial oxygen demand.(136–138) Although CFR is a continuous variable, by convention, CMD is defined as CFR (or CFVR) <2, provided that there is no concurrent obstructive CAD.(93)

II.4 Pharmacological vasodilators for coronary flow reserve testing

A variety of different pharmacological agents are available to induce hyperaemia. Their properties are summarised in table 1-2.

Papaverine is an opiate derivative that acts as a phosphodiesterase inhibitor, leading to increased cyclic adenosine monophosphate and a direct relaxant effect on vascular smooth muscle.(139) It is administered predominantly as an intracoronary agent and produces coronary hyperaemia without a significant effect on systemic HR or BP. The hyperaemic effect of papaverine is similar to both adenosine and dipyridamole.(140,141) However, it is rarely used in modern practice due to its association with ventricular arrhythmias.(142)

Dipyridamole is another mainly historic agent. It acts as an indirect adenosine receptor agonist by inhibiting the enzyme adenosine deaminase, leading to reduced cellular reuptake of endogenous adenosine. Due to its indirect action, it has a slower onset of action than adenosine but a similar side effect profile, albeit with fewer respiratory side effects.(143) It is not widely used in the UK.

Adenosine is the most widely used agent in the UK. It is a naturally occurring endogenous purine nucleoside consisting of an adenine molecule attached to a ribose sugar moiety.(144) Intracellular adenosine occurs both due to de novo synthesis and breakdown of adenosine tri-phosphate. Adenosine acts directly on 4 specific adenosine receptors – A1, $A2_A$, $A2_B$ and A3 – which are expressed widely on endothelial and smooth muscle cells throughout almost all tissues and organs in the body.(137,145) It has the highest affinity for the $A2_A$ and $A2_B$ receptors, activation of which causes potent vasodilatation in most vascular beds including the coronary microcirculation.(137,146)

Adenosine has several properties that make it an ideal agent for use in CFR testing. It is widely available, cheap and has a short onset and offset of action (half-life <30 seconds).(137,143) Additionally, its delivery as an infusion allows the dose to be increased in the case of inadequate response. Furthermore, it predominantly causes vasodilation in vessels with diameter <150µm, where the majority of coronary flow resistance occurs.(147) This makes adenosine a suitable agent for CFVR agent testing, as the epicardial coronary arteries remain a constant size during hyperaemia.

Adenosine use is associated with a number of different side effects, due to its non-selective agonism of all adenosine receptor subtypes. The Adenoscan multicentre registry of over 9000 patients undergoing adenosine single photon emission computed tomography (SPECT) identified side effects in 81% of patients. The most common reported side effects were flushing (36.5%), dyspnoea (35.2%), chest pain (34.6%), gastrointestinal discomfort (14%), and headache (11%).(148) However, this is offset

by adenosine's short half-life, which allows rapid resolution of side-effects within 30 seconds of cessation of the drug.(143)

Regadenoson is a newer agent that is increasingly used in pharmacological stress testing. It acts directly on the $A2_A$ receptor to cause vasodilatation. Importantly, it has little effect on the $A2_B$ receptor, which is expressed on mast cells, and activation of which can cause bronchoconstriction.(143,149) In general, the side effect profile of regadenoson is more favourable than that of adenosine, with a lower incidence of common side-effects – dyspnoea (24%), chest discomfort (16%), and chest pain (13%), headache (16%) and gastrointestinal upset (6%).(150) However, this comes at the expense of a longer duration of action (initial half-life of 4 minutes followed by intermediate phase with average half-life of 30 minutes which coincides with loss of pharmacodynamic effect) and increased cost.(143)

Both stress perfusion CMR and myocardial perfusion imaging studies have shown similar results using adenosine and regadenoson.(151,152) The main safety concern with use of these agents is the development of atrio-ventricular block. In a meta-analysis of 34 studies using adenosine or regadenoson for pharmacological stress in myocardial perfusion imaging, overall rates of high grade atrio-ventricular block were low (1.93%). However, rates were higher with adenosine compared to regadenoson (5.21%; 95% CI 2.81%–8.30% vs 0.05%; 95% CI <.001%–0.19% respectively, $p<0.001$).(153) Thus, both agents are safe in clinical practice, although appropriate electrocardiographic (ECG) and BP monitoring are necessary.

Table 1-2: Properties of pharmacological agents used for vasodilator stress

Agent	***Class***	***Mechanism of action***	***Mode of administration***	***Dose***	***Half life***	***Increase in coronary blood flow***
Adenosine	Direct vasodilator	Non-selective adenosine receptor agonist	IC bolus or IV infusion	30-60mcg (LCA) 20-30mcg (RCA) 140mcg/kg/min (iv)	<30s	3.5-4 times baseline
Dipyridamole	Indirect vasodilator	Non-selective adenosine receptor agonist	IV infusion	142mcg/kg/min	30-45 min	3.8-7 times baseline
Papaverine	Direct vasodilator	Phosphodiesterase inhibitor	IC bolus	12mg (LCA) 8mg (RCA)	2 min	4-5 times baseline
Regadenoson	Direct vasodilator	Selective adenosine $A2_A$ receptor agonist	IV bolus	400mcg	Initial 2–4 min; intermediate 30 min; Terminal 2 h	2.5 times baseline

IC: intracoronary, IV: intravenous, LCA: left coronary artery, RCA: right coronary artery, s: seconds, min: minutes.

II.5 Modalities for measurement of coronary microvascular function

Coronary microvascular function can be measured using many different parameters and modalities – table 1-3. An overview of the available techniques is provided in this section. There is no widely accepted “gold standard” technique for the measurement of CFR and choice of modality depends on local resources and expertise.

II.5.1 Invasive angiography

Coronary flow reserve can be assessed at the time of invasive coronary angiography. The use of coronary angiography allows definitive exclusion of CAD, but its invasive nature exposes patients to infrequent but significant risks including vascular injury, stroke, contrast nephropathy and death. Two distinct techniques exist for the measurement of CFR by invasive angiography.

Firstly, CFVR can be assessed using intracoronary Doppler ultrasound. An angioplasty wire tipped with a high frequency piezoelectric Doppler transducer is used to measure flow velocities in a coronary artery at rest and at hyperaemia – figure 1-10. Coronary flow velocity reserve is the ratio of hyperaemic/resting coronary flow velocities.(154) The technique can be challenging to perform, with Everaars *et al.* showing that only 57% of intracoronary Doppler tracings were good quality on visual inspection.(155) Furthermore, reproducibility of the technique is poor, with a small study of 26 patients showing relatively high limits of agreement (LOA 27-39%) for repeat intracoronary Doppler measurements.(156)

Coronary flow reserve can also be measured by intracoronary thermodilution using a pressure wire positioned in the distal third of the target vessel. The properties of the wire allow the shaft to act as a proximal thermistor, while a sensor at its tip acts as a distal thermistor. After injection of a bolus of normal saline at room temperature down the coronary artery being assessed, coronary flow can be assessed using the formula:

$$F = V \times T_{MN}$$

where F is flow, V is the volume of blood between the tip of the guide catheter and the distal thermistor, and T_{MN} is the transit time derived from the thermodilution curve. Coronary flow reserve is the ratio of hyperaemic/baseline flow.

Fearon *et al.* used a pig model to compare both intracoronary Doppler and intracoronary thermodilution against a reference standard (CFR_{FLOW}) consisting of an external flow probe that measured flow directly in the left anterior descending (LAD) artery. Although both techniques correlated well with CFR_{FLOW}, the correlation was stronger with the thermodilution technique (r=0.85 vs r=0.72). Furthermore, the thermodilution technique had a closer agreement with CFR_{FLOW} than intracoronary Doppler.(157) Similarly, Everaars *et al.* also demonstrated that CFR by thermodilution was more likely to produce good quality data, and had lower inter-observer variability than intracoronary Doppler, suggesting that the thermodilution technique may be the more reliable invasive technique for measurement of CFR.(155)

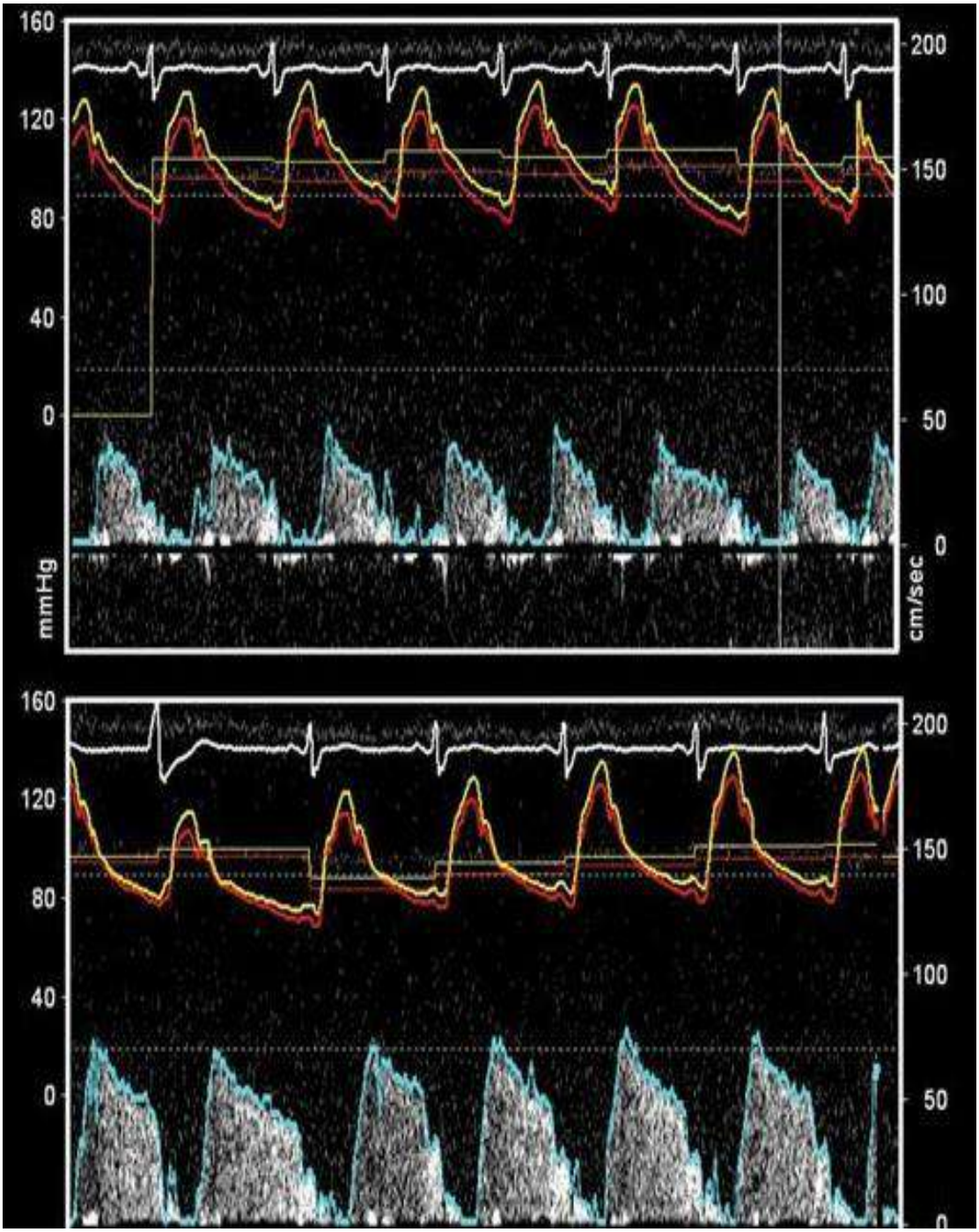


Figure 1-10: Resting (top) and hyperaemic (bottom) coronary flow velocity by intracoronary Doppler. Adapted with permission from Amier *et al*.(158)

II.5.2 Quantitative positron emission tomography

Positron emission tomography (PET) involves the use of a gamma camera to detect photons released by the breakdown of a radionucleotide in a tissue of interest.(159) It is the most well studied non-invasive modality for quantifying myocardial blood flow (MBF) and has been validated against microsphere blood flow studies in animal models.(160) To measure MBF, a flow tracer that increases linearly with coronary flow, has high first pass extraction and no significant recirculation is required.(159) Examples include ^{15}O-water, ^{13}N-ammonia and ^{82}Rb.

Absolute values of MBF at rest and during hyperaemia can be calculated with PET. Coronary flow reserve is calculated as MBF hyperaemia/ MBF rest – figure 1-11. In addition to CFR, PET allows assessment of regional blood flow, myocardial scar and myocardial ischaemia.(159) Quantification of MBF and calculation of CFR by PET has excellent inter-observer reproducibility in experienced centres, with an intra-class correlation coefficient (ICC) of 0.94 (95% CI 0.88–0.98 reported by Murthy *et al.* in their series of 2783 studies from Boston.(161) The technique also correlates with CFVR by intracoronary Doppler ($r=0.82$, $p<0.001$) and CFR by thermodilution ($r=0.55$, $p<0.001$).(155)

Disadvantages of PET include exposure to ionising radiation, high cost, difficulties in procuring radio-isotopes and the relative unavailability of quantitative PET in the UK.

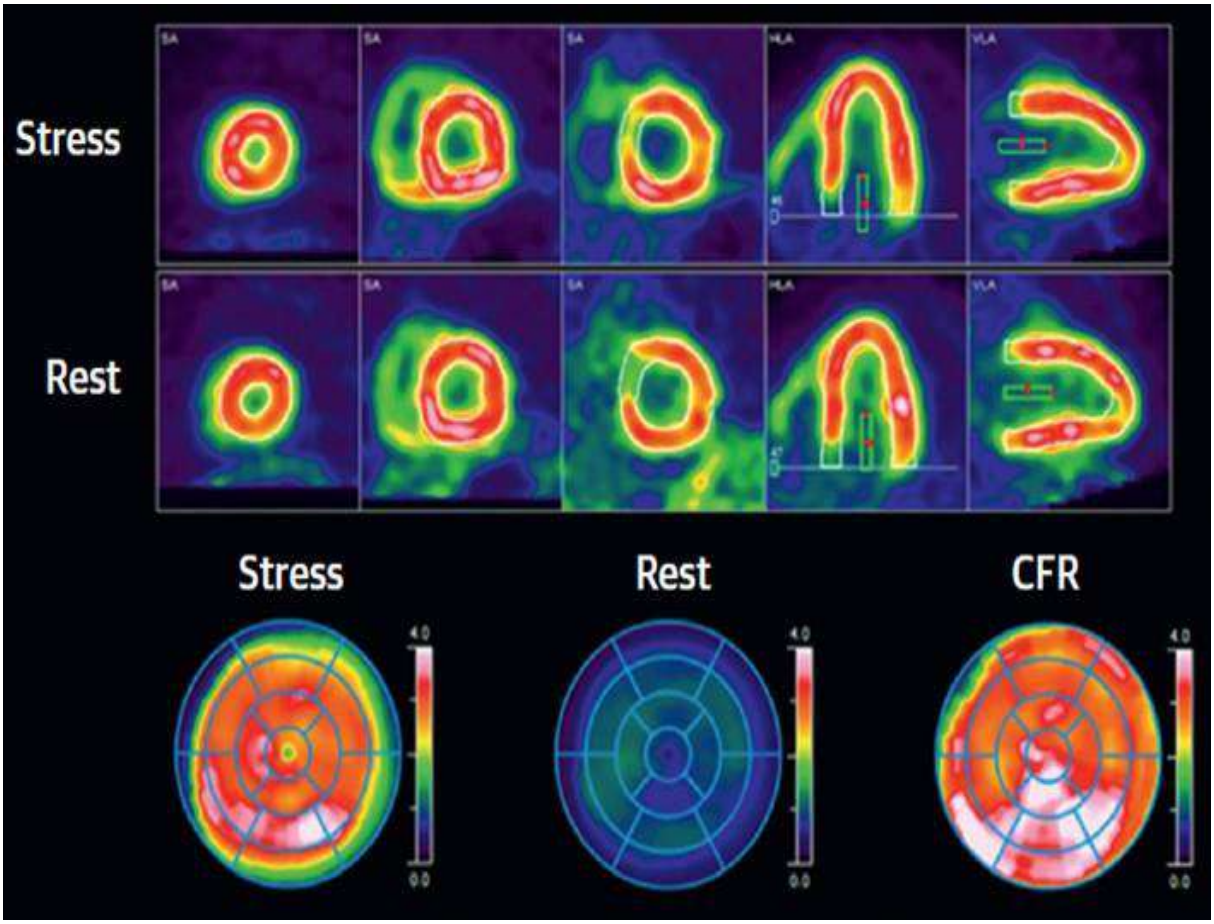


Figure 1-11: Positron emission tomography images at rest and at stress to allow calculation of coronary flow reserve. Adapted with permission from Feher *et al.*(159)

II.5.3 Cardiac magnetic resonance imaging

A number of different methods of assessing CFR by CMR exist. The most well established CMR technique involves stress perfusion imaging. After administration of gadolinium, first pass imaging of the myocardium is performed to assess myocardial perfusion in basal, mid-ventricular and apical short axis slices at rest and during vasodilator stress. Relative upslope (RU) is calculated as the ratio of the maximum upslope of the myocardial segment divided by the maximum upslope of the left ventricular cavity – figure 1-12.

Coronary flow reserve is represented by stress RU/rest RU.(162,163) In addition to calculating CFR, stress perfusion CMR can localise coronary artery lesions and myocardial viability can be assessed using late gadolinium enhancement. The technique shows good intra- and inter-observer reproducibility (ICC 0.89 and 0.80 respectively) and also correlates well with intracoronary Doppler (r=0.8).(164,165)

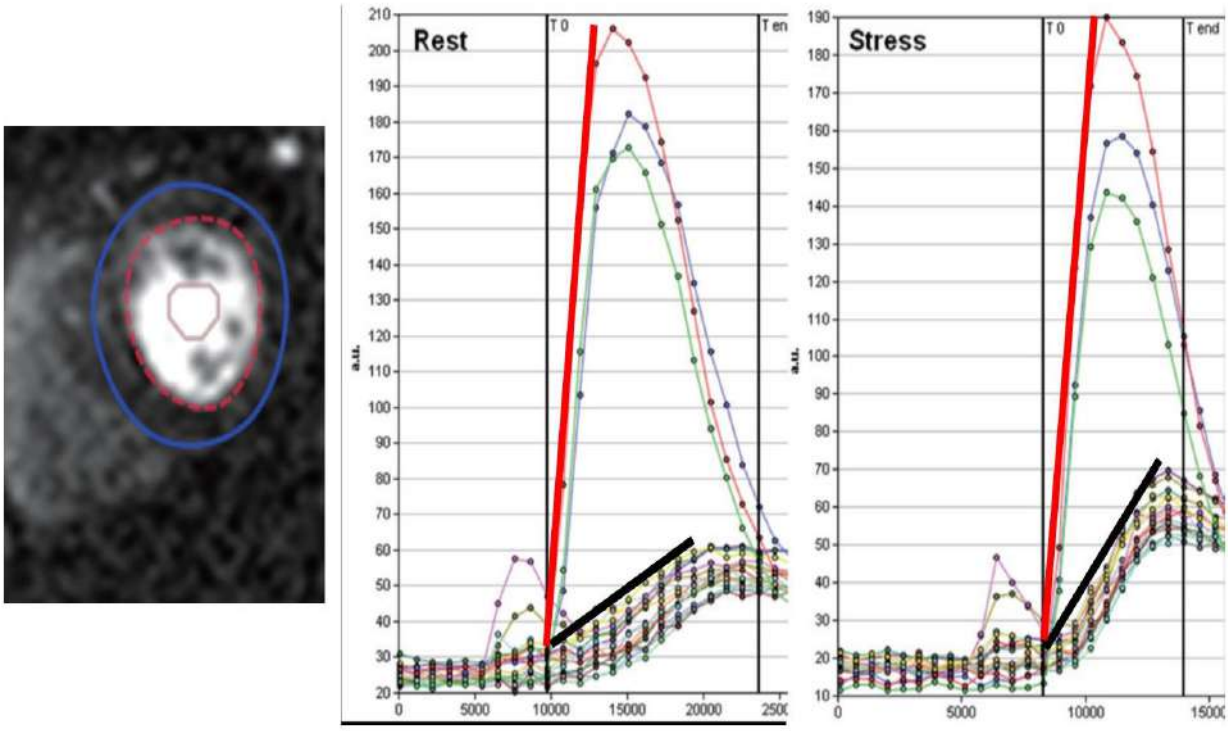
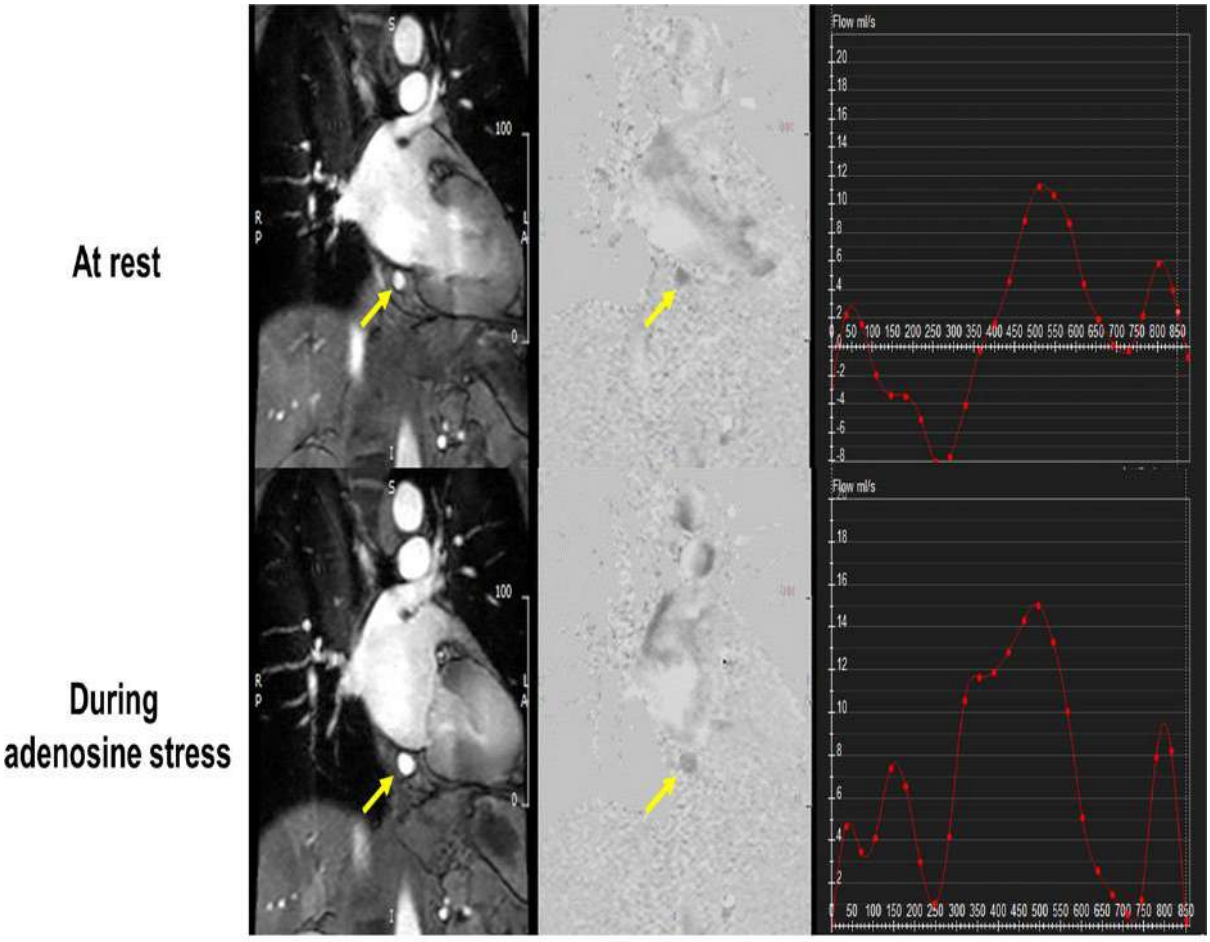


Figure 1-12: Coronary flow reserve calculated by first pass perfusion. Left panel shows epicardial and endocardial contours used to generate time-intensity curves. Middle and right panels show time-intensity curves at rest and stress. Relative upslope is calculated as the ratio of the maximum upslope of the myocardial segments (thick black line) divided by maximum upslope of left ventricular cavity curve (thick red line). The slope of the stress perfusion curve is steeper than that of rest perfusion. Adapted with permission from Shufelt *et al.*(163)

Coronary flow reserve can also be assessed by measuring coronary sinus flow (CSF) – figure 1-13. This relies on the principle that the majority of blood from the epicardial ventricular veins drain into the coronary sinus. Therefore, changes in CSF are an indirect measure of MBF. The coronary sinus can be visualised on CMR using velocity

encoded cine sequences. Coronary sinus flow is the product of the cross-sectional area of the coronary sinus and the mean velocity of blood in it. Measuring CSF at rest and at hyperaemia allows calculation of CFR, with good intra- (ICC 0.88, 95% CI 0.6-0.97) and inter-observer (ICC 0.83, 95% CI 0.46-0.96) reproducibility of the technique seen in the study Nakamori *et al.*(166)



$$\text{Global CFR} = \frac{\text{Stress MR coronary sinus flow (mL/min)}}{\text{Rest MR coronary sinus flow (mL/min)}}$$

Figure 1-13: Coronary sinus flow at rest and during adenosine stress to calculate coronary flow reserve. CFR – coronary flow reserve. MR – magnetic resonance. Reproduced with permission from Nakamori *et al*.(166)

II.5.4 Doppler transthoracic echocardiography

Assessment of CFVR by Doppler TTE is a well-established non-invasive method of assessing coronary microvascular function. After identification of the distal LAD, coronary flow velocity (CFV) is measured at rest and at maximal hyperaemia to calculate CFVR.

Advantages of the technique include its portability, low cost and lack of ionising radiation.(93) Doppler TTE correlates well with both invasive and non-invasive measures of CFR. Lethen *et al.* showed an excellent correlation between Doppler TTE and intracoronary Doppler measurements ($r=0.8$, $p<0.001$).(167) When CFVR was categorised as a binary variable (normal or abnormal), Caiati *et al.* demonstrated a 97% agreement between Doppler TTE and intracoronary Doppler.(154) Similarly, multiple studies have demonstrated significant correlations between Doppler CFVR and CFR by PET, with correlation coefficients ranging from 0.5-0.9 in the different studies.(168,169)

Although poor acoustic windows and reduced image quality are limitations of ultrasound based imaging modalities, assessment of CFVR by Doppler TTE has high feasibility even in obese patients, due to the superficial position of the LAD.(117,169) Feasibility of Doppler CFVR is further increased through the use of contrast agents that aid identification of the LAD, as well as improving the quality of spectral Doppler traces. Success rates of up to 97% have been reported with the use of contrast agents.(170) The technique will be further described in Chapter 2. Reproducibility of the technique will be discussed in Chapter 3.

II.5.5 Myocardial contrast echocardiography

Myocardial contrast echocardiography (MCE) is a rapid, easy to perform and safe technique for the bedside assessment of myocardial perfusion. Through the use of contrast microbubbles that have a lower diameter than red blood cells and remain intravascular in the circulation, direct quantification of myocardial perfusion is possible.(171) After destruction of microbubbles with a high intensity ultrasound beam, MBF and CFR can be calculated by assessing the rate and pattern of microbubble replenishment. Calculation of MBF by quantitative MCE correlates closely with measurements made using radiolabelled microspheres and PET.(172,173) A single small study compared CFR by MCE with CFVR by intracoronary Doppler but did not show any significant correlation between the two techniques. However, the study only included 10 patients, and may have been underpowered to investigate agreement between the techniques.(174)

Although predominantly used as a research tool, MCE has been shown to be of benefit in diagnosing CAD, with good concordance with SPECT and invasive coronary angiography for the localisation of coronary lesions.(171,175) It also has a role in assessing myocardial viability and predicting prognosis in CAD and heart failure.(176–178) Combination of MCE with traditional stress echocardiography and wall motion assessment can provide a comprehensive structural and functional cardiac assessment.(179) Quantitative MCE will be described further in Chapter 2. Reproducibility of the technique will be discussed in Chapter 3.

Table 1-3: Modalities for assessing coronary flow reserve and coronary flow velocity reserve

Technique	*Parameter*	*Advantages*	*Disadvantages*
Coronary sinus flow	CFR	1. Non-invasive 2. Sequences and analysis quick to perform	1. Contraindications to MRI limits its utility 2. Limited availability and expertise with the technique
Doppler transthoracic echo	CFVR	1. Non-invasive 2. Cheap 3. Portable 4. Feasible even with poor image quality	1. Only validated for LAD territory
First pass perfusion	CFR	1. Non-invasive 2. Can assess myocardial ischaemia and scar 3. Can assess myocardial viability	1. Contraindications to MRI limits its utility 2. Requires gadolinium limiting its use in CKD 3. Scan sequences can be lengthy to perform and analyse 4. Limited availability and expertise with the technique
Invasive angiography Doppler Thermodilution	 CFVR CFR	1. Definitive exclusion of epicardial coronary artery disease 2. Widely available	1. Invasive procedure 2. Ionising radiation
Myocardial contrast echo	CFR	1. Non-invasive 2. Cheap 3. Portable 4. Allows calculation of regional and global myocardial blood flow	1. Requires good acoustic windows
Positron emission tomography	CFR	1. Non-invasive 2. Can assess myocardial ischaemia and scar 3. Can assess myocardial viability 4. Allows calculation of global and regional flow	1. Ionising radiation 2. Shortage of radio-isotopes 3. Limited availability in UK

CFR: coronary flow reserve, CFVR: coronary flow velocity reserve, HMR: hyperaemic microcirculatory resistance, IMR: index of microcirculatory resistance, UK: United Kingdom, MRI: magnetic resonance imaging, LAD: left anterior descending artery

II.6 Coronary microvascular dysfunction in cardiovascular disease

Coronary microvascular dysfunction is extremely common in many different cardiac conditions, where it is independently associated with an adverse prognosis.

A number of studies have demonstrated a high prevalence of CMD in HCM. Coronary flow reserve is inversely correlated with degree of LVH, and is lower in patients with left ventricular outflow tract obstruction.(180) Coronary microvascular dysfunction is also an adverse prognostic marker in HCM, with Nemes *et al*. showing that a CFVR <2.35 by Doppler TTE was associated with a significantly greater risk of cardiovascular death at 9 years follow-up [HR 4.21 (95% CI 1.01–19.22, p< 0.05)].(181) A larger PET study by Cecchi *et al*. demonstrated that being in the lowest tertile of CFR was associated with an age-adjusted hazard ratio of 9.6 for cardiovascular death.(182)

As described previously, there is a high prevalence of CMD among patients with HFpEF. The PROMIS HFpEF study identified the presence of CMD in 75% of a multinational cohort of patients with HFpEF. However, the study used a cut-off value of CFVR <2.5 to define CMD, which may have overestimated the prevalence compared to studies using the traditional cut-off value of CFVR <2. A more recent invasive angiography study by Rush *et al.* in a smaller cohort of Scottish patients showed that among patients with HFpEF, who had no angiographic evidence of obstructive CAD, 81% had evidence of CMD, suggesting a truly high prevalence of CMD among patients with HFpEF.(183) One year follow-up data from the PROMIS HFpEF study has shown that patients with CMD had a significantly increased risk of cardiovascular death or

heart failure hospitalisation than the cohort with normal microvascular function, suggesting that CMD is also an adverse prognostic marker in this population.(184)

Similarly, among patients with dilated cardiomyopathy (DCM), there is a significant reduction in CFR compared to healthy controls. The degree of CMD is related to severity of left ventricular systolic impairment.(185) Abnormal CFR in DCM patients is an independent predictor of major adverse cardiovascular events (MACE)(186), and preserved CFR prior to implant predicts response to cardiac resynchronisation therapy.(187)

Epicardial and microvascular coronary disease often co-exist. There is a high prevalence of CMD in patients investigated for chest pain, with multiple studies suggesting that the presence of CMD is an adverse prognostic marker, even in the absence of significant CAD. Westergren *et al.* showed that among non-diabetic patients with normal myocardial perfusion scans, CFVR <2 was associated with a significantly higher incidence of MACE.(188) Similarly, Cortigiani *et al.* showed that CFVR <2 was a strong and independent predictor of mortality among patients investigated for chest pain who had no wall motion abnormalities on stress echo.(189) Among patients with non-obstructive CAD on coronary angiography, normal LV function and no stress inducible ischaemia, the presence of CMD identified by Doppler TTE was associated with an increased risk of MACE at 48-months follow-up.(190) Murthy *et al.* showed that diabetic patients with CMD but no CAD had rates of cardiac death comparable to non-diabetic patients with CAD.(191) Among patients who underwent invasive angiography in the Women's Ischemia Syndrome Evaluation

study, women with CFR <1.6 had a significantly increased risk of MACE even after adjustment for cardiovascular risk factors, despite significantly higher rates of non-obstructive CAD.(192) These studies highlight the significant morbidity and mortality associated with microvascular disease.

Indeed, there is some evidence that the presence of CMD may be a worse prognostic marker in cardiovascular disease than the presence of obstructive CAD. Van de Hoef *et al.* performed a retrospective analysis of 157 patients with intermediate coronary lesions, who had percutaneous coronary intervention (PCI) deferred. At 10-years follow-up, patients with CMD and non-obstructive CAD had a worse prognosis than patients with obstructive CAD and normal microvascular function – figure 1-14.(193) The DEFINE FLOW study was an open label multinational unblinded study that measured both CFVR and fractional flow reserve (FFR) in 430 patients undergoing coronary angiography. Fractional flow reserve is a parameter used to assess the significance of an epicardial coronary artery stenosis. A value ≤0.8 indicates a flow-limiting lesion. Patients were divided into 4 groups on the basis of their coronary physiology – FFR >0.8 & CFVR ≥2 (medical therapy), FFR >0.8 & CFVR <2 (medical therapy), FFR ≤0.8 & CFVR ≥2 (medical therapy), FFR ≤0.8 & CFVR <2 (revascularisation by PCI). The aim of the study was to show non-inferiority at 2-year follow-up in the FFR ≤0.8 & CFVR ≥2 group compared to the FFR >0.8 & CFVR ≥2 group, both of which were treated with medical therapy. However, a significantly higher MACE rate was seen in the FFR ≤0.8 & CFVR ≥2 group (difference in MACE rate 5%, p value for non-inferiority =0.065). Interestingly, the 2-year MACE rate was higher among patients with normal FFR and abnormal CFVR (FFR >0.8 & CFVR <2) than

among those with abnormal FFR and normal CFVR (FFR ≤0.8 & CFVR ≥2) – 12.4% vs 10.8%.(194) Although the study was not powered to assess the significance of this difference, this once again suggests that microvascular dysfunction may be a worse prognostic marker than obstructive CAD.

Thus, it is clear that CMD is prevalent across a wide spectrum of CVD. Similar to these populations, patients with CKD have a number of substrates that predispose to CMD including comorbidities (hypertension, diabetes), structural abnormalities (LVH) and functional abnormalities (endothelial dysfunction, impaired LV systolic and diastolic function.) Coronary microvascular dysfunction provides a compelling and plausible explanation for the pathogenesis and consequences of cardiovascular disease in CKD. The current evidence for CMD in CKD will be discussed in section III.

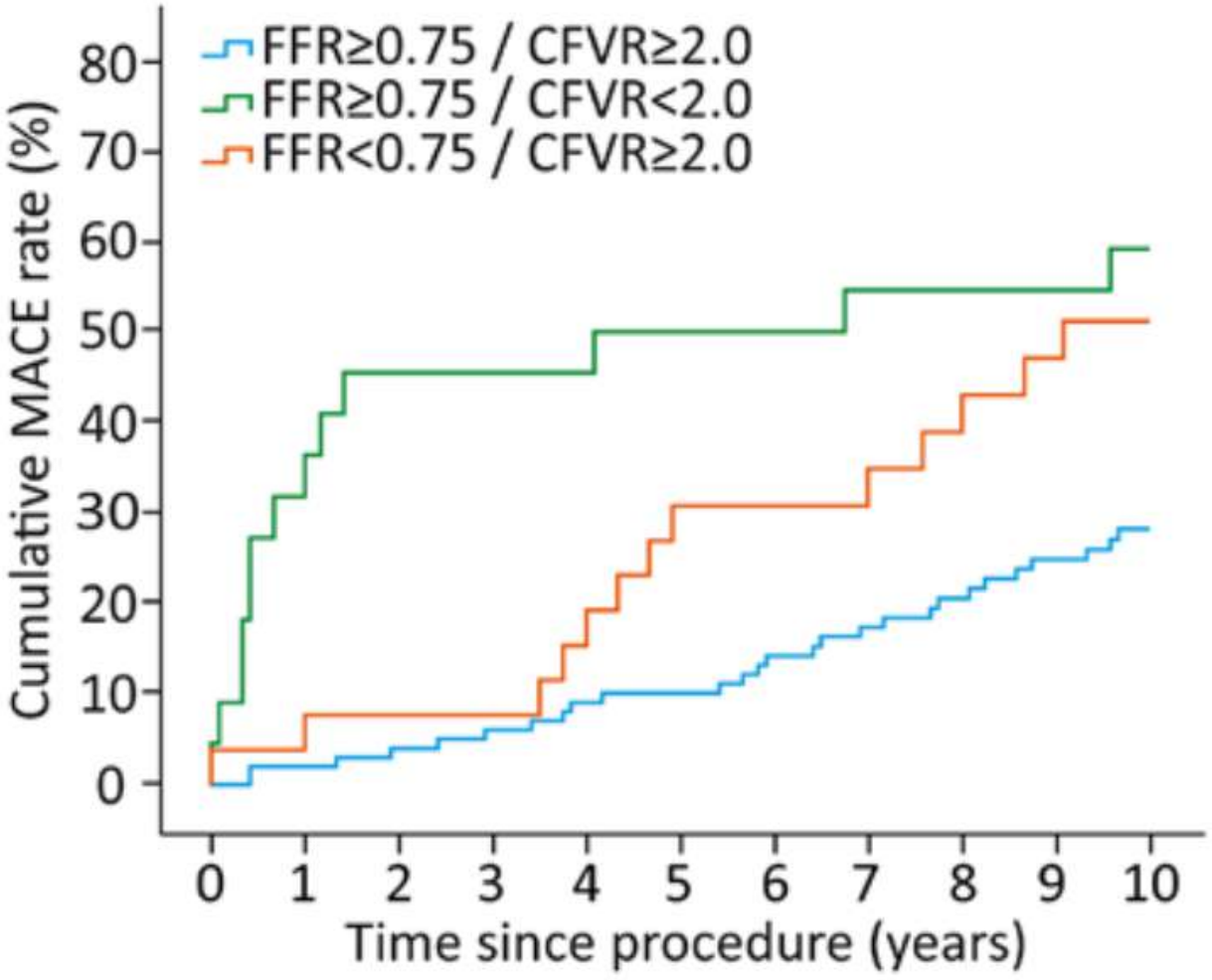


Figure 1-14: Cumulative rates of major adverse cardiovascular events in patients with intermediate coronary lesions who had invasive coronary physiology parameters measured and subsequent deferral of revascularisation. Patients with concordant FFR and CFVR had the best prognosis. Patients with non-obstructive coronary artery disease but microvascular dysfunction had the worst prognosis. Adapted from van de Hoef *et al.*(193)

Section III: Coronary microvascular dysfunction in chronic kidney disease

III.1 Preface

In this section, I explore the pathophysiological changes to the coronary microcirculation that are seen in patients with CKD. I also describe the results of a narrative literature review detailing the current evidence for CMD in CKD. Some of the work presented in this section has been previously published in a review article.(93) I was responsible for the literature search, the writing of the text and the design of the figures in that publication, which are also presented in this thesis.

III.2 Abnormalities of the coronary microcirculation in chronic kidney disease

The mechanisms of CMD in CKD are not fully understood. However, a number of structural and functional abnormalities of the microcirculation are seen in CKD, that may predispose to CMD.

In addition to the accelerated atherosclerosis and arteriosclerosis seen in larger vessels, capillary abnormalities are also present in CKD. Amann *et al.* demonstrated in an animal model that rats who underwent subtotal nephrectomy developed LVH and had reduced capillary length and density compared with rats who underwent a sham procedure.(195) Similar findings were seen in a human post-mortem study that showed myocyte-capillary mismatch and reduced LV capillary density in the hearts of patients on dialysis compared to hypertensive and normotensive controls.(196) This reduced capillary density increases oxygen diffusion distance from the centre of the capillary to the cardiomyocyte, and may contribute to tissue hypoxia and ischaemia.(195,197) A

consequence of this tissue hypoxia and ischaemia may be the development of myocardial fibrosis, which has been shown in animal models, with rats who underwent uni-nephrectomy demonstrating increased rates of myocardial fibrosis and diastolic dysfunction compared to animals that underwent a sham procedure.(198)

Functional abnormalities of the microcirculation also occur in CKD. Impaired MBF autoregulation has been shown in animal models.(199) The endothelium, which plays a key role in the response of coronary resistance vessels to metabolic stimuli, is also impaired in CKD.(200) Endothelial dysfunction occurs for a number of reasons in CKD, including NO deficiency and oxidative stress secondary to a chronic inflammatory state.(201) Yilmaz *et al.* studied 159 non-diabetic patients with CKD stages 1-5 and demonstrated that plasma malondialdehyde (a marker of oxidative stress), as well as ADMA, were significantly increased among patients with higher stage CKD, and were independently associated with endothelial dysfunction measured by flow-mediated dilatation.(202) Morris *et al.* showed that there was a reduced vasodilatory response to acetylcholine in the resistance arteries of uraemic patients, consistent with endothelial dysfunction secondary to reduced NO activity.(203) Nitric oxide deficiency is common in CKD, and occurs due to multiple pathways including lower availability of the substrate L-arginine due to reduced renal synthesis and increased levels of circulating NO synthase inhibitors such as ADMA.(204) Multiple studies have also demonstrated that markers of endothelial activation and dysfunction including von-Willebrand factor, thrombomodulin, C-reactive peptide (CRP) and cell adhesion molecules such as iCAM-1 and E-sel are elevated across all stages of CKD.(205–207) Studies directly measuring endothelial function have shown that endothelial function is

impaired in both HD and PD patients compared to controls.(206,208,209) There is also evidence that endothelial function is significantly impaired in early stage CKD and pre-dialysis patients and that it is worse in patients with greater albuminuria.(206,210,211)

Endothelial dysfunction also has a prognostic value in CKD. In the Hoorn population cohort study, there was an independent association between mild renal failure and markers of endothelial activation. Each 5ml/min/1.73m^2 decrease in eGFR was associated with a 22% increase in relative risk of cardiovascular mortality over a median follow-up period of 12.5 years. However, after adjustment for markers of endothelial dysfunction, the strength of this association was markedly attenuated, suggesting that endothelial dysfunction contributes significantly to the cardiovascular mortality seen in early stage CKD.(212)

Abnormal coronary microvascular function in CKD may also result as a consequence of interventions for CKD. The creation of an arterio-venous fistula in pre-dialysis patients has been shown to reduce endothelium-dependent vasodilatation in the fistula arm. Interestingly, there was also a reduction in endothelium-dependent vasodilation in the contralateral arm, suggesting that localised changes to the vasculature can lead to systemic changes in the microcirculation.(213)

Thus, there are plausible mechanisms by which the maladaptive structural and functional changes seen in CKD, in combination with cardiovascular risk factors such as hypertension, hyperglycaemia, chronic inflammation and oxidative stress, may lead to CMD and subsequently the development of uraemic cardiomyopathy.

III.3 Aim of literature review

The aim of this review was to summarise the current evidence for CMD in CKD and to identify gaps in the literature. Due to a number of factors (small sample sizes, significant heterogeneity between study populations and imaging modalities, varying definitions of CKD and CMD), a narrative review of the literature was performed.

III.4 Methods

A structured PubMed database search was carried out using the keywords “coronary flow reserve”, “coronary flow velocity reserve”, “myocardial perfusion reserve” or “coronary microvascular dysfunction” combined with” chronic kidney disease”, “end-stage kidney disease”, “end-stage renal disease” or “uraemic cardiomyopathy”. The reference lists of identified studies were also searched for any relevant titles. Studies were included if they assessed CFR or CFVR in CKD, involved adult patients, and were published in the English language. The definition of CKD was deliberately kept broad to maximise the number of studies identified and included the full spectrum of patients with CKD (early stage, pre-dialysis, dialysis, transplant including multi-organ transplant). Animal studies were excluded. Studies including patients with obstructive CAD or that performed CFR assessment for diagnosis of CAD were also excluded. The search was initially carried out in May 2019 and repeated in July 2021. Based on the search strategy, 598 articles were screened. After removal of inappropriate studies and duplicates, 40 studies were considered relevant to the review. Included studies are summarised in table 1-4.

III.5 Results

Much of the current data on CMD in CKD are limited and conflicting, with inadequate exclusion of CAD a limitation of many of these studies. Coronary microvascular dysfunction in CKD appears common, with prevalence rates of 24-90%. However, there is significant variation among studies depending on the definition of CMD used and the severity of renal disease in their included populations.(214–219) In addition, many studies have included patients with diabetes and hypertension, both of which independently affect CFR.(220,221)

III.5.1 Pre-dialysis

The data on CFR in early stage and pre-dialysis CKD are conflicting. An interesting study by Turkmen *et al.* examined 30 normotensive adults with polycystic kidney disease and preserved kidney function (median creatinine clearance 95ml/min) and showed a significantly reduced CFVR compared to healthy controls of similar age, renal function and BP. This appears to suggest that even patients with CKD stage 1 had evidence of impaired microvascular function.(222) However, further studies of CFR in early CKD have provided mixed results.

A number of studies have compared CFR in CKD versus non-CKD patients, using a cut-off value to define CKD of eGFR <60ml/min/1.73m^2. Sakamoto *et al.* studied 13 CKD and 60 non-CKD subjects using Doppler angiography and showed that patients with CKD had a significantly reduced CFVR. On multivariate regression, age, eGFR and diabetes were independent predictors of low CFVR, although a relatively high cut-off value of CFVR <2.8 was used to define CMD.(223) Similar findings were seen in

PET and SPECT studies.(224–226) In patients with essential hypertension, Bezante *et a*l. showed that co-existent nephropathy was associated with a significant (10-fold) increased risk of CMD.(214) However, the largest angiographic study by Chade *et al*. examined CFVR in 605 patients. Although unadjusted analysis suggested a reduced CFVR in CKD, eGFR <60ml/min/1.73m^2 was not an independent predictor of CFVR after correction for age, gender and comorbidities including hypertension and diabetes.(227) This suggests that the small differences in CFVR seen in the smaller studies may be due to confounding factors such as hypertension, diabetes and LVH.

Several studies have examined CFR across stages of CKD. Again, their results are conflicting. A trend towards reduced CFR with worsening renal function was shown in a retrospective PET study (the RAMPART study) of 435 non-diabetic patients with early CKD (stages 1-3), although this was not significant in an adjusted analysis.(228) A small Finnish PET study of 10 controls and 22 patients with later stage CKD (stages 3-5) also showed a trend towards reduced CFR with increasing stage of CKD, although this was again not statistically significant.(229) A large prospective Doppler TTE study by Kashioulis *et al.* showed significantly reduced CFVR in patients with CKD stages 3-4 compared to controls. However, in an adjusted model, only age and 24 hour ambulatory systolic BP were independent predictors of CFVR <2.5.(230)

Imamura *et al.* conducted the largest cross-sectional prospective Doppler TTE study of CFVR in CKD. The relationship between albuminuria and CMD was investigated in 175 Japanese patients with CKD stages 1-5. There was a significant relationship between increasing CKD stage and decreasing CFVR from CKD stage 3 onwards, and

patients with albuminuria had significantly lower CFVR – figure 1-15.(231) This is consistent with other data that proteinuria is an independent marker of adverse cardiovascular outcomes in CKD.(13) Mohandas *et al.* also showed that eGFR is an independent predictor of CFVR and each 10-unit decrease in eGFR associated with 0.04-unit decrease in CFVR. However, the association between eGFR and CFVR was only seen in patients >60 years of age.(232) The largest study to date of CFR across CKD stages was a PET study conducted by Charytan *et al*. Analysis of 1892 patients who had no regional perfusion defect (thus excluding significant CAD) demonstrated a significant decrease in CFR as renal function declined, with the largest drop being in patients with CKD stage 5 and a small increase in CFR for patients on dialysis.(233) This relationship was independent of age, gender, race and cardiovascular risk factors. Despite its retrospective design, the large number of patients studied provide strong evidence that CFR falls as CKD stage increases.

Thus, the data appears to show that early-stage CKD is not associated with significant reductions in CFR, with an important decrease in CFR from CKD stage 3 onwards. This appears consistent with large prognostic studies in CKD that showed that cardiovascular events and mortality appears to increase significantly once eGFR falls below 60ml/min/1.73m^2.(27)

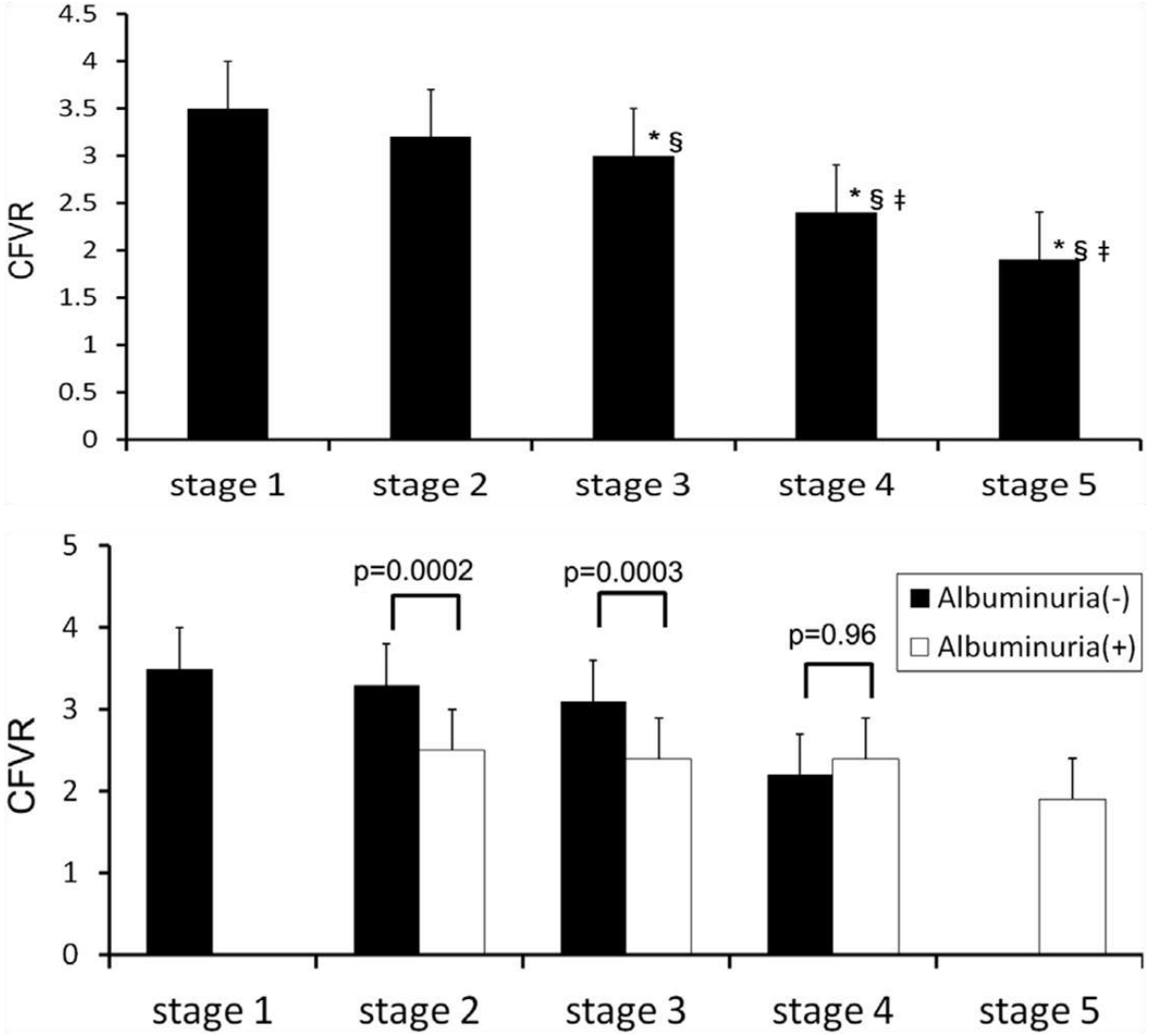


Figure 1-15: Coronary flow velocity reserve across stages of chronic kidney disease (top). *p <0.05 vs stage 1; §p <005 versus stage 2; ǂp <0.05 vs stage 3. Incremental reduction in coronary flow velocity reserve with albuminuria (bottom). Reproduced with permission from Imamura *et al.*(231)

III.5.2 End-stage renal disease

The evidence for CMD in ESRD is stronger. A small angiographic study of 64 patients by Ragosta *et al.* showed that patients with diabetes and ESRD had significantly reduced CFVR compared to healthy controls and diabetic patients without nephropathy.(234) Simlarly, Nelson *et al.* also used Doppler angiography to show that CFVR was reduced in ESRD compared to healthy controls.(235) A number of studies have examined CFR in dialysis patients. Small Doppler TTE studies have shown that CFVR is reduced in HD patients compared to controls.(215,236–238) There is a high prevalence of CMD among patients on HD, with reported rates of 36-52%.(239,240) There are no studies directly comparing patients on PD to healthy controls. However, Caliskan *et al.* have studied CFVR using Doppler TTE in patients with PD and demonstrated a high prevalence of CMD (58-78% of cases), which was associated with serum biomarkers including troponin T and ghrelin.(241,242)

It is possible that some of the reduced CFR seen in these studies may be due to occult CAD that has not been fully excluded. Among patients with ESRD undergoing cardiovascular assessment for renal transplant, 59% of patients had a CFR <2. However, even in those patients without any feature of infarction or ischaemia, CFR was abnormal in 63% of cases.(219)

Thus, there is strong evidence that ESRD is associated with a high prevalence of CMD, which is understandable, given the inverse linear relationship between CFR and eGFR seen in earlier stages of CKD.

III.5.3 Kidney transplant recipients

There has been limited investigation into the effects of kidney transplantation on CFR. Coronary microvascular dysfunction appears to persist after kidney transplantation with a number of studies showing reduced CFR in kidney transplant recipients compared to healthy and hypertensive control subjects, with mean time from kidney transplantation ranging from 33-100 months.(243–245) The reduction in CFR persists despite adequate restoration of kidney function with mean creatinine clearance in these studies approximately 70ml/min. This was also seen in a recent CMR study which showed reduced CFR compared to controls in transplant recipients with eGFR consistent with CKD stages 2-3.(246)

As kidney function is partially restored, one would expect an improvement in coronary microvascular function after kidney transplantation. Cross-sectional data from the RAMPART sub-study of patients with early stage CKD showed that each 10ml/min increase in creatinine clearance was associated with an increase in CFR of 0.11.(228) However, there are no longitudinal studies on the impact of renal transplant on CFR. Improvement in CFR after kidney transplantation is suggested from cross sectional data showing that CFR may be higher in transplant recipients compared to patients with ESRD.(215,216) Possible explanations are that some of the microvascular changes seen in ESRD are reversible or alternatively that repeated haemodialysis causes microvascular dysfunction.

Despite this, rates of CMD remain high after kidney transplant, with 8-73% of renal transplant recipients having a CFR <2, suggesting that the process is not fully

reversible despite restoration of kidney function.(243,244,247,248) Furthermore, a single study showed that patients with a failed kidney transplant have worse microvascular function than patients on HD with no history of renal transplant (mean CFVR 1.6 ± 0.2 vs 1.75 ± 0.3, p=0.028).(249)

III.5.4 The prognostic role of coronary flow reserve in chronic kidney disease

Several studies have assessed the relationship between reduced CFR and prognosis in CKD. Murthy *et al.* performed a retrospective analysis of stress PET studies in 866 patients with moderate to severe CKD. In this population, CFR below the median (<1.5) was associated with a 2.1-fold increased risk of cardiovascular mortality even after adjustment for clinical risk factors, LV systolic function, extent of ischaemia and scar.(250) A further retrospective study by the same group examined a cohort of 186 patients with dialysis-dependent ESRD. Over a median 3 year follow-up period, CFR <1.4 (the median value in the study) was associated with a significantly increased risk of all-cause and cardiovascular mortality.(217) A further retrospective PET study by Bajaj *et al.* showed that among patients with eGFR <60ml/min/1.73m^2, CFR <1.5 but not eGFR, was an independent predictor of MACE at median follow-up of 4.4 years.(226) Nakanishi *et al.* performed a prospective Doppler TTE study of 139 patients with eGFR <60ml/min/1.73m^2. Patients with CFVR<2 had a significantly higher mortality rate compared to patients with CFVR>2, with the Kaplan-Meier curves diverging early within the first year of follow up. Coronary flow velocity reserve was an independent predictor of mortality even after adjustment for cardiovascular risk factors.(218)

Coronary flow velocity reserve also has a prognostic role in renal transplant recipients. Lakkas *et al.* showed that CFVR <2 at baseline post-transplant was associated with an increased risk of adverse cardiac and renal events at 3 years follow-up.(248) Similarly, Tona *et al.* studied 48 patients who underwent simultaneous kidney/pancreas transplant and showed that a CFVR <2 following transplantation was associated with increased risk of MACE.(251) However, the authors do not present data on the improvement of glycaemic control with pancreas transplant, which is a potential confounder. Furthermore, the small sample sizes and low cardiovascular event rates in these studies make it difficult to draw any meaningful conclusions.

Thus, there is strong evidence that, similar to the situation in CVD, the presence of CMD in CKD is associated with an adverse prognosis. Whether persistent CMD after kidney transplant also confers an adverse prognosis is less clear but is nevertheless suggested by the current literature.

III.6 Limitations to the current evidence

Three broad areas of uncertainty remain in the literature. Firstly, to date, there are no studies investigating the effect of living kidney donation on CFR. Given the cardiovascular changes in LKD described in section I, and the concerns raised about the long-term implications of living kidney donation, it is important to know if coronary microvascular function is also reduced in otherwise healthy LKD.

Secondly, there are limited data on whether initiation of dialysis improves or worsens microvascular function. Charytan *et al.* suggested an improvement in CFR in dialysis patients compared to pre-dialysis.(233) However, no longitudinal studies have been performed examining coronary microvascular function pre- and post-initiation of dialysis.

Thirdly, the effect of kidney transplantation on coronary microvascular function is not known. Although cross sectional data suggests that CMD is reduced after kidney transplantation, there are no longitudinal studies examining coronary microvascular function pre- and post-kidney donation. If kidney transplant does not reverse CMD, this may partly explain the persistently increased cardiovascular mortality seen among kidney transplant recipients.

III.7 Conclusions and aims of thesis

The current evidence demonstrates that CMD is relatively rare in early-stage CKD, becomes more common once eGFR falls below 60ml/min/1.73m^2, and is endemic among patients with ESRD. Regardless of the stage of CKD, the presence of CMD is an adverse prognostic marker. However, important gaps in the literature remain. In this thesis, I aim to address some of the lacunae in the current literature regarding CMD in CKD. Through the clinical studies described in subsequent chapters, I plan to address the following topics:

1. Is CFVR reduced in LKD compared to healthy controls? – Chapter 4.
2. The prevalence of CMD in potential kidney transplant recipients and what factors are associated with CMD in this population? – Chapter 5.
3. The effect of kidney transplantation on CFVR – Chapter 6.
4. The relationship between CFVR and myocardial fibrosis in advanced CKD – Chapter 7.

Table 1-4: Summary of studies of coronary microvascular dysfunction in chronic kidney disease

Study	Year	Country	Design	Population	Modality	Findings
Pre-dialysis						
Chade *et al.*(227)	2006	USA	Prospective	GFR >60ml/min/1.73m^2 (n=481) GFR <60ml/min/1.73m^2 (n=124)	Doppler angiography	Non-significant trend towards reduced CFVR as eGFR falls
Turkmen *et al.*(222)	2008	Turkey	Prospective	Controls (n=30) APKD with CKD stage 1 (n=30)	Doppler TTE	Significantly lower CFVR in APKD than controls despite preserved renal function.
Bezante *et al.*(214)	2009	Italy	Prospective	Hypertensive patients with normal renal function (n=64) Hypertensive patients with renal impairment (n=12)	Doppler TTE	Significantly lower CFVR in hypertensive patients with renal impairment.
Koivuviita *et al.*(229)	2009	Finland	Prospective	Controls (n=10) CKD stages 3-5 (n=22)	PET	Non-significant trend towards reduced CFR as eGFR falls
Charytan *et al.*(228)	2010	USA	Retrospective	CKD stages 1-3 (n=435)	PET	Non-significant trend towards reduced CFR as eGFR falls
Fukushima *et al.*(224)	2012	USA/ Germany	Retrospective	eGFR >60ml/min/1.73m^2 (n=42) eGFR <60ml/min/1.73m^2 (n=40)	PET	Significantly reduced CFR in patients with eGFR <60ml/min/1.73m^2.
Sakamoto *et al.*(223)	2012	Japan	Prospective	eGFR >60ml/min/1.73m^2 (n=60) eGFR <60ml/min/1.73m^2 (n=13)	Doppler angiography	Significantly reduced CFVR in patients with eGFR <60ml/min/1.73m^2.
Imamura *et al.*(231)	2014	Japan	Prospective	Controls (n=15) CKD stages 1-5 (n=175)	Doppler TTE	Significant decrease in CFVR as eGFR falls. Incremental reduction in CFVR with albuminuria
Mohandas *et al.*(232)	2015	USA	Retrospective	eGFR<89ml/min/1. 73m^2 (n=102) eGFR≥89 ml/min/1. 73m^2 (n=96)	Doppler angiography	eGFR independent predictor of CFVR. Each 10-unit decrease in eGFR associated with 0.04-unit decrease in CFVR

Tsuda *et al.* (225)	2018	Japan	Prospective	eGFR >60ml/min/1.73m^2 (n=46) eGFR <60ml/min/1.73m^2 (n=46)	SPECT	Significantly reduced CFR in patients with eGFR <60ml/min/1.73m^2.
Kashioulis *et al.* (230)	2020	Sweden	Prospective	Controls (n=33) CKD stages 3-4 (n=49)	Doppler TTE	Significantly reduced CFVR in CKD compared to controls.
Xiao *et al.* (252)	2020	China	Prospective	CKD stages 1-4 (n=243)	SPECT	Increased CMD as severity of intra-renal arterial lesions increases.
End-stage renal disease						
Ragosta *et al.*(234)	2004	USA	Prospective	Controls (n=32) Diabetic patients with no kidney disease (n=11) Diabetic patients ESRD (n=21)	Doppler angiography	Significantly lower CFVR in patients with diabetic nephropathy compared to other 2 groups
Tok *et al.*(237)	2005	Turkey	Prospective	Controls (n=14) Patients on HD (n=10)	Doppler TTE	Significantly lower CFVR in HD patients
Caliskan *et al.* (215)	2008	Turkey	Prospective	Controls (n=39) HD (n=48) Renal transplant recipients (n=27)	Doppler TTE	Significantly lower CFVR in ESRD and in renal transplant recipients. Lower CFVR in ESRD than renal transplant recipients.
Niizuma *et al.*(236)	2008	Japan	Prospective	Controls (n=20) Patients on HD (n=21)	Doppler TTE	Significantly lower CFVR in HD patients
Bozbas *et al.*(216)	2009	Turkey	Prospective	Controls (n=26) ESRD (n=30) Renal transplant recipients (n=30)	Doppler TTE	Significantly lower CFVR in ESRD and in renal transplant recipients. Lower CFVR in ESRD than renal transplant recipients.
Caliskan *et al.*(242)	2009	Turkey	Prospective	Patients on PD (n=24)	Doppler TTE	CFVR <2 in 58% of patients
Yelken *et al.*(253)	2009	Turkey	Prospective	Dialysis dependent patients with failed renal transplant (n=26)	Doppler TTE	CFVR significantly lower in Hepatitis C positive patients compared to Hepatitis C negative patients.

Caliskan *et al.*(241)	2012	Turkey	Prospective	Patients on PD (n=37)	Doppler TTE	CFVR <2 in 78% of patients and correlated with troponin T.
Paz *et al.*(219)	2017	USA	Prospective	ESRD awaiting transplant (n=131)	PET	CFR <2 in 58.8% of patients with ESRD
Honda *et al.*(238)	2019	Japan	Retrospective	HD (n=20) Non-HD (n=141)	Doppler TTE	Lower CFVR in HD patients compared to non-HD patients post-CABG with LAD grafting. No significant difference in magnitude of increase in CFVR between pre- and post-CABG
Nelson *et al.*(235)	2019	Australia/ USA	Prospective	Controls (n=15) ESRD (n=15)	Doppler angiography	Significantly reduced CFVR in ESRD compared to controls
Malak *et al.* (240)	2020	Canada/ USA	Retrospective	Controls (n=100) HD (n=188) PD (n=120) Pre-dialysis (n=20)	PET	CFR <2 in of 30% of ESRD compared to 23% of controls
Papamichail *et al.*(239)	2020	Greece	Prospective	Patients on HD (n=29)	Doppler TTE	CFVR <1.5 in 52% of patients.
Gkirdis *et al.*(254)	2020	Greece	Prospective	HD (n=21) PD (n=22)	Doppler TTE	No significant difference in CFVR between HD and PD
Renal transplant						
Vigano *et al.*(243)	2007	Italy	Prospective	Controls (n=16) Renal transplant recipients (n=25)	Doppler TTE	CFVR impaired in half of cases
Turiel *et al.*(244)	2009	Italy	Prospective	Controls (n=25) Renal transplant recipients (n=25)	Doppler TTE	Significantly lower CFVR in renal transplant recipients compared to controls
Gorgulu *et al.*(249)	2010	Turkey	Prospective	HD (n=40) Failed renal transplant (n=43)	Doppler TTE	Significantly lower CFVR in failed renal transplants compared to patients on HD
Akagun *et al.*(247)	2011	Turkey	Prospective	Renal transplant recipients (n=20)	Doppler TTE	CFVR <2 in 65% of patients
Parnham *et al.* (245)	2015	Australia	Prospective	Renal transplant recipients (n=20) Liver transplant recipients (n=15) Hypertensive controls (n=10)	Stress perfusion CMR	Significantly lower CFR in renal transplant recipients compared to hypertensive controls

Paivarinta *et al.*(246)	2020	Finland	Prospective	Controls (n=10) Kidney transplant recipients (n=19)	Stress perfusion CMR	Reduced CFR in transplant recipients
Prognosis						
Murthy *et al.*(250)	2012	USA	Retrospective	eGFR <60ml/min/1.73m^2 (n=866)	PET	CFR <1.5 associated with increased risk of cardiac mortality
Tona *et al.*(251)	2016	Italy	Prospective	Simultaneous kidney pancreas transplant recipients (n=48)	Doppler TTE	Lower CFVR associated with worse cardiovascular outcomes
Shah *et al.*(217)	2016	USA	Retrospective	Dialysis dependent patients (n=168)	PET	CFR <1.5 associated with increased risk of cardiac mortality
Nakanishi *et al.*(218)	2016	Japan	Prospective	eGFR <60ml/min/1.73m^2 (n=139)	Doppler TTE	CFVR <2 associated with worse cardiovascular outcomes
Charytan *et al.*(233)	2018	USA	Retrospective	Controls (n=198) CKD stages 1-5 (n=3748)	PET	Significant decrease in CFR as CKD stage increases
Lakkas *et al.*(248)	2020	Greece	Prospective	Renal transplant recipients (n=45)	Doppler TTE	CFVR <2 in 24% of patients. Baseline CMD associated with future CV/renal events at 3-year follow up.
Bajaj *et al.*(226)	2020	USA	Retrospective	eGFR >60ml/min/1.73m^2 (n=236) eGFR <60ml/min/1.73m^2 (n=112)	PET	Significantly reduced CFR in patients with eGFR <60ml/min. CFR but not eGFR an independent predictor of death, myocardial infarction or heart failure hospitalisation.
Wenning *et al.*(255)	2020	Germany	Prospective	Dialysis dependent patients (n=39)	PET	CFR<2 associated with reduced cardiovascular event free survival

APKD: adult polycystic kidney disease, CABG: coronary artery bypass grafting, CFVR: coronary flow velocity reserve, CKD: chronic kidney disease, CMD: coronary microvascular dysfunction, CMR: cardiac magnetic resonance imaging, eGFR: estimated glomerular filtration rate, ESRD: end-stage renal disease, HD: haemodialysis, LAD: left anterior descending artery, PD: peritoneal dialysis, PET: positron emission tomography, SPECT: single photon emission computed tomography, TTE: transthoracic echocardiogram.

CHAPTER 2: METHODS

This chapter describes in detail the methods used in the clinical studies described in this thesis. Table 2-1 lists the major data collection methods employed and the key outcomes of interest.

Table 2-1: Summary of major data collection methods and outcomes of interest

Method	***Outcome***
Electrocardiogram	Evidence of ischaemia or conduction disease
Transthoracic echocardiography	Left ventricular mass Left ventricular systolic function Left ventricular diastolic function Global longitudinal strain
Doppler echocardiography	Coronary flow velocity reserve
Myocardial contrast echocardiography	Wall motion assessment Perfusion assessment Coronary flow reserve
BPTru	Office blood pressure
SphygmoCor	Pulse wave velocity Pulse wave analysis
Bloods	Haemoglobin, urea, creatinine, eGFR, albumin, phosphate, calcium, PTH, hsCRP, NT-proBNP, uric acid, iron
Urine	Albumin-creatinine ratio
Multiplex immunoassay	Biomarkers of inflammation, atrial stretch, cardiac fibrosis, kidney injury and left ventricular hypertrophy

eGFR – estimated glomerular filtration rate, PTH – parathyroid hormone, hsCRP – high sensitivity C-reactive peptide, NT-proBNP – N-terminal pro-B-type natriuretic peptide

2.1 Transthoracic echocardiography

A comprehensive transthoracic echocardiogram was performed on all subjects using an iE33 machine (Philips, Eindhoven, Netherlands) and a S5-1 transducer. All studies were performed and analysed by me [a British Society of Echocardiography (BSE) TTE accredited physician]. Scans were performed with patients in the left lateral decubitus position. Continuous ECG monitoring was performed to allow timing of cardiac cycles. Retrospective acquisition of at least two consecutive cardiac cycles was performed for each view. Measurements were performed according to BSE guidelines.(256) Studies were stored under an anonymous code, to allow for blinded analysis. Unblinding was performed once image analysis had been completed for all study participants. All images were analysed offline using commercially available software (Intellispace Cardiovascular, Philips, Eindhoven, Netherlands).

2.1.1 Left ventricular dimensions and mass

Left ventricular internal diameters in diastole (LVIDd) and systole (LVIDs) and diastolic septal (SWTd) and posterior wall thickness (PWTd) were measured in the parasternal long axis view at the mitral chordal level using 2-dimensional echocardiography. Left ventricular mass was estimated using the Cube formula(257):

$$\text{LV mass} = 0.8 \times (1.04[(\text{LVIDd} + \text{PWTd} + \text{SWTd})^3 - (\text{LVIDd})^3]) + 0.6\text{g}$$

and indexed for body surface area (BSA) to calculate LVMI. Increased LVMI was defined as LVMI > 99g/m^2 in women or >110g/m^2 in men.(258) Further characterisation of the nature of the left ventricular remodelling was performed by calculation of the relative wall thickness (RWT) using the formula:

$$\text{RWT} = (\text{PWTd} \times 2)/\text{LVIDd}.(258)$$

Left ventricular geometry was categorised based on LVMI and RWT – figure 2-1. There are limitations to acknowledge with echocardiographic measures of LV mass, which were historically validated against autopsy specimens of normal hearts.(257) Thus, they rely on certain geometric assumptions that may not be valid in patients with CKD who are prone to chronic volume overload and LVH. Volume overload in particular can lead to overestimation of LV mass by TTE as the effect of chamber dilatation on LV mass estimation is amplified by the cubing of values in the formula.(259) Cardiac magnetic resonance imaging is the gold standard non-invasive method for calculation of LV mass due to its comprehensive volumetric analysis which does not rely on geometric assumptions. However, as the studies presented in this thesis were designed as echocardiographic studies, TTE was used to measure LV mass in all subjects.

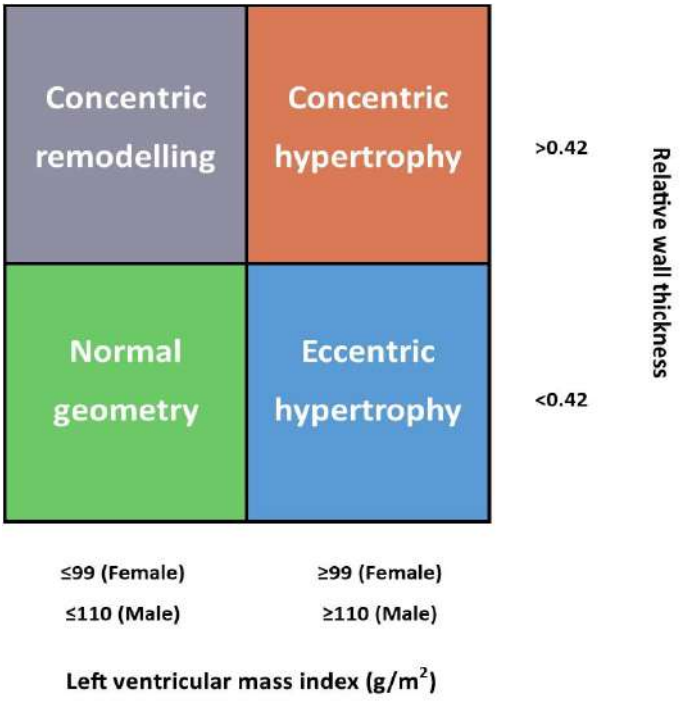


Figure 2-1: Characterisation of left ventricular geometry based on left ventricular mass index and relative wall thickness.

2.1.2 Left ventricular ejection fraction

Left ventricular ejection fraction was calculated using the Simpson's biplane method.(256,260) The endocardial contours of the left ventricle were manually traced in end-diastole and end-systole in the apical 4-chamber and apical 2-chamber views to calculate end-diastolic volume (EDV) and end-systolic volume (ESV) – figure 2-2. End-diastole was defined as when the LV cavity was at its largest. End-systole was defined as when the LV cavity was at its smallest. Both EDV and ESV were indexed to BSA.

Left ventricular ejection fraction was calculated by the formula:

$$LVEF = (EDV-ESV)/EDV$$

Where the Simpson's biplane method was not possible due to poor endocardial definition, visual estimation of LVEF was performed. Left ventricular ejection fraction was classified as normal (LVEF ≥55%), borderline low (LVEF 50-54%), impaired (LVEF 36-49%) or severely impaired (LVEF ≤35%) based on BSE normal reference values.(258)

The Simpson's biplane method assumes that the LV cavity visualised in the apical 4- and 2-chamber views is representative of the entire LV cavity, which may not be true in ventricles with regional wall motion abnormalities. Furthermore, volume loading will affect EDV, and thus will also affect the LVEF calculated by the Simpson's biplane method. These limitations can be overcome by techniques which provide a volumetric analysis of LVEF (3D TTE or CMR). 3D TTE was not available for my studies so LVEF was assessed with 2D TTE in all subjects. A small proportion of subjects also underwent CMR (methods and results presented in Chapter 7).

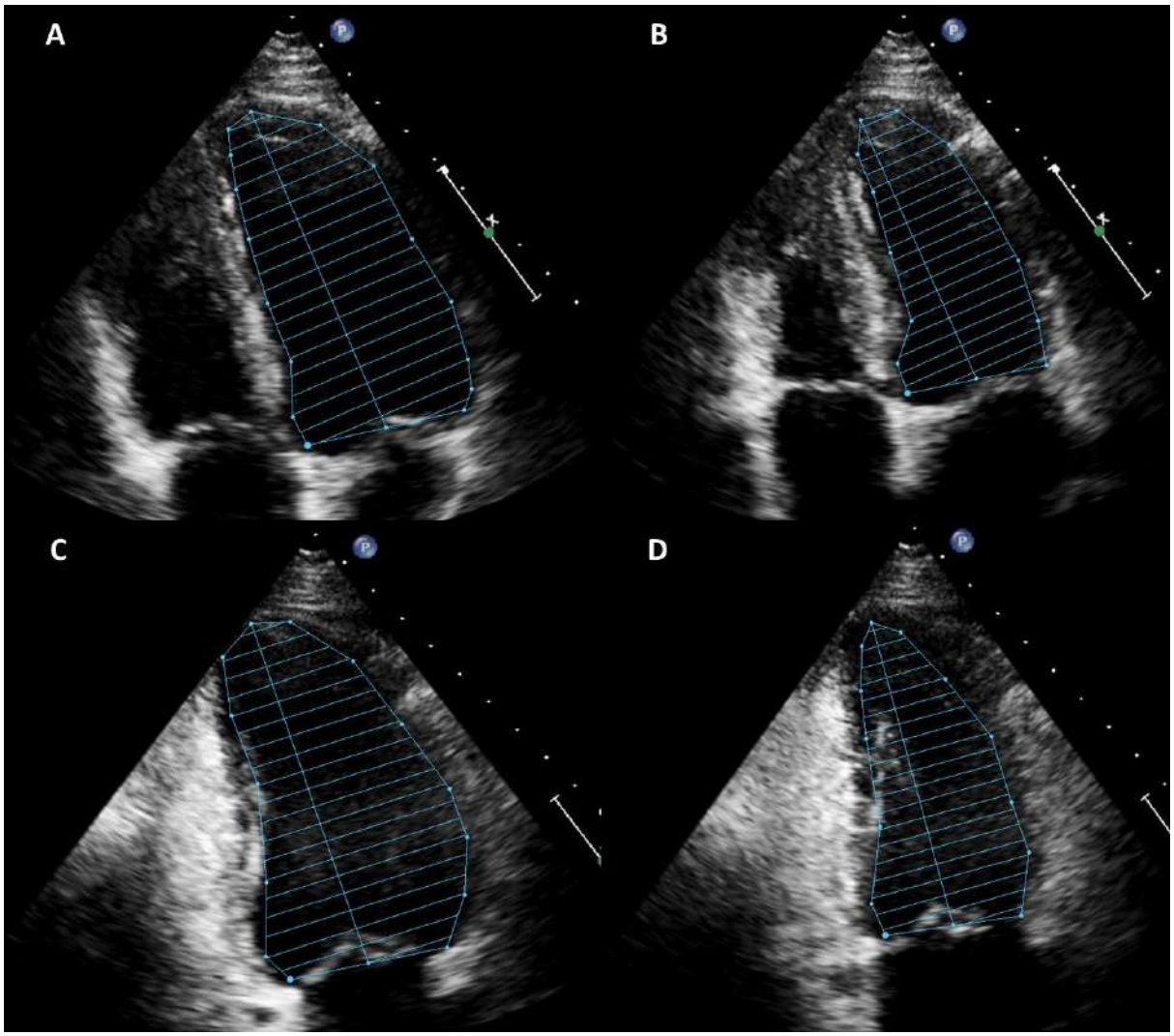


Figure 2-2: Calculation of left ventricular ejection fraction by Simpson's biplane method. A – Apical 4-chamber end-diastolic volume. B – Apical 4-chamber end-systolic volume. C – Apical 2-chamber end-diastolic volume. D – Apical 2-chamber end-systolic volume.

2.1.3 Left ventricular diastolic function

Left ventricular diastolic function was assessed from multiple parameters including mitral valve inflow, tissue Doppler imaging of the lateral and septal LV walls, pulmonary vein Doppler flow and left atrial size.(261) Diastolic dysfunction was graded according to the algorithm suggested by the BSE – figure 2-3.

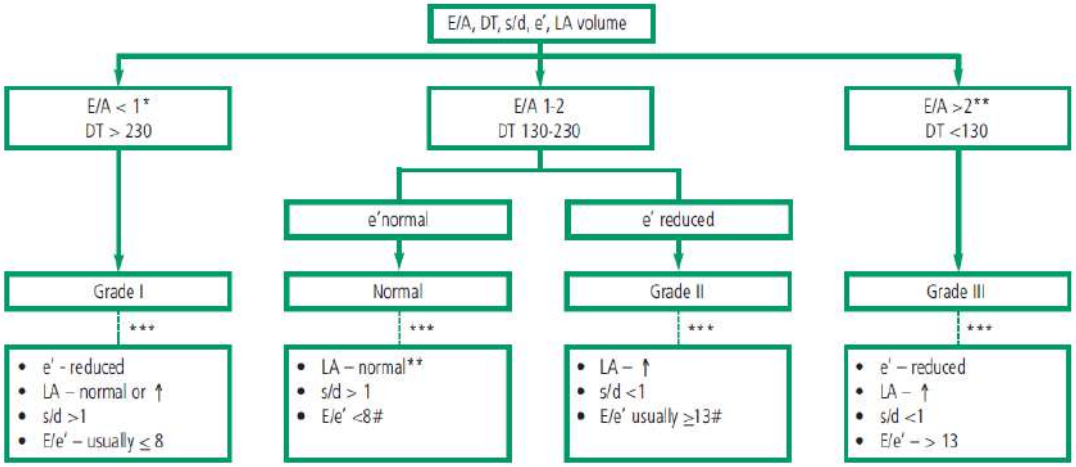


Figure 2-3: British Society of Echocardiography guidelines for assessment of diastolic function by transthoracic echocardiography. Reproduced with permission from 2013 British Society of Echocardiography diastolic dysfunction guidelines.(261)

2.1.4 Strain imaging

Global longitudinal strain – a measure of myocardial deformation - was measured using speckle tracking echocardiography. Images of the left ventricle were obtained from the apical 4-, 3- and 2-chamber views, taking care to avoid foreshortening of the left ventricle.(262) Sector width was minimised and the depth of the ultrasound beam was adjusted to ensure that minimal structures other than the LV myocardium were included in the field of view. The frame rate was maintained >60 frames per minute, to minimise the risk of under-sampling, especially in patients with tachycardia.(263)

Images were analysed offline using commercially available software (Qlab, Philips, Eindhoven, Netherlands). A region of interest (ROI) was drawn in the myocardium in each of the apical views – figure 2-4. The entire myocardium was included in the ROI in three sectors: (i) an endocardial border tracing the inner border of the myocardium, (ii) an epicardial border tracing the outer border of the myocardium and (iii) a middle region in between the inner and outer ROI.(264) After tracing the ROI, moving images were reviewed to ensure the myocardium was tracked appropriately throughout the entire cardiac cycle. The ROI was adjusted to optimise tracking of the myocardium during the cardiac cycle if required. Segments with inadequate tracking were excluded. If regional tracking was suboptimal in ≥2 segments in the same view, that view was excluded for GLS assessment. The QLAB software automatically calculated strain in each of the apical views as well as a GLS value. A bullseye plot was also automatically generated by the software to allow calculation and visualisation of segmental strain values – figure 2-5.

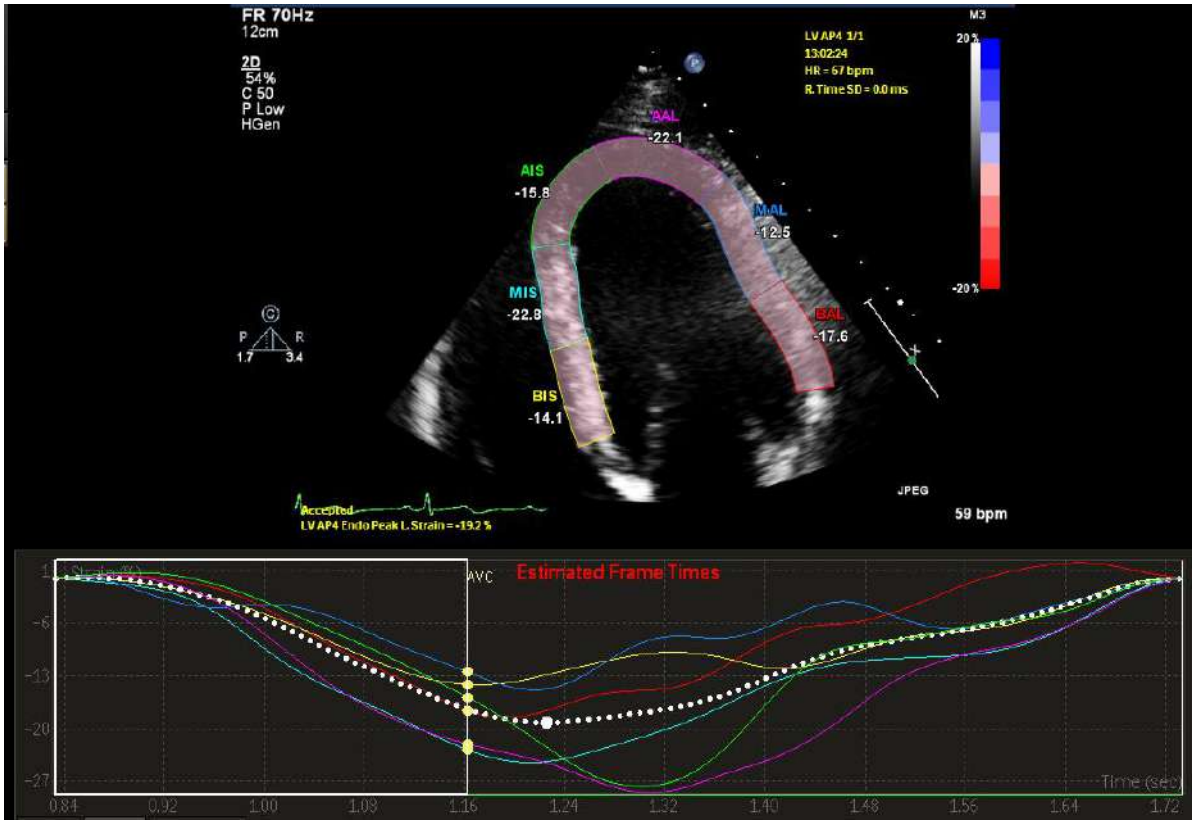


Figure 2-4: An example of strain imaging using speckle tracking in the apical 4-chamber view.

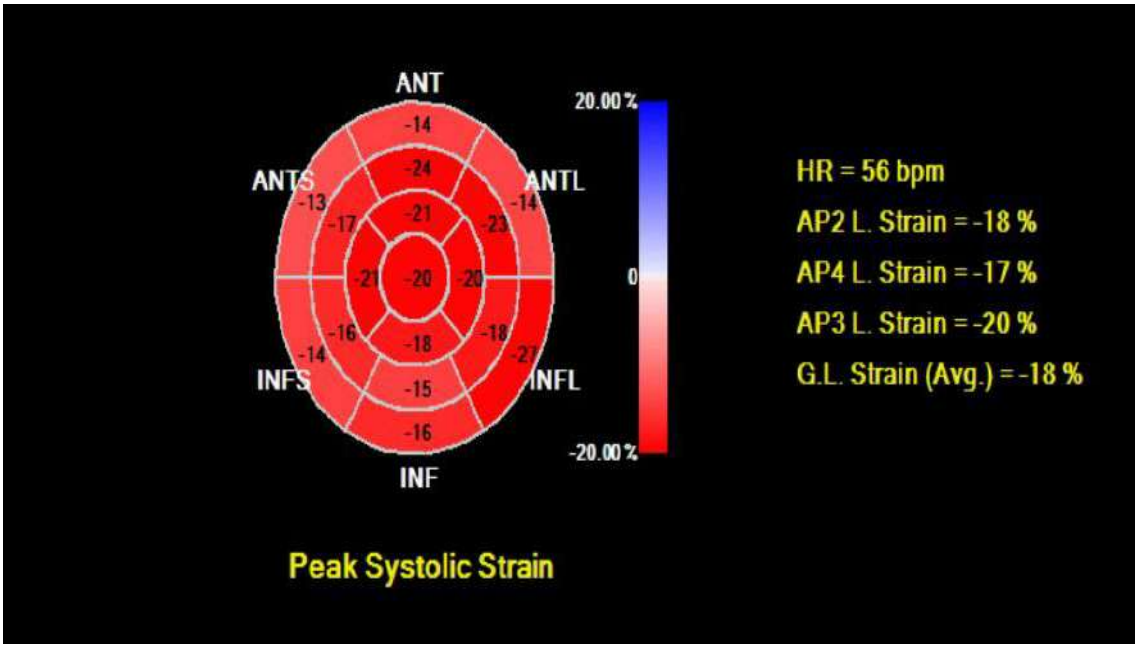


Figure 2-5: An example of a bullseye plot showing segmental strain values.

2.2 Doppler coronary flow velocity reserve

I performed all Doppler CFVR studies included in this thesis. To learn the technique, I attended a training course organised by Dr Sara Svedlund and Professor Li Min Gan at the Department of Clinical Physiology, Sahlgrenska University Hospital, Gothenburg, Sweden. This was supplemented by practising the technique on patients undergoing adenosine myocardial perfusion scans at the Queen Elizabeth Hospital Birmingham (QEHB), as they were already receiving pharmacological vasodilator stress for clinical reasons. The technique is described below.

Studies were performed using an iE33 machine (Philips, Eindhoven, Netherlands) and a high frequency S8-3 transducer (frequency range 3-8MHz). Patients were advised to abstain from caffeine for 24 hours prior to CFVR assessment. Firstly, the distal LAD was identified in the anterior inter-ventricular sulcus – figure 2-6. The acoustic window was in the region of the 4^{th} to 5^{th} intercostal space in the midclavicular line with the patient in the left lateral decubitus position and the transducer was tilted cranio-medially to identify the anterior inter-ventricular sulcus.(265) The LAD was visualised using colour flow Doppler with machine settings optimised to accentuate low velocity flow (frequency 3.5-4MHz, colour filter high, gain 80%). If it was not possible to identify the distal LAD, the mid-distal LAD was identified in a modified low parasternal short axis view. If the LAD could still not be identified, then Sonovue contrast agent (Bracco Diagnostics Inc, Milan, Italy) was used to accentuate colour flow Doppler signals.

Once the LAD was identified, a PW Doppler sample volume of 3mm was placed in the vessel to capture its flow pattern, which consists of a small systolic component and a

larger diastolic component. The position, angle and rotation of the probe were optimised for measuring the diastolic component of the spectral Doppler trace. Coronary flow velocity was calculated by measuring the peak velocity of the diastolic component of the spectral Doppler trace.

Coronary flow velocity (unit = cm/s) was measured at rest and at maximal hyperaemia – figures 2-7 and 2-8. As far as possible, the same probe position and Doppler angle were maintained during rest and hyperaemic measurements. Maximal hyperaemia was induced with an infusion of intravenous adenosine, via a 20G intravenous cannula in the antecubital fossa, at a rate of 140micrograms/kg/min for a minimum of 3 minutes. Hyperaemia was confirmed by the presence of symptoms and/or haemodynamic changes (an increase in HR >10% from baseline and/or a drop in systolic blood pressure >10% from baseline). Continuous ECG monitoring and regular non-invasive BP monitoring were performed during the procedure to ensure safety. The infusion was stopped once satisfactory hyperaemic images were obtained or if the patient found the side effects of adenosine intolerable. Coronary flow velocity reserve was calculated by the formula:

$$\text{CFVR} = \text{hyperaemic CFV}/\text{resting CFV}$$

For each variable in the CFVR calculation, the highest values of 3 cardiac cycles were averaged. As CFVR is a ratio, it does not have units. Analysis of CFVR was performed offline by me, blinded to study group. Repeatability and intra-observer variability of Doppler CFVR will be discussed in Chapter 3.

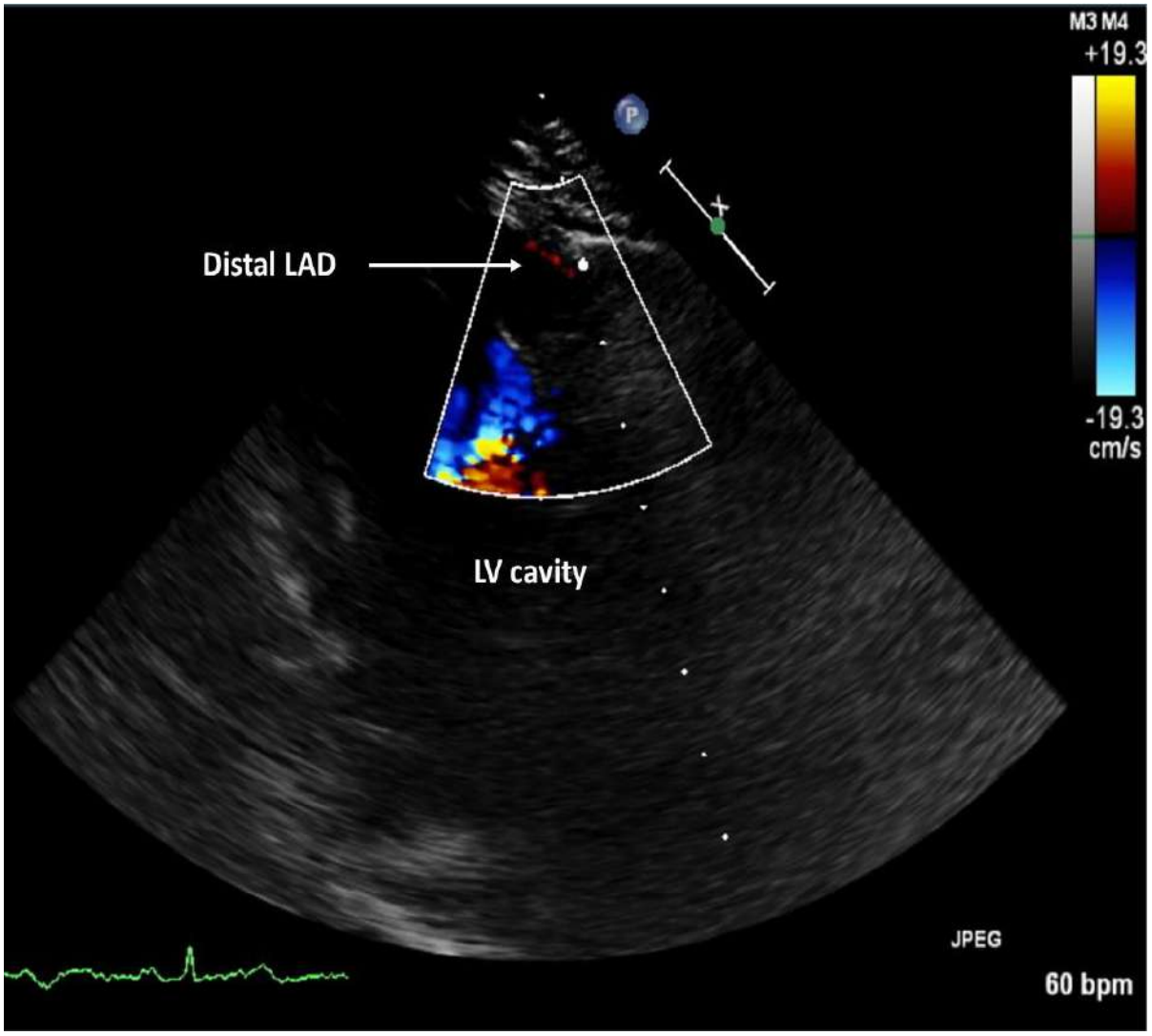


Figure 2-6: Colour flow Doppler identifying the distal left anterior descending artery. LAD – left anterior descending artery, LV – left ventricular.

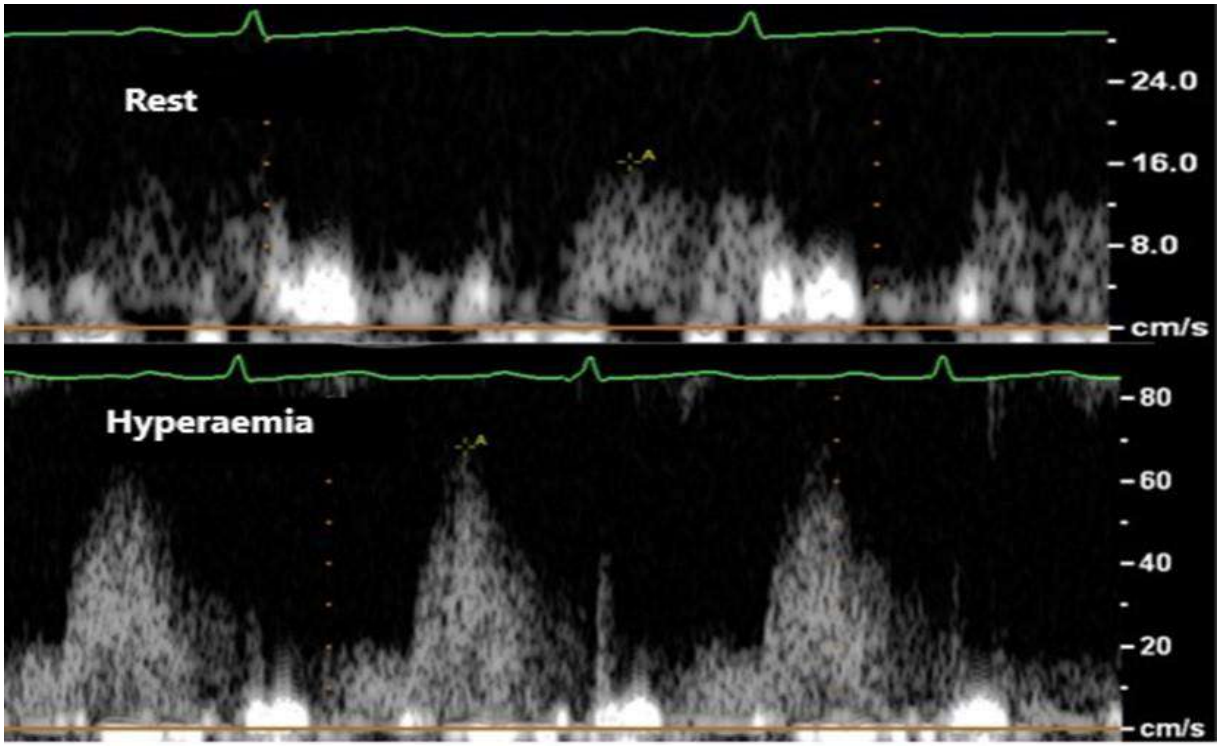


Figure 2-7: Coronary flow velocity at rest and at hyperaemia in an individual with normal microvascular function. Coronary flow velocity reserve = 4.4.

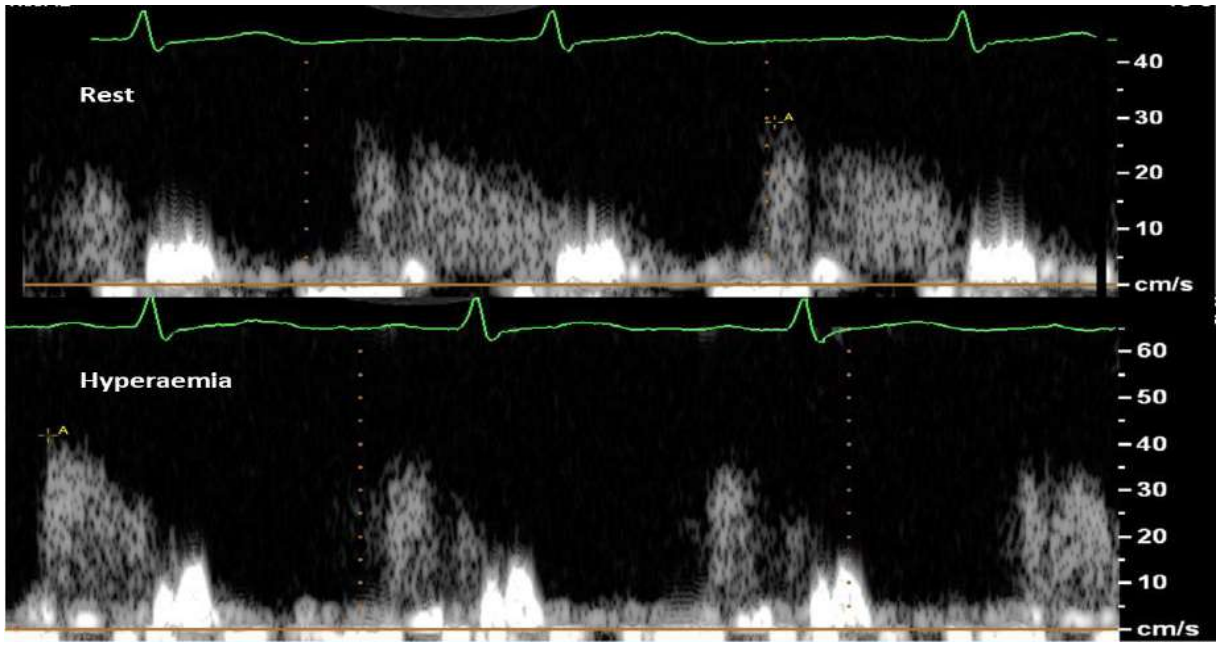


Figure 2-8: Coronary flow velocity at rest and at hyperaemia in an individual with coronary microvascular dysfunction. Coronary flow velocity reserve = 1.5.

2.3 Myocardial contrast echocardiography

I performed all MCE studies included in this thesis. I was trained in the technique by Reinette Hampson and Prof Roxy Senior at the Cardiac Research Unit, Northwick Park Hospital, Harrow, London, UK. This was supplemented by practising the technique on clinical stress echocardiography lists at QEHB. Doppler CFVR was performed before MCE CFR in each case. If adenosine was poorly tolerated, then Doppler CFVR was prioritised as it was the primary imaging modality.

2.3.1 Qualitative myocardial contrast echocardiography

Both real time and triggered MCE were performed as described by Senior *et al*.(176) Real time imaging allows for simultaneous assessment of wall thickening abnormalities. Triggered imaging, allows for more sensitive assessment of myocardial perfusion.(266) The protocol for MCE image acquisition is described below:

1. Firstly, a continuous infusion of SonoVue (Bracco Diagnostics Inc, Milan, Italy) was administered via a 20G cannula in the antecubital fossa to allow myocardial opacification. The infusion was started at an initial rate of 70-100ml/hr using an infusion pump that oscillates gently throughout the infusion to ensure that microbubbles remain in suspension (Vueject, Bracco Diagnostics Inc, Milan, Italy). The infusion rate was adjusted to ensure adequate myocardial opacification without attenuation.
2. Echo windows were optimised to allow adequate visualisation of the entire myocardium in each of the 3 apical views. The focus was set at the level of the

mitral valve but moved towards the apex to avoid near-field artefact. Images were prospectively acquired in continuous loops with ECG gating.

3. Real time imaging was performed with mechanical index (MI) 0.1 and frame rate 40 frames/second. After image optimisation, triggered high MI (1.0) flash echocardiography at end-systole (determined by the T wave on ECG) was performed to destroy microbubbles in the myocardium and to observe microbubble replenishment – figure 2-9.
4. Triggered imaging (using a 1 beat ECG trigger) was performed at MI 0.1 and frame rate 1 frame/second. After image optimisation, triggered high MI (1.0) flash echocardiography at end-systole was performed to destroy microbubbles in the myocardium and to observe microbubble replenishment. End-systolic frames of up to 10 cardiac cycles were prospectively acquired.
5. In each apical view (4-chamber, 2-chamber and 3-chamber), real time and then triggered imaging was performed.
6. Images were visually assessed to ensure adequate microbubble destruction. If inadequate microbubble destruction occurred, images were repeated using a combination of increased flash frames, reduced gain or increased MI as necessary to ensure sufficient microbubble destruction.
7. Once satisfactory real time and triggered images at rest were acquired, the entire sequence was repeated during maximal hyperaemia which was induced with an infusion of intravenous adenosine as described in section 2.2.

Rest and stress real time images were reviewed offline for any wall motion abnormalities. Rest and stress triggered images were examined for any sub-endocardial perfusion defects. The combination of normal wall motion at rest and stress and lack of perfusion defects on vasodilator MCE was deemed sufficient to exclude any significant CAD.

2.3.2 Quantitative myocardial contrast echocardiography

Triggered images were analysed offline using the QLab system (Philips, Eindhoven, Netherlands) to quantify MCE. The left ventricle was segmented using a 16-segment model.(260) The frame after flash echocardiography, which has maximal microbubble destruction, was used as the background frame. Regions of interest were placed across the entire thickness of the myocardium in the 10 mid and apical segments, taking care to exclude the high-intensity endocardial and epicardial borders. Basal segments were excluded as they are more prone to artefact. Each end-systolic frame was reviewed and the ROI adjusted to ensure that it was in a similar position in the myocardium. Segments were excluded from analysis if there was artefact, inadequate microbubble destruction, attenuation, or a wide variation in contrast intensity. A minimum of 6 quantifiable segments was necessary for the study to be included in analysis.

From the selected ROIs, the QLab software automatically generated background-subtracted plots of contrast intensity vs time which were fitted to an exponential function $y=A(1-e-\beta^{t})$. From this, peak myocardial contrast intensity (A - representing myocardial blood volume, unit = mean intensity) and the slope of the replenishment

curve (β - depicting mean microbubble velocity, unit = 1/s) could be derived – figure 2-10. The product of Axβ equals MBF (unit = mean intensity/s).

LAD MBF (defined as the average of mid anteroseptal, apical septal, mid anterior and apical anterior segments) and global MBF (defined as the average of all ten segments) were calculated at rest and at stress. Coronary flow reserve was calculated as MBF_{stress}/MBF_{rest}.(177) As CFR is a ratio, it does not have units. Intra-observer variability of quantitative MCE will be discussed in Chapter 3.

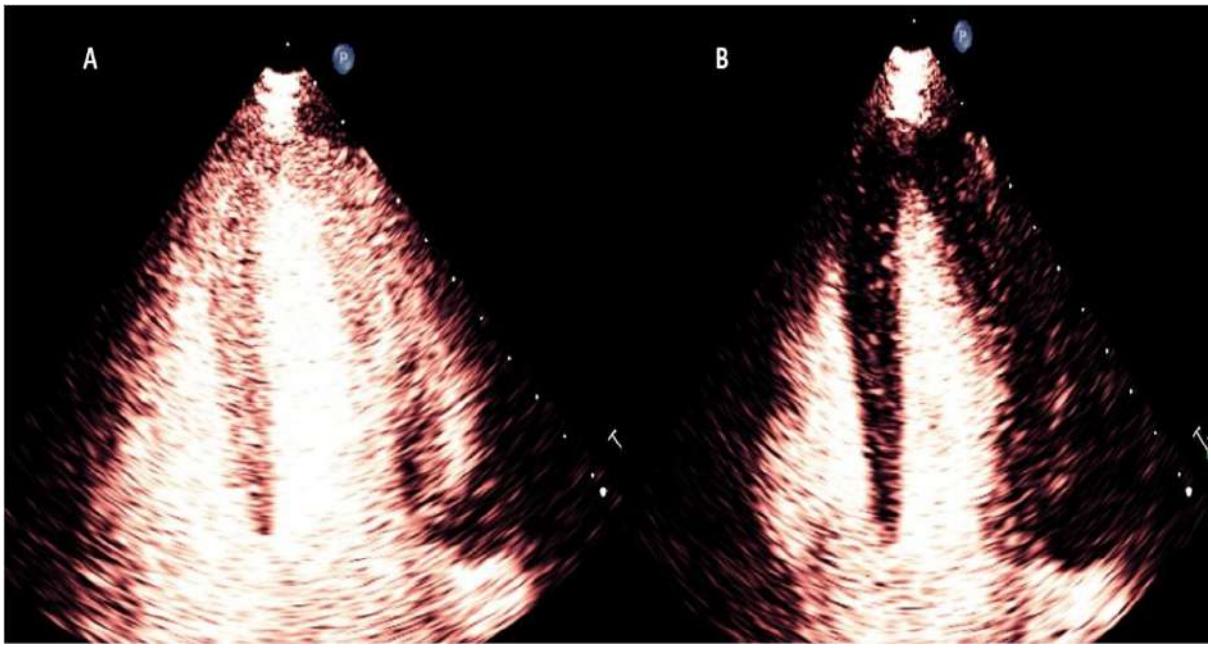


Figure 2-9: Myocardial contrast echocardiography images of left ventricle in the apical 4-chamber view. A – showing myocardial contrast opacification. B – showing microbubble destruction in the myocardium after flash echocardiography.

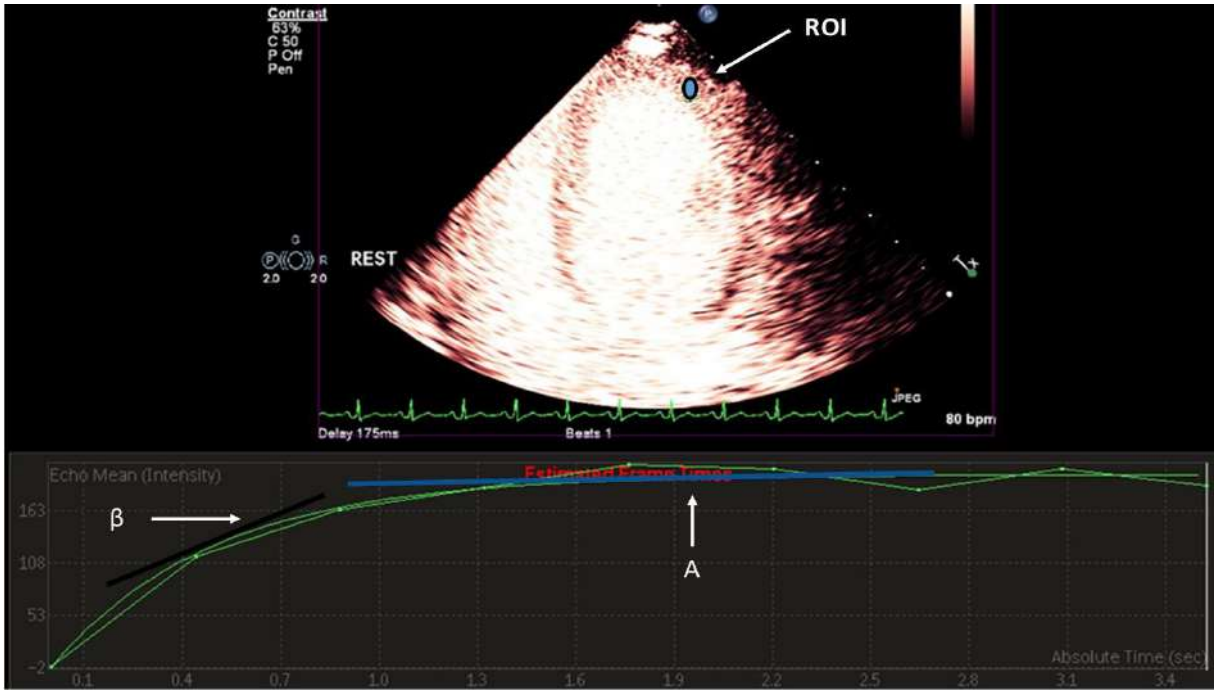


Figure 2-10: Example of myocardial contrast echocardiography quantification output from the Philips QLab system. ROI – region of interest.

2.4 Assessment of blood pressure

Supine and sitting BP was measured using an automatic BP monitor (British Hypertension Society approved BpTRU device – VSM Medtech, Coquitlam, BC, Canada). Measurements were taken from the non-dominant arm using an appropriately sized cuff after a minimum of five minutes of seated rest. Five readings were recorded by the device at one-minute intervals over five minutes. The mean of the five recordings was taken.(267)

2.5 Assessment of arterial stiffness

2.5.1 Pulse wave velocity

Carotid-femoral pulse wave velocity (PWV) was measured by applanation tonometry using the SphygmoCor device (AtCor Medical, Sydney, Australia) as previously described.(108,268) Subjects were studied in a quiet room, after 15 minutes of supine rest. Pressure waveforms from the carotid and femoral arteries were sequentially obtained using a high fidelity micromanometer (SPC-301, Millar Instruments, TX, USA), with simultaneous 3-lead ECG monitoring.

Pulse wave velocity was calculated by the formula:

$$PWV = distance/time.$$

Distance was determined by measuring the distance between the sternal notch and the palpable femoral artery pulse minus the distance between the sternal notch and the palpable carotid artery pulse. Time was determined by the time between the ECG R wave and the foot of the carotid and femoral pulse waveforms respectively. Using this information, PWV was automatically calculated by the Sphygmocor software. The

average of 3 readings was used. A representative output from the Sphygmocor software is show in figure 2-11.

Figure 2-11: Representative output of pulse wave velocity from the SphygmoCor device.

Aortic PWV is dependent on BP and therefore PWV values should be adjusted for mean arterial pressure (MAP) and HR.(269) Adjustment was made based on a linear regression model of PWV with PWV as the dependent variable and MAP and HR as independent variables. Values obtained from this regression model were used to derive predicted PWV based on the MAP and HR of the cohort using the formula:

Predicted PWV = Constant (from regression equation) + [(Unstandardized β coefficient (from regression model equation) x average MAP of cohort)] + [(Unstandardized β coefficient (from regression model equation) x average HR of cohort)]

Adjusted PWV (PWVadj) was then calculated using the formula:

PWVadj = Predicted PWV + unstandardised residuals from the regression model.

2.5.2 Pulse wave analysis

Pulse wave analysis (PWA) was also measured by applanation tonometry using the SphygmoCor device (AtCor Medical, Sydney, Australia) as previously described.(108,268) A high fidelity micromanometer (SPC-301, Millar Instruments, TX, USA) was used to flatten, but not occlude, the radial artery, resulting in the generation of a peripheral waveform from which a central waveform can be automatically derived by the SphygmoCor software – figure 2-12. Central BP and augmentation index [AIx – the difference between the second and first peaks of the central waveform (augmentation pressure) expressed as percentage of pulse pressure] were then automatically calculated using transfer functions.(270)

The Sphygmocor outputs were visually inspected for quality. Individuals with a type C waveform, where the peak systolic pressure precedes the reflected wave and thus leads to a negative AIx, were excluded from analysis as AIx from type C waveforms have been shown to correlate poorly with wave intensity analysis and other forms of wave reflection analysis.(271,272) As AIx is influenced by HR, it is commonly corrected for a HR of 75 beats per minute.(273) For each individual, the average of 3 readings was used. Representative outputs of PWA from the SphgmoCor device are shown in figure 2-12.

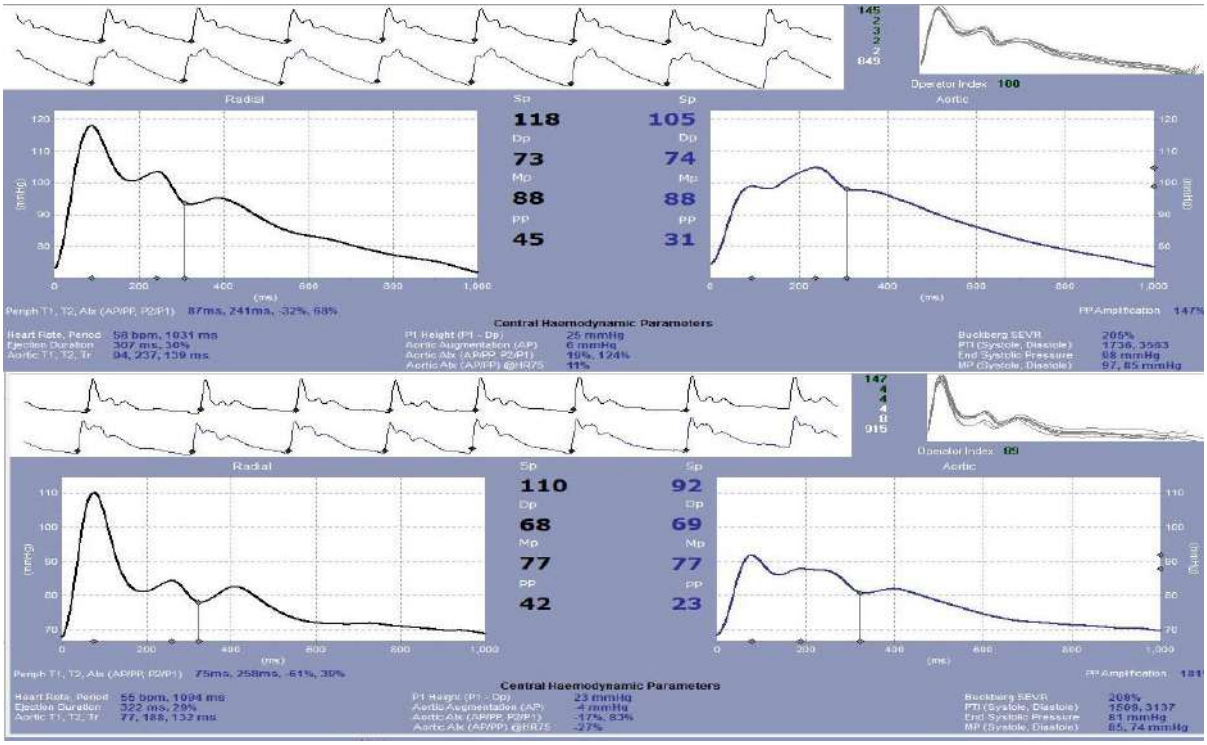


Figure 2-12: Representative outputs of pulse wave analysis showing type A waveform with corrected augmentation index of 11% (top) and type C waveform with corrected augmentation index of -27% (bottom).

2.6 Biomarker analysis

Full blood count, serum calcium, phosphate and uric acid were measured by standard automated methods. Estimated glomerular filtration rate was calculated from serum creatinine using the CKD-EPI formula.(274) Urinary albumin-creatinine ratio (ACR) was determined from a spot morning urine sample. Plasma intact parathyroid hormone (PTH) was measured by a sandwich immunoassay method (Roche Diagnostics. Reference range, 1.6-7.2 pmol/L). N-terminal pro-B-type natriuretic peptide (NT-proBNP) was assayed using the Alere point of care assay (Alere, Massachusetts, USA. Reference range 0-400ng/L). High sensitivity C-reactive peptide (hsCRP) was performed by Mohammed Shaikh (Birmingham Heartlands Hospital) using the

Architect MULTIGENT CRP Vario assay (Abbott, Illinois, USA. Reference range 0-5mg/L).

Renin and aldosterone were measured in healthy controls and LKD. Renin was measured using the iSYS direct renin mass immunoassay (Immunodiagnostic Systems Holdings, Tyne and Wear, UK). Aldosterone was measured using a liquid chromatography – tandem mass spectrometry method. Samples were taken after the patient was seated for 15 minutes and transported immediately to the QEHB laboratory for analysis. Patients' usual medications were not discontinued prior to blood sampling. However, only one control subject was taking anti-hypertensive medication (perindopril). Renin and aldosterone were not measured in patients with CKD due to the high prevalence of anti-hypertensive medication in this cohort.

A panel of 16 biomarkers (table 2-2) were measured using multiplex immunoassay. These particular biomarkers were chosen as this panel was already available from its use in the PhD project of Dr Anna Price. The individual biomarkers also relate to factors that are potential mediators of CMD (inflammation, LVH, fibrosis), and thus were felt to be relevant to my studies of CMD in different populations of renal disease. Ideally, FGF-23 would also have been measured due to its association with LVH and cardiovascular mortality in CKD. However, I was unable to procure the assay for measurement during my research period. A metabolomics or proteomics approach was also considered to study a wide array of potential mediators. However, the cost of such an approach was prohibitive and there was a lack of expertise with the techniques within the Birmingham Cardio-Renal Group. Therefore, a multiplex immunoassay was

used due to its availability, cost-effectiveness and ease. The technique is described further below.

2.6.1 Multiplex immunoassay

The Luminex® multi-analyte assay increases the ease with which multiple biomarkers of interest can be measured and allows for panel testing on small volumes of serum or plasma. This technique uses magnetic microparticles fixed with fluorophores, a biotinylated antibody cocktail specific to the analytes of interest and a streptavidin-phycoerythrin conjugate (Streptavidin-PE) which binds to the antibody – figure 2-13. The fluorescence responses of several biomarkers of interest can then be assessed in a single sample using multiplexed sandwich enzyme-linked immunosorbent assays.(275)

Multiplex immunoassay analysis was performed on frozen serum samples by Dr Anna Price, with my assistance, at the University of Birmingham laboratories using the Human Magnetic Luminex® multi-analyte assay (Catalogue number LXSAHM-04 and LXSAHM-12. Lot:1573578 and Lot: L133365. R&D Systems, Minneapolis, MN, USA). The protocol is described below:

2.6.1.1 Blood sample preparation

Venous blood was drawn from an antecubital vein into serum separator tube blood bottles. These were transported immediately to the Wellcome Trust Clinical Research Facility laboratory at QEHB. Sample preparation was performed by laboratory staff at the clinical research facility. After being allowed to clot for 30 minutes, samples were

spun in a refrigerated centrifuge for 15 minutes at 1500g at a temperature of 4^{o}C. Serum was then pipetted into cryotubes for storage in a -80^{o}C freezer until required for multiplex immunoassay analysis.

2.6.1.2 Sample and reagent preparation

All reagents and components were made according to manufacturer's instructions.

1. Frozen serum samples were thawed slowly on ice prior to use and then centrifuged at 16,000 x g for 4 minutes.
2. A series of 6 standard cocktails were used. Each standard cocktail provided by the manufacturer was reconstituted with Calibrator Diluent RD6-52 to form a 10x concentrate. 100µL of each reconstituted standard cocktail was added to a polypropylene tube with Calibrator Diluent RD6-52 to create a total volume of 1000µL – standard 1. Standard 1 then underwent a 3-fold serial dilution process to create standards 2-6. Standard 1 served as the highest standard. A final standard contained Calibrator Diluent RD6-52 only and served as the blank standard.
3. The microparticle cocktail was centrifuged at 1000 x g for 30 seconds, sonicated and vortexed before 500µL of microparticle cocktail was diluted using 5ml Diluent RD2-1 and stored in foil to protect it from light.
4. The Biotin-Antibody cocktail was centrifuged at 1000 x g for 30 seconds, sonicated and vortexed before 500µL of Biotin-Antibody cocktail was diluted with 5ml of Diluent RD2-1.

5. Streptavidin-PE was centrifuged for 30 seconds, sonicated and vortexed at 1000 x g and then diluted with 5.35ml of wash buffer and stored in foil to protect it from light.

2.6.1.3 Assay procedure

All samples were assayed on the same day under identical laboratory conditions using the same kits as detailed above. Anonymised study codes were used to identify samples and to allow blinding of the investigator to patient group allocation (controls, LKD or CKD).

1. A 96-well plate was used. 50µL of standard or sample was added to each well using a prespecified plate layout.
2. 50µL of microparticle cocktail was added to each well. The plate was covered with foil then incubated for 2 hours at room temperature on a horizontal microplate shaker set at 800rpm (Corning® LSE™ Digital microplate shaker).
3. The plate was washed three times using a magnetic wash station (Bio-Plex Pro™ Wash station, Bio-Rad, California) and wash buffer.
4. 50µL of diluted Biotin-Antibody cocktail was added to each well and the plate was covered with foil and then incubated for a further 1 hour at room temperature on a shaker set at 800rpm.
5. A repeat wash was performed, as per step 3.
6. 50µL of diluted Streptavidin-PE was added to each well. The plate was covered with foil and incubated for 30 minutes at room temperature on a shaker set at 800rpm.
7. A repeat wash was performed, as per step 3.

8. 100µL of wash buffer was added to each well. The plate was covered with foil and then incubated for 2 minutes at room temperature on a shaker set at 800rpm.

2.6.1.4 Analysis

The Bio-RAD Bio-Plex™ Luminex200® system was used for analysis and the plate was read immediately. Concentrations were calculated using the Bio-Plex Software Manager™ (version 6.1) generated standard curves and a 5PL logistic curve fitting technique – figure 2-14. The software automatically generated observed concentrations of analytes based on the fluorescence intensity of the samples compared to the standards.

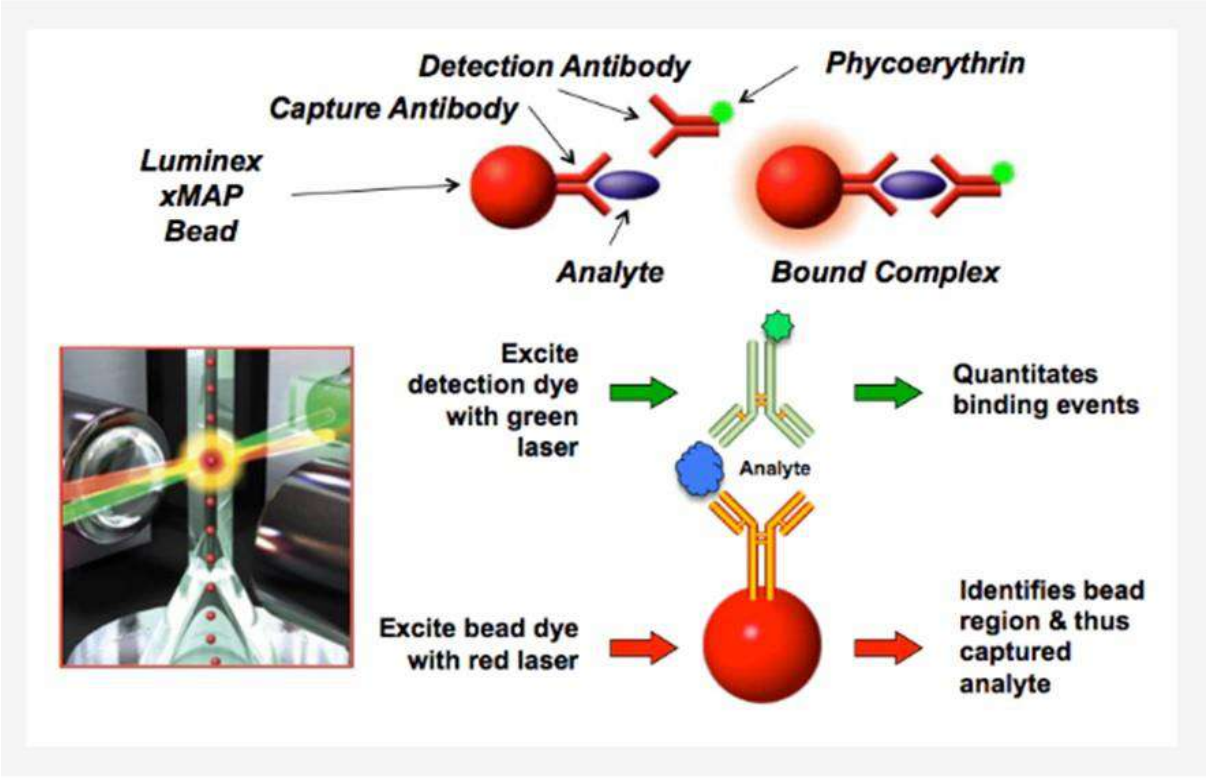


Figure 2-13: Schematic representation of the Luminex assay. Reproduced from Thermofisher.com

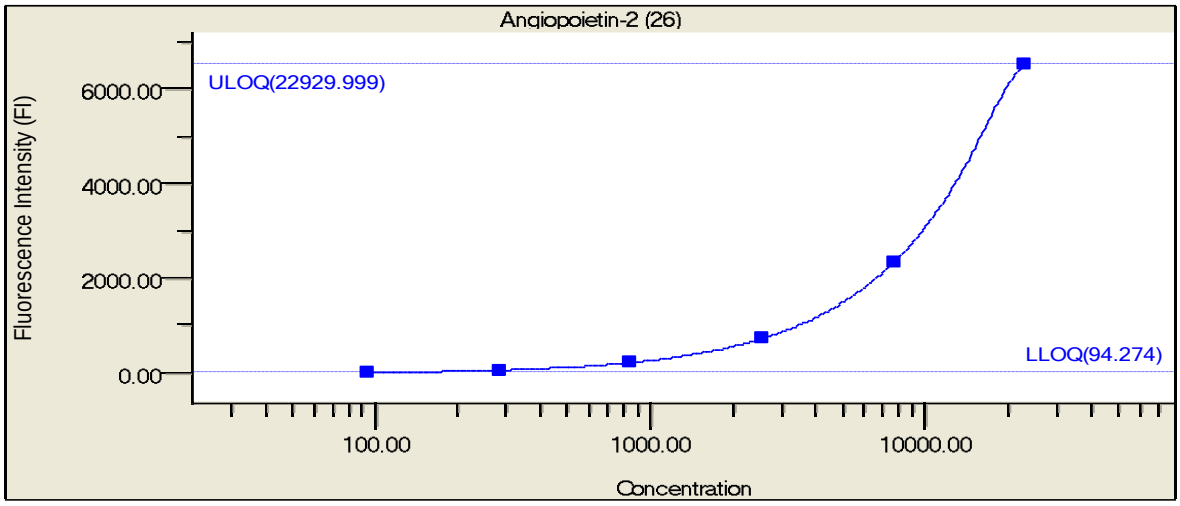


Figure 2-14: Example of Bio-Plex Software Manager™ (version 6.1) generated standard curve.

2.7 Statistical methods

All statistical analyses presented in this thesis were performed using SPSS version 26 (SPSS Inc, Chicago, Illinois). Data normality was assessed using the Shapiro-Wilk test due to the relatively small sample sizes. Continuous variables are expressed as mean ± standard deviation (SD) for parametric data or median (interquartile range - IQR) for non-parametric data. Unpaired group comparisons for continuous data were made using the unpaired t-test for parametric data or the Mann-Whitney U test for non-parametric data. Linear regression analysis was performed to determine the relationship between independent variables and a continuous dependent variable. Binary logistic regression was performed to determine the relationship between independent variables and a binary dependent variable. Categorical data are presented as frequency (percentage) and were analysed using Fisher's exact test. Correlation was assessed using the Pearson correlation coefficient for parametric data and the Spearman correlation coefficient for non-parametric data. Statistical tests were 2-tailed, and a p value <0.05 was considered statistically significant.

Table 2-2: Summary of biomarkers assayed by the multiplex immunoassay	
Biomarker	**Standard concentration (pg/ml)**
Inflammation	
Interleukin-1 receptor antagonist	6820
Interleukin-10	880
Interleukin-6	1130
Interleukin-8	830
Leptin	122930
Monocyte chemoattractant protein-1	7160
Tumour necrosis factor α	2240
Uromodulin	171190
Fibrosis and angiogenesis	
Galactin-3	4460
Matrix metallopeptidase-9	30190
Suppression of tumorigenicity 2	130950
Vascular endothelial growth factor	1640
Kidney injury	
Kidney injury molecule 1	16480
Neutrophil gelatinase associated lipocalin	22230
Left ventricular hypertrophy	
Angiopoietin-2	22930
Atrial stretch	
Atrial natriuretic peptide	49740

2.8 Regulatory approvals and authorisations

Data presented in this thesis were generated from subjects recruited to the Chronic Renal Impairment in Birmingham Coronary Flow Reserve (CRIB-FLOW) study and the Prospective Study of the Effects of Renal Transplantation on Uraemic Cardiomyopathy using Magnetic Resonance Imaging (RETRACT) echocardiogram sub-study. Further details about these studies are presented in Chapter 4 (CRIB-FLOW) and Chapter 5 (RETRACT). Both studies were conducted in accordance with the principles of the Declaration of Helsinki. Written informed consent was obtained prior to any study procedures being conducted.

Professor Jonathan N Townend was the designated principal investigator for the CRIB-FLOW study. The study was reviewed and approved by the West Midlands – Solihull Research Ethics Committee (19/WM/0066) and the Health Research Authority (HRA). The study was given local Research and Development approval from University Hospitals Birmingham NHS Foundation Trust, who acted as the sponsor (reference RRK6607).

Professor Charles J Ferro was the designated principal investigator for the RETRACT study. The study was reviewed and approved by the West Midlands – Black Country Research Ethics Committee (18/WM/0287) and the HRA. The study was given local Research and Development approval from University Hospitals Birmingham NHS Foundation Trust, who acted as the sponsor (reference RRK6458).

2.9 Funding

My salary funding for the two years of research towards this MD thesis was jointly provided by Birmingham Health Partners, University Hospitals Birmingham NHS Foundation Trust and the Institute of Cardiovascular Sciences, University of Birmingham, The CRIB-FLOW study was funded by research grants from University Hospitals Birmingham Charity and the Metchley Park Medical Society. The RETRACT study was funded by a British Heart Foundation Clinical Research Training Fellowship awarded to Dr Luke Pickup (FS/18/29/33554). The RETRACT echocardiogram sub-study was funded by research grants from University Hospitals Birmingham Charity and the Metchley Park Medical Society.

CHAPTER 3: REPEATABILITY AND VARIABILITY OF IMAGING TECHNIQUES TO ASSESS CORONARY MICROVASCULAR FUNCTION

3.1 Introduction

In this chapter, repeatability and intra-observer variability of the primary imaging techniques for assessment of CFVR and CFR are reported. Repeatability and variability of both Doppler CFVR and MCE CFR were assessed in healthy controls and LKD. Due to smaller subject numbers, and to avoid research participant fatigue as many subjects with ESRD were also participating in the RETRACT study, repeatability was not performed in this population. However, intra-observer variability of the offline measurement of Doppler CFVR and MCE CFR were also assessed in subjects with ESRD.

3.2 Methods

3.2.1 Repeatability

Repeatability is a measure of the variation in repeat measurements made on the same individual under identical conditions.(276) Doppler CFVR was repeated by me in 11 subjects (8 controls and 3 LKD). Repeat acquisitions of images for Doppler CFVR were made after a rest period of 10 minutes, to allow sufficient time for adenosine washout. Participants underwent repeatability studies if they tolerated the initial infusion of adenosine without excessive symptoms or haemodynamic compromise, and they agreed to undergo repeat testing.

3.2.2 Intra-observer variability

To assess intra-observer variability of my offline analysis, I repeated blinded offline analysis, after 1 week, of the original Doppler CFVR and MCE CFR images in a random subset of subjects. For MCE CFR, intra-observer variability of A, β and CFR were calculated. As I performed and analysed all echocardiographic studies, inter-observer variability was not assessed.

3.2.3 Statistical analysis

Repeatability was assessed by calculating the repeatability coefficient (estimated as 1.96 x $\sqrt{2}$ x within-subject SD). Future measurements made by a particular observer on a particular subject would be expected to fall within this absolute difference on 95% of occasions.(276) Further assessment of agreement between measurements for repeatability and intra-observer variability were performed using a two-way mixed effects model ICC looking for absolute agreement. A p value <0.05 was considered statistically significant. Agreement was categorised as poor (ICC <0.5), moderate (ICC 0.5-0.75), good (ICC 0.76-0.9) or excellent (ICC >0.9).(277) Finally, repeatability and intra-observer variability were also tested by calculating mean bias (defined as the mean of the differences between measure 1 and measure 2) and LOA (defined as 1.96 x SD of the differences between measure 1 and measure 2) from Bland-Altman analyses.(278)

3.3 Results

3.3.1 Repeatability of Doppler coronary flow velocity reserve

Repeatability of Doppler CFVR was assessed in 11 subjects (8 controls and 3 LKD). There was a moderate correlation between the two measurements (r=0.7, p=0.026) – figure 3-1. Overall, repeatability of Doppler CFVR was moderate - ICC 0.587 (95% CI 0.044-0.867, p=0.009). Bland-Altman analysis revealed a mean bias of -0.3 (95% CI -0.03 to -0.65) and LOA of 0.9 (95% CI 0.34-1.46). The repeatability coefficient was calculated at 1.1.

To assess improvements in operator technique with increased experience, sub-analysis of repeatability was performed on the 5 studies that were performed furthest in time order from the commencement of data collection for this thesis. Mean time from onset of data collection to these studies was 257 ± 25 days. When restricted to these 5 studies there was strong correlation between the two measurements, although due to the small sample size this was not statistically significant (r=0.9, p=0.065). The strength of the ICC remained moderate – ICC 0.604 (95% CI -0.110; 0.947, p=0.015). However, there was an improvement in the LOA calculated by Bland-Altman analysis as well as an improvement in the repeatability coefficient: mean bias -0.4 (95% CI -0.1; -0.6), LOA 0.4 (95% CI -0.1; 0.8), repeatability coefficient 0.5.

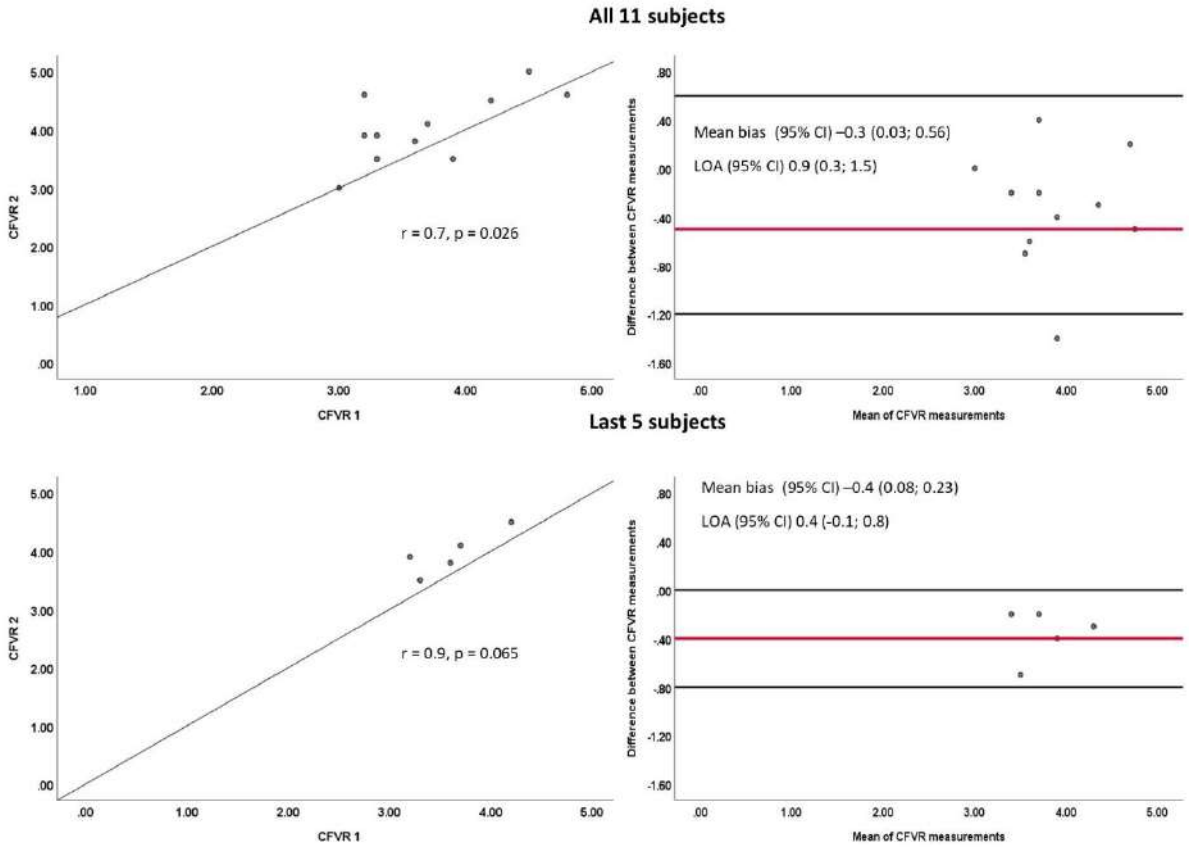


Figure 3-1: Correlation of Doppler CFVR repeatability measurements in all 11 subjects (top left). Bland-Altman analysis of Doppler CFVR repeatability measurements in all 11 subjects (top right). Correlation Doppler CFVR repeatability measurements in 5 subjects with furthest time from onset of data collection (bottom left). Bland-Altman analysis of Doppler CFVR repeatability measurements in in 5 subjects with furthest time from onset of data collection (bottom right). CFVR – coronary flow velocity reserve, CI – confidence interval, LOA – limits of agreement.

3.3.2 Repeatability of coronary flow reserve by myocardial contrast echocardiography

Only 2/11 subjects agreed to further adenosine administration to allow repeat measurement of MCE CFR. Their data are not presented as the sample size is too small to draw any meaningful conclusions.

3.3.3 Intra-observer variability of Doppler coronary flow velocity reserve

Repeat blinded offline analysis of Doppler CFVR in controls and LKD was performed in 12/48 subjects (6 controls and 6 LKD). Combined results for controls and LKD are presented here. However, there were no significant differences in intra-observer variability between the controls and LKD. There was an excellent correlation between the two measurements ($r=0.98$, $p<0.001$) – figure 3-2. There was also a very low intra-observer variability for Doppler CFVR – ICC 0.976 (95% CI 0.923-0.993, $p<0.001$). Bland-Altman analysis revealed a mean bias of 0.01 (95% CI -0.07 to 0.1) and LOA of 0.3 (95% CI 0.1-0.4).

Intra-observer variability was also assessed in 6/24 subjects with ESRD. There was an excellent correlation between the two measurements ($r=1.0$, $p<0.001$). Intra-observer variability for Doppler CFVR in subjects with ESRD was also very low – ICC 0.996 (95% CI 0.972-0.999, $p<0.001$). Bland-Altman analysis revealed a mean bias of -0.1 (95% CI -0.2 to 0.1) and LOA of 0.2 (95% CI 0-0.5).

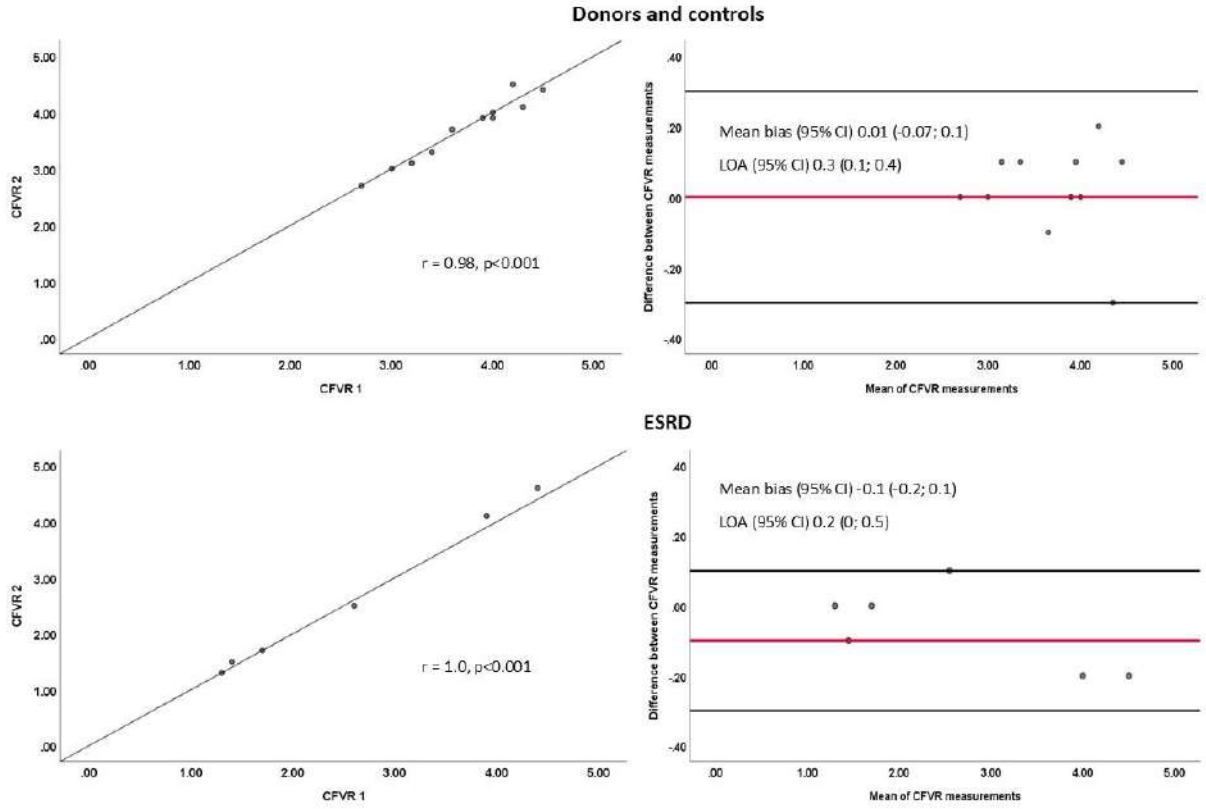


Figure 3-2: Correlation between repeat offline measurements of Doppler CFVR in donors and controls (top left). Bland Altman analysis of repeat offline measurements of Doppler CFVR in donors and controls (top right). Correlation between repeat offline measurements of Doppler CFVR in ESRD (bottom left). Bland Altman analysis of repeat offline measurements of Doppler CFVR in ESRD (bottom right). CFVR – coronary flow velocity reserve, CI – confidence interval, ESRD – end-stage renal disease, LOA – limits of agreement.

3.3.4 Intra-observer variability of quantitative myocardial contrast echocardiography

Repeat blinded offline analysis of quantitative MCE in controls and LKD was performed in 12/48 subjects (6 controls and 6 LKD) to assess intra-observer variability. Combined data for controls and LKD are presented. Results were similar between the two groups. Intra-observer variability for A and β were tested in 94 segments at rest and during hyperaemia. Results of the individual parameters are summarised in table 3-1.

Table 3-1: Summary of intra-observer variability for coronary flow reserve in controls and living kidney donors (n=12)

Parameter (Unit)	ICC (95% CI, p value)	Mean bias (95% CI)	LOA (95% CI)
A at rest (intensity)	0.531 (0.369-0.662, p<0.001)	-2 (-10; 7)	84 (69-100)
β at rest (1/seconds)	0.618 (0.474-0.729, p<0.001)	-0.16 (-0.02; -0.3)	1.35 (1.11-1.6)
A at hyperaemia (intensity)	0.81 (0.727-0.870, p<0.001)	-3 (-7; 1)	38 (31-44)
β at hyperaemia (1/seconds)	0.481 (0.309-0.623, p<0.001)	0.04 (-0.39; 0.46)	4.05 (3.32-4.78)
CFR LAD	0.822 (0.506-0.945, p<0.001)	0.3 (-0.1; 0.7)	1.2 (0.5-1.9)
CFR Global	0.699 (0.265-0.901, p=0.004)	0.3 (-0.3; 0.9)	1.8 (0.8-2.9)

ICC – intra-class correlation coefficient, CI – confidence interval, LOA – limits of agreement, CFR – coronary flow reserve, LAD – left anterior descending artery

There was a wide range of intra-observer variability for the different parameters in controls and LKD. β was less variable at rest whereas A was less variable at hyperaemia. β at hyperaemia showed the highest variability while CFR LAD showed the lowest variability. However, the LOA for both CFR LAD and CFR Global were relatively high on Bland-Altman analyses.

Intra-observer variability of quantitative MCE was also performed in 6/24 subjects with ESRD. Intra-observer variability for A and β were tested in 44 segments at rest and during hyperaemia. Results of the individual parameters are summarised in table 3-2.

Table 3-2: Summary of intra-observer variability for coronary flow reserve in end-stage renal disease (n=6)

Parameter (Unit)	ICC (95% CI, p value)	Mean bias (95% CI)	LOA (95% CI)
A at rest (intensity)	0.824 (0.679-0.904, p<0.001)	-9 (-2; -9)	43 (31-54)
β at rest (1/seconds)	0.323 (0.048-0.558, p=0.011)	0.3 (0.02-0.62)	1.9 (1.4-2.4)
A at hyperaemia (intensity)	0.479 (0.214-0.678, p<0.001)	-3 (-15; 9)	75 (55-96)
β at hyperaemia (1/seconds)	0.379 (0.103-0.603, p=0.004)	0.4 (-0.1; 0.9)	3.3 (2.4-4.2)
CFR LAD	0.916 (0.504-0.988, p=0.003)	0 (-0.4; 0.4)	0.8 (0-1.5)
CFR Global	0.85 (0.297-0.977, p=0.01)	-0.1 (-0.5; 0.3)	0.7 (0-1.4)

ICC – intra-class correlation coefficient, CI – confidence interval, LOA – limits of agreement, CFR – coronary flow reserve, LAD – left anterior descending artery

Similar to the findings in controls and LKD, there was a wide range of intra-observer variability for the different parameters that comprise CFR. A at rest and β at rest and at hyperaemia all showed high variability. However, variability of CFR was lower in ESRD than in donors and controls, with CFR LAD having low variability and CFR Global having moderate variability, as well as narrower LOA on Bland-Altman analyses.

3.4 Discussion

Previous studies using TTE have shown good repeatability for Doppler CFVR. Olsen *et al.* repeated CFVR measurements in 21 individuals and found good agreement between the measurements – ICC 0.84 (95% CI 0.65-093) and LOA 0.7, with a calculated repeatability coefficient of 0.7.(169) Similarly Michelsen *et al.* studied repeatability of Doppler CFVR in 20 individuals and found an excellent agreement between the 2 measures – ICC 0.96 (0.92-0.99), LOA 0.4.(279) The repeatability data presented here showed only moderate repeatability, with wider LOA and repeatability coefficient compared to these studies. This may be partly due to the smaller sample size making the data more vulnerable to skew from outliers, particularly as both LOA and repeatability coefficient depend on the SD of measurements for calculation. Furthermore, there is a steep learning curve with Doppler CFVR and it is likely that the superior repeatability quoted in these studies is due to greater operator experience with the technique. Analysis of my last 5 subjects with repeat measurements, which were performed after an additional 8 months personal experience with the technique, showed marked improvement in both LOA and repeatability coefficient, which were now within the range reported by these other authors. This suggests that repeatability of Doppler CFVR can be improved with increased operator experience.

The intra-observer variability of Doppler CFVR analysis reported here is low, and is consistent with the results reported by other authors.(280) This is true for controls, LKD and subjects with ESRD, suggesting that the offline analysis technique is consistent across many study populations. This is likely to be due to the ease of offline analysis and the straightforward calculation required to compute CFVR.

It was not possible to perform repeatability of MCE CFR. This was due to the study protocol which required two separate administrations of adenosine to generate hyperaemia for Doppler CFVR and MCE CFR respectively. However, as the two techniques require different transducers and machine protocols, it was not feasible to perform both hyperaemic measurements in one stretch without an excessively long infusion of adenosine. Thus, assessing repeatability of MCE CFR would have necessitated a 4th administration of adenosine. An alternative solution would have been to perform the repeatability studies on a separate visit. However, this would have increased the likelihood that repeat tests were not performed under identical conditions to the original study. Furthermore, participants were reluctant to return for a further study visit and one of the reasons for the good recruitment to my studies prior to the COVID-19 outbreak was that many participants only required a single visit as part of their involvement in this research.

The intra-observer variability of MCE CFR was higher than that of Doppler CFVR, with lower ICC and higher LOA. Quantitative MCE is particularly dependent on good image quality to ensure accurate tracking of the myocardium. The use of adenosine, which can cause uncomfortable dyspnoea and chest wall movement, can compromise the image quality needed for optimal MCE quantification, and may have influenced these results. However, adenosine was used as the vasodilator of choice as it does not cause coronary artery vasodilatation at hyperaemia, and thus improves the accuracy of Doppler CFVR.(137) Previous studies have used intravenous dipyridamole, which has fewer respiratory side effects, but was not available in our hospital.(177) Furthermore,

dipyridamole can cause coronary artery vasodilatation, and thus may lead to underestimation of CFVR due to an artificially low hyperaemic CFV.(133)

The intra-observer variability of the individual parameters A and β was higher than that of CFR. This may be because CFR is an average of several segments, which minimises bias from individual segments. CFR LAD was consistently less variable than CFR Global. One possible reason for this is that CFR LAD is mainly calculated from septal segments, that are well visualised on TTE. Interestingly, CFR LAD and CFR Global were less variable in subjects with ESRD compared to controls or LKD. This may be a chance finding. However, subjects with ESRD have a higher incidence of LVH and it is possible that the increased wall thickness seen in ESRD aids consistent tracking of a ROI through the myocardium. Overall my intra-observer variability for CFR is consistent with that of other authors who also found low to moderate intra-test variability for A, β and CFR.(281) The variability of quantitative MCE can be reduced through the use of computer-assisted methods as described by Li *et al.*(282) However, this algorithm remains in development and was not available for use.

3.5 Conclusions

In this chapter, I have discussed the main imaging techniques used in my studies for assessment of coronary microvascular function. Both Doppler CFVR and MCE CFR have advantages and disadvantages, and required dedicated training and practice. On balance, Doppler CFVR was chosen as the primary imaging modality for the studies described in this thesis due to its relative ease, higher feasibility and lower variability compared with MCE CFR.

CHAPTER 4: CORONARY FLOW VELOCITY RESERVE IN CONTROLS AND LIVING KIDNEY DONORS

4.1 Preface

Some of the work presented in this chapter has been previously published.(283) I was responsible for the collection of data, the writing of the text and the design of the figures in that publication, which are also presented in this thesis.

4.2 Introduction

It is recognised that kidney transplantation is the most effective form of renal replacement therapy for patients with CKD, and is associated with significant health benefits for the recipient, including improved BP control, and lower all-cause and cardiovascular mortality.(284) However, there is a long-standing shortage of sufficient cadaveric donors. As a result, there has been a steady increase in rates of living kidney donation worldwide, with the procedure accounting for approximately 30% of kidney transplants in the UK in 2018.(285)

After unilateral nephrectomy, most LKD will have an eGFR consistent with CKD stages 2-3.(286) Reductions in CFVR have been demonstrated in patients with CKD that have this level of eGFR.(228,231,233) However, patients with CKD often have comorbidities such as hypertension and diabetes that affect coronary microvascular function. The use of LKD as a model of kidney disease allows one to study the isolated effect of reduced eGFR without confounding comorbidities. The effects of uni-nephrectomy on coronary microvascular function are not known, and CFVR in LKD has not been studied to date. Given the increasing numbers of LKD worldwide, and the concerns

raised about increased long-term cardiovascular mortality in LKD,(287) it is important to assess whether unilateral nephrectomy is associated with impaired microvascular function, which may have long-term implications for cardiovascular risk in LKD.

4.3 Aims and hypothesis

The aim of the Chronic Renal Impairment in Birmingham Coronary Flow Reserve (CRIB-FLOW) study was to assess coronary microvascular function in LKD and to look for associations between CFVR and markers of inflammation and fibrosis. The hypothesis was that CFVR is reduced in LKD compared to healthy controls of a similar age and gender.

4.4 Methods

4.4.1 Study design

The CRIB-FLOW Study was a single-centre cross-sectional observational study of coronary microvascular function in controls and LKD.

4.4.2 Study population

Between May 2019 and February 2020, 23 LKD and 25 healthy controls were enrolled in the CRIB-FLOW study at QEHB – figure 4-1. All participants were >18 years of age and provided written informed consent. Donors were recruited from the living kidney donor registry at QEHB. Healthy controls, of a similar age and gender, were recruited from staff members at QEHB and control subjects from the CRIB Donor study. Full Inclusion and exclusion criteria are shown in table 4-1.

4.4.3 Study investigations

All subjects underwent study investigations as described in Chapter 3. Serum was frozen for subsequent analysis of hsCRP and other biomarkers.

4.4.4 Blinded analysis

Echocardiograms were stored under an anonymous code and analysed offline using commercially available software (IntelliSpace Cardiovascular, Philips, Eindhoven, Netherlands). The TTE, CFVR and MCE studies were all analysed by me, blinded to study group.

4.4.5 Endpoints and sample size justification

The primary endpoint was the difference in mean Doppler CFVR between controls and LKD. There were no prior data on CFVR in LKD on which to base a power calculation. Using previous data by Imamura *et al.*(231) [CFVR for controls (3.8 ± 0.4), CFVR for CKD stage 2 (3.2 ± 0.7), CFVR for CKD stage 3 (3.0 ± 0.6)] - I estimated that 30 patients in each group would provide 90% power with an alpha value of 0.05 to demonstrate a difference in Doppler CFVR of 0.6 between controls and LKD. I planned to recruit a total of 70 subjects in case Doppler CFVR measurement was not possible in some subjects. The onset of the COVID-19 pandemic meant that recruitment to this study was prematurely curtailed. However, a revised power calculation estimated that a sample size of 22 patients in each group would be sufficient to provide 80% power to demonstrate the pre-specified difference in Doppler CFVR of 0.6 between controls and LKD. Difference in CFR by MCE was the secondary endpoint.

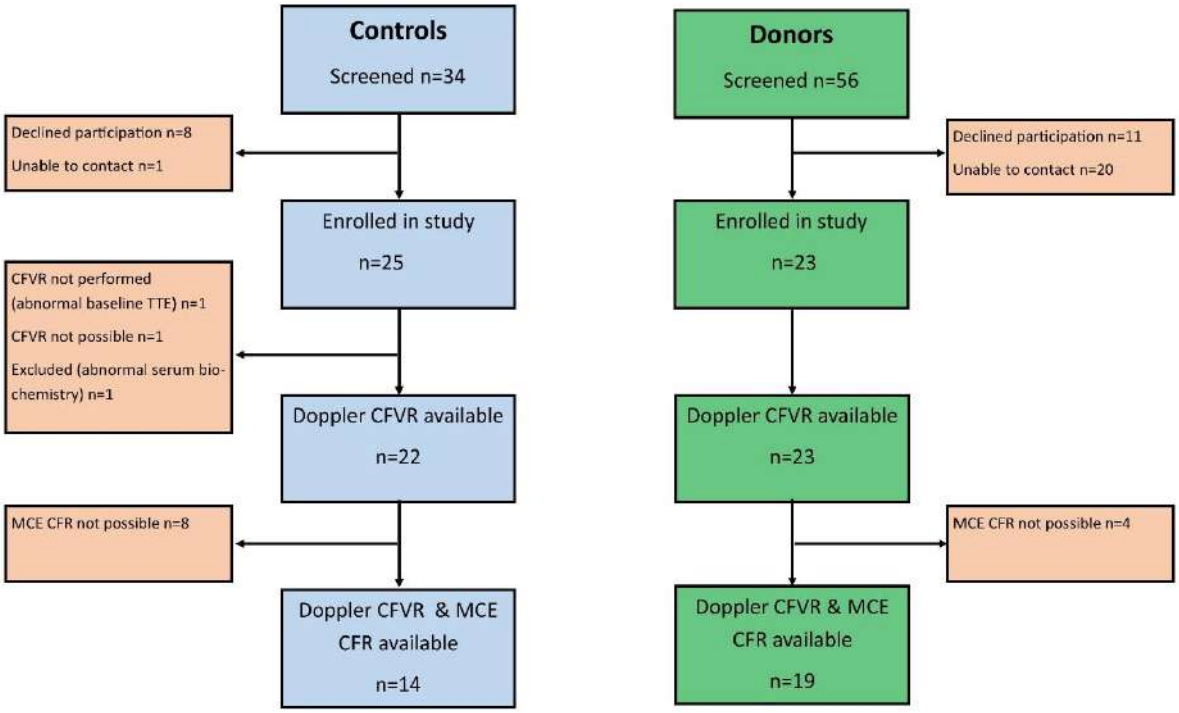


Figure 4-1: CONSORT statement for the CRIB-FLOW study. TTE – transthoracic echocardiogram, CFVR – coronary flow velocity reserve, CFR – coronary flow reserve, MCE – myocardial contrast echocardiogram. Reproduced with permission from Radhakrishnan *et al.* (283)

Table 4-1: Inclusion and exclusion criteria for the CRIB-FLOW study

Inclusion criteria	***Exclusion criteria***
Age >18 years Able to provide written informed consent Donors: >12 months from living kidney donation Controls: eGFR >90ml/min/1.73m^2 or eGFR 60-90ml/min/1.73m^2 and no significant proteinuria or signs of kidney damage	Pregnancy Diabetes mellitus Uncontrolled hypertension Ischaemic heart disease Moderate/severe valvular heart disease Contraindication to adenosine or Sonovue

4.5 Results

4.5.1 Subject characteristics

A total of 48 subjects (25 controls, 23 LKD) were enrolled in the CRIB-FLOW study. Baseline demographic, laboratory and haemodynamic data are presented in table 4-2. Median time from donation in LKD was 30 months (IQR 24-67 months). Baseline demographic characteristics were similar between controls and LKD. The majority of participants were male and Caucasian. One donor was on anti-hypertensive therapy. 21/48 (44%) of the entire cohort had total cholesterol >5mmol/L. Two controls and 1 donor were on statin therapy. Of the remaining 18 participants with total cholesterol >5mmol/L, only 1 donor and 2 controls met UK criteria for primary prevention statin therapy (QRISK3 10 year risk >10%).(288)

There was a significant difference in renal function between controls and LKD. As expected, serum creatinine was significantly higher and eGFR was significantly lower in LKD. 20/23 (87%) donors had eGFR consistent with CKD stage 2, while the remainder had eGFR in the range of CKD stage 3. Serum phosphate was significantly lower in LKD. However, other markers of bone mineral metabolism such as calcium and PTH were similar between the groups. Detectable CRP and median hsCRP were both significantly higher in LKD.

Table 4-2: Demographic, laboratory and haemodynamic variables in controls and living kidney donors

	Controls (n=25)	***Donors (n=23)***	***p value***
Demographics			
Age (years)	41 ± 10	46 ± 10	0.098
Male n(%)	18 (72)	16 (70)	0.853
Caucasian n(%)	15 (60)	18 (78)	0.173
BMI (kg/m^2)	25.6 ± 2.3	26.8 ± 4.2	0.230
Smoker n(%) – Current Ex Never	2 (8) 5 (20) 18 (72)	3 (13) 4 (17) 16 (70)	0.905
Hypertension n(%)	1 (4)	1 (4)	1.0
Hypercholesterolaemia n(%)	8 (32)	13 (57)	0.145
ACE inhibitors n(%)	0 (0)	1 (4)	0.479
Statin therapy n(%)	2 (8)	1 (4)	1.0
Time from donation (months)	n/a	30 (24-67)	n/a
Laboratory data			
Haemoglobin (g/L)	146 ± 11	141 ± 10	0.198
Urea (mmol/L)	5.0 ± 1.3	5.7 ± 1.1	0.061
Creatinine (µmol/L)	**80 ± 17**	**107 ± 15**	**<0.001**
eGFR (ml/min/1.73m^2)	**99 (91-112)**	**68 (64-72)**	**<0.001**
ACR (mg/mmol)	0.9 (0-2.1)	0.9 (0-1.8)	0.298
Phosphate (mmol/L)	**1.13 ± 0.17**	**1.03 ± 0.17**	**0.042**
Corrected calcium (mmol/L)	2.33 ± 0.08	2.36 ± 0.08	0.152
PTH (µmol/L)	5.7 ± 2.1	6.6 ± 2.0	0.237
Total cholesterol (mmol/L)	4.6 (4.0-5.2)	5.1 (4.8-5.6)	0.06
LDL cholesterol (mmol/L)	2.7 ± 1.0	3.2 ± 0.8	0.06
NT-proBNP (ng/L)	40 (22-69)	54 (24-95)	0.391
Detectable CRP n(%)	**7 (29)**	**18 (73)**	**0.01**
hsCRP (mg/L)	**0.63 (0.41-0.86)**	**1.31 (0.92-2.0)**	**0.006**
Urate (µmol/L)	332 ± 84	366 ± 82	0.158
Renin (mIU/L)	21.2 (16.9-35.6)	17.9 (13.4-35.5)	0.324
Aldosterone (µmol/L)	161 (129-225)	129 (44-222)	0.156
Haemodynamic data			
Systolic BP (mmHg)	116 ± 11	115 ± 12	0.835
Diastolic BP (mmHg)	76 ± 10	76 ± 10	0.816
Heart rate (bpm)	71 ± 12	65 ± 11	0.066

Data are presented as mean ± SD or median (IQR). BMI – body mass index, ACE – angiotensin converting enzyme, eGFR – estimated glomerular filtration rate, ACR – albumin creatinine ratio, PTH – parathyroid hormone, LDL – low density lipoprotein, NT-proBNP – N-terminal pro-B-type natriuretic peptide, CRP – C-reactive peptide, hsCRP – high sensitivity C-reactive peptide, BP – blood pressure.

Echocardiographic parameters are presented in table 4-3. Controls and LKD had similar LVMI, LV volumes and systolic and diastolic function. One control subject had previously undiagnosed severe aortic regurgitation detected on baseline TTE.

Table 4-3: Echocardiographic parameters in controls and living kidney donors

	Controls (n=25)	***Donors (n=23)***	***p value***
IVSD (mm)	10 (9-11)	10 (8-11)	0.106
LVIDD (mm)	44 ± 4	44 ± 5	0.946
PWD (mm)	9 (8-10)	9 (8-10)	0.732
LVIDS (mm)	28 ± 3	29 ± 4	0.470
Fractional Shortening (%)	36 (31-38)	32 (31-36)	0.201
LVEDVi (ml/m^2)	46 ± 8	47 ± 10	0.716
LVESVi (ml/m^2)	17 (14-19)	18 (13-22)	0.713
EF (%)	62 (60-65)	61 (57-65)	0.305
TAPSE (mm)	21 ± 3	20 ± 3	0.168
GLS (%)	-19 ± 3	-19 ± 3	0.849
LV mass index (g/m^2)	71 (62-88)	69 (57-76)	0.307
LV geometry n(%) – normal geometry concentric remodelling eccentric hypertrophy concentric hypertrophy	17 (68) 6 (24) 1 (4) 1 (4)	14 (61) 9 (39)	0.439
Left atrial volume index (ml/m^2)	19.3 ± 4.3	20.5 ± 6.8	0.477
E/A ratio	1.2 ± 0.3	1.1 ± 0.2	0.184
E/e’	6 (5-8)	6 (6-7)	0.655

Data are presented as mean ± SD or median (IQR). IVSD – interventricular septal diameter, LVIDD – left ventricular internal diameter diastole, PWD – posterior wall diameter, LVIDS – left ventricular internal diameter systole, LVEDVi – indexed left ventricular end diastolic volume, LVESVi – indexed left ventricular end systolic volume, EF – ejection fraction, TAPSE – tricuspid annular plane systolic excursion, GLS – global longitudinal strain, LV – left ventricular

4.5.2 Doppler coronary flow velocity reserve

Doppler CFVR measurement was successful in 46/47 (99%) of subjects in which it was attempted. The control subject with newly identified severe aortic regurgitation on baseline TTE did not undergo Doppler CFVR assessment. One control was unable to tolerate adenosine and thus no hyperaemic measurements were possible. One subject was subsequently excluded from CFVR analysis due to the new finding of thyrotoxicosis on serum biochemistry. Final Doppler TTE CFVR data for 22 controls and 23 LKD are presented. SonoVue was used in 31/45 (69%) cases.

There was an adequate and similar haemodynamic effect from adenosine in both groups. The HR response was more prominent than the BP response. The mean percent increase in HR from baseline was 44% ± 16% in controls and 45% ± 16% in donors, p=0.802. The median percent change in systolic BP from baseline was -11% (-7; -15) in controls and -7% (0; -13) in donors, p=0.157.

Resting CFV was numerically but not statistically higher in donors [median CFV 19.9cm/s (17.4-22.2) vs 18.1cm/s (15.6-20.4), p=0.114]. Hyperaemic CFV did not differ between the groups (mean CFV 70.2cm/s ± 14.6 vs 70.5cm/s ± 13.8, p=0.944) – figure 4-2. Doppler CFVR was significantly reduced in LKD compared to controls (mean CFVR 3.4 ± 0.7 vs 3.8 ± 0.6, mean difference 0.4 95% CI 0.03-0.8, p=0.036) – figure 4-3. No subjects in this study had CFVR <2. However, 6/23 (26%) LKD had CFVR ≤2.7 (the lowest CFVR value in controls). There was a modest significant correlation between eGFR and CFVR (r=0.3 p=0.034).

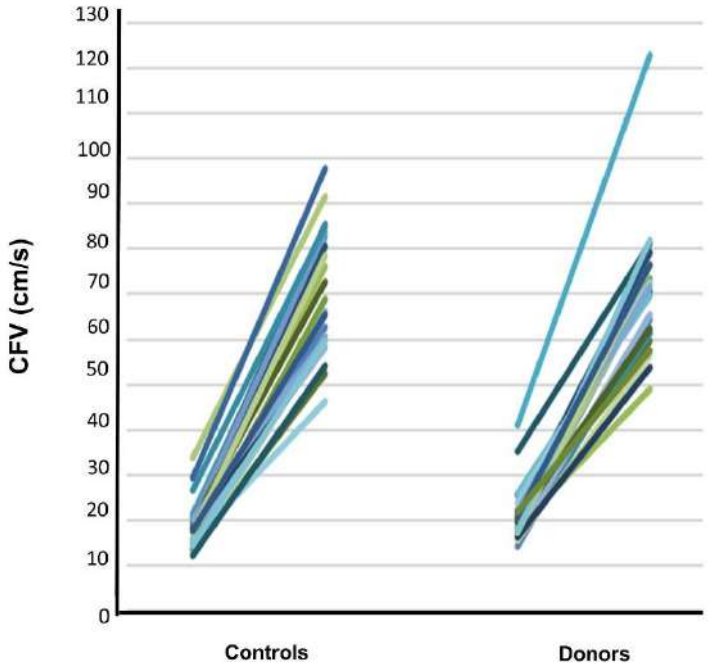


Figure 4-2: Coronary flow velocity at rest and at hyperaemia in controls and living kidney donors. CFV – coronary flow velocity. Reproduced with permission from Radhakrishnan *et al.*(283)

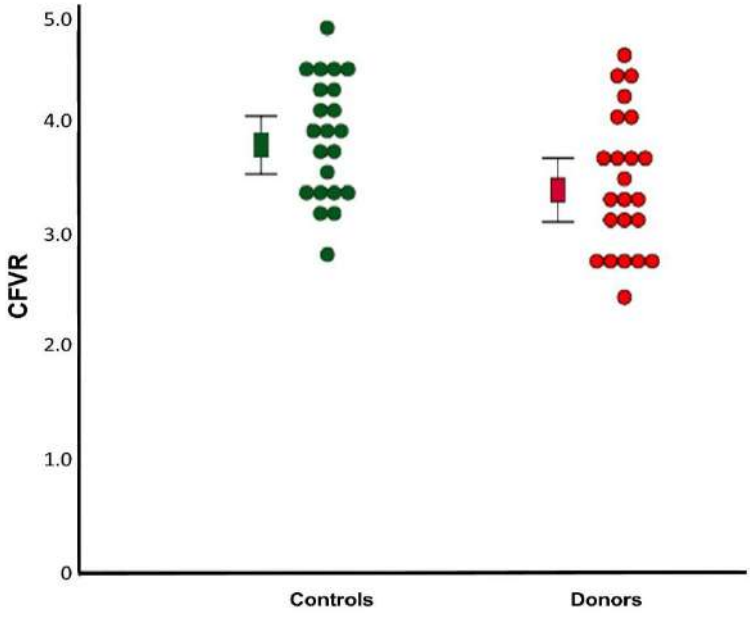


Figure 4-3: Doppler coronary flow velocity reserve in controls and living kidney donors. Squares represent mean. Error bars represent 95% confidence intervals. Circles represent individual CFVR measurements. CFVR – coronary flow velocity reserve. Reproduced with permission from Radhakrishnan *et al.*(283)

4.5.3 Myocardial contrast echocardiography

No subjects had stress induced wall motion abnormalities or perfusion defects on MCE. Quantitative MCE was possible in 19/23 LKD and 14/25 controls. Both LAD CFR and global CFR were numerically but not statistically lower in LKD compared to controls – LAD CFR [median CFR 2.7 (IQR 2.2-3.9) vs 3.4 (IQR 2.6-5.0), p=0.212] and global CFR [median CFR 3.0 (IQR 2.3-4.2) vs 3.4 (IQR 2.2-3.8), p=1.0].

4.5.4 Arterial stiffness measurements

Pulse wave analysis was performed in 14/23 LKD and 15/25 controls. The remaining 19 subjects declined PWA measurement. 3 controls and 3 LKD had their PWA results excluded from analysis due to the presence of type C waveforms. Analysis of the remaining subjects showed no significant difference in corrected PWA between LKD and controls (mean AIx 17% ± 11% vs 18% ± 12%, p=0.862).

Pulse wave velocity was measured in 13/23 LKD and 15/25 controls. It could not be measured in 1 donor due to equipment malfunction. The remaining 19 subjects declined PWV measurement. There was no significant difference in PWVadj between LKD and controls [mean PWVadj 4.8 ± 0.9 vs 5.4 ± 1.3, p=0.217).

4.5.5 Multiplex immunoassay

The results of the Multiplex immunoassay are shown in table 4-4. One control did not provide blood for immunoassay analysis. There were no significant differences between controls and LKD in the assays tested, apart from uromodulin which was significantly lower in LKD.

Table 4-4: Results of multiplex immunoassay in controls and living kidney donors

Assay	***Controls (n=24)***	***Donors (n=23)***	***p value***
Angiopoetin-2 (pg/ml)	1518 (1260-2006)	1348 (1143-1865)	0.322
Atrial natriuretic peptide (pg/ml)	4730 (3449-6145)	5778 (3653-8248)	0.268
Detectable IL-10 n(%)	11 (44)	11 (48)	0.790
Detectable KIM-1 n(%)	9 (36)	11 (48)	0.406
Galectin-3 (ng/ml)	0.9 (0.8-1.2)	1.1 (0.8-1.3)	0.317
IL-1ra (pg/ml)	522 (356-655)	503 (340-703)	0.807
IL-6 (pg/ml)	1.26 (0.82-1.86)	1.26 (0.97-1.81)	0.661
IL-8 (pg/ml)	12.3 (8.4-25.5)	11.3 (8-29.1)	0.992
Leptin (ng/ml)	5.7 (3.0-11.1)	4.9 (3.2-8.5)	0.865
MCP-1 (pg/ml)	378 (298-537)	391 (325-480)	0.670
MMP-9 (pg/ml)	9118 (6465-13292)	9928 (7374-19628)	0.360
NGAL (ng/ml)	15.5 (14.0-16.6)	16.7 (14.4-18.3)	0.187
ST2 (ng/ml)	12 (9-16)	10 (6-18)	0.444
TNFα (pg/ml)	3.5 (2.53-4.22)	3.37 (2.59-4.28)	0.924
Uromodulin (ng/ml)	**98 ± 43**	**67 ± 35**	**0.009**
VEGF (pg/ml)	48 (24-60)	65 (41-93)	0.101

Data are presented as mean ± SD or median (IQR). IL-10 – interleukin-10, KIM-1 – kidney injury molecule 1, IL-1ra – interleukin 1 receptor antagonist, IL-6 – interleukin-6, IL-8 – interleukin-8, MCP-1 – monocyte chemoattractant protein, MMP-9 – matrix metallopeptidase 9, NGAL – neutrophil gelatinase associated lipocalin, ST2 - suppression of tumorigenicity 2, TNFα – tumour necrosis factor alpha, VEGF – vascular endothelial growth factor.

4.6 Discussion

This was the first study to investigate CFVR in LKD. The majority of LKD in this study had eGFR consistent with CKD stage 2. My data demonstrate that despite only small reductions in eGFR, LKD had a small but statistically significant reduction in Doppler CFVR compared to controls of similar age, gender and comorbidity. The magnitude of difference in CFVR seen here between controls and LKD is similar to the previously demonstrated difference between controls and subjects with CKD stage 2 in the study by Imamura *et al*.(231) These findings suggest that loss of renal function alone may influence parameters of coronary microvascular function. Interestingly, the reduction in CFVR occurred despite the absence of any significant proteinuria, which is an independent risk factor for cardiovascular risk in CKD.(13) Reassuringly, no individual in this cohort had CFVR <2, which is known to be a poor prognostic marker among subjects with CKD.(218) This is consistent with the studies described in Chapter 1, that show that rates of CMD are low until eGFR falls below 60 ml/min/1.73m^2.

I was unable to detect a significant difference in CFR by MCE between controls and LKD. This may be because the lower feasibility and wider variances in CFR among my subjects meant that the study was underpowered for this secondary endpoint. Coronary flow reserve by MCE was measurable in 69% of my cohort, which is consistent with previous studies showing that quantitative MCE using adenosine is feasible in only 33-75% of patients.(289,290) Nevertheless, there was a trend towards reduced CFR in LKD, which is consistent with the Doppler CFVR data. The difference in CFR between the groups was also of similar magnitude to that seen with Doppler CFVR.

There were no significant differences between controls and LKD in measures of arterial stiffness. Although my sample size was limited, this is consistent with the results of the largest study to date of arterial stiffness in LKD and controls, which also did not show any difference in PWA or PWV between controls and LKD at 12 months post-nephrectomy.(108)

4.6.1 Mechanisms of reduced coronary flow velocity reserve in living kidney donors

The pathophysiology of microvascular dysfunction in LKD are not clear but abnormalities of both structure and function may be present. As discussed in Chapter 1, animal models have demonstrated reduced capillary length and density in the hearts of rats who underwent subtotal nephrectomy and evidence of fibrosis and diastolic dysfunction in rats after uni-nephrectomy.(195,198)

Living kidney donors in this study had higher baseline CFV with similar maximal hyperaemic values, leading to a reduced CFVR. Elevated resting CFV is seen in CKD and has been linked to factors such hypertension, LVH and diastolic dysfunction, which all increase resting myocardial oxygen demand.(231,291) Elevated resting CFV may also reflect increased SNS activity which causes vasoconstriction of vascular smooth muscle cells, increases coronary vascular resistance and decreases coronary perfusion pressure.(292) Increased activation of the SNS is seen in early CKD but to date, there are no studies of this phenomenon in LKD.(293) In addition, the reduced CFVR in LKD also reflects a diminished hyperaemic response to adenosine. As adenosine predominantly exerts its vasodilatory effect on the coronary microcirculation,(137) and adenosine-induced vasodilatation is at least partially

mediated by NO release from the endothelium,(294) endothelial dysfunction may be a contributory mechanism to CMD in LKD. Endothelial dysfunction is common in early CKD, where eGFR is similar to that seen in LKD, and is associated with poor prognosis.(210,212) Although not directly tested in this study, the reduced serum phosphate in LKD seen here may be related to increased FGF-23, elevated levels of which have been previously shown in LKD by Moody *et al.*(107) Increased FGF-23 activity has been previously suggested as a cause of endothelial dysfunction in CKD by Verkaik *et al.* In their elegant animal study, arteries of mice who underwent partial nephrectomy showed impaired endothelium-mediated vasodilatation to acetylcholine compared to mice who underwent a sham procedure. *In vivo* injections of FGF-23 induced impaired endothelium-mediated vasodilatation in the sham group and injections of FGF-23 blocking antibodies reversed these findings in the partial nephrectomy group.(295) Thus, FGF-23 may play a causative role in endothelial dysfunction in CKD, and it is plausible that it may also contribute to endothelial dysfunction in LKD. To date, there are no studies of endothelial function in LKD but the CENS study, which is currently recruiting, will provide a comprehensive assessment of endothelial function in LKD.(296)

4.6.2 Inflammation as a cardiovascular risk factor

High sensitivity C-reactive peptide was significantly higher in LKD, although median values were within the normal range in both groups. Similarly, the prevalence of detectable CRP was also significantly higher in LKD. The significance of this small increase in inflammatory markers in LKD is unclear. An inflammatory response in LKD has been shown in the early post-operative phase, with an 80-fold increase in CRP in

the first week after nephrectomy.(297) Longer term data on chronic inflammation in LKD are conflicting. Huan *et al.* showed no increase in inflammatory markers in LKD at 6 months post donation.(298) However, Moody *et al.* showed that LKD had an increase in hsCRP at 12 months post-donation.(107) The elevated hsCRP seen here suggests that a pattern of subclinical chronic inflammation may be present in LKD. As median time from donation in this study was 30 months, the elevated hsCRP cannot be solely attributed to post-operative changes. Chronic kidney disease is characterised by systemic inflammation, with numerous studies showing that patients with CKD have a high prevalence of circulating inflammatory biomarkers, including hsCRP, tumour necrosis factor alpha (TNFα) and interleukin-6 (IL-6).(299) Levels of inflammatory biomarkers are higher as CKD stage increases and elevated levels are independently associated with CKD progression and death.(299–303) It is plausible that subjects with reduced kidney function due to uni-nephrectomy might also exhibit a pro-inflammatory state.(304)

Inflammation is increasingly being recognised as a key component of the pathogenesis of CVD, and particularly of atherosclerotic disease. Multiple studies have demonstrated that elevated biomarkers of inflammation are associated with an increased risk of ischaemic heart disease.(305,306) Cardiovascular events are also increased after processes that produce a systemic inflammatory response, such as infection or non-cardiac surgery.(307,308) Furthermore, anti-inflammatory therapy has been shown to reduce cardiovascular events in patients with ischaemic heart disease.(309,310) A link between elevated inflammatory markers and increased MACE in LKD has not been demonstrated to date. Nevertheless, given the well-established association between

inflammation and CVD, my finding of increased inflammatory biomarkers among LKD may be clinically important.

4.6.3 The role of inflammation in coronary microvascular dysfunction

There is ample evidence that chronic inflammation is associated with CMD. Osto *et al.* showed that young patients with psoriasis had impaired CFVR compared to healthy controls and patients with CMD (defined as CFVR ≤2.5) had higher Psoriasis Area Severity Index, indicating more severe disease.(311) Coronary microvascular dysfunction appears prevalent across the spectrum of rheumatological disease, with a meta-analysis of 21 studies showing that CFR is consistently and significantly lower in rheumatological disease compared to healthy controls (standardised mean difference = −1.51, 95% CI −1.91 to −1.11; p<0.001).(312) Coronary microvascular dysfunction is also seen in non-rheumatological inflammatory conditions. Kruse *et al.* used PET to study 32 individuals with known or suspected cardiac sarcoidosis. In patients with normal perfusion but positive ^{18}F-fluorodeoxyglucose uptake (suggesting active cardiac sarcoidosis), CFR was significantly reduced compared to patients who were ^{18}F-fluorodeoxyglucose negative.(313) Among patients with suspected myocarditis, patients with biopsy-proven inflammatory infiltrates had significantly lower CFR than biopsy-negative patients.(314)

Inflammation may also play a role in subclinical microvascular dysfunction, a context more similar to that seen among the LKD in this study. A twin study compared the association between PET CFR and inflammatory markers among male monozygotic and dizygotic twins. Within each pair of twins, the twin with lower CFR had significantly

higher inflammatory markers, despite similar genetic history and cardiovascular risk factors, suggesting an association between subclinical inflammation and microvascular dysfunction, even in asymptomatic individuals.(315) .

The mechanism of CMD in chronic inflammation is likely to be related to endothelial dysfunction. A high correlation has been shown between indices of inflammation (IL-6, TNFα) and markers of endothelial activation (von Willebrand factor, circulating endothelial cells) among patients with acute myocardial infarction.(316) Similar associations between inflammatory cytokines and markers of endothelial activation have been demonstrated in rheumatoid arthritis. After an inflammatory stimulus, endothelial cells undergo activation that is characterised by an upregulation of inflammatory cytokines, disrupted vascular tone, and inflammatory reactions within the blood vessel wall, which in turn promotes further inflammatory damage to the endothelium.(317)(318) This increase in inflammatory cytokines is associated with reduced flow-mediated dilatation (endothelium-dependent), reduced nitroglycerine-mediated dilatation (endothelium-independent), and impaired CFVR.(316,319) The endothelial inflammatory process can also result in microvascular rarefaction, leading to reduced coronary microvascular density, further impairing oxygen delivery to the myocardium.(320)

Uromodulin, a glycoprotein secreted by the thick ascending limb of the loop of Henle, was significantly reduced in LKD in this study, reflecting the loss of nephrons from uni-nephrectomy. In a normally functioning kidney, uromodulin is thought to have a protective anti-inflammatory role through neutralisation of urinary cytokines. In the

presence of tubular damage, as seen in CKD, the reduction in uromodulin may have a pro-inflammatory effect by activating NLRP3 dependent IL-1β secretion and subsequent induction of other pro-inflammatory cytokines.(321) Uromodulin may have additional protective properties, with animal studies have shown that it also inhibits TNFα mediated pro-calcific signalling, leading to a reduction in vascular calcification.(322) Thus, reductions in uromodulin may contribute to chronic inflammation in LKD, which in turn may predispose them to the development of inflammation-mediated subclinical microvascular dysfunction. Although plausible, this hypothesis cannot be proven by my data.

The effect of reducing systemic inflammation has shown mixed results in improving coronary microvascular function. Mouse models of type 2 diabetes have shown that TNFα knockout mice have improved endothelial function compared to mice with intact TNFα gene expression.(323) Kellermair *et al.* studied CFVR using Doppler TTE in 14 patients with acute myocarditis. Coronary microvascular dysfunction was present in 57% of cases and associated with higher levels of troponin T and larger areas of late gadolinium enhancement on CMR. At 3-month follow-up, when the myocarditis had resolved, CFR normalised in all patients.(324) In patients with severe psoriasis, treatment with TNFα inhibitors for 6 months led to an improvement in CFVR from 1.88 ± 0.3 to 2.74 ± 0.5 ($p < 0.0001$). However, this was a non-randomised trial with no placebo control.(325) By contrast, a study of Tocilizumab (an anti-IL-6 receptor antibody) in patients with non ST-elevation myocardial infarction showed no effect on CFR compared to placebo.(326) A single small study has examined the effect of surgery for inflammatory bowel disease on CFVR, with multiple linear regression

analysis showing that reduction of hsCRP was independently associated with improvement of CFVR at 1-year post surgery.(327)

4.6.4 Clinical relevance of reduced coronary flow velocity reserve in living kidney donors

The clinical significance of my findings needs further investigation. It is possible that the small reduction in CFVR seen in LKD is a type 1 statistical error given the small sample size as my study did not recruit sufficient subjects to meet the initial power calculation. All LKD had a CFVR within normal limits so it is likely that this small reduction in CFVR in LKD has no clinical sequelae and may be an epi-phenomenon related to chronic low-grade inflammation after uni-nephrectomy. My results should stimulate larger, adequately powered studies of CMD in LKD, to detect whether there truly is a significant difference in CFVR between LKD and appropriate controls. The role of inflammation after nephrectomy also warrants further research.

My findings also highlight the importance of long-term follow up and monitoring of LKD, to allow early detection of cardiovascular problems and ensure aggressive risk factor management. Long-term follow up of LKD in the UK is poor, with only 42% of LKD followed up at 10 years. This is mainly due to logistical reasons as LKD are usually asymptomatic, of working age and may be geographically remote from transplant centres, which in the UK are linked to the recipient.(285) However, it is vital that the importance of long term follow-up is emphasised to LKD, and structural institutional changes are necessary to improve follow-up rates of this important patient population.

4.7 Limitations

Similar to other non-invasive studies of CFVR, I could not fully exclude CAD in this cohort without coronary angiography (either computed tomography or invasive). However, all subjects were asymptomatic, had a normal ECG and normal vasodilator MCE – a highly sensitive and specific technique for the diagnosis of flow limiting CAD.(328) Thus, there is strong indirect evidence that there was no myocardial ischemia due to obstructive CAD in this cohort.

My cohort was predominantly male and Caucasian. This limits the generalisability of my findings to the wider LKD population. However, the majority of UK LKD are Caucasian(285), and it has previously been shown that there are similar rates of CMD among men and women.(329)

Finally, my study was cross-sectional in design, meaning that a causal link between uni-nephrectomy and the reduced CFVR seen in LKD in this study cannot be definitively demonstrated. Future longitudinal work examining CFVR pre- and post-nephrectomy is needed to confirm the observations seen here.

4.8 Conclusions

The CRIB-FLOW study has shown that despite only mild renal impairment and no cardiovascular risk factors, there was a small reduction in Doppler CFVR in LKD compared to healthy controls. This suggests that mild renal impairment from uni-nephrectomy may influence parameters of coronary microvascular function. Larger scale studies are required to confirm this finding. Although current data suggests that living kidney donation remains extremely safe, my study highlights the importance of long-term follow-up and aggressive risk factor management to detect early cardiovascular changes and to minimise any future cardiovascular morbidity and mortality in this population. The role of chronic inflammation in LKD also needs further examination.

CHAPTER 5: CORONARY MICROVASCULAR DYSFUNCTION AMONG POTENTIAL KIDNEY TRANSPLANT RECIPIENTS

5.1 Preface

Some of the work presented in this chapter has been previously published.(330) I was responsible for the collection of data, the writing of the text and the design of the figures in that publication, which are also presented in this thesis.

5.2 Introduction

As discussed in Chapter 1, CMD is common in advanced CKD, with prevalence rates of 30-78% depending on the population studied.(240,241) Patients with CKD stage 5 represent a heterogenous population which includes pre-dialysis patients, as well as PD and HD patients of varying vintage. However, it is recognised that there are significant differences between transplant candidates and those ineligible for transplant. Patients on the kidney transplant waiting list are often younger, have fewer comorbidities, and have a reduced risk of death compared to ESRD patients not suitable for kidney transplantation.(331) Although a high prevalence of CMD has been demonstrated in the wider ESRD population, the data among patients suitable for kidney transplant are less clear.

Furthermore, the mechanisms of CMD in ESRD are not fully understood. Uraemic cardiomyopathy is a clinical syndrome, characterised by LVH, diffuse interstitial fibrosis, systolic and diastolic dysfunction and an increased risk of SCD. The syndrome starts in early CKD but is most pronounced in ESRD, where it is associated with adverse cardiovascular outcomes.(91,332,333) There appears to be a significant

overlap between uraemic cardiomyopathy and HFpEF, another myocardial disorder characterised by a high burden of CMD.(117) Both diseases are characterised by left ventricular stiffness, diastolic function, left atrial dilatation, elevated NTpro-BNP and a high frequency of LVH. The current paradigm for both conditions suggest that common underlying conditions such as diabetes, obesity, hypertension and renal dysfunction lead to a systemic pro-inflammatory state that causes adverse cardiovascular consequences including CMD.(334) Factors such as diabetes and hypertension, that contribute to the development of uraemic cardiomyopathy, have been linked with CMD in CKD.(214,234) However, a number of other mediators, including anaemia, bone mineral disease and chronic inflammation, are thought to be important in the aetiology of uraemic cardiomyopathy. Their impact on the development of CMD in advanced CKD remain unknown.

5.3 Aims

This chapter provides a descriptive cross-sectional analysis of the baseline cohort of patients recruited for the longitudinal study of CFVR in renal transplant recipients described in Chapter 6. The aims of this cross-sectional analysis were threefold. Firstly, I aimed to explore the prevalence of CMD among a population of potential kidney transplant recipients with CKD stage 5. Secondly, I sought to assess how many of this cohort met proposed diagnostic criteria for HFpEF. Finally, I wished to examine the association between CMD and markers of anaemia, bone mineral disease and chronic inflammation in this population.

5.4 Methods

5.4.1 Study population

Between March 2019 and March 2020, subjects with CKD stage 5 were recruited to either the CRIB-FLOW study or the RETRACT echocardiogram sub-study at QEHB – figure 5-1. The RETRACT study is a prospective longitudinal study examining the effects of successful kidney transplantation on parameters including LVMI, arterial stiffness and biomarkers of bone mineral disease. A proportion of patients in the main RETRACT study were enrolled in an echocardiographic sub-study where coronary microvascular function was also assessed.

Patients were recruited from the kidney transplant waiting list at QEHB. All participants were >18 years of age and provided written informed consent. Full inclusion and exclusion criteria are shown in table 5-1. As QEHB has a large PD population, and there are limited data on CFVR in PD patients, recruitment of subjects with CKD stage 5 to the RETRACT echocardiographic sub-study was restricted to those who were pre-dialysis or on PD. Haemodialysis patients were excluded as there are some data that the presence of arterio-venous fistulae produces local and systemic changes to the microcirculation, which may have affected our CFVR results.(213) Furthermore, echocardiography in patients on HD is heavily influenced by volume status, and studies should ideally be performed on the day post-dialysis.(335) Due to logistical reasons around access to the machine required to perform the TTE and CFVR studies, it would not have been consistently possible to perform CFVR studies on the post-dialysis day, which would have resulted in the exclusion of many HD patients.

I planned to recruit 70 patients with CKD stage 5 in total, based on the power calculation for the longitudinal study of CFVR in kidney transplant recipients described in Chapter 6 (see Chapter 6.3.4). However, the outbreak of the global COVID-19 pandemic meant that recruitment was severely curtailed. Twenty-four patients had been recruited by 23rd March 2020. On this date the UK entered the first national lockdown related to the pandemic and non-essential trips outside the home were banned. As patients advanced CKD are particularly susceptible to serious morbidity and mortality from COVID-19 infection, and with the anticipated prolonged disruption of routine clinical and research activity at QEHB, it was decided that further recruitment of patients to the study was not feasible. The data for the patients recruited prior to the first national lockdown are presented in this chapter.

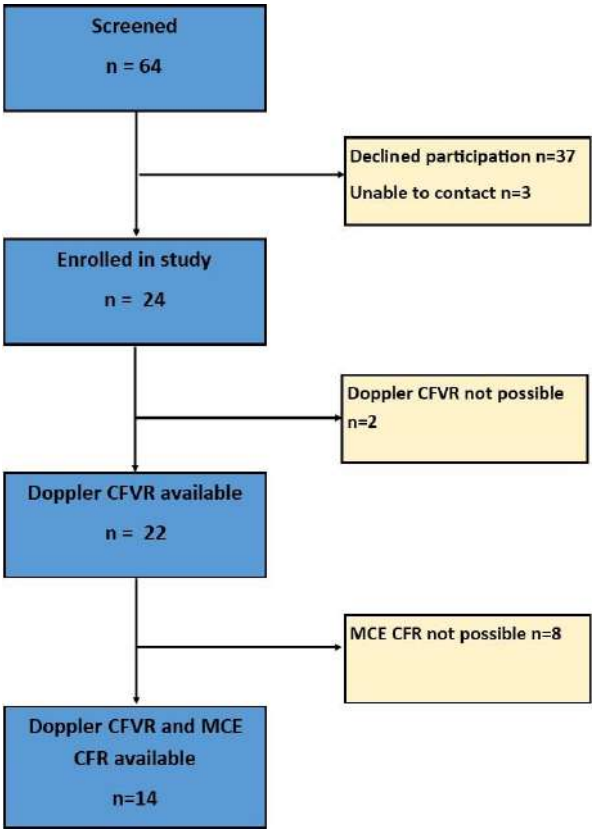


Figure 5-1: CONSORT statement. TTE – transthoracic echocardiogram, CFVR – coronary flow velocity reserve, CFR – coronary flow reserve, MCE – myocardial contrast echocardiogram.

Table 5-1: Inclusion and exclusion criteria

Inclusion criteria	***Exclusion criteria***
Age >18 years Able to provide written informed consent eGFR <15ml/min/1.73m^2 Pre-dialysis or peritoneal dialysis	Pregnancy Haemodialysis Diabetes mellitus Uncontrolled hypertension Ischaemic heart disease Moderate/severe valvular heart disease Contraindication to adenosine or Sonovue

5.4.2 Study investigations

All subjects underwent the study investigations described in Chapter 2. Serum was frozen for subsequent analysis of hsCRP and other biomarkers. The HFA-PEFF score was calculated in all participants with LVEF >50% to assess the likelihood of a diagnosis of HFpEF. The HFA-PEFF score is a diagnostic algorithm devised by the Heart Failure Association of the European Society of Cardiology (ESC) as a means of assessing the probability of a diagnosis of HFpEF. A combined score is assigned from 3 echocardiographic and biomarker domains. Each domain can contribute a maximum of 2 points to the overall score, with the points awarded if any of the criteria within it are met. Thus, a maximum score of 6 points is possible. Scores ≥5 points suggest a high probability of HFpEF and a score ≤1 makes the diagnosis unlikely. Individuals with a score of 2-4 points have an intermediate probability of HFpEF and require further testing to confirm or refute the diagnosis – figure 5-2.(336)

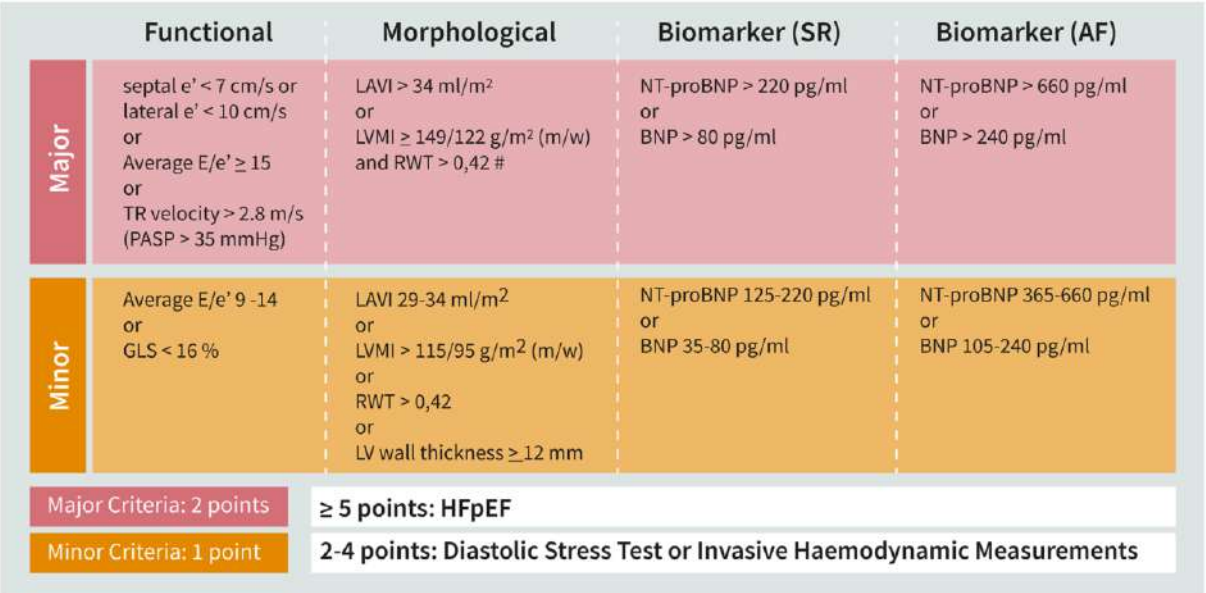


Figure 5-2: HFA-PEFF score. Reproduced with permission from Pieske *et al.*(336)

5.4.3 Blinded analysis

Echocardiograms were stored under an anonymous code and analysed offline using commercially available software (IntelliSpace Cardiovascular, Philips, Eindhoven, Netherlands). The TTE, CFVR and MCE studies were all analysed by me, blinded to study group.

5.4.4 Statistical analysis

Data normality was assessed using the Shapiro-Wilk test. Continuous variables are expressed as mean ± SD for parametric data or median (IQR) for non-parametric data. Unpaired group comparisons for continuous data were made using the unpaired t-test or the Mann-Whitney U test. Unpaired categorical data were compared using Fisher's exact test. Correlation was assessed using the Pearson correlation coefficient. Univariable and multivariable linear regression models were performed with CFVR as the dependent variable. Factors previously shown to influence CFVR (age, systolic BP, LVMI), markers of anaemia (haemoglobin, iron), bone mineral disease (calcium, phosphate, PTH) and inflammation (hsCRP, TNFα, IL-6, IL-8, IL-10) were included as independent variables in the regression model. Binary logistic regression was also performed, with CFVR <2 as the dependent variable, and the parameters listed above as independent variables. Parameters that were significant in univariable analysis were entered into multivariable regression models. A variance inflation factor >5 was taken to represent collinearity. Statistical tests were 2-tailed, and a p value <0.05 was considered statistically significant.

5.5 Results

5.5.1 Subject characteristics

Doppler CFVR was attempted in 24 subjects. It was not possible to identify the LAD in one individual. One subject did not tolerate adenosine and therefore no hyperaemic CFV measurements were obtained. The results for the 22 subjects who successfully underwent CFVR measurement are presented in this chapter. The aetiology of CKD was: glomerulonephritis (45%), polycystic kidney disease (23%), hypertension (9%), obstructive uropathy (9%), pyelonephritis (9%) and idiopathic (5%). No participants reported symptoms of ischaemic heart disease or heart failure at study enrolment. 14/22 (64%) had been previously investigated for CAD as part of the transplant recipient cardiac work-up protocol at QEHB using myocardial perfusion scintigraphy (n = 11), exercise stress echocardiography (n = 2) or invasive coronary angiography (n = 1). Median time from cardiovascular assessment to study enrolment for these individuals was 18 months (IQR 3–33 months). The remaining 8 participants did not require cardiovascular assessment as per the QEHB transplant protocol.

Using the cut-off value of CFVR <2 to signify CMD, 7/22 (32%) of the cohort had CMD. Mean CFVR for subjects with CMD was 1.6 ± 0.2. Mean CFVR for subjects without CMD was 3.2 ± 0.9 – figure 5-3. Demographic, laboratory and haemodynamic data for subjects with and without CMD are shown in table 6-2. There were no significant demographic or haemodynamic differences between the 2 groups. There were similar numbers of patients on PD in both groups, although PD patients with CMD had a trend towards longer dialysis vintage.

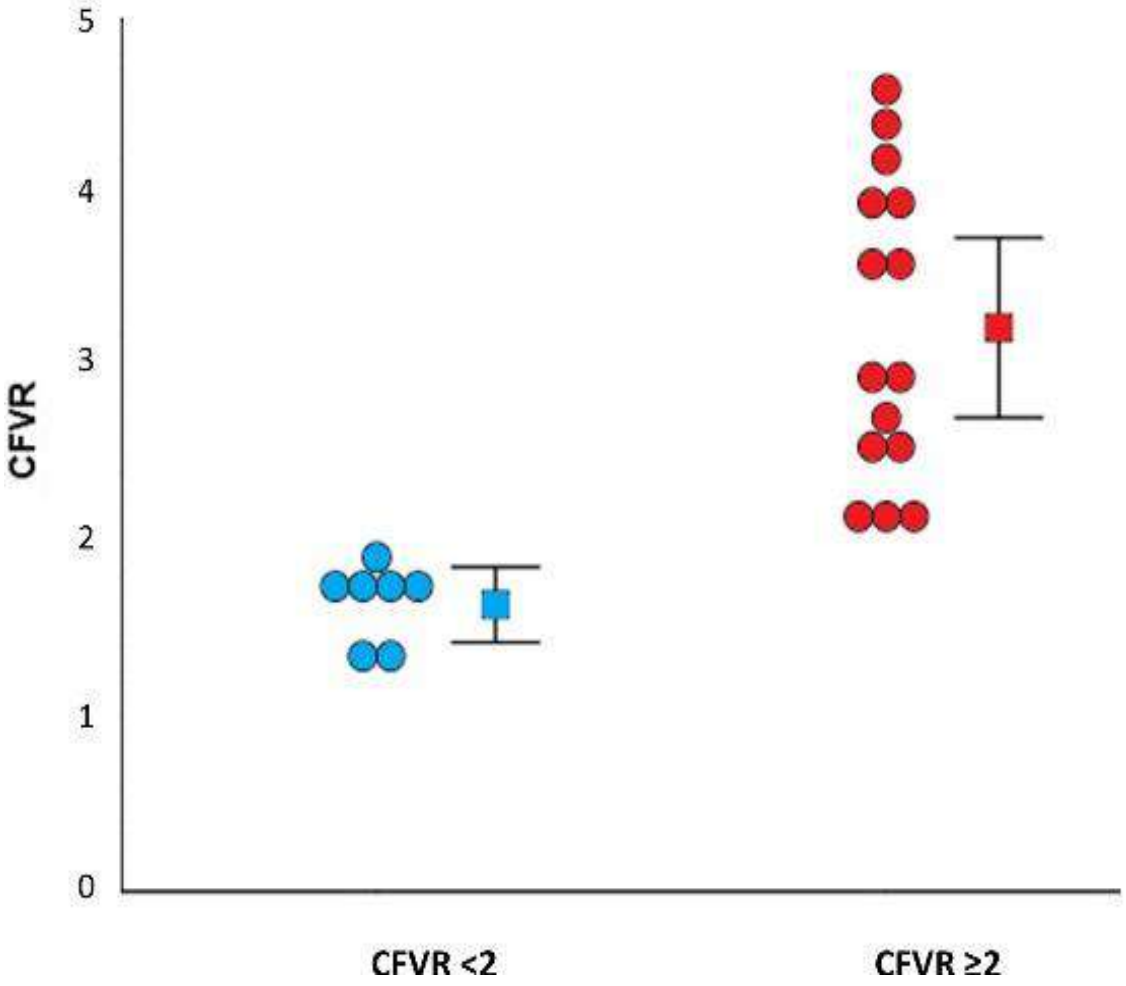


Figure 5-3: Coronary flow velocity reserve in subjects with and without coronary microvascular dysfunction. Squares represent mean. Error bars represent 95% confidence intervals. Circles represent individual CFVR measurements. CFVR – coronary flow velocity reserve.

Table 5-2: Demographic, laboratory and haemodynamic variables in subjects with and without coronary microvascular dysfunction

	CFVR <2 (n=7)	***CFVR ≥2 (n=15)***	***p value***
Demographics			
Age (years)	47 ± 15	55 ± 10	0.177
Male n(%)	3 (43)	8 (53)	1.0
Caucasian n(%)	5 (71)	12 (80)	1.0
BMI (kg/m^2)	26.3 ± 4.4	27.7 ± 4.9	0.527
Smoker n(%) – Ex Never Current	1 (14) 6 (86) 0 (0)	4 (27) 10 (67) 1 (6)	0.744
Hypertension n(%)	6 (86)	14 (93)	1.0
Hypercholesterolaemia n(%)	4 (57)	11 (73)	0.630
Peritoneal dialysis n(%)	5 (71)	9 (60)	1.0
Duration of dialysis (months)	5 (4-48)	6 (4-9)	0.797
ACE inhibitor n(%)	1 (14)	4 (27)	1.0
ARB n(%)	1 (14)	3 (20)	1.0
Statin n(%)	1 (14)	8 (53)	0.165
Loop diuretic n(%)	5 (71)	5 (33)	0.172
Calcium channel blocker n(%)	5 (71)	9 (60)	1.0
Beta blocker n(%)	2 (29)	3 (20)	1.0
Alpha blocker	3 (43)	4 (27)	0.630
Erythropoietin treatment n(%)	5 (71)	4 (27)	0.074
Laboratory data			
Haemoglobin (g/L)	**102 ± 12**	**117 ± 11**	**0.008**
Haematocrit (%)	**31.2 ± 3.3**	**35.4 ± 3.7**	**0.019**
Mean cell volume (fl)	88.9 ± 3.3	91.6 ± 3.7	0.118
Urea (mmol/L)	21.8 ± 6.2	22.1 ± 5.6	0.902
Creatinine (µmol/L)	673 ± 300	606 ± 192	0.534
eGFR (ml/min/1.73m^2	7 (5-11)	9 (7-10)	0.837
ACR (mg/mmol)	204 (109.3-277.8)	77.4 (62.8-199.4)	0.239
Ferritin (µmol/L)	271 ± 178	303 ± 204	0.731
Iron (µmol/L)	11.8 (9.5-13)	12.9 (9.4-16)	0.494
Transferrin (g/L)	1.92 ± 0.54	2.06 ± 0.4	0.525
Albumin (g/L)	35 ± 6	40 ± 7	0.125
Corrected calcium (mmol/L)	2.45 ± 0.13	2.33 ± 0.17	0.123
hsCRP (mg/L)	1.9 (1-3.6)	2.8 (1.9-8)	0.312
NT pro-BNP (ng/L)	1900 (522-4597)	441 (342-643)	0.416
Phosphate (mmol/L)	1.71 (1.55-2.07)	1.59 (1.53-1.69)	0.312
PTH (µmol/L)	41.7 ± 23.2	30.5 ± 16.9	0.271
Total cholesterol (mmol/L)	4.8 ± 1.7	5.0 ± 1.4	0.772
Haemodynamic data			
Systolic BP (mmHg)	129 ± 25	137 ± 20	0.398
Diastolic BP (mmHg)	83 ± 14	85 ± 8	0.798

Heart Rate (bpm)	72 ± 14	66 ± 8	0.156

Data are presented as mean ± SD or median (IQR). CFVR – coronary flow velocity ratio, BMI – body mass index, ACE – angiotensin converting enzyme, ARB – angiotensin receptor blocker, eGFR – estimated glomerular filtration rate, ACR – albumin creatinine ratio, hsCRP – high sensitivity C-reactive peptide, NT-proBNP – N-terminal pro-B-type natriuretic peptide, PTH – parathyroid hormone, BP – blood pressure, bpm – beats per minute.

5.5.2 Coronary flow reserve by myocardial contrast echocardiography

Coronary flow reserve by MCE was feasible in 14 individuals. CFR LAD was lower in the CMD group, compared to subjects with CFVR ≥2, although this was not statistically significant - CFR LAD 1.5 ± 0.7 vs 2.5 ± 1.5, p=0.177. CFR Global did not differ between the two groups – 1.8 (1.2-2.3) vs 1.8 (1.5-2.8), p=0.606.

5.5.3 HFA-PEFF score

Two individuals had LVEF <50% and thus the HFA-PEFF score was not calculated. The HFA-PEFF score was calculated in the remaining 20 subjects – figure 5-4. No subject had a score ≤1. 5/20 (25%) had a HFA-PEFF score of 2-4 points, suggesting an intermediate probability of HFpEF. NTpro-BNP data was not available in 1 of these individuals, which may have underestimated their total HFpEF score. 15/20 (75%) of individuals had a HFA-PEFF score ≥5, suggesting a high probability of HFpEF.

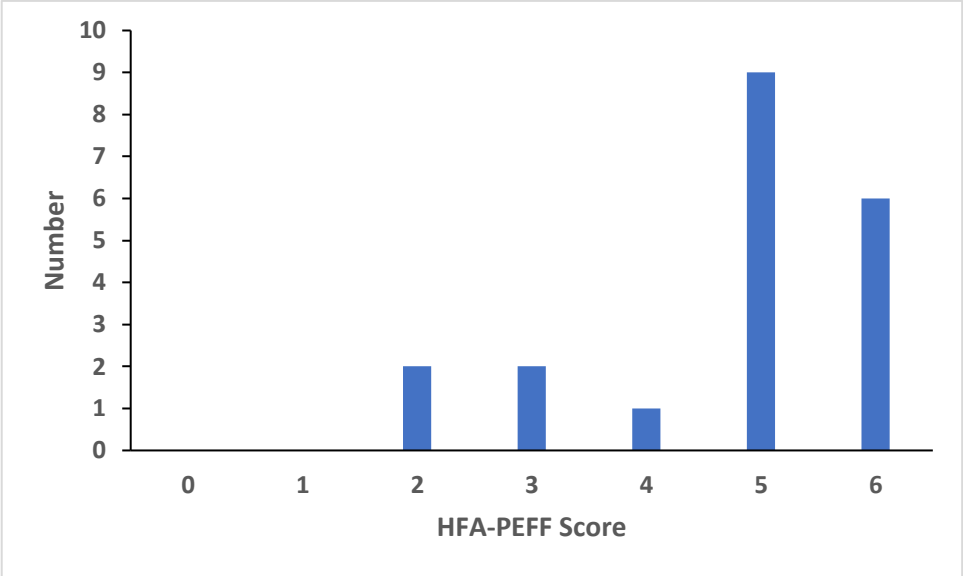


Figure 5-4: Breakdown of HFA-PEFF scores among the cohort

5.5.4 Anaemia

Haemoglobin concentration was significantly lower in patients with CMD compared to those without CMD (102g/L ± 12 vs 117g/L ± 11, mean difference 15g/L, 95% CI 4-26, p=0.008) – figure 5-5. There was a corresponding significantly lower haematocrit among subjects with CMD (31.2% ± 3.1 vs 35.4% ± 3.7, mean difference 4.2%, 95% CI 0.8-7.8, p=0.019). There were moderate positive correlations between CFVR and haemoglobin (r=0.7, p=0.001) and between CFVR and haematocrit (r=0.5, p=0.011) – figure 5-6.

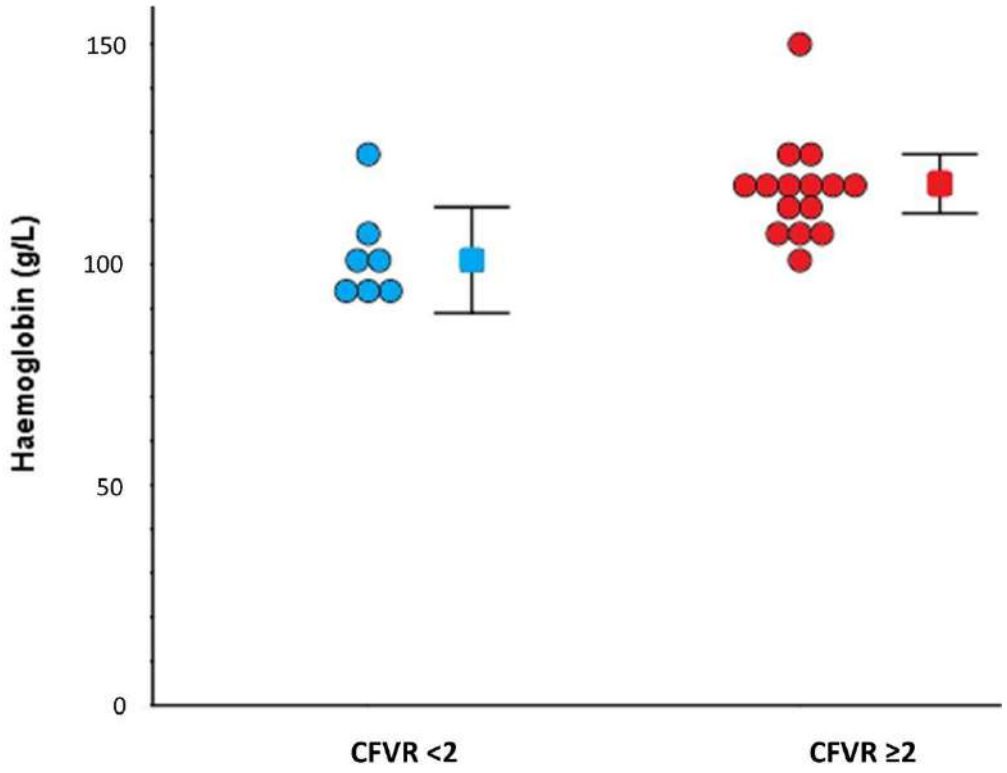


Figure 5-5: Haemoglobin in subjects with CFVR <2 and CFVR ≥2. Circles represent individual measurements. Squares represent mean. Error bars represent 95% confidence intervals of the mean. CFVR – coronary flow velocity reserve. Reproduced with permission from Radhakrishnan *et al.*(326)

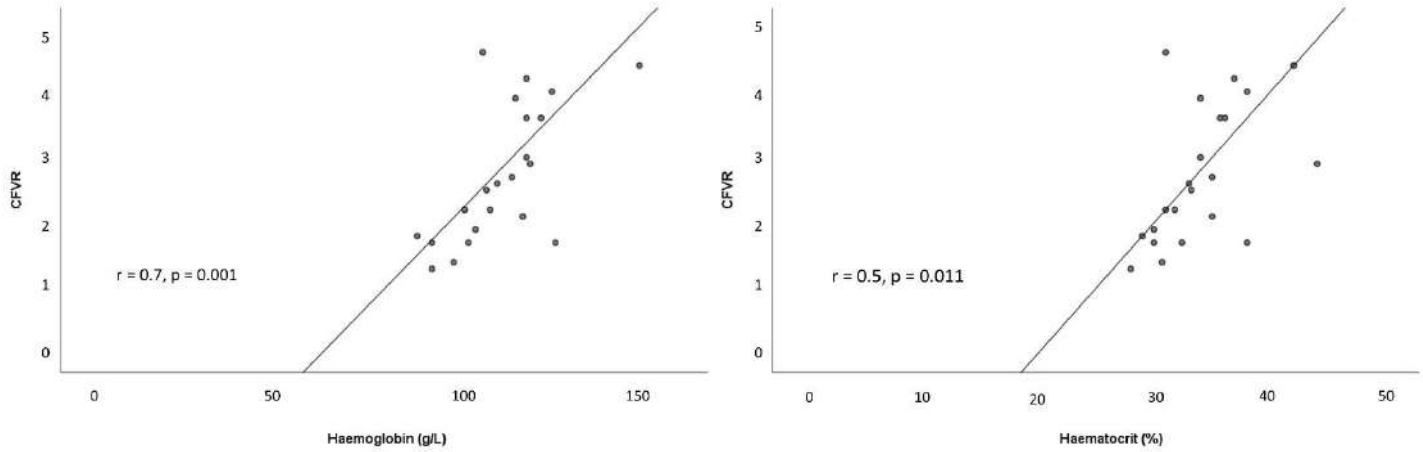


Figure 5-6: Correlation between coronary flow velocity reserve and haemoglobin (left) and haematocrit (right). CFVR – coronary flow velocity reserve. Reproduced with permission from Radhakrishnan *et al.*(326)

5.5.5 Bone mineral disease

Markers of CKD bone mineral disease did not differ significantly between the two groups. Calcium, phosphate and PTH were all numerically higher in patients with CMD, but this did not reach statistical significance.

5.5.6 Multiplex immunoassay

One subject with CMD did not provide blood for immunoassay analysis. Inflammatory markers were similar among subjects with CMD and those with normal microvascular function – table 5-3. Analysis of the remaining biomarkers studied by multiplex immunoassay also did not show any significant differences between the two groups.

Table 5-3: Results of multiplex immunoassay in subjects with and without coronary microvascular dysfunction

Assay	***CFVR <2 (n=6)***	***CFVR ≥2 (n=15)***	***p value***
Angiopoetin-2 (pg/ml)	3274 (1000-5136)	3051 (2230-4053)	0.850
Atrial natriuretic peptide (pg/ml)	25836 ± 9520	20568 ± 11210	0.329
Detectable KIM-1 n(%)	2 (33)	5 (31)	1.0
Galectin-3 (ng/ml)	1.3 (1-1.3)	1.3 (1-1.4)	0.791
IL-1ra (pg/ml)	667 (526-742)	515 (384-729)	0.850
IL-6 (pg/ml)	2.09 ± 1.3	2.69 ± 1.35	0.371
IL-8 (pg/ml)	6.1 (4.2-11.5)	11.4 (8-23)	0.132
IL-10 (pg/ml)	2.5 (0.9-4.1)	1.4 (0.9-3.4)	1.0
Leptin (ng/ml)	17.7 (6.6-20.6)	13.2 (4.2-50.4)	0.910
MCP-1 (pg/ml)	396 ± 221	375 ± 102	0.770
MMP-9 (pg/ml)	10614 (4955-11509)	9880 (6244-13648)	1.0
NGAL (ng/ml)	26.3 ± 8.9	26.6 ± 4.8	0.898
ST2 (ng/ml)	14 (10-33)	12 (9-19)	0.850
TNFα (pg/ml)	6.1 (4.5-8.1)	5.7 (5.1-6.7)	0.850
Uromodulin (ng/ml)	18 ± 9	21 ± 10	0.53
VEGF (pg/ml)	52 ± 26	75 ± 25	0.108

Data are presented as mean ± SD or median (IQR). CFVR – coronary flow velocity ratio, KIM-1 – kidney injury molecule 1, IL-1ra – interleukin 1 receptor antagonist, IL-6 – interleukin-6, IL-8 – interleukin-8, IL-10 – interleukin-10, MCP-1 – monocyte chemoattractant protein, MMP-9 – matrix metallopeptidase 9, NGAL – neutrophil gelatinase associated lipocalin, ST2 - suppression of tumorigenicity 2, TNFα – tumour necrosis factor alpha, VEGF – vascular endothelial growth factor.

5.5.7 Echocardiographic data

Echocardiographic data are reported in table 5-4. There were no significant differences in left ventricular dimensions, LVMI and systolic or diastolic function between the two groups. Cardiac output was significantly higher in subjects with CMD (6.1l/min ± 0.8 vs 4.7l/min ± 1.4, mean difference 1.4l/min, 95% CI 0.3-2.5l/min, p=0.02). This was the result of higher stroke volume and higher HR in subjects with CMD. There was a trend toward lower GLS among subjects with CMD. No subjects had regional wall motion abnormalities or perfusion defects on MCE.

Table 5-4: Echocardiographic parameters in subjects with and without coronary microvascular dysfunction

	CFVR <2 (n=7)	***CFVR ≥2 (n=15)***	***p value***
IVSD (mm)	12 ± 1	11 ± 2	0.610
LVIDD (mm)	46 ± 9	47 ± 6	0.679
PWD (mm)	10 ± 2	11 ± 2	0.789
LVIDS (mm)	31 (29-36)	30 (28-35)	0.535
FS (%)	33 ± 9	35 ± 5	0.639
LVEDVi (ml/m^2)	55 (49-69)	44 (39-51)	0.115
LVESVi (ml/m^2)	21 (18-28)	18 (16-21)	0.275
EF (%)	59 ± 7	59 ± 4	0.923
Stroke volume (ml)	87 ± 25	72 ± 20	0.182
Cardiac output (L/min)	**6.1 ± 0.8**	**4.7 ± 1.4**	**0.02**
GLS (%)	-16 ± 3	-19 ± 2	0.107
TAPSE (mm)	21 ± 4	21 ± 5	0.875
LV mass index (g/m^2)	99 ± 31	98 ± 28	0.936
LV geometry n(%) – normal geometry concentric remodelling eccentric hypertrophy concentric hypertrophy	2 (29) 3 (43) 1 (14) 1 (14)	4 (27) 1 (7) 3 (20) 7 (46)	0.237
LA volume index (ml/m^2)	31.3 (26-44.1)	28.8 (20-38.3)	0.630
E/A ratio	1.1 (0.9-1.2)	0.8 (0.7-1.1)	0.340
E/e’	9 (8-11)	8 (7-10)	0.123

Data are presented as mean ± SD or median (IQR). CFVR – coronary flow velocity ratio, IVSD – interventricular septal diameter, LVIDD – left ventricular internal diameter diastole, PWD – posterior wall diameter, LVIDS – left ventricular internal diameter systole, LVEDVi – indexed left ventricular end diastolic volume, LVESVi – indexed left ventricular end systolic volume, EF – ejection fraction, GLS – global longitudinal strain, TAPSE – tricuspid annular plane systolic excursion, LV – left ventricular.

5.5.8 Arterial stiffness measurements

Pulse wave analysis was performed in 4/7 patients with CMD and 13/15 patients without CMD. The remaining 5 subjects declined PWA measurement. 1 patient with CMD and 1 patient without CMD had their PWA results excluded from analysis due to the presence of type C waveforms. Analysis of the remaining subjects showed no significant difference in corrected PWA between patients with and without CMD (mean AIx 31% ± 17% vs 32% ± 8%, p=0.841).

Pulse wave velocity was measured in 4/7 patients with CMD and 11/15 patients without CMD. PWVadj was lower in patients with CMD, although this was not statistically significant – median PWVadj 7.1m/s (6.9-7.6) vs 8.7m/s (8.0-8.8), p=0.104).

5.5.9 Regression analysis

The variables tested in univariable regression analysis and their results are summarised in table 5-5. In univariable linear regression analysis, haemoglobin and iron were independent predictors of CFVR – haemoglobin (β=0.051 95% CI 0.023-0.079, p=0.001) and iron (β=0.094 95% CI 0.003-0.185, p=0.044). However, in multivariable analysis, only haemoglobin was an independent predictor of CFVR (β=0.041 95% CI 0.012-0.071, p=0.009). In univariable binary logistic regression, haemoglobin was an inverse predictor of CFVR <2 (Odds ratio 0.85 95% CI 0.74-0.98, p=0.022). No other parameters showed a significant association with CFVR <2. Therefore, multivariable binary logistic regression was not performed.

Table 5-5: Univariable regression analysis

	Linear regression (CFVR)			Logistic Regression (CFVR <2)		
Parameter	**β**	**95% CI**	**p value**	**OR**	**95% CI**	**p value**
Age	0.003	-0.037, 0.42	0.89	0.95	0.87-1.03	0.176
LVMI	-0.009	-0.025, 0.008	0.277	1.00	0.97-1.03	0.932
SBP	0.006	-0.16, 0.29	0.549	0.98	0.94-1.03	0.382
Haemoglobin	**0.051**	**0.023, 0.079**	**0.001**	**0.85**	**0.74-0.98**	**0.022**
Iron	**0.094**	**0.003, 0.185**	**0.044**	0.85	0.64-1.13	0.267
Calcium	-1.826	-4.604, 0.953	0.186	125.57	0.23-69927.42	0.134
Phosphate	-0.99	-2.36, 0.38	0.145	6.16	0.29-129.35	0.242
PTH	-0.004	-0.31, 0.22	0.729	1.03	0.98-1.09	0.264
hsCRP	0.016	-0.77, 0.109	0.722	0.91	0.73-1.14	0.419
TNFα	-0.112	-0.290, 0.066	0.203	1.03	0.71-1.48	0.895
IL-6	0.04	-0.322, 0.401	0.821	0.69	0.31-1.53	0.356
IL-8	-0.011	-0.044, 0.023	0.517	0.95	0.85-1.07	0.389
IL-10	-0.111	-0.290, 0.069	0.208	0.95	0.62-1.45	0.8

CFVR – coronary flow velocity reserve, CI – confidence interval, OR – odds ratio, LVMI – left ventricular mass index, SBP – systolic blood pressure, PTH – parathyroid hormone, hsCRP – high sensitivity C-reactive peptide, TNFα – tumour necrosis factor alpha, IL-6 – interleukin 6, IL-8 – interleukin 8, IL-10 – interleukin 10.

5.6 Discussion

This study has confirmed that, similar to an unselected population with advanced CKD, there is a high prevalence of CMD in subjects with CKD stage 5 who are potential kidney transplant candidates. Previous work has shown that among patients with ESRD undergoing evaluation for kidney transplant, CMD was present in 59% of patients, and was more common in subjects with diabetes or LV systolic dysfunction.(219) Unlike this study, my cohort did not include individuals with diabetes or uncontrolled hypertension, both of which independently influence CFVR.(214,234) Despite this, nearly a third of this cohort of kidney transplant candidates had CFVR <2. As CMD is an adverse prognostic marker in CKD,(218,233) the high prevalence of CMD among transplant candidates may partly explain the high mortality even among the least comorbid patients with advanced CKD.

5.6.1 Uraemic cardiomyopathy and heart failure with preserved ejection fraction – two sides of the same coin?

The diagnosis of HFpEF remains clinically challenging, with a variety of definitions in clinical use. A broad definition consists of symptoms and signs of clinical heart failure, LVEF >50% and evidence of raised cardiac filling pressures on invasive or non-invasive testing.(336) The HFA-PEFF score represents an attempt to standardise the diagnosis of HFpEF and provides a simple non-invasive algorithm for the diagnosis of the condition. It also highlights that myocardial disease in HFpEF is characterised by abnormalities in multiple domains including cardiac structure (LVH, left atrial enlargement), cardiac function (diastolic dysfunction) and myocardial stretch (elevated NTpro-BNP). The score has been validated in 2 independent prospective cohorts in the Netherlands and the United States, where it was shown to have high sensitivity (99%) and specificity (93%) for the diagnosis of HFpEF.(337) These cohorts were relatively small (228 and 459 patients respectively) and further validation in larger cohorts is required.

Using the HFA-PEFF score, all subjects in my cohort with EF >50% had intermediate or high probability of HFpEF. These data provide further evidence of the significant overlap between uraemic cardiomyopathy and HFpEF. The two conditions share a number of similarities including common mediators (hypertension, diabetes, obesity, chronic inflammation), and related cardiac abnormalities (LVH, diastolic dysfunction, increased filling pressures).(93) Furthermore, CKD is the most common non-cardiac comorbidity seen in patients with HFpEF.(338) Both conditions also share a high prevalence of CMD, with multiple studies showing rates of CMD up to 75% in HFpEF,

and this is associated with an increased risk of cardiovascular death and first heart failure hospitalisation.(117,184,339) The HFA-PEFF score has not been validated to date in patients with advanced CKD but my data suggest that the majority of patients with CKD stage 5 meet diagnostic criteria for HFpEF. If this is confirmed in larger cohorts of patients with advanced CKD, it may be reasonable, and indeed useful, to consider uraemic cardiomyopathy as a form of HFpEF, as this serves to highlight the multifactorial nature of the condition and the adverse prognosis associated with it.

5.6.2 Anaemia and coronary microvascular dysfunction in end-stage renal disease

To my knowledge, this is the first study to suggest an association between CMD and anaemia in patients with advanced CKD. Despite comparable kidney function and iron stores and a higher prevalence of EPO treatment, subjects with CMD had a small but statistically significant reduction in haemoglobin and haematocrit compared to those with CFVR ≥2. I have also shown an association between haemoglobin and CFVR, that is independent of traditional factors thought to influence CFVR such as hypertension, diabetes and LVH.

Although causation in either direction cannot be confirmed, it seems unlikely that anaemia could be caused by CMD. However, there are biologically plausible reasons why anaemia may lead to CMD in ESRD. Anaemia can cause a number of maladaptive changes to the cardiovascular system that may predispose to CMD. Animal studies have shown that in order to maintain adequate myocardial oxygen supply, there is an increase in resting myocardial blood flow compared to non-anaemic controls,

predominantly due to capillary widening and reduced blood viscosity.(340) Thus, in anaemia, the microcirculation operates in a state of supra-normal vasodilation at rest, which may limit its ability to vasodilate further during hyperaemia. Anaemia is also associated with abnormal red cell function and reduced NO bioactivity, which further impairs endothelium-dependent vasodilation in the microcirculation.(341,342) It is plausible that the combination of increased basal myocardial blood flow and a submaximal hyperaemic response leads to reduced CFVR in conditions of chronic anaemia – a pattern suggested among the subjects with CMD in this cohort.

There are several potential confounders to consider regarding my finding of an association between anaemia and CMD in advanced CKD. Firstly, a common causative factor may be responsible for both anaemia and CMD in this population. Possibilities include systemic inflammation and malnutrition. There was no strong evidence that patients with CMD in this cohort had higher levels of inflammatory markers. Markers of nutritional status such as body mass index (BMI), albumin and cholesterol were numerically lower among subjects with CMD, but this was not statistically significant. However, it is certainly possible that the small sample size meant that my study was underpowered to find a difference in markers of inflammation or nutritional status which would also explain the difference in haemoglobin between the groups. Secondly it is possible that the reduced haemoglobin in the CMD group may be dilutional as these individuals had features of volume overload with a trend towards higher LV and atrial volumes and markers of myocardial stretch (atrial natriuretic peptide and NTpro-BNP), as well as a significantly increased cardiac output which may be related to an elevated preload from fluid retention. These changes may

reflect more advanced cardiac disease, or be the consequence of the longer dialysis vintage in this group, and could also contribute to the reduced haemoglobin seen in patients with CMD in this cohort.

To date there are no other studies examining the effect of anaemia on CFVR in CKD. However, there is some evidence from other conditions of an association between anaemia and CMD. A Japanese study of 337 patients who underwent PCI for ST-elevation myocardial infarction showed that incomplete ST segment resolution (defined as ST segment improvement <50%) despite adequate epicardial coronary artery flow was more common in patients with pre-procedure anaemia. This finding was attributed to microvascular dysfunction by the authors, possibly as a result of impaired NO bioavailability in patients with anaemia, although this was not directly measured.(343) In patients with beta thalassemia minor, Doppler CFVR was significantly lower compared to control subjects matched for age, gender and BMI.(344) A single study also included a group of patients with iron deficiency anaemia but did not demonstrate any reduction in CFVR compared to healthy controls.(345)

5.6.3 Additional factors related to coronary microvascular dysfunction in end-stage renal disease

Unlike with markers of anaemia, it was not possible to demonstrate any significant association between markers of bone mineral metabolism or inflammation and CFVR in ESRD. Again, due to the smaller than anticipated sample size, it is possible that the study was underpowered to detect these differences.

5.6.4 Clinical significance

The recognition of the significant overlap between uraemic cardiomyopathy and HFpEF is of clinical interest and may highlight therapeutic targets for reducing the morbidity and mortality associated with uraemic cardiomyopathy. In the future, it is conceivable that therapeutic agents that are useful in HFpEF may also be beneficial in uraemic cardiomyopathy. Unfortunately, traditional pharmacological therapies for heart failure have had disappointing results in HFpEF, with only carvedilol (reduced mortality) and spironolactone (reduced heart failure hospitalisation) showing any benefit in randomised trials.(113,346,347) Furthermore, renal dysfunction in advanced CKD often precludes the use of many of the drug classes traditionally used in heart failure management, including angiotensin converting enzyme (ACE) inhibitors and angiotensin receptor blockers (ARBs). Two novel drug classes have shown some promise in the treatment of HFpEF. Firstly sodium-glucose-cotransporter-2 (SGLT-2) inhibitors have been shown to reduce heart failure hospitalisation in patients with HFpEF, although no mortality benefit was seen. This beneficial effect of SGLT-2 inhibitors was more marked in patients with renal impairment.(348) The selective nonsteroidal mineralocorticoid receptor antagonist, finerenone, has also been shown to reduce heart failure hospitalisation in HFpEF, again with no mortality benefit being demonstrated.(349) However, data for its use in patients with advanced CKD are lacking. At present, specific drug therapies for HFpEF and uraemic cardiomyopathy are unavailable, and management of both conditions consists predominantly of aggressive risk factor modification and symptom management.

The clinical significance of my finding of lower haemoglobin in patients with CMD is unclear. Current guidelines recommend a target haemoglobin range of 100-120g/L in patients with CKD.(350) Mean haemoglobin levels in the CMD group were within this target range. Previous studies of aggressive anaemia treatment in CKD have had disappointing results, with no improvement in cardiovascular outcomes and possibly an increased risk of harm from correcting haemoglobin to a higher threshold.(351) Therefore it is unlikely that more aggressive treatment of anaemia would be recommended at the current time, especially as there are no longitudinal studies examining the impact of improving haemoglobin on CFVR. .

5.7 Limitations

The main limitation of this analysis was the small sample size, which may have limited the possibility of detecting small differences in markers of bone mineral metabolism and inflammation between the two groups. Furthermore, I only assessed haemoglobin concentration at a single time-point and do not have data on chronicity of anaemia among the subjects in this study. All patients were eligible for kidney transplant, and patients with diabetes or on HD were excluded. These tight inclusion criteria improve the validity of the findings in the population studied, but limit the generalisability of these findings to the wider CKD population. Finally, the study was cross-sectional in design, meaning that a causative link between low haemoglobin and CMD cannot be demonstrated. Further longitudinal work would be necessary to confirm if there is truly a causal link between anaemia and CMD.

5.8 Conclusions

Among kidney transplant candidates with CKD stage 5, there is a high prevalence of CMD, even in the absence of traditional risk factors such as diabetes, uncontrolled hypertension or significant LVH. Furthermore, the majority of patients with CKD stage 5 in this cohort met diagnostic criteria for HFpEF, suggesting a significant overlap between the two conditions. My findings suggest that anaemia may be associated with CMD in patients with CKD stage 5 who are eligible for kidney transplant. However, larger studies, powered to exclude the effect of confounding factors such as inflammation or malnutrition, are required to confirm this association. If this association is confirmed in larger studies, then studies investigating the effect of more aggressive treatment of anaemia on CMD may be considered.

CHAPTER 6: THE EFFECT OF KIDNEY TRANSPLANTATION ON CORONARY FLOW VELOCITY RESERVE

6.1 Introduction

Kidney transplantation is the most effective form of renal replacement therapy and is associated with improved quality of life and reduced mortality in the recipient.(284,352) Cardiovascular mortality is reduced 10-fold in kidney transplant recipients compared to patients on dialysis.(25) Despite this, cardiovascular mortality in kidney transplant recipients remains several times higher than the general population.(353) Furthermore, the impact of kidney transplantation on cardiovascular structure and function remains poorly understood.

A number of cardiovascular parameters are potentially improved with kidney transplantation. Blood pressure is better controlled after kidney transplantation.(284) However, hypertension remains prevalent and is seen in up to 90% of kidney transplant recipients, and may be partly due to transplant related factors such as immunosuppressive agents.(354) The prevalence of anaemia, with its potentially deleterious effects on coronary microvascular function as demonstrated in Chapter 5, is also reduced after kidney transplantation.(355) By contrast, the evidence for improvements in LV mass after renal transplant is less conclusive. A number of small uncontrolled echocardiographic studies showed significant reductions in LVMI after kidney transplantation.(356–358) However, this was not confirmed in a recent meta-analysis of four studies (both TTE and CMR) that included control groups of non-transplanted patients.(359) The RETRACT study, which is currently recruiting at

QEHB, will hopefully provide a definitive answer to the question of whether kidney transplantation is associated with regression of LVH.

Several cross-sectional studies have examined CFVR in renal transplant recipients and demonstrated reduced CFVR compared to healthy or hypertensive controls.(243–246) Other studies have shown that CFVR in kidney transplant recipients is higher than the values seen among patients with ESRD.(215,216) Despite this, CMD remains common among renal transplant recipients.(93) This is understandable, as many of the substrates thought to predispose to CMD in ESRD, including diabetes and hypertension, remain prevalent among renal transplant recipients. To date, there are no longitudinal studies that have examined whether CFVR is improved by kidney transplantation.

6.2 Aim and hypothesis

The aim of this chapter was to examine whether CFVR improves after successful kidney transplantation. The hypothesis was that CFVR would significantly increase among kidney transplant recipients at 1-year post-transplant, whereas no significant change in CFVR would be seen among non-transplanted control subjects with CKD stage 5. One year follow-up was chosen based on the previous work by Moody *et al.* which showed that increased LVMI was seen among LKD at 1-year after unilateral nephrectomy.(107) It is plausible that a change in LVMI and CFVR would also be evident at 1-year post renal transplant.

6.3 Methods

6.3.1 Study design

The study was envisaged as a single-centre prospective longitudinal observational study examining the effects of kidney transplantation on CFVR in subjects with CKD stage 5. The subjects described in Chapter 5 represent the baseline cohort for the longitudinal study, and were recruited between March 2019 and March 2020. The aim was to repeat CFVR in these individuals at 12 months, to compare the change in CFVR from baseline between the transplant cohort and the non-transplanted control cohort. The global COVID-19 pandemic led to termination of recruitment in March 2020. This was partly due to restrictions on recruitment to non-COVID research studies at QEHB. In addition, the study cohort were particularly vulnerable to adverse outcomes from COVID-19, with a French series of 1216 kidney transplant recipients showing that infection with COVID-19 was associated with a 24-fold increased risk of mortality.(360) Therefore, a clinical decision was made by the research team to suspend further study visits for these high-risk patients. I had to return to full time clinical practice in September 2020 due to the cessation of my research funding and out of programme time. Furthermore, there was ongoing reluctance from patients with ESRD to attend hospital for study visits due to concerns regarding nosocomial COVID-19 infection. As a result of these factors, it was not possible to conduct the longitudinal aspect of this study and no follow-up data are available for any of the patients recruited to this study. Baseline data comparing the transplant and non-transplant cohorts are reported in this chapter.

6.3.2 Study investigations

All subjects underwent the study investigations described in Chapter 2. Serum was frozen for subsequent analysis of hsCRP and other biomarkers.

6.3.3 Blinded analysis

Echocardiograms were stored under an anonymous code and analysed offline using commercially available software (IntelliSpace Cardiovascular, Philips, Eindhoven, Netherlands). The TTE, CFVR and MCE studies were all analysed by myself, blinded to study group.

6.3.4 Endpoint and sample size calculation

The primary endpoint to be assessed would have been the difference in mean change in CFVR between transplanted patients and non-transplanted controls at 12 months. After kidney transplant, most recipients have an eGFR consistent with CKD stage 3.(361) Data from Imamura *et al.* showed a difference in CFVR of 1.1 (SD 0.6) between CKD stage 3 and CKD stage 5, which equates to a difference of approximately 60%.(231) I felt it was unlikely that there would be an improvement in CFVR of this magnitude after kidney transplantation. Therefore, I estimated that 30 patients in each group would provide 90% power with an alpha value of 0.05 to demonstrate a difference in mean change of Doppler CFVR of 0.5 (approximately 25%) between transplants and controls at 12 months. I planned to recruit 70 patients to the longitudinal study to allow for drop-out. Difference in mean change in CFR by MCE between the groups was the planned secondary endpoint.

6.4 Results

6.4.1 Subject characteristics

Twenty-two patients are included in this baseline analysis (7 patients who subsequently had a transplant and 15 who were non-transplanted controls). One individual subsequently received a kidney transplant from a cadaveric donor. The remaining 6 individuals subsequently received transplants from LKD. Baseline demographic, laboratory and haemodynamic parameters are shown in table 6-1. There were no significant baseline differences between the groups apart from age which was significantly lower in the transplant cohort.

Echocardiographic data are presented in table 6-2. There were no significant differences between the two groups at baseline.

6.4.2 Parameters of coronary microvascular function

Baseline Doppler CFVR was performed in all 22 individuals. Baseline Doppler CFVR results were similar between the two groups – CFVR in transplant recipients 2.8 ± 1.2 vs 2.7 ± 1.0 in non-transplanted controls, p=0.929. A similar proportion of patients in each group had CMD at baseline – 3/7 transplants (43%) vs 4/11 controls (27%), p=0.63.

Baseline MCE CFR was possible in 5 transplant patients and 9 non-transplant controls. There were no significant differences in baseline CFR between the two groups – CFR LAD (2.6 ± 1.5 vs 1.9 ± 1.2, p=0.346) and CFR Global [2.4 (2.2-2.7) vs 1.5 (1.1-1.7), p=0.298].

Table 6-1: Baseline demographic, laboratory and haemodynamic variables in transplant recipients and non-transplanted controls

	Transplant recipients (n=7)	***Non-transplanted (n=15)***	***p value***
Demographics			
Age (years)	**45 ± 11**	**56 ± 11**	**0.041**
Male n(%)	1 (14)	10 (67)	0.063
Caucasian n(%)	7 (100)	10 (67)	0.135
BMI (kg/m^2)	26 ± 4	28 ± 5	0.393
Smoker n(%) – Ex Never Current	3 (43) 4 (57) 0 (0)	2 (13) 1 (7) 12 (80)	0.267
Hypertension n(%)	6 (86)	14 (93)	1.0
Hypercholesterolaemia n(%)	4 (57)	11 (73)	0.63
Peritoneal dialysis n(%)	2 (29)	12 (80)	0.052
ACE inhibitor n(%)	2 (29)	3 (20)	1.0
ARB n(%)	1 (14)	3 (20)	1.0
Statin n(%)	2 (29)	7 (47)	0.648
Loop diuretic n(%)	2 (29)	8 (53)	0.381
Calcium channel blocker n(%)	4 (57)	10 (67)	1.0
Beta blocker n(%)	2 (29)	3 (20)	1.0
Alpha blocker	3 (43)	4 (27)	0.63
Laboratory data			
Haemoglobin (g/L)	119 ± 18	109 ± 10	0.1
Urea (mmol/L)	23 ± 6	21 ± 6	0.52
Creatinine (µmol/L)	508 ± 173	683 ± 231	0.09
eGFR (ml/min/1.73m^2	10 ± 3	7 ± 3	0.078
ACR (mg/mmol)	164.7 (62.4-193.4)	81.2 (66-287.4)	0.735
Albumin (g/L)	40 ± 7	38 ± 6	0.446
Corrected calcium (mmol/L)	2.39 ± 0.13	2.36 ± 0.18	0.68
hsCRP (mg/L)	1.8 (1.4-8)	2.8 (2.1-5.5)	0.757
NT pro-BNP (ng/L)	365 (316-432)	643 (367-1577)	0.127
Phosphate (mmol/L)	1.6 (1.5-1.7)	1.6 (1.5-1.9)	0.643
PTH (µmol/L)	28.6 ± 27.7	35.5 ± 15.2	0.502
Total cholesterol (mmol/L)	5 ± 1.2	4.9 ± 1.6	0.988
Haemodynamic data			
Systolic BP (mmHg)	130 ± 16	137 ± 24	0.459
Diastolic BP (mmHg)	84 ± 9	84 ± 11	0.991
Heart Rate (bpm)	67 ± 8	68 ± 11	0.895

Data are presented as mean ± SD or median (IQR). CFVR – coronary flow velocity ratio, BMI – body mass index, ACE – angiotensin converting enzyme, ARB – angiotensin receptor blocker, eGFR – estimated glomerular filtration rate, ACR – albumin creatinine ratio, hsCRP – high sensitivity C-reactive peptide, NT-proBNP – N-terminal pro-B-type natriuretic peptide, PTH – parathyroid hormone, BP – blood pressure, bpm – beats per minute.

Table 6-2: Baseline echocardiographic parameters in transplant recipients and non-transplanted controls

	Transplant recipients (n=7)	***Non-transplanted (n=15)***	***p value***
IVSD (mm)	11 ± 1	11 ± 2	0.556
LVIDD (mm)	44 ± 9	48 ± 5	0.178
PWD (mm)	9 ± 2	11 ± 2	0.097
LVIDS (mm)	28 ± 6	32 ± 5	0.09
Fractional Shortening (%)	37 ± 6	34 ± 6	0.233
LVEDVi (ml/m^2)	48 (43-54)	49 (42-64)	0.904
LVESVi (ml/m^2)	19 (16-21)	19 (18-25)	0.841
EF (%)	61 ± 3	58 ± 5	0.168
TAPSE (mm)	22 ± 4	21 ± 5	0.541
GLS (%)	-19 ± 3	-16 ± 3	0.181
LV mass index (g/m^2)	84 ± 33	105 ± 24	0.094
LV geometry n(%) – normal geometry concentric remodelling eccentric hypertrophy concentric hypertrophy	3 (43) 2 (29) 2 (29) 0 (0)	3 (20) 2 (13) 2 (13) 8 (53)	0.118
Left atrial volume index (ml/m^2)	27.1 (22.2-32.6)	28.9 (23.2-39.8)	0.332
E/A ratio	0.8 (0.8-1.1)	1 (0.7-1.1)	0.799
E/e’	7.9 (7.2-8.4)	9.2 (7.5-11.1)	0.21

Data are presented as mean ± SD or median (IQR). IVSD – interventricular septal diameter, LVIDD – left ventricular internal diameter diastole, PWD – posterior wall diameter, LVIDS – left ventricular internal diameter systole, LVEDVi – indexed left ventricular end diastolic volume, LVESVi – indexed left ventricular end systolic volume, EF – ejection fraction, TAPSE – tricuspid annular plane systolic excursion, GLS – global longitudinal strain, LV – left ventricular

6.4.3 Arterial stiffness measurements

Baseline PWA data are available for 5/7 transplant patients and 10/15 non-transplanted controls. The remaining 7 subjects declined PWA measurement. Two non-transplanted controls had their PWA results excluded from analysis due to the presence of type C waveforms. Analysis of the remaining subjects showed no significant difference in corrected PWA between transplant and non-transplanted patients (mean AIx 25% ± 6% vs 30% ± 9%, p=0.311).

Pulse wave velocity data are available in 4/7 transplant patients and 13/15 patients non-transplanted controls. PWVadj was lower in transplant patients, although this was not statistically significant – median PWVadj 7.5m/s (7.3-8.0) vs 8.6m/s (7.8-8.9), p=0.316).

6.4.4 Multiplex immunoassay

The results of multiplex immunoassay are shown in table 6-3. Tumour necrosis factor alpha was significantly higher in the non-transplanted controls at baseline. No other parameters showed any significant differences between the groups.

Table 6-3: Baseline results of multiplex immunoassay in transplant recipients and non-transplanted controls

Assay	***Transplant recipients (n=7)***	***Non-transplanted (n=15)***	***p value***
Angiopoetin-2 (pg/ml)	3348 (2350-4228)	2658 (2143-4101)	0.488
Atrial natriuretic peptide (pg/ml)	19927 ± 9997	23345 ± 11368	0.513
Detectable KIM-1 n(%)	0 (0)	6 (40)	0.121
Galectin-3 (ng/ml)	1.2 (1-1.3)	1.3 (1-1.4)	0.585
IL-1ra (pg/ml)	513 (403-582)	698 (476-905)	0.149
IL-6 (pg/ml)	2.06 ± 1.79	2.75 ± 1.06	0.28
IL-8 (pg/ml)	6.6 (5.4-10.7)	10.3 (8.2-25.2)	0.094
IL-10 (pg/ml)	1.8 (0.9-2.5)	0.9 (0.9-4.7)	1.0
Leptin (ng/ml)	21 (13.9-58.3)	10.7 (2.8-43)	0.488
MCP-1 (pg/ml)	371 ± 206	386 ± 102	0.822
MMP-9 (pg/ml)	10742 (5681-17756)	9551 (5685-12753)	0.913
NGAL (ng/ml)	25.9 ± 5.5	26.9 ± 6.4	0.724
ST2 (ng/ml)	9 (8-10)	17 (11-21)	0.056
TNFα (pg/ml)	**4.2 (2.7-5.7)**	**5.8 (5.5-7.6)**	**0.02**
Uromodulin (ng/ml)	18 ± 6	21 ± 11	0.53
VEGF (pg/ml)	81 ± 20	64 ± 28	0.306

Data are presented as mean ± SD or median (IQR). CFVR – coronary flow velocity ratio, KIM-1 – kidney injury molecule 1, IL-1ra – interleukin 1 receptor antagonist, IL-6 – interleukin-6, IL-8 – interleukin-8, IL-10 – interleukin-10, MCP-1 – monocyte chemoattractant protein, MMP-9 – matrix metallopeptidase 9, NGAL – neutrophil gelatinase associated lipocalin, ST2 - suppression of tumorigenicity 2, TNFα – tumour necrosis factor alpha, VEGF – vascular endothelial growth factor.

6.5 Discussion

My baseline data highlight that patients with CKD stage 5 are a heterogenous population. Despite all included patients being eligible for kidney transplant, there were clear differences between patients who subsequently underwent renal transplant and those who remained on the waiting list. Kidney transplant recipients were significantly younger and had lower markers of inflammation. They also demonstrated a trend towards better blood pressure control, higher haemoglobin and LVEF, and improved markers of arterial stiffness. When assessing the impact of kidney transplantation on longitudinal changes in CFVR, these baseline differences are likely to be important, as several of these factors have been linked to CMD in other settings. My work on LKD described in Chapter 4 has demonstrated that increased inflammatory markers are associated with a reduced CFVR.(283) Similarly, previous work among potential kidney transplant recipients showed that CFR was lower among patients with pre-existing LV dysfunction.(219) It is likely that the findings seen in these other populations also apply to patients with advanced CKD. It would not be ethical to perform a prospective randomised controlled trial of kidney transplantation compared to conservative treatment. Therefore, any prospective observational studies to assess the longitudinal impact of kidney transplant on CFVR would need to ensure that both transplant recipients and controls have minimal baseline differences by matching them for variables including age, haemoglobin, blood pressure and inflammatory markers.

My data are consistent with the published literature, which also highlights that kidney transplant recipients differ from those who remain on the transplant waiting list. Large registry data from the United States have shown that, compared to patients who

remained on dialysis, patients who underwent kidney transplant were more likely to be younger and Caucasian and less likely to have diabetes.(331) Furthermore, multiple studies have shown that patients eligible for kidney transplant have significantly fewer comorbidities and significantly lower mortality than the general ESRD population.(331,362) This limits the generalisability of findings in kidney transplant recipients to the ESRD population as a whole.

There is some evidence that CMD may be reversible, with multiple pharmacological agents including ACE inhibitors, ARBs, vasodilating beta blockers, statins and SGLT-2 inhibitors showing benefit at improving parameters of microvascular function in small studies.(363–367) The aim of this study was to examine whether restoration of renal function by successful kidney transplantation also improves CFVR. Due to the outbreak of the COVID-19 pandemic and its effects on patient recruitment and follow-up as outlined above, this study could not be performed as planned as I was unable to perform the 1-year scans for any of the participants. Therefore, I was unable to test the hypothesis that CFVR is improved by kidney transplantation. Longitudinal data are required to confirm or refute this hypothesis. It would also be important to assess whether any improvement in CFVR after kidney transplant is directly due to an increase eGFR, or whether it is due to indirect factors associated with kidney transplant such as improved BP control, regression of LVH, reduction in anaemia or better glycaemic control. Thus, future studies in this area should be adequately powered to adjust for these potential confounders.

6.6 Conclusions

Due to my lack of follow-up data, I was unable to test the hypothesis that CFVR is improved after successful kidney transplantation. However, it is plausible that kidney transplantation, which is associated with an improved cardiovascular risk factor profile, may improve coronary microvascular function. Longitudinal studies, similar to the planned design of this study, are needed to test this hypothesis, so that this important gap in the current literature can be addressed.

CHAPTER 7: THE RELATIONSHIP BETWEEN CORONARY FLOW VELOCITY RESERVE AND MYOCARDIAL FIBROSIS IN CHRONIC KIDNEY DISEASE

7.1 Introduction

Myocardial fibrosis is a hallmark feature of uraemic cardiomyopathy, with both diffuse interstitial fibrosis and coarse replacement fibrosis commonly seen in endomyocardial biopsy specimens and in post-mortem studies of patients with ESRD.(79,368) This fibrotic process can also be detected non-invasively on CMR through T1 mapping; a technique that quantifies the relaxation time of protons on inversion recovery prepared images (T1 times) by using analytical expression of image-based signal intensities.(369,370) Although there have been no studies correlating T1 times in CKD with histological markers of fibrosis from endomyocardial biopsy specimens, the parameter is widely accepted as a surrogate non-invasive measure of myocardial fibrosis. This stems from the histological correlation that has been demonstrated in other conditions such as HCM and valvular heart disease.(371) The related parameter of T2 relaxation times, which are prolonged by myocardial water content, provides a useful discriminant of myocardial oedema, which is also present in CKD and can impact T1 times.(372)

Although more extensive in ESRD, myocardial fibrosis also occurs in early CKD, with elevated T1 times documented in patients with CKD stages 2-3 compared with age- and sex-matched controls.(81) Furthermore, previous work by the Birmingham Cardio-Renal Group has shown that there is a linear relationship between myocardial T1 times and CKD stage and this is associated with serum markers of myocardial stretch, injury and fibrosis as well as reductions in exercise capacity on cardiopulmonary exercise

testing, highlighting that myocardial fibrosis has functional consequences.(82) Despite the high prevalence of both myocardial fibrosis and CMD in ESRD, the relationship between these two pathologies has not been well studied to date.

7.2 Aim and hypothesis

The aim of this chapter was to examine whether there is an association between CMD (assessed by Doppler CFVR) and myocardial fibrosis (assessed by T1 times) in patients with CKD stage 5. The hypothesis was that there is a negative correlation between CFVR and native T1 times.

7.3 Methods

Data presented in this chapter were obtained from patients enrolled in the RETRACT echocardiogram sub-study who underwent both Doppler CFVR assessment as well as measurement of native T1 and T2 times by CMR. Fifteen subjects with CKD stage 5 are included in this analysis. Patients were divided into CMD and no CMD groups using CFVR <2 as a cut-off point. Subjects underwent the study investigations detailed in Chapter 3. In addition, ventricular volumes and mass, and native T1 and T2 times were assessed by CMR as described below.

7.3.1 Cardiac magnetic resonance image acquisition

All CMR studies were performed at 3 Tesla on a Magnetom Skyra machine (Siemens, Erlangen, Germany). Standard sagittal, axial and coronal localiser images were used for sequence planning. Retrospective ECG-gated steady state free precession (SSFP) cine imaging of the vertical long axis and horizontal long axis of both the right and left

ventricle were used to pilot the LV short axis stack, from which a contiguous series of cine images of the left ventricle were taken from the atrioventricular junction to the apex. Typical scan parameters were as follows: repetition time 45.48ms; echo time 1.69; flip angle 65°; field of view 340mm with a slice thickness of 7mm with a 3mm gap over 25 phases per cardiac cycle.

A breath-held SSFP motion corrected modified Look-Locker inversion recovery (MOLLI) sequence was used for native T1 mapping at the basal and mid ventricular short axis levels. A sampling scheme of 5(3)3 was used with a total breath hold of 11 R-R intervals. Parameters for MOLLI were: repetition time 280.56ms, echo time 1.12ms, flip angle 35°, field of view 360mm. For T2 mapping 3 single shot images of the same basal and mid ventricular short axis levels were taken at the following T2 preparation times, 0ms, 30ms and 55ms.

7.3.2 Cardiac magnetic resonance imaging analysis

Volumetric assessment was performed offline from the LV short axis stack using commercially available software (CVi 42, Circle Vascular Imaging, Calgary, Alberta, Canada). End-diastole and end-systole were determined by selecting images with the largest and smallest cavity size respectively. Delineation of trabeculations and papillary muscles were performed using thresholding to determine the endocardial border. Papillary muscles were excluded from blood pool volumes but were included in calculations of LV mass as previously described.(112) The epicardial border was drawn manually then smoothed. To assess right ventricular volumes, manual contours

of the right ventricle were traced in end-systole and end-diastole. Left ventricular mass was calculated automatically by the software using the formula:
(total epicardial volume – endocardial volume) x 1.05g/ml, with 1.05g/ml representing the specific density of myocardium.(373)

T1 and T2 times were measured from the software generated parametric maps after delineation of endocardial and epicardial borders. A 20% offset was used to avoid blood pool contamination. After defining the right ventricular insertion point, the basal and mid-ventricular short axis slices were segmented using semi-automated methods into 12 segments using the American Heart Association 17-segment model – figure 7-1. Segments involving the left ventricular outflow tract or those with artefact were excluded from analysis. The software performed a three-parameter curve fitting of the data to automatically generate T1 and T2 times. By convention, septal or global values are reported as they are more reproducible than values from the artefact-prone LV free wall myocardium.(374) Basal septal (average of basal anteroseptal and basal inferoseptal segments), mid-septal (average of mid anteroseptal and mid inferoseptal segments), and global (average of all 12 basal and mid-ventricular segments) T1 and T2 times were calculated as previously described.(81,375) Only native T1 times are reported as gadolinium based contrast agents were not administered as all patients had eGFR <30ml/min/1.73m^2. Analysis of the CMR data was performed by Dr Luke Pickup.

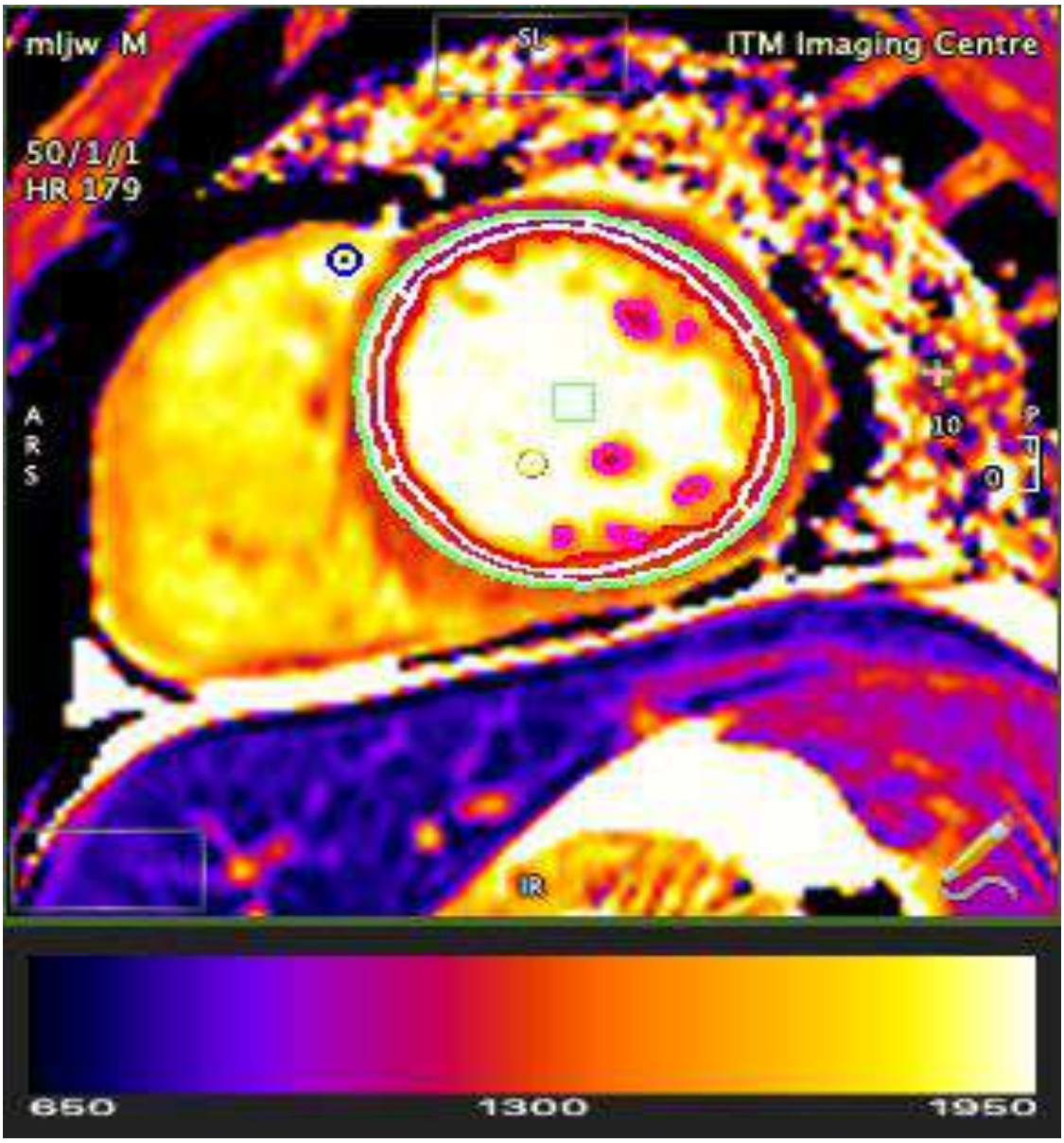


Figure 7-1: Example of a native T1 map in a patient with chronic kidney disease stage 5

7.4 Results

Using the cut-off value of CFVR <2 to signify CMD, 5 subjects had CMD and 10 subjects had normal coronary microvascular function. Mean CFVR in subjects with CMD was 1.7 ± 0.2. Mean CFVR in subjects without CMD was 3.1 ± 0.8.

Baseline demographic, laboratory and haemodynamic parameters are shown in table 7-1. There were no significant differences between patients with and without CMD, apart from lower albumin and higher calcium in patients with CMD.

Echocardiographic parameters are shown in table 7-2. There were no significant differences between the two groups, apart from GLS, which was significantly lower in subjects with CMD.

Cardiac magnetic imaging resonance parameters are shown in table 7-3. Similar to the echocardiographic data, there were no significant differences in LV mass, volumes or ejection fraction between the 2 groups. Basal septal T1 times were significantly higher in subjects with CMD – figure 7-2 and table 7-3. There was a trend towards increased mid-septal and global T1 times in the CMD group. There were significant negative correlations between CFVR and basal septal ($r = -0.7$, $p=0.003$), mid-septal ($r = -0.5$, $p=0.037$) and global ($r = -0.6$, $p=0.012$) T1 times – figure 7-3. T2 times did not differ between subjects with and without CMD.

Table 7-1: Demographic, laboratory and haemodynamic variables in patients with chronic kidney disease stage 5

	CFVR <2 (n=5)	***CFVR ≥2 (n=10)***	***p value***
Demographics			
Age (years)	43 (42-44)	42 (41-44)	1.0
Male n(%)	2 (40)	5 (50)	1.0
Caucasian n(%)	5 (100)	8 (80)	0.524
BMI (kg/m^2)	27.1 ± 4.9	26.1 ± 3.8	0.647
Peritoneal dialysis n(%)	4 (80)	4 (40)	0.282
Hypertension n(%)	4 (80)	9 (90)	1.0
Hypercholesterolaemia n(%)	1 (20)	2 (20)	1.0
ACE inhibitor n(%)	0 (0)	3 (30)	0.505
Alpha blocker	3 (60)	3 (30)	0.329
ARB n(%)	0 (0)	3 (30)	0.505
Beta blocker n(%)	1 (20)	2 (20)	1.0
Calcium channel blocker n(%)	4 (80)	7 (70)	1.0
Loop diuretic n(%)	4 (80)	2 (20)	0.089
Statin n(%)	1 (20)	6 (60)	0.282
Laboratory data			
Haemoglobin (g/L)	105 ± 14	119 ± 13	0.072
Urea (mmol/L)	22.7 ± 6.3	24.1 ± 5.0	0.638
Creatinine (µmol/L)	731 ± 325	558 ± 182	0.201
eGFR (ml/min/1.73m^2)	8 ± 4	9 ± 2	0.722
ACR (mg/mmol)	185.2 ± 110.7	139.4 ± 107.9	0.498
Albumin (g/L)	**37 ± 5**	**43 ± 5**	**0.048**
Corrected calcium (mmol/L)	**2.49 ± 0.09**	**2.30 ± 0.15**	**0.021**
hsCRP (mg/L)	1.0 (1.0-3.8)	2.1 (1.7-2.6)	0.606
NTpro-BNP (ng/L)	1900 (937-12519)	424 (328-594)	0.154
Phosphate (mmol/L)	1.7 (1.6-2.1)	1.6 (1.5-1.7)	0.371
PTH (µmol/L)	34.5 (27.9-47)	16.4 (15.2-27.5)	0.199
Urate (µmol/L)	437 ± 178	420 ± 54	0.843
Total cholesterol (mmol/L)	4.7 ± 1.6	5.1 ± 1.5	0.684
Haemodynamic data			
Systolic BP (mmHg)	139 ± 21	135 ± 13	0.65
Diastolic BP (mmHg)	89 ± 12	83 ± 7	0.313
Heart Rate (bpm)	69 (66-77)	68 (61-74)	1.0

Data are presented as mean ± SD or median (IQR). CFVR – coronary flow velocity reserve, BMI – body mass index, ACE – angiotensin converting enzyme, ARB – angiotensin receptor blocker, eGFR – estimated glomerular filtration rate, ACR – albumin creatinine ratio, hsCRP – high sensitivity C-reactive peptide, NT-proBNP – N-terminal pro-B-type natriuretic peptide, PTH – parathyroid hormone, BP – blood pressure.

Table 7-2: Echocardiographic parameters in patients with chronic kidney disease stage 5

	CFVR <2 (n=5)	***CFVR ≥2 (n=10)***	***p value***
IVSD (mm)	12 ± 1	11 ± 1	0.318
LVIDD (mm)	45 ± 11	46 ± 7	0.827
PWD (mm)	10 ± 1	10 ± 2	0.912
LVIDS (mm)	30 ± 9	30 ± 5	0.977
FS (%)	35 ± 7	36 ± 5	0.739
LVEDVi (ml/m^2)	55 (44-67)	44 (37-51)	0.190
LVESVi (ml/m^2)	21 (17-26)	18 (15-19)	0.364
EF (%)	61 ± 4	61 ±2	0.953
GLS (%)	**-17 ± 1**	**-20 ± 2**	**0.02**
TAPSE (mm)	21 ± 4	23 ± 5	0.597
LV mass index (g/m^2)	95 ± 33	99 ± 29	0.791
LV geometry n(%) – normal geometry concentric remodelling eccentric hypertrophy concentric hypertrophy	1 (20) 2 (40) 1 (20) 1 (20)	3 (30) 2 (20) 2 (20) 3 (30)	0.861
LA volume index (ml/m^2)	26.5 (25.5-31.3)	31.4 (22.5-38.5)	0.768
E/A ratio	1.0 (0.8-1.4)	0.9 (0.7-1.1)	0.733
E/e’	9 ± 2	8 ± 3	0.441

Data are presented as mean ± SD or median (IQR). CFVR – coronary flow velocity reserve, IVSD – interventricular septal diameter, LVIDD – left ventricular internal diameter diastole, PWD – posterior wall diameter, LVIDS – left ventricular internal diameter systole, LVEDVi – indexed left ventricular end diastolic volume, LVESVi – indexed left ventricular end systolic volume, EF – ejection fraction, GLS – global longitudinal strain, TAPSE – tricuspid annular plane systolic excursion, LV – left ventricular.

Table 7-3: Cardiac magnetic resonance imaging parameters in patients with chronic kidney disease stage 5

	CFVR <2 (n=5)	***CFVR ≥2 (n=10)***	***p value***
LVEDVi (ml/m^2)	91 (88-110)	89 (83-97)	0.859
LVESVi (ml/m^2)	32 (30-42)	34 (32-36)	0.768
LVSVi (ml/m^2)	59 (59-68)	55 (52-64)	0.594
LVEF (%)	64 (62-65)	64 (63-66)	0.679
LVMI (g/m^2)	73 ± 14	70 ± 15	0.747
RVEDVi (ml/m^2)	90 (83-96)	88 (74-96)	0.679
RVESVi (ml/m^2)	26 (26-39)	31 (25-38)	0.768
RVSVi (ml/m^2)	57 (57-64)	53 (50-58)	0.371
RVEF (%)	67 ± 9	64 ± 5	0.493
Basal septal T1 (ms)	**1309 (1301-1313)**	**1292 (1281-1295)**	**0.028**
Mid septal T1 (ms)	1319 ± 29	1285 ± 49	0.173
Global T1 (ms)	1299 ± 25	1262 ± 38	0.073
Basal septal T2 (ms)	43 ± 1	43 ± 3	0.940
Mid septal T2 (ms)	43 ± 3	42 ± 3	0.386
Global T2 (ms)	42 ± 2	44 ± 3	0.222

Data are presented as mean ± SD or median (IQR). CFVR – coronary flow velocity reserve, LVEDVi – indexed left ventricular end-diastolic volume, LVESVi – indexed left ventricular end-systolic volume, LVSVi – indexed left ventricular stroke volume, LVEF – left ventricular ejection fraction, LVMI – left ventricular mass index, RVEDVi – indexed right ventricular end-diastolic volume, RVESVi – indexed right ventricular end-systolic volume, RVSVi – indexed right ventricular stroke volume, RVEF – right ventricular ejection fraction.

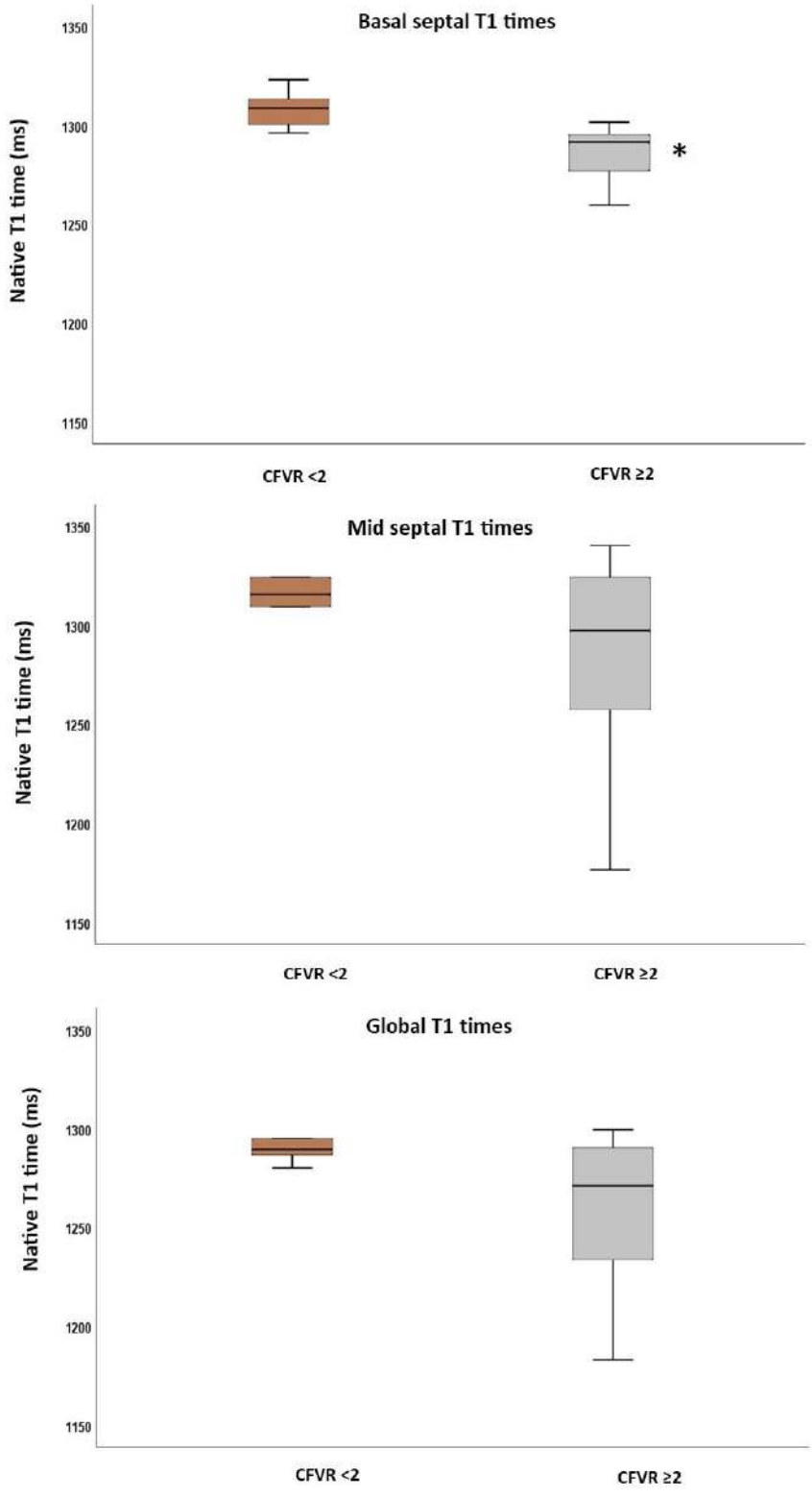


Figure 7-2: Native T1 times in subjects with and without coronary microvascular dysfunction. Solid line represents median. Box represents interquartile range. Error bars represent 95% confidence intervals. * - p<0.05 compared to CFVR <2.

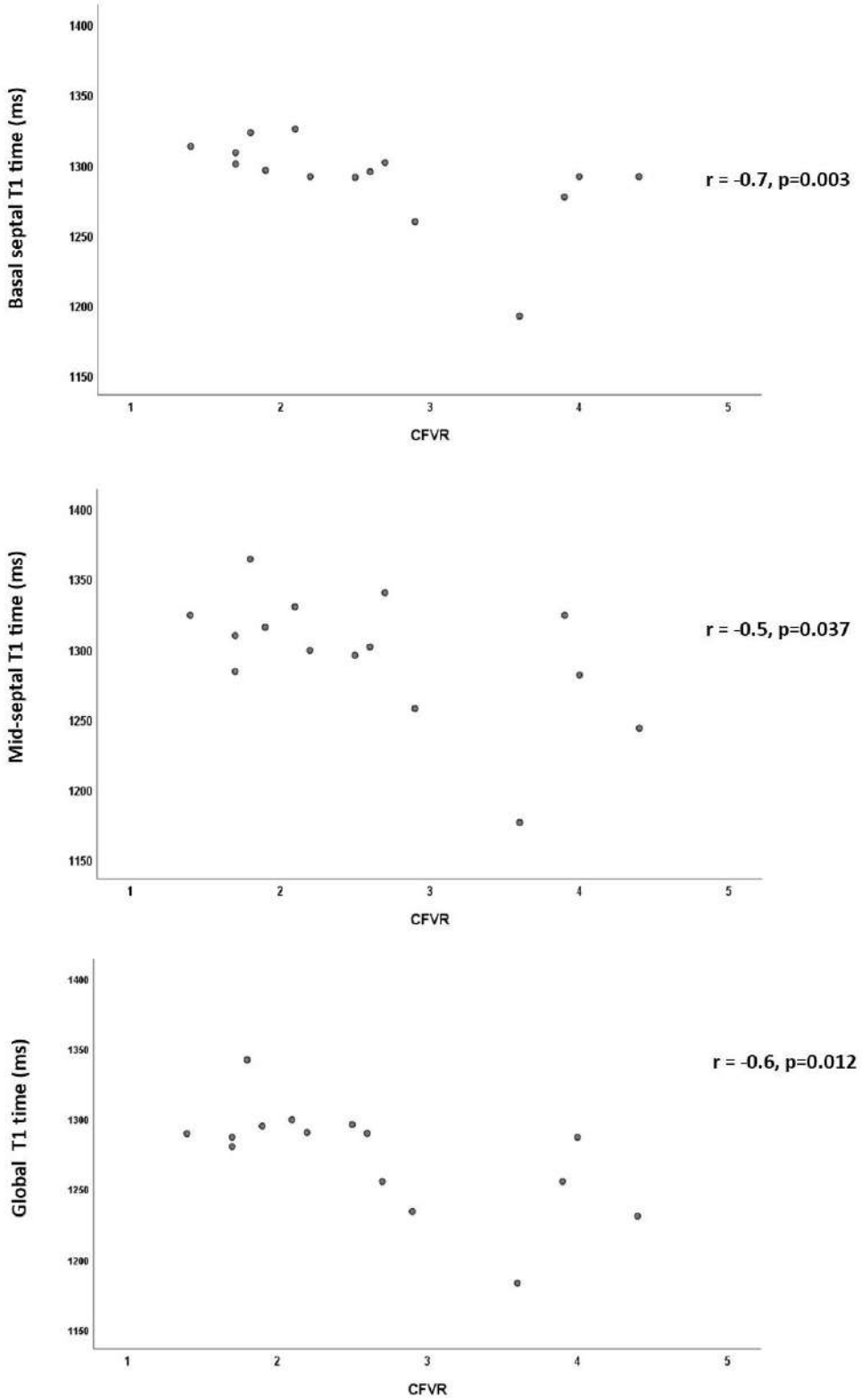


Figure 7-3: Correlations between coronary flow velocity reserve (CFVR) and basal septal T1 time (top panel), mid-septal T1 time (middle panel) and global T1 time (bottom panel) in subjects with end-stage renal disease.

The results of serum multiplex immunoassay in patients with ESRD is shown in table 7-4. There were no significant differences between the groups in any of the 16 biomarkers that were assessed.

Table 7-4: Results of multiplex immunoassay in patients with chronic kidney disease stage 5

Assay	***CFVR <2 (n=5)***	***CFVR ≥2 (n=10)***	***p value***
Angiopoetin-2 (pg/ml)	3651 ± 3864	3166 ± 1012	0.795
Atrial natriuretic peptide (pg/ml)	27379 ± 9769	20463 ± 11899	0.291
Detectable KIM-1 n(%)	2 (40)	3 (30)	1.0
Galectin-3 (ng/ml)	1.1 ± 0.5	1.2 ± 0.2	0.533
IL-1ra (pg/ml)	647 (485-688)	515 (415-725)	0.859
IL-6 (pg/ml)	2.18 ± 1.48	2.43 ± 1.53	0.769
IL-8 (pg/ml)	6.4 (5.9-13.3)	11.4 (7.8-19.8)	0.31
IL-10 (pg/ml)	4.1 (2.5-4.1)	1.8 (0.9-6.4)	1.0
Leptin (ng/ml)	16.1 (3.5-21.0)	13.2 (5.6-42.6)	0.859
MCP-1 (pg/ml)	336 (264-351)	382 (327-405)	0.513
MMP-9 (pg/ml)	13214 (8013-16274)	9692 (5376-17270)	0.768
NGAL (ng/ml)	25.6 ± 9.7	26.3 ± 5.2	0.855
ST2 (ng/ml)	18 (9-38)	13 (8-20)	0.679
TNFα (pg/ml)	5.5 (4.2-6.7)	5.7 (5.1-7.1)	0.679
Uromodulin (ng/ml)	17.1 ± 9.7	22.7 ± 9.3	0.334
VEGF (pg/ml)	56 ± 28	71 ± 29	0.432

Data are presented as mean ± SD or median (IQR). CFVR – coronary flow velocity reserve, KIM-1 – kidney injury molecule 1, IL-1ra – interleukin 1 receptor antagonist, IL-6 – interleukin-6, IL-8 – interleukin-8, IL-10 – interleukin-10, MCP-1 – monocyte chemoattractant protein, MMP-9 – matrix metallopeptidase 9, NGAL – neutrophil gelatinase associated lipocalin, ST2 - suppression of tumorigenicity 2, TNFα – tumour necrosis factor alpha, VEGF – vascular endothelial growth factor.

7.5 Discussion

These data add to the current evidence regarding T1 times in ESRD. A number of studies have been previously carried out at both 1.5 Tesla and 3 Tesla, which have compared native T1 times in control subjects to patients with ESRD. Several authors have shown that native T1 times are significantly elevated in HD patients compared to controls.(332,376–378) Similarly, Zhou *et al.* showed that PD patients also have elevated native T1 times compared to controls.(379) Although I did not directly compare T1 times in my CKD stage 5 cohort to control subjects, the T1 times seen here are higher than previously published native T1 times in control subjects studied by the Birmingham Cardio-Renal Group using the same 3T scanner.(112) This finding again suggests the presence of increased myocardial fibrosis among subjects with advanced CKD, which is consistent with the endomyocardial biopsy study by Aoki *et al.* demonstrating a high burden of myocardial fibrosis among dialysis patients.(79) A strength of this study is the clinical exclusion of patients with diabetes and those with significant CAD or valvular heart disease, which all influence T1 times.(370)

Unlike in patients with HCM or valvular heart disease, there have been no studies in CKD comparing T1 times to histological specimens from biopsy or post-mortem studies. Recently, there has been some data suggesting that increased T1 times in CKD do not wholly represent myocardial fibrosis. In HD patients, 2 CMR studies have demonstrated significant reductions in post-dialysis native T1 times compared to pre-dialysis values – a time frame in which myocardial fibrosis could not have regressed.(380,381) A possible explanation is that the changes in T1 times with dialysis are due to changes in myocardial water content. In keeping with this

hypothesis, myocardial T2 times (a marker of myocardial oedema) were also significantly reduced post-dialysis in these 2 studies. Ventricular volumes by CMR and TTE in my cohort were similar between patients with and without CMD. Similarly, there were no significant differences in T2 times between the groups. This suggests that differences in volume status were not responsible for the increased T1 times seen among patients with CMD in my cohort. Although impossible to confirm without histological validation, my findings suggest an increased incidence of myocardial fibrosis among CKD subjects with CMD.

7.5.1 The relationship between myocardial fibrosis and coronary microvascular dysfunction.

To my knowledge, this is the first study to demonstrate a relationship between non-invasive measures of myocardial fibrosis and CMD among patients with advanced kidney disease. A negative correlation was seen between native T1 relaxation times and CFVR in patients with CKD stage 5. Among patients with CKD and CMD, only basal septal T1 times were significantly elevated compared to patients without CMD. This finding may partly be explained by the presence of LVH, which is extremely common in advanced CKD, and is a known driver of CMD.(93) The basal septum is particularly vulnerable to LVH as it subject to higher wall stress than other segments due to the presence of larger muscle fibres. It is also the last segment of the ventricle to be electrically activated, and thus it is subject to transmitted wall stress from other LV segments.(382) However, there was no correlation between basal septal T1 times and LVMI by TTE (r=0.09, p=0.75) or by CMR (r=0.359, p=0.189) in this cohort of CKD patients. Nevertheless, elevated basal septal T1 times and the associated trend

towards increased mid-septal and global T1 times among subjects with CMD, suggests that there is a true inverse relationship between myocardial fibrosis and CMD in advanced CKD, which warrants further investigation. My findings contrast with the only previous study using the combination of these two imaging techniques. That study of 64 women with angina and non-obstructive coronary arteries also examined T1 times by CMR and CFVR by Doppler TTE and found no correlation between the two measures (r^2=0.004, p=0.61).(383) However, none of the patients included in that study had evidence of focal fibrosis on gadolinium imaging, so it is not unexpected that there was no correlation between T1 times and CFVR.

The significance of these findings is not fully clear. As seen in Chapter 1, CMD is associated with adverse prognosis in CKD. Similarly, myocardial fibrosis is an adverse prognostic marker in other cardiomyopathic conditions including non-ischaemic cardiomyopathy, HFpEF and HCM.(384–387) The evidence for a poor prognosis associated with myocardial fibrosis is not as clear in CKD. However, Aoki *et al.* demonstrated that HD patients with extensive fibrosis (>30%) on LV biopsy specimens had a 2-fold increased risk of cardiovascular death compared to those with less widespread fibrosis.(79) It is likely that the combined presence of CMD and myocardial fibrosis in an individual with CKD confers additional cardiovascular risk.

7.5.2 Myocardial fibrosis – the chicken or the egg?

There are plausible reasons why myocardial fibrosis and CMD may be inter-related. Current cross-sectional data suggests that as CKD stage progresses, there is a stepwise decline in coronary microvascular function. In parallel, there is an increase in

native T1 times - a surrogate marker of myocardial fibrosis.(82,93) However, which condition is the primary pathology is not clear. One possibility is that CMD leads to microvascular rarefaction and remodelling, resulting in myocardial ischaemia due to impaired myocardial oxygen supply, causing chronic scar formation and the development of myocardial fibrosis.(119) However, it is equally plausible that myocardial fibrosis is the initial insult, with fibrosis leading to perivascular deposition of scar tissue, which increases the oxygen diffusion distance to the cardiomyocyte, leading to myocardial ischaemia and the development of CMD.(388) Finally, it is also possible that both myocardial fibrosis and CMD are unrelated, and a common factor such as inflammation causes them to decline in parallel. In the iPOWER sub-study described above, patients did not have myocardial fibrosis despite a high prevalence of CMD.(383) Although not directly comparable to CKD, this raises the intriguing possibility that CMD may actually be the primary pathology in this relationship. However, the most likely scenario is that there is a reciprocal relationship between myocardial fibrosis and CMD, which is exacerbated by common factors such as inflammation, triggering a vicious cascade of progressive ischaemia and myocardial dysfunction leading to the increased heart failure, arrhythmia and SCD seen in uraemic cardiomyopathy. The cross-sectional nature of this study does not allow one to draw any conclusions about whether myocardial fibrosis or CMD is the causative factor in the relationship described, and further longitudinal work is required to answer this question.

7.6 Limitations

The main limitation of this study is the small sample size due to recruitment being heavily curtailed by the COVID-19 pandemic as previously described. Despite this I have shown a significant negative correlation between CFVR and T1 times. It is likely that the study was underpowered to find any significant differences in mid-septal and global T1 times between CKD patients with and without CMD. Similarly, it is not possible to draw any firm conclusions about the relationship between these parameters and biochemical or immunoassay biomarkers.

Gadolinium was not administered as all patients had an eGFR <30ml/min/1.73m^2, and gadolinium administration in patients with this degree of renal impairment is not recommended due to the increased risk of nephrogenic systemic fibrosis. Thus, late gadolinium enhancement imaging, which would have provided additional information on degree and pattern of myocardial fibrosis, was not possible. Similarly, calculation of extra-cellular volume, which also relies on gadolinium administration, was not possible. Extracellular volume is more reproducible, has better correlation with histological collagen volume fraction and has a stronger association with outcome measures than native T1 times.(389–391) Although a recent meta-analysis by Woolen *et al.* has suggested that the true risk of nephrogenic systemic fibrosis after administration of second generation gadolinium based contrast agents in patients with CKD stage 4 or 5 is very low (<0.07%), QEHB guidelines do not recommend gadolinium administration in patients with advanced CKD without a strong clinical indication.(392) Consequently, only native T1 mapping was performed in this study as it does not require gadolinium

administration and is the main non-invasive method of assessing myocardial fibrosis that can be performed safely in patients with ESRD.

7.7 Conclusions

This is the first study to suggest a possible relationship between CMD and myocardial fibrosis in ESRD. The combination of CMD and myocardial fibrosis, which are both associated with an adverse prognosis in other cardiovascular conditions, may help to explain the exceedingly high cardiovascular morbidity and mortality seen in advanced kidney disease. Further longitudinal studies are needed to assess which condition is the primary pathology, as this may aid the development of therapeutic strategies to slow the progression of cardiac disease in CKD.

CHAPTER 8: CONCLUSIONS AND FUTURE WORK

8.1 Summary of main findings

In this thesis, I have made several observations that further our understanding of the prevalence and implications of CMD in patients with CKD.

Firstly, in Chapter 4, I have demonstrated that asymptomatic LKD have a small but statistically significant reduction in CFVR compared to healthy control subjects of a similar age and gender. Although this finding is of unclear clinical significance, it raises the possibility that the mild loss of renal function associated with uni-nephrectomy may have an impact on the coronary microcirculation, My data should be seen in the context of previous long-term follow-up studies of Norwegian LKD, which have shown an increased risk of ischaemic heart disease, cardiovascular death, ESRD and all-cause mortality in LKD compared to highly selected control populations eligible for kidney donation.(102,393) If my finding is reproduced and replicated in other adequately powered, longitudinal studies of LKD, it would raise the question as to whether some of the increased long-term cardiovascular risk in LKD is related to CMD. My data are hypothesis-generating only and should not change current recommendations on the safety of living kidney donation or dissuade people from donating. However, they should prompt larger studies on the cardiovascular safety of the procedure and ensure longer-term follow-up of these individuals, to allow regular assessment and treatment of their cardiovascular risk factors.

Secondly, in Chapter 5, I have examined the prevalence of CMD among patients with CKD stage 5 who are potential kidney transplant candidates. Although a high

prevalence of CMD has been identified in patients with ESRD, this is one of the few studies examining its prevalence in potential kidney transplant candidates, who differ significantly from the wider ESRD population. The exclusion of patients with CAD, uncontrolled hypertension and diabetes, which are all recognised substrates for CMD, was a strength of this study. Despite this, CMD was seen in nearly a third of cases. Given the adverse prognosis associated with CMD, this represents a significant burden of cardiovascular disease, even among the least comorbid patients with advanced CKD.

Thirdly, this thesis has also shed light on potential drivers of CMD in CKD. In Chapter 4, I have shown that reduced CFVR in LKD is associated with subclinical inflammation (elevated CRP and hsCRP, reduced uromodulin). A clear association between inflammation and CMD has been shown in other populations so it is plausible that chronic inflammation related to uni-nephrectomy can affect microvascular function in LKD. Given the duration from uni-nephrectomy in my study population, this finding cannot be attributed directly to the operation itself. Rather it suggests that uni-nephrectomy may be associated with a pattern of prolonged subclinical inflammation that may have adverse effects on microcirculatory function, a finding that warrants further investigation. Furthermore, I have demonstrated in Chapter 5 that there may be an association between anaemia and CMD in patients with CKD stage 5 – an observation that has also not been demonstrated previously. Despite no significant differences in traditional risk factors for CMD (age, BP, LVMI), subjects with CMD had significantly lower haemoglobin concentration than those without CMD. My observational data do not prove that anaemia causes CMD in this population. Neither

can the effect of potential confounders such as inflammation or malnutrition be wholly excluded. Nevertheless, if there truly is a causal link between anaemia and CMD, then more aggressive treatment of anaemia in advanced CKD could be considered to reduce the burden of CMD and its associated adverse prognosis.

Finally, in Chapter 7, I have demonstrated a negative correlation between CFVR and native T1 times in patients with advanced CKD. This suggests a complex interplay between CMD and myocardial fibrosis and it is plausible, and indeed likely, that the two pathologies act synergistically to increase cardiovascular risk in ESRD.

8.2 Implications of findings

There is increasing recognition of the importance of CMD and its associated adverse prognosis. The 2019 ESC Guidelines on chronic coronary syndromes recommend investigation for microvascular disease in patients with typical angina and non-obstructive CAD.(394) However, this still rarely occurs in clinical practice. Screening for CMD in non-coronary cardiovascular disease is even rarer. My work has highlighted that uraemic cardiomyopathy shares many phenotypical similarities with other myocardial diseases such as HFpEF, and is also associated with a high burden of CMD. One of the strengths of the data presented here is the demonstration that Doppler CFVR is a feasible, reliable and safe technique for measuring coronary microvascular function across a range of populations. Thus, it may be a potentially useful non-invasive technique to screen for CMD in high-risk populations.

My work has also shown that the aetiology of CMD is multifactorial and that it is likely to be a manifestation of poorly controlled systemic disease. There are no recognised treatments directly targeted at CMD and current therapeutic strategies rely on management of cardiac risk factors to prevent the development of CMD. This highlights the importance of aggressive screening and treatment of cardiovascular risk factors in patients at high risk of developing CMD, such as those with CKD, something which unfortunately is often poorly done.

8.3 Potential future work

My data should lay the foundation for future studies that would further explore the mechanisms of CMD in patients with CKD and address gaps in the current literature. Some potential studies are described below.

In LKD, larger, adequately powered cross-sectional studies are required to confirm my finding of a significant difference in CFVR between LKD and controls. Furthermore, it would be important to assess the impact of time from donation on CFVR. Previous long-term follow-up studies of LKD have shown increased cardiovascular risk mainly after 10 years of follow-up.(102) It is possible that the small difference in CFVR seen in my study may be amplified as time from donation increases. Thus, it would be important to compare donors of varying vintage to appropriate control groups matched for age, gender and comorbidity, to assess the impact of time from donation on CFVR Mechanistic studies are also required, to identify mediators that might be responsible for any differences in CFVR. My study identified inflammation as a potential mediator of reduced CFVR in LKD. The utilisation of a metabolomic or proteomic approach

would be useful in this respect as it would allow assessment of a large number of potential mediators of CMD in LKD. A key aspect of any study involving CFVR testing in LKD would be long-term follow-up, as to date, there are no data on the prognostic role of CMD in LKD. Given the adverse prognosis with CMD seen in CKD, it is plausible that a similar association may be seen in LKD, but this remains to be proven. As it is already recommended that all LKD have long-term clinical follow-up, this should be feasible through the use of routinely collected clinical information.

If a difference in CFVR is confirmed in larger cross-sectional studies, then a prospective, longitudinal, parallel group, blinded endpoint design comparing CFVR in LKD pre- and post-nephrectomy to a population of control subjects who meet eligibility criteria for living kidney donation is required. This would allow confirmation of a causal link between uni-nephrectomy and reductions in CFVR. If a reduced CFVR is confirmed in LKD post-nephrectomy, with no similar reduction in eligible controls who do not donate a kidney, this would provide further evidence that a loss of renal function directly causes a reduction in CFVR, independent of other cardiovascular risk factors. This would have important implications for cardiovascular risk in LKD, and would prompt the need for further investigation and detailed counselling of potential LKD on the possible risks to their cardiovascular health from kidney donation.

The reverse hypothesis should also be tested, i.e., does restoration of kidney function with a successful kidney transplant improve CFVR. This was the ultimate aim of the study described in Chapter 6, but could not be tested due to the lack of longitudinal follow-up data. However, this remains an important study to perform, because if CFVR

does improve after kidney transplant, this would provide further welcome and reassuring evidence that CMD is reversible.

The effect dialysis on coronary microvascular function is another area of uncertainty in the literature. There have been very few cross-sectional studies comparing the effects of dialysis modality on coronary microvascular function and it is not clear whether HD or PD is associated with better cardiovascular outcomes. As PD is the initial modality of renal replacement therapy in approximately 20% of patients in the UK, this is an important question that needs addressing.(23) Some studies have suggested that the initial survival of patients with ESRD but no CAD is better among PD patients than those that commence HD.(395) This may be due to the continuous ultrafiltration and maintenance of urine output seen with PD.(396) However, other studies appear to show long term cardiovascular mortality is higher in patients treated with PD, particularly in diabetic patients or those with pre-existing CAD.(395,397,398) Adequately powered cross-sectional studies are necessary to assess whether there is any significant difference in rates of CMD between PD and HD patients. These groups would need to be well matched for comorbidities, given the high prevalence of conditions that contribute to CMD, such as hypertension and diabetes, in patients with ESRD. Again, it would be important to study the impact of dialysis vintage on CMD as the beneficial effects of PD appear to correspond with duration of dialysis, with an increased cardiovascular mortality reported after 1 year of treatment with PD.(398) This finding can be partly be understood by the properties of the peritoneum as a dialysate membrane. Compared to an artificial dialysis membrane, the peritoneum usually has a smaller number of pores but a larger surface area.(399) This may result

in better clearance of uraemic toxins in the short term. However, with long term PD use, failure of the peritoneal membrane leads to a loss of if its filtration capacity, leading to inadequate removal of toxins and may lead to increased mortality.(399) Paired groups of HD and PD patients of varying dialysis vintage should be studied. It is recognised that the clearance of specific toxins varies depending on the property of the dialysate membrane in question. Thus, a metabolomic approach would be particularly helpful in this setting to identify any specific molecules that differ between HD and PD patients, and which may be important in the development of CMD in these differing dialysis populations. The data from such a potential study would further support decision making for clinicians and patients on which is the best modality of renal replacement therapy to initiate from a cardiovascular perspective.

Finally, both myocardial fibrosis and CMD are widespread in advanced CKD but which is the causative pathology is not known. To address this, a prospective longitudinal study with serial assessment of CFVR and non-invasive measures of myocardial fibrosis by CMR across stages of CKD is required to ascertain whether CMD or myocardial fibrosis develops first as CKD stage progresses. This would provide valuable insight into the pathogenesis of uraemic cardiomyopathy, and may pave the way for therapeutic treatments to halt its onset. It would be important to include patients with early-stage CKD as this would permit the administration of gadolinium-based contrast agents, allowing late gadolinium enhancement imaging and calculation of extracellular volume to be performed, thereby providing a more comprehensive assessment of myocardial fibrosis and its relationship with CMD. A study design such as this would require patients with a progressive but predictable decline in renal

function. Although there are significant variations between individuals, the average rate of decline in eGFR per year is in the order of 1-5ml/min/1.73m^2.(400,401) Therefore, a very long study duration would be necessary to ensure sufficient patients developed ESRD. Such a study design would have significant challenges as it would be associated with high funding costs and potentially a high attrition rate if patients found repeated CFVR and CMR studies onerous. However, without a prospective longitudinal study design, it would not be possible to determine whether CMD precedes or follows the onset of myocardial fibrosis in patients with CKD.

8.4 Coronary microvascular dysfunction – is this the key intermediary step in the development of uraemic cardiomyopathy?

The aetiology of uraemic cardiomyopathy and its associated cardiac risk remains poorly understood. Although the studies described in this thesis are not mechanistic, taken together, they suggest that CMD may play an important role in the pathogenesis and consequences of uraemic cardiomyopathy.

Coronary microvascular dysfunction is a heterogenous condition, which is influenced by a variable combination of factors including endothelial dysfunction, smooth muscle hyperreactivity, SNS activation, arterial and arteriolar remodelling and increased aortic stiffness. These abnormalities are all commonly seen in CKD and are caused by mediators including diabetes, hypertension, LVH, anaemia and chronic inflammation. Thus, the common end-point of these disparate mediators in CKD may be the development of CMD. Furthermore, there is a possible link between myocardial fibrosis, a hallmark feature of uraemic cardiomyopathy, and CMD, although a causal

relationship in either direction between the two has not been established. The current literature demonstrates that rates of CMD increase as CKD stage progresses, mirroring the elevated cardiac risk seen with increasing renal dysfunction. Given the well documented adverse prognosis associated with CMD, it is possible that the onset of CMD is a key step in the pathogenesis of uraemic cardiomyopathy. It is plausible that the development of CMD then promotes a cycle of deleterious cardiac changes including myocardial ischaemia, micro-infarction, chronic inflammation, oxidative stress and scar formation, leading to the adverse consequences of myocardial fibrosis, systolic and diastolic dysfunction and an increased risk of SCD.

Ultimately, this hypothesis would be difficult to prove until an effective method of reversing or preventing CMD is identified. If reversing CMD delayed or prevented the development of uraemic cardiomyopathy, this would prove a causal role for CMD in the pathogenesis of the condition. Unfortunately, no such “magic bullet” currently exists. However, with increasing understanding of the importance of CMD in patients with CKD, greater recognition, identification, and ultimately treatment for CMD, should help to reduce the significant health burden posed by cardiovascular disease in CKD.

I hope that the observational studies described in this thesis will inspire the further work needed to confirm whether the mediators associated with CMD are indeed causative and whether therapy can improve or even regress the harmful effects of CMD in patients with CKD.

CHAPTER 9: LIST OF REFERENCES

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APPENDIX I: LIST OF ABSTRACTS RELATED TO THIS PERIOD OF RESEARCH

Poster communications

- **Radhakrishnan A,** Pickup LC, Price A, Law JP, Fabritz L, Senior R, Steeds RP, Ferro CJ, Townend JN. Myocardial fibrosis is associated with reduced coronary flow velocity reserve in end-stage renal disease. *Heart,* Jun 2021;107(Supplement 1):A123-125.

- **Radhakrishnan A,** Pickup LC, Price AM, Law JP, McGee KC, Fabritz L, Senior R, Steeds RP, Ferro CJ, Townend JN. Anaemia and coronary microvascular dysfunction in end-stage renal disease. *Eur Heart J Cardiovasc Imaging,* Jan 2021;22:Supplement 1:jeaa356.099

- **Radhakrishnan A,** Pickup LC, Price AM, Law J, Fabritz L, Steeds R, Ferro C, Townend J. Coronary flow reserve is reduced in asymptomatic living kidney donors – results of the Chronic Renal Impairment in Birmingham Coronary Flow Reserve (CRIB-FLOW) Study. *Heart,* Jul 2020;106:Supplement 2:A84-A85

- Price A, Moody W, Stoll V, Vijapurapu R, Hayer M, Biasiolli L, Weston C, Webster R, Wesolowski R, McGee K, Edwards N, Liu B, Baig S, Pickup L, **Radhakrishnan A,** Law J, Chue C, Steeds R, Ferro C, Townend J. Cardiovascular effects of living kidney donation: a five year longitudinal study. *Heart,* Jul 2020;106:Supplement 2:A94-95.

- Price A, Stoll V, Vijapurapu R, McGee K, Weslowski R, Hayer M, Moody W, Edwards N, Liu B, Kaur A, Pickup L, **Radhakrishnan A,** Law J, Steeds R, Ferro C, Townend J. Myocardial tissue characterization in living kidney donors 5 years after nephrectomy. *Nephrol Dial Transplant,* June 2020;35:559

- Price A, Moody W, Stoll V, Vijapurapu R, Hayer M, Biasiolli L, Weston CJ, Webster R, Wesolowski R, McGee K, Kaur A, Edwards N, Liu B, Pickup L, **Radhakrishnan A**, Law J, Chue C, Steeds R, Ferro C, Townend J. Cardiovascular effects of unilateral nephrectomy in living kidney donors at 5 years. *Nephrol Dial Transplant*, June 2020;35:118

- Price A, Law J, Hayer M, Pickup L, **Radhakrishnan A**, Moody W, Edwards N, Ferro C, Townend J. Medium term haemodynamic and blood pressure effects of living kidney donation. *J Hum Hypertens*, Sep 2019;33:12

- Price A, Hayer M, **Radhakrishnan A**, Pickup L, Moody W, Edwards N, Steeds R, Ferro C, Townend J. Weight gain and office blood pressure in living kidney donors: a five year follow up study. *Nephrol Dial Transplant*, June 2019;34:405

APPENDIX II: LIST OF PUBLICATIONS ARISING DIRECTLY FROM THIS WORK

- **Radhakrishnan A,** Pickup LC, Price AM, Law JP, McGee KC, Fabritz L, Senior R, Steeds RP, Ferro CJ, Townend JN. Coronary microvascular dysfunction is associated with degree of anaemia in end-stage renal disease. *BMC Cardiovasc Disord* 2021;21:211:1-9.

- **Radhakrishnan 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;320:141-147.

- **Radhakrishnan A**, Pickup LC, Price AM, Law JP, Edwards NC, Steeds RP, Ferro CJ, Townend JN. Coronary microvascular dysfunction: a key step in the development of uraemic cardiomyopathy? *Heart* 2019;105:17:1302-1309.

BMC Cardiovascular Disorders

RESEARCH

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Coronary microvascular dysfunction is associated with degree of anaemia in end-stage renal disease

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Abstract

Background: Coronary microvascular dysfunction (CMD) is common in end-stage renal disease (ESRD) and is an adverse prognostic marker. Coronary flow velocity reserve (CFVR) is a measure of coronary microvascular function and can be assessed using Doppler echocardiography. Reduced CFVR in ESRD has been attributed to factors such as diabetes, hypertension and left ventricular hypertrophy. The contributory role of other mediators important in the development of cardiovascular disease in ESRD has not been studied. The aim of this study was to examine the prevalence of CMD in a cohort of kidney transplant candidates and to look for associations of CMD with markers of anaemia, bone mineral metabolism and chronic inflammation.

Methods: Twenty-two kidney transplant candidates with ESRD were studied with myocardial contrast echocardiography, Doppler CFVR assessment and serum multiplex immunoassay analysis. Individuals with diabetes, uncontrolled hypertension or ischaemic heart disease were excluded.

Results: 7/22 subjects had CMD (defined as CFVR < 2). Demographic, laboratory and echocardiographic parameters and serum biomarkers were similar between subjects with and without CMD. Subjects with CMD had significantly lower haemoglobin than subjects without CMD (102 g/L ± 12 vs. 117 g/L ± 11, p = 0.008). There was a positive correlation between haemoglobin and CFVR (r = 0.7, p = 0.001). Similar results were seen for haematocrit. In regression analyses, haemoglobin was an independent predictor of CFVR (β = 0.041 95% confidence interval 0.012–0.071, p = 0.009) and of CFVR < 2 (odds ratio 0.85 95% confidence interval 0.74–0.98, p = 0.022).

Conclusions: Among kidney transplant candidates with ESRD, there is a high prevalence of CMD, despite the absence of traditional risk factors. Anaemia may be a potential driver of microvascular dysfunction in this population and requires further investigation.

Keywords: Coronary flow velocity reserve, Anaemia, End-stage renal disease, Coronary microvascular dysfunction

Background

Coronary microvascular dysfunction (CMD) is common among patients with chronic kidney disease (CKD) [1]. With each increase in CKD stage, there is a corresponding rise in rates of CMD, with the highest prevalence among patients with end-stage renal disease (ESRD) [2, 3]. The presence of CMD is a poor prognostic marker and may partly explain the excessive cardiac risk associated

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with CKD [3–5]. Coronary flow velocity reserve (CFVR) is a recognised measure of microvascular function. It reflects the ability of the coronary microcirculation to respond to vasodilatory stimuli and can be reliably detected using Doppler transthoracic echocardiography [6]. In individuals with normal coronary microvascular function, coronary flow should at least double at maximal hyperaemia. Therefore, CFVR < 2, in the absence of obstructive coronary artery disease (CAD), is widely accepted to signify CMD [1].

The syndrome of uraemic cardiomyopathy, characterised by left ventricular hypertrophy (LVH), diffuse interstitial fibrosis, systolic and diastolic dysfunction and an increased risk of sudden cardiac death, represents advanced cardiac disease in ESRD, and is associated with significantly worse cardiovascular outcomes [7, 8]. Factors such as diabetes and hypertension, that contribute to the development of uraemic cardiomyopathy, have been linked with CMD in CKD [9, 10]. A number of other mediators, including anaemia, bone mineral disease and chronic inflammation, are important in the aetiology of uraemic cardiomyopathy. Their impact on the development of CMD in ESRD remain unknown. The aim of this hypothesis generating study was to examine the prevalence of CMD among a population of potential kidney transplant recipients, and to look for associations between CMD and markers of anaemia, bone mineral disease and chronic inflammation.

Methods

Study population

Twenty-two kidney transplant candidates with ESRD who successfully underwent CFVR assessment at the Queen Elizabeth Hospital, Birmingham (QEHB), United Kingdom between March 2019 and March 2020 were included in this analysis. These individuals were research participants in the Chronic Renal Impairment in Birmingham Coronary Flow Reserve (CRIB-FLOW) study or the Prospective Study of the Effects of Renal Transplantation on Uraemic Cardiomyopathy using Magnetic Resonance Imaging (RETRACT) echocardiogram substudy, both of which examined CFVR in patients with ESRD.

Participants were >18 years old, considered suitable for kidney transplantation by the renal transplant team at QEHB, had estimated glomerular filtration rate (eGFR) < 15 ml/min/1.73 m^2 and were pre-dialysis or on peritoneal dialysis (PD). Exclusion criteria were: pregnancy, haemodialysis (HD), diabetes mellitus, uncontrolled hypertension, known ischaemic heart disease, moderate/severe valvular heart disease and contraindication to adenosine or sulphur hexafluoride contrast agent (SonoVue, Bracco, Milan, Italy).

Blood pressure

Office blood pressure (BP) was measured using an automated BP monitor (BpTRU, VSM Medtech, Coquitlam, BC, Canada), which takes 6 BP readings over 6 min. After exclusion of the first reading, an average of the remaining 5 readings was used to represent office BP.

Transthoracic echocardiography (TTE)

All subjects underwent comprehensive two-dimensional echocardiography by a British Society of Echocardiography accredited physician (AR). Studies were performed on a Philips iE33 machine (Philips, Eindhoven, Netherlands) using a S5-1 transducer for TTE and myocardial contrast echocardiogram (MCE) studies and a S8-3 transducer for CFVR measurements. Echocardiograms were stored under an anonymous code and analysed offline by a single investigator (AR) using commercially available software (IntelliSpace Cardiovascular, Philips, Eindhoven, Netherlands).

Left ventricular mass was estimated using the Cube formula and indexed for body surface area [11]. The Simpson's biplane method was used to measure left ventricular volumes and ejection fraction [11]. Diastolic function was quantified using multiple parameters [12]. Global longitudinal strain (GLS) was assessed in the 3 standard apical views using speckle tracking.

Doppler coronary flow velocity reserve

Doppler CFVR assessment was performed as previously described [13]. The left anterior descending artery (LAD) was identified on colour Doppler in the anterior interventricular sulcus. Pulse wave Doppler signals of LAD flow were recorded to measure coronary flow velocity (CFV) at rest and at hyperaemia. SonoVue was used, if necessary, to identify LAD flow and to improve the spectral Doppler trace. Hyperaemia was induced by an infusion of adenosine at a rate of 140micrograms/kg/min for 3 min. Subjects were advised to abstain from caffeine for 24 h prior to adenosine administration. CFVR was calculated as hyperaemic CFV/rest CFV. For each variable in the CFVR calculation, the highest values of 3 cardiac cycles were averaged.

Myocardial contrast echocardiography

Myocardial contrast echocardiography was performed as previously described [13]. Images were taken in the 3 apical views using low-power continuous MCE at a mechanical index (MI) of 0.1. Sonovue was continually infused using an oscillating infusion pump that maintains microbubbles in suspension (Vueject, Bracco, Milan, Italy). The infusion rate was started at 70-100ml/hr but adjusted to ensure sufficient myocardial opacification without

excessive contrast attenuation. Triggered high MI (1.0) flash echocardiography was performed at end-systole, where the myocardium is at its thickest, to destroy microbubbles in the myocardium and to observe replenishment. The sequence was initially performed at rest and then repeated after adenosine vasodilator stress as above. The absence of regional wall motion abnormalities or sub-endocardial perfusion defects on vasodilator MCE was deemed sufficient to exclude flow limiting CAD.

Laboratory analysis

N-terminal pro-brain natriuretic peptide (NTpro-BNP) was assayed using the Alere point of care assay (Alere, Massachusetts, USA). High sensitivity C-reactive peptide was assayed using the Architect MULTIGENT CRP Vario assay (Abbott, Illinois, USA). The remaining laboratory parameters were assayed using standardised automated methods. The fluorescence responses of 16-analytes of inflammation, atrial stretch, cardiac fibrosis, kidney injury and LVH were obtained using Human Magnetic Luminex® Asssays (R&D Systems, Minneapolis, MN, USA) and the Bio-RAD Bio-Plex™ 200 system for analysis. Concentrations were calculated using the Bio-Plex Software Manager™ (version 6.1) generated standard curves and a 5PL logistic curve fitting technique as per the manufacturer's instructions.

Statistical analysis

Statistical analysis was performed using SPSS version 26 (SPSS Inc, Chicago, Illinois). The Shapiro–Wilk test was used to assess data normality. Continuous variables are expressed as mean ± standard deviation for parametric data or median (interquartile range—IQR) for non-parametric data. Unpaired group comparisons for continuous data were made using the unpaired t-test or the Mann-Whitney U test. Unpaired categorical data were compared using Fisher's exact test. Correlation was assessed using the Pearson correlation coefficient. Univariable and multivariable linear regression models were performed with CFVR as the dependent variable. Factors known to influence CFVR (age, systolic BP, left ventricular mass index) as well as markers of anaemia (haemoglobin, iron), bone mineral disease [calcium, phosphate, parathyroid hormone (PTH)] and inflammation (high sensitivity C-reactive peptide, tumour necrosis factor-α, interleukin-6, interleukin-8, interleukin-10) were included as independent variables in regression models. Binary logistic regression was also performed, with CFVR < 2 as the dependent variable, and the parameters listed above as independent variables. Parameters that were significant in univariable analysis were entered into multivariable regression models. A variance inflation factor > 5 was taken to represent collinearity. Statistical tests were 2-tailed, and a p value < 0.05 was considered statistically significant.

Results

Subject characteristics

Twenty-two kidney transplant candidates with ESRD (8 pre-dialysis and 14 PD) were included. The aetiology of ESRD was: glomerulonephritis (45%), polycystic kidney disease (23%), hypertension (9%), obstructive uropathy (9%), pyelonephritis (9%) and idiopathic (5%). No participants had symptoms of ischaemic heart disease or heart failure at study enrolment. 14/22 (64%) had undergone prior cardiovascular assessment for CAD as part of the transplant recipient cardiac work-up protocol at the Queen Elizabeth Hospital, Birmingham using myocardial perfusion scintigraphy (n = 11), exercise stress echocardiography (n = 2) or invasive coronary angiography (n = 1). Median time from cardiovascular assessment to study enrolment for these individuals was 18 months (IQR 3–33 months). The remaining 8 participants did not require cardiovascular assessment as per our institutional protocol.

Using CFVR < 2 to signify CMD, 7/22 (32%) of our cohort with ESRD had CMD. Mean CFVR for subjects with CMD was 1.6 ± 0.2. Mean CFVR for subjects without CMD was 3.2 ± 0.9. Previously published data by our group demonstrated a reference value of CFVR in healthy controls of 3.8 ± 0.6 [13]. Baseline demographic, laboratory and haemodynamic data for subjects with and without CMD are shown in Table 1. There were no significant demographic or haemodynamic differences between the 2 groups. There were similar numbers of PD patients in both groups. Hypertension and hypercholesterolaemia (defined as total cholesterol > 5 mmol/L or statin therapy) were common in the entire cohort, but the prevalence of these comorbidities was not significantly higher in subjects with CFVR < 2.

Anaemia

Anaemia (defined as < 120 g/L in females and < 130 g/L in males) [14] was present in 17/22 (77%) of the whole cohort, and was normocytic in all cases. Haemoglobin concentration was significantly lower in patients with CMD compared to those without CMD [102 g/L ± 12 vs. 117 g/L ± 11, mean difference 15 g/L, 95% confidence interval (CI) 4–26, p = 0.008]—Fig. 1. There was a corresponding significantly lower haematocrit among subjects with CMD (31.2% ± 3.1 vs. 35.4% ± 3.7, mean difference 4.2%, 95% CI 0.8–7.8, p = 0.019). There were positive correlations between CFVR and haemoglobin (r = 0.7, p = 0.001) and between CFVR and haematocrit (r = 0.5, p = 0.011)—Fig. 2.

Table 1 Demographic, laboratory and haemodynamic variables

	CFVR < 2 (n = 7)	CFVR ≥ 2 (n = 15)	p value
Demographics			
Age (years)	47 ± 15	55 ± 10	0.177
Male n (%)	3 (43)	8 (53)	1.0
Caucasian n (%)	5 (71)	12 (80)	1.0
BMI (kg/m^2)	26.3 ± 4.4	27.7 ± 4.9	0.527
Smoker n (%)—Ex Never Current	1 (14) 6 (86) 0 (0)	4 (27) 10 (67) 1 (6)	0.744
Hypertension n (%)	6 (86)	14 (93)	1.0
Hypercholesterolaemia n (%)	4 (57)	11 (73)	0.630
Peritoneal dialysis n (%)	5 (71)	9 (60)	1.0
Duration of dialysis (months)	5 (4–48)	6 (4–9)	0.797
ACE inhibitor n (%)	1 (14)	4 (27)	1.0
ARB n (%)	1 (14)	3 (20)	1.0
Statin n (%)	1 (14)	8 (53)	0.165
Loop diuretic n (%)	5 (71)	5 (33)	0.172
Calcium channel blocker n (%)	5 (71)	9 (60)	1.0
Beta blocker n (%)	2 (29)	3 (20)	1.0
Alpha blocker	3 (43)	4 (27)	0.630
Erythropoietin treatment n (%)	5 (71)	4 (27)	0.074
Laboratory data			
Haemoglobin (g/L)	**102 ± 12**	**117 ± 11**	**0.008**
Haematocrit (%)	**31.2 ± 3.3**	**35.4 ± 3.7**	**0.019**
Mean cell volume (fl.)	88.9 ± 3.3	91.6 ± 3.7	0.118
Urea (mmol/L)	21.8 ± 6.2	22.1 ± 5.6	0.902
Creatinine (μmol/L)	673 ± 300	606 ± 192	0.534
ACR (mg/mmol)	204 (109.3-277.8)	77.4 (62.8-199.4)	0.239
Iron (μmol/L)	11.8 (9.5–13)	12.9 (9.4–16)	0.494
Transferrin (g/L)	1.92 ± 0.54	2.06 ± 0.4	0.525
Albumin (g/L)	35 ± 6	40 ± 7	0.125
Corrected calcium (mmol/L)	2.45 ± 0.13	2.33 ± 0.17	0.123
hsCRP (mg/L)	1.9 (1-3.6)	2.8 (1.9-8)	0.312
NT pro-BNP (ng/L)	1900 (522–4597)	441 (342–643)	0.416
Phosphate (mmol/L)	1.71 (1.55–2.07)	1.59 (1.53–1.69)	0.312
PTH (μmol/L)	41.7 ± 23.2	30.5 ± 16.9	0.271
Total cholesterol (mmol/L)	4.8 ± 1.7	5.0 ± 1.4	0.772
Haemodynamic data			
Systolic BP (mmHg)	129 ± 25	137 ± 20	0.398
Diastolic BP (mmHg)	83 ± 14	85 ± 8	0.798
Heart Rate (bpm)	72 ± 14	66 ± 8	0.156

Data are presented as mean ± SD or median (IQR). Variables highlighted in bold demonstrated a significant difference between the two groups

CFVR, coronary flow velocity reserve; BMI, body mass index; ACE, angiotensin converting enzyme; ARB, angiotensin receptor blocker; ACR, albumin creatinine ratio; hsCRP, high sensitivity C reactive peptide; NT-proBNP, N terminal pro brain natriuretic peptide; PTH, parathyroid hormone; BP, blood pressure; bpm, beats per minute

Bone mineral disease

Markers of CKD bone mineral disease were similar between the two groups. Calcium, phosphate and PTH were all numerically higher in patients with CMD, but this was not statistically significant.

Inflammatory markers

One subject with CMD did not provide stored blood for serum multiplex immunoassay. Inflammatory markers were similar among subjects with CMD and those with normal coronary microvascular function—Table 2.

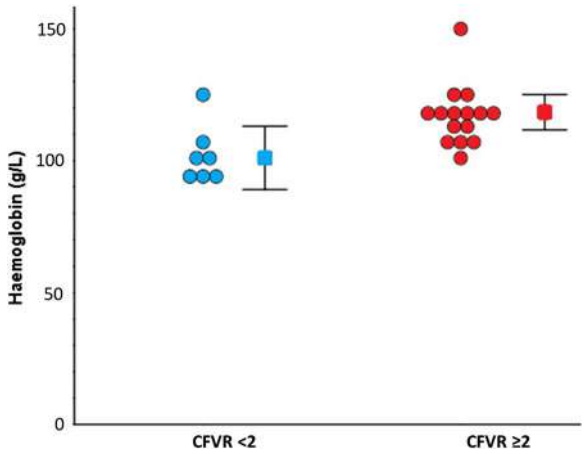

Fig. 1 Haemoglobin in subjects with CFVR < 2 and CFVR ≥ 2. Circles represent individual measurements. Squares represent mean. Error bars represent 95% confidence intervals of the mean. CFVR, coronary flow velocity reserve

Analysis of the remaining biomarkers studied by multiplex immunoassay also did not show any significant differences between the two groups.

Echocardiographic data

Echocardiographic data are reported in Table 3. Left ventricular dimensions, mass index and systolic and diastolic function were similar between the two groups. Cardiac output was significantly higher in subjects with CMD (6.1 L/min ± 0.8 vs. 4.7 L/min ± 1.4, mean difference 1.4 L/min, 95% CI 0.3-2.5 L/min, p = 0.02). No subjects had regional wall motion abnormalities or perfusion defects on MCE.

Regression analysis

In univariable linear regression analysis, haemoglobin and iron were independent predictors of CFVR—haemoglobin (β = 0.051 95% CI 0.023–0.079, p = 0.001) and iron (β = 0.094 95% CI 0.003–0.185, p = 0.044). However, in multivariable analysis, only haemoglobin was an independent predictor of CFVR (β = 0.041 95% CI 0.012–0.071, p = 0.009). In univariable binary logistic regression, haemoglobin was a negative predictor of CFVR < 2 (Odds ratio 0.85 95% CI 0.74–0.98, p = 0.022). No other parameters showed a significant association with CFVR < 2.

Discussion

This study has confirmed a high prevalence of CMD in subjects with ESRD. To our knowledge, it is also the first study to suggest an association between CMD and anaemia in this population. It is recognised that patients on the kidney transplant waiting list are often younger, have fewer comorbidities, and a reduced risk of death compared to ESRD patients not suitable for kidney transplantation [15]. However, previous work has shown that CMD was present in 59% of patients with ESRD undergoing evaluation for kidney transplant, and was more common in those with diabetes or left ventricular systolic dysfunction [16]. Unlike this study, our cohort did not include individuals with diabetes and uncontrolled hypertension, both of which independently influence CFVR [9, 10]. Despite this, nearly a third of our cohort of potential kidney transplant candidates had CFVR < 2.

The presence of anaemia in patients with CKD is associated with a significantly increased risk of cardiovascular and all-cause mortality [17]. Thus, our novel finding that subjects with ESRD and CMD have lower haemoglobin than patients with normal CFVR raises the possibility that this adverse association with prognosis may be in part related to the presence of CMD. Despite comparable kidney function and iron stores, and a higher prevalence of erythropoietin treatment, subjects with CMD had significantly lower haemoglobin and haematocrit than

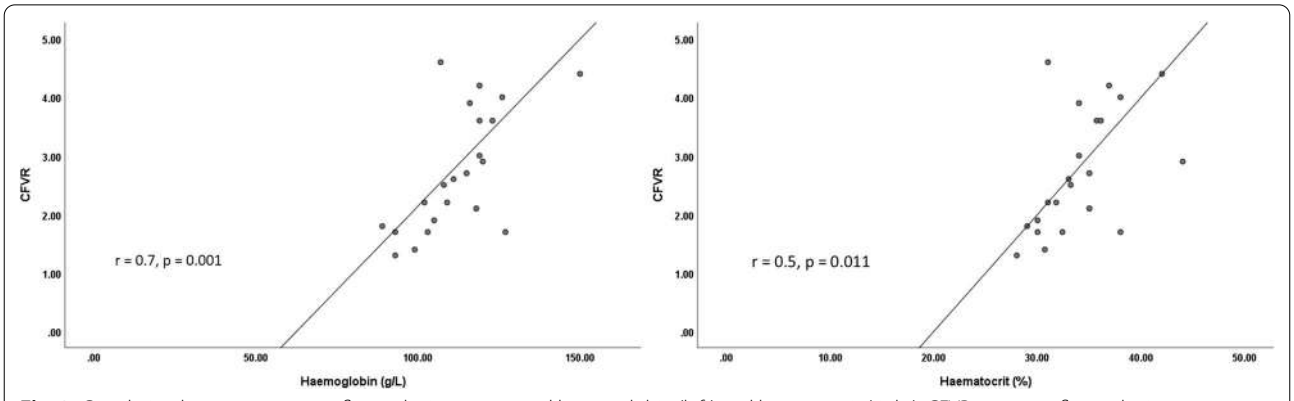

Fig. 2 Correlation between coronary flow velocity reserve and haemoglobin (left) and haematocrit (right). CFVR, coronary flow velocity reserve

Table 2 Results of human magnetic luminex assay

Assay	CFVR < 2 (n = 6)	CFVR ≥ 2 (n = 15)	p value
Angiopoetin-2 (pg/ml)	3274 (1000–5136)	3051 (2230–4053)	0.850
Atrial natriuretic peptide (pg/ml)	25,836 ± 9520	20,568 ± 11,210	0.329
Detectable KIM-1 n (%)	2 (33)	5 (31)	1.0
Galectin-3 (ng/ml)	1.3 (1-1.3)	1.3 (1-1.4)	0.791
IL-1ra (pg/ml)	667 (526–742)	515 (384–729)	0.850
IL-6 (pg/ml)	2.09 ± 1.3	2.69 ± 1.35	0.371
IL-8 (pg/ml)	6.1 (4.2–11.5)	11.4 (8–23)	0.132
IL-10 (pg/ml)	2.5 (0.9–4.1)	1.4 (0.9–3.4)	1.0
Leptin (ng/ml)	17.7 (6.6–20.6)	13.2 (4.2–50.4)	0.910
MCP-1 (pg/ml)	396 ± 221	375 ± 102	0.770
MMP-9 (pg/ml)	10,614 (4955–11,509)	9880 (6244–13,648)	1.0
NGAL (ng/ml)	26.3 ± 8.9	26.6 ± 4.8	0.898
ST2 (ng/ml)	14 (10–33)	12 (9–19)	0.850
TNFα (pg/ml)	6.1 (4.5–8.1)	5.7 (5.1–6.7)	0.850
Uromodulin (ng/ml)	18 ± 9	21 ± 10	0.53
VEGF (pg/ml)	52 ± 26	75 ± 25	0.108

Data are presented as mean ± SD or median (IQR)

CFVR, coronary flow velocity reserve; KIM-1, kidney injury molecule 1; IL-1ra, interleukin 1 receptor antagonist; IL-6, interleukin-6; IL-8, interleukin-8; IL-10, interleukin-10; MCP-1, monocyte chemoattractant protein; MMP-9, matrix metallopeptidase 9; NGAL, neutrophil gelatinase associated lipocalin; ST2, suppression of tumorigenicity 2; TNFα, tumour necrosis factor alpha; VEGF, vascular endothelial growth factor.

those with CFVR ≥ 2. We have also shown an association between haemoglobin and CFVR, that is independent of traditional factors thought to influence CFVR such as hypertension, diabetes and left ventricular hypertrophy. As anaemia is extremely prevalent in ESRD, low haemoglobin maybe an important driver of microvascular dysfunction and the increased cardiovascular mortality seen in this population. Furthermore, patients with CKD have additional risk factors for CMD, which may be exacerbated by anaemia.

These findings are of potential importance. While we cannot assume causation in either direction, it seems unlikely that anaemia could be caused by CMD. Our findings are also unlikely to be related to the methodology of our imaging technique since measurement of CFVR by Doppler TTE is not conventionally adjusted for haemoglobin as the pulse wave Doppler velocity signal is independent of haemoglobin concentration [18]. There are biologically plausible reasons why anaemia may lead to CMD in ESRD. Anaemia causes a number of maladaptive changes to the cardiovascular system that may predispose to CMD. Chronic anaemia can induce a form of high output cardiac failure, that leads to adverse cardiac remodelling including left ventricular dilatation, volume overload and LVH [19, 20]. This is suggested in our cohort, where subjects with CMD had an increased cardiac output, as well as a trend towards increased left ventricular and atrial volumes and markers of myocardial stretch. Animal studies in anaemia have shown that in order to maintain adequate myocardial oxygen supply, there is an increase in resting myocardial blood flow compared to non-anaemic controls, predominantly due to capillary widening and reduced blood viscosity [20]. Thus, in anaemia, the microcirculation operates in a state of supra-normal vasodilation at rest, which may limit its ability to vasodilate further during hyperaemia. Anaemia is also associated with abnormal red cell function and reduced nitric oxide bioactivity, which further impairs endothelium-dependent vasodilation in the microcirculation [21]. It is plausible that the combination of increased basal myocardial blood flow and a submaximal hyperaemic response leads to reduced CFVR in conditions of chronic anaemia—a pattern seen among our subjects with CMD.

Alternatively, a common causative factor may result in both anaemia and CMD. Possibilities include systemic inflammation and malnutrition, which are both commonly found in chronic disease states. We found no strong evidence that patients with CMD had higher levels of inflammatory markers. Markers of nutritional status such as body mass index, albumin and cholesterol were numerically lower among subjects in our cohort with CMD, but this was not statistically significant. It is possible that the small sample size means that we were unable to detect subtle differences in these variables.

To date there are no other studies examining the effect of anaemia on CFVR in CKD. However, there is some evidence from other conditions of an association between anaemia and CMD. In patients with beta thalassemia

Table 3 Echocardiographic parameters

	CFVR < 2 (n = 7)	CFVR ≥ 2 (n = 15)	p value
IVSD (mm)	12 ± 1	11 ± 2	0.610
LVIDD (mm)	46 ± 9	47 ± 6	0.679
PWD (mm)	10 ± 2	11 ± 2	0.789
LVIDS (mm)	31 (29–36)	30 (28–35)	0.535
FS (%)	33 ± 9	35 ± 5	0.639
LVEDVi (ml/m^2)	55 (49–69)	44 (39–51)	0.115
LVESVi (ml/m^2)	21 (18–28)	18 (16–21)	0.275
EF (%)	59 ± 7	59 ± 4	0.923
Stroke volume (ml)	87 ± 25	72 ± 20	0.182
Cardiac output (L/min)	**6.1 ± 0.8**	**4.7 ± 1.4**	**0.02**
GLS (%)	-16 ± 3	-19 ± 2	0.107
TAPSE (mm)	21 ± 4	21 ± 5	0.875
LV mass index (g/m^2)	99 ± 31	98 ± 28	0.936
LV geometry n (%)— normal geometry Concentric remodelling Eccentric hypertrophy Concentric hypertrophy	2 (29) 3 (43) 1 (14) 1 (14)	4 (27) 1 (7) 3 (20) 7 (46)	0.237
LA volume index (ml/m^2)	31.3 (26-44.1)	28.8 (20-38.3)	0.630
E/A ratio	1.1 (0.9–1.2)	0.8 (0.7–1.1)	0.340
E/e′	9 (8–11)	8 (7–10)	0.123

Data are presented as mean ± SD or median (IQR) .Variables highlighted in bold demonstrated a significant difference between the groups

CFVR, coronary flow velocity reserve; IVSD, interventricular septal diameter; LVIDD, left ventricular internal diameter diastole; PWD, posterior wall diameter; LVIDS, left ventricular internal diameter systole; LVEDVi, indexed left ventricular end diastolic volume; LVESVi, indexed left ventricular end systolic volume; EF, ejection fraction; GLS, global longitudinal strain; TAPSE, tricuspid annular plane systolic excursion; LV, left ventricular.

minor, Doppler CFVR was significantly lower compared to control subjects matched for age, gender and BMI [22]. Similarly, in patients with sickle cell disease, studies have demonstrated impaired coronary microvascular function compared to healthy controls. However, the aetiology of CMD in sickle cell disease is likely to be different to that seen in CKD and may be related to microvascular obstruction from vaso-occlusive events [23, 24]. A single study also included a group of patients with iron deficiency anaemia but did not demonstrate any reduction in CFVR compared to healthy controls [23].

The clinical significance of our findings requires further investigation. Current guidelines recommend aiming for a haemoglobin concentration > 90 g/L in patients on dialysis and > 100 g/L in non-dialysis CKD patients [14]. We have demonstrated that significant reductions in CFVR are present even above these treatment thresholds. Previous studies of aggressive anaemia treatment in CKD have had disappointing results, with no improvement in cardiovascular outcomes and possibly an increased risk of harm from correcting haemoglobin to a higher threshold [25]. To date, there are no studies examining the impact of improving haemoglobin concentration on CFVR.

Limitations

The main limitation of our study is the small sample size, which was limited by the outbreak of the global COVID-19 pandemic. Despite the small sample size, we found a high prevalence of CMD among this cohort. Furthermore, the size of the difference in haemoglobin and the strength of the relationship between haemoglobin and CFVR in multivariable analysis suggests that this is a true finding. Our study was underpowered to find small differences in the other variables tested.

Similar to other non-invasive studies of CFVR, we could not fully exclude occult CAD among our population. However, the majority of subjects in our study had undergone prior screening for CAD. Furthermore, all subjects were asymptomatic, had normal electrocardiograms and no coronary distribution perfusion defect or regional wall motion abnormality on vasodilator MCE—a highly sensitive and specific technique for the diagnosis of CAD [26]. This provides strong indirect evidence that there was no obstructive CAD in our cohort.

We included only patients eligible for kidney transplantation in this study. We also excluded patients on HD, as echocardiographic measurements in this population are more volume dependent [27]. These tight inclusion criteria improve the validity of our findings in the population we studied but limits the generalisability of our findings to the wider ESRD population.

Finally, our study was cross-sectional in design, meaning that causation cannot be definitively demonstrated. Future longitudinal work examining the role of anaemia and its correction on CFVR is needed.

Conclusions

Among patients suitable for kidney transplantation, there is a high prevalence of CMD, even in the absence of traditional risk factors such as diabetes, uncontrolled hypertension or significant LVH. In this population, we have shown that CMD is associated with low haemoglobin and an increased cardiac output—findings that require further investigation and independent confirmation. Together, they suggest that anaemia is a possible driver of CMD in ESRD. If this association is confirmed in larger studies, then correction of anaemia may represent a potential therapeutic target to improve microvascular function in ESRD.

Abbreviations
BP: Blood pressure; CAD: Coronary artery disease; CFVR: Coronary flow velocity reserve; CFV: Coronary flow velocity; CI: Confidence interval; CKD: Chronic kidney disease; CMD: Coronary microvascular dysfunction; CRIB-FLOW: Chronic Renal Impairment in Birmingham Coronary FLOW Reserve study; eGFR: Estimated glomerular filtration rate; ESRD: End-stage renal disease; GLS: Global longitudinal strain; IQR: Inter-quartile range; LAD: Left anterior descending artery; MCE: Myocardial contrast echocardiogram; MI: Mechanical index; NTpro-BNP: N-terminal pro-brain natriuretic peptide; PTH: Parathyroid hormone; QEHB: Queen Elizabeth Hospital Birmingham; RETRACT: Prospective Study of the Effects of Renal Transplantation on Uraemic Cardiomyopathy using Magnetic Resonance Imaging; TTE: Transthoracic echocardiogram.

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Authors' contributions
AR, CJF and JNT developed the research idea and study design. AR, LCP and AMP acquired the study data. AR performed the data and statistical analysis. AR and JNT wrote the original draft of the manuscript. All authors revised the manuscript and have read and approved the final version.

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Availability of data and materials
The datasets used and analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
Patients included in this analysis were recruited from studies reviewed and approved by a National Health Service Research Ethics Committee [CRIB-FLOW study approved by the West Midlands—Solihull Research Ethics Committee (19/WM/0066), RETRACT study approved by the West Midlands—Black Country Research Ethics Committee (18/WM/0287)]. The studies were carried out in accordance with the principles of the Declaration of Helsinki. All participants provided written informed consent.

Consent for publication
Not applicable.

Competing interests
LF has received institutional research grants and non-financial support from the European Union, British Heart Foundation, Medical Research Council (UK), DFG and several biomedical companies. LF is listed as an inventor on two patents held by the University of Birmingham (Atrial Fibrillation Therapy WO 2015140571, Markers for Atrial Fibrillation WO 2016012783). No other authors have any competing interests to declare.

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Coronary flow velocity reserve and inflammatory markers in living kidney donors

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ABSTRACT

Background: Coronary microvascular dysfunction is prevalent in chronic kidney disease (CKD), and may contribute to the development of myocardial dysfunction in CKD. Coronary flow velocity reserve (CFVR) is a marker of coronary microvascular function and falls with increasing CKD stage. Living kidney donors have renal function consistent with early stage CKD and concern has been raised about their cardiovascular risk. No studies to date have investigated the presence of coronary microvascular dysfunction in living kidney donors.

Methods: 25 healthy controls and 23 living kidney donors were recruited and underwent assessment with transthoracic echocardiography, Doppler CFVR, myocardial contrast echocardiography and serum multiplex immunoassay panels.

Results: Doppler CFVR was significantly reduced in living kidney donors compared to controls (mean CFVR 3.4 ± 0.7 vs 3.8 ± 0.6, mean difference 0.4 95% confidence interval 0.03–0.8, $p = .036$). Quantitative myocardial contrast echocardiography showed a trend towards reduced coronary flow reserve in living kidney donors. Compared to controls, living kidney donors had higher serum high sensitivity C reactive peptide (hsCRP) and lower levels of uromodulin.

Conclusions: This is the first study of CFVR in living kidney donors. We have shown that the modest drop in estimated glomerular filtration rate in living kidney donors is associated with lower values of Doppler CFVR compared to controls, suggesting that isolated reductions in renal function may lead to altered microvascular function. The increase in hsCRP and reduction in uromodulin suggests that chronic subclinical inflammation may contribute to altered microvascular function in this population.

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1. Introduction

Kidney transplantation is the most effective form of renal replacement therapy and is associated with significant health benefits for the recipient, including improved blood pressure (BP) control, and reduced all-cause and cardiovascular mortality [1]. Given the shortage of cadaveric donors, there is a worldwide drive to increase rates of living kidney donation, which now accounts for approximately 30% of transplants in the United Kingdom (UK) [2]. Living kidney donors (LKD) provide a unique model of reduced estimated glomerular filtration rate (eGFR) without progressive kidney disease or confounding comorbidities. After unilateral nephrectomy, most donors will have an eGFR consistent with stage 2–3 chronic kidney disease (CKD) [3]. Although long term evidence shows that living kidney donation is safe, the possible

Abbreviations: BP, Blood pressure; CAD, Coronary artery disease; CFR, Coronary flow reserve; CFVR, Coronary flow velocity reserve; CFV, Coronary flow velocity; CKD, Chronic kidney disease; CMD, Coronary microvascular dysfunction; CRIB-Donor, Chronic Renal Impairment in Birmingham - Donor study; CRIB-FLOW, Chronic Renal Impairment in Birmingham Coronary FLOW Reserve study; CRP, C reactive peptide; ECG, Electrocardiogram; eGFR, Estimated glomerular filtration rate; IL-1ra, Interleukin-1 receptor antagonist; IL-6, Interleukin-6; IL-8, Interleukin-8; hsCRP, High sensitivity C reactive peptide; LKD, Living kidney donors; LVH, Left ventricular hypertrophy; MCE, Myocardial contrast echocardiogram; MI, Mechanical index; SD, Standard deviation; SNS, Sympathetic nervous system; TNFα, Tumour necrosis factor α; TTE, Transthoracic echocardiogram; QEHB, Queen Elizabeth Hospital Birmingham; UK, United Kingdom.

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cardiovascular risks of living kidney donation remain unclear. Previous studies of LKD have shown small but significant changes in cardiovascular structure and function at 1 year after donation [4,5]. Although the majority of studies, including a recent meta-analysis, have not shown any increased mortality compared to the general population [6,7], Mjoen et al raised concerns about long term mortality in LKD when compared to a highly selected control group who met the eligibility criteria for living kidney donation [8].

There is growing interest in the role that coronary microvascular dysfunction (CMD) may play in the increased cardiovascular risk seen in CKD [9]. Coronary flow reserve (CFR) is a widely reported parameter of microvascular function and is primarily a measure of the ability of the microcirculation to respond to vasodilatory stimuli. In normal subjects, coronary flow should at least double with hyperaemia, so a CFR <2 is considered abnormal [9]. Multiple studies have shown a graded inverse relationship between CFR and CKD stage, and this has prognostic significance [9–14]. Both CFR and its surrogate marker CFVR (coronary flow velocity reserve) can be reliably measured using non-invasive contrast enhanced echocardiography techniques [15–17].

Reduced CFR is seen even in early CKD (stages 1–3), a level of eGFR often present in LKD [10–12]. Given the increasing numbers of LKD worldwide, it is important to assess whether unilateral nephrectomy is associated with impaired microvascular function, which may have long term implications for cardiovascular risk in donors. The Chronic Renal Impairment in Birmingham Coronary Flow Reserve (CRIB-FLOW) study was designed to assess coronary microvascular function in LKD and to look for associations between CFVR and markers of inflammation and fibrosis.

2. Methods

2.1. Study population

Between May 2019 and February 2020, 23 LKD and 25 healthy controls were enrolled in the CRIB-FLOW study at the Queen Elizabeth Hospital, Birmingham (QEHB) – Supplementary Fig. 1. Participants were >18 years of age and provided written informed consent. The study was carried out in accordance with the principles of the Declaration of Helsinki. Donors were recruited from the LKD registry at QEHB. Healthy controls, of a similar age and gender, were recruited from staff members and control subjects from the Chronic Renal Impairment in Birmingham – Donor (CRIB-Donor) study [4].

Kidney donors were >12 months post-donation. Healthy controls had eGFR >90ml/min/1.73m^2 or eGFR 60-90ml/min/1.73m^2 and no significant proteinuria or signs of kidney damage. The Chronic Kidney Disease Epidemiology Collaboration formula was used to calculate eGFR [18]. Exclusion criteria were: pregnancy, diabetes mellitus, uncontrolled hypertension, ischaemic heart disease, moderate/severe valvular heart disease and contraindication to adenosine or sulfur hexafluoride contrast agent (SonoVue, Bracco, Milan, Italy). The study was reviewed and approved by the West Midlands – Solihull Research Ethics Committee (19/WM/0066) and registered with ClinicalTrials.gov (NCT04014127).

2.2. Blood pressure

Supine and sitting office BP were measured using an automatic BP monitor. The average of five readings taken over five minutes was used.

2.3. Transthoracic echocardiography (TTE)

Two-dimensional echocardiography was performed by a single experienced cardiologist (AR) using a Philips iE33 machine (Philips, Eindhoven, Netherlands) with S5–1 transducer for TTE and myocardial contrast echocardiogram (MCE) studies and S8–3 transducer for CFVR measurements.

Left ventricular mass was estimated using the Cube formula and indexed for body surface area [19]. Left ventricular volumes and ejection fraction were measured using the Simpson's biplane method [19]. Diastolic function was quantified using multiple parameters [20]. Global longitudinal strain was assessed in the 3 standard apical views using speckle tracking.

2.4. Doppler coronary flow velocity reserve

Subjects were asked to abstain from caffeine for 24 hours prior to the study. The left anterior descending artery (LAD) was identified on colour Doppler in the anterior inter-ventricular sulcus using a modified apical 2-chamber view (distal LAD) or a low parasternal short axis view (mid LAD) as previously described [15]. Pulse wave Doppler signals of LAD flow were recorded at rest and at hyperaemia, maintaining an identical probe position and angle. SonoVue was used, if needed, to identify LAD flow and accentuate Doppler signals. Adenosine was infused, with BP and electrocardiogram (ECG) monitoring, at a rate of 140micrograms/kg/min for 3 minutes to induce hyperaemia. Peak diastolic coronary flow velocity (CFV) was calculated at rest and hyperaemia – Supplementary Fig. 2. CFVR was calculated as hyperaemic CFV/rest CFV. For each variable in the CFVR calculation, the highest values of 3 cardiac cycles were averaged.

2.5. Myocardial contrast echocardiography

Myocardial contrast echocardiography was performed as previously described [17]. Briefly, images were taken in the 3 standard apical views using low-power continuous MCE at a mechanical index (MI) of 0.1. SonoVue was infused at a rate of 70-100 ml/h using an infusion pump that oscillates gently throughout the infusion to ensure that microbubbles remain in suspension (Vueject, Bracco, Milan, Italy). The infusion rate was adjusted to ensure adequate myocardial opacification without attenuation. The focus was set at the level of the mitral valve but moved towards the apex to avoid near-field artefact. Triggered high MI (1.0) flash echocardiography at end-systole was performed to destroy microbubbles in the myocardium and to observe replenishment. End-systolic frames of up to 10 cardiac cycles were captured in each view. Rest and adenosine vasodilator stress images were recorded. Stress images were reviewed for any regional wall motion abnormalities or any subendocardial perfusion defects suggesting myocardial ischaemia.

2.6. Quantitative myocardial contrast echocardiography

The QLab system (Philips, Eindhoven, Netherlands) was used to quantify MCE. The left ventricle was segmented using a 16-segment model [19]. Regions of interest were placed across the entire thickness of the myocardium in the 10 mid and apical segments, taking care to exclude the high-intensity endocardial and epicardial borders. Basal segments were excluded due to high rates of artefact. Segments were also excluded if there was artefact, inadequate microbubble destruction, attenuation, or a wide variation in contrast intensity. A minimum of 6 quantifiable segments was necessary for the study to be included in analysis.

The QLab software automatically generated background-subtracted plots of contrast intensity vs time which were fitted to an exponential function $y = A(1 - e^{-\beta t})$. From this, peak myocardial contrast intensity (A - representing myocardial blood volume) and the slope of the replenishment curve (β - depicting mean microbubble velocity) could be derived. The product of Aβ equals myocardial blood flow (MBF). LAD MBF (average of mid anteroseptal, apical septal, mid anterior and apical anterior segments) and global MBF (average of all ten segments) were calculated at rest and at stress. CFR was calculated as MBF_{stress}/MBF_{rest} [17].

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2.7. Blinded analysis

Echocardiograms were stored under an anonymous code and analysed offline using commercially available software (IntelliSpace Cardiovascular, Philips, Eindhoven, Netherlands). The TTE, CFVR and MCE studies were all analysed by a single investigator (AR) blinded to study group. Ten randomly selected studies had repeat blinded Doppler CFVR analysis by the same investigator to assess intra-observer variability.

2.8. Serum biomarkers

Serum biomarkers of inflammation, myocardial stretch, cardiac fibrosis and markers associated with left ventricular hypertrophy (LVH) were tested in both LKD and controls. N-terminal pro brain natriuretic peptide was assayed using the Alere point of care assay (Alere, Massachusetts, USA). High sensitivity C-reactive peptide (hsCRP) was assayed using the Architect MULTIGENT CRP Vario assay (Abbott, Illinois, USA). The fluorescence responses of 16-analytes were obtained using Human Magnetic Luminex® Asssays (R&D Systems, Minneapolis, MN, USA) and the Bio-RAD Bio-Plex™ 200 system for analysis. Concentrations were calculated using the Bio-Plex Software Manager™ (version 6.1) generated standard curves and a 5PL logistic curve fitting technique as per the manufacturer's instructions [21].

2.9. Endpoints & sample size justification

The primary endpoint was difference in mean Doppler CFVR between controls and LKD. Based on previous data by Imamura et al [10] [CFVR for controls (3.8 ± 0.4), CFVR for CKD stage 2 (3.2 ± 0.7), CFVR for CKD stage 3 (3.0 ± 0.6)] - we estimated that 22 patients in each group would provide 80% power with an alpha value of 0.05 to demonstrate a difference in Doppler CFVR of 0.6 between controls and LKD. Difference in CFR by MCE was the secondary endpoint.

2.10. Statistical analysis

Statistical analysis was carried out using SPSS version 26 (SPSS Inc., Chicago, Illinois). Data normality was assessed using the Shapiro-Wilk test. Continuous variables are expressed as mean ± standard deviation (SD) for parametric data or median (interquartile range) for non-parametric data. Unpaired group comparisons for continuous data were made using the unpaired *t*-test or the Mann-Whitney *U* test. Unpaired categorical data were compared using Fisher's exact test. Correlation was assessed using the Pearson correlation coefficient. Statistical tests were 2-tailed, and a p value <.05 was considered statistically significant.

3. Results

3.1. Subject characteristics

Baseline demographic, laboratory and haemodynamic data are presented in Table 1. Median time from donation in LKD was 30 months (interquartile range 24–67 months). There were no significant differences in demographic variables between controls and LKD. One LKD was on anti-hypertensive therapy. Two controls and 1 LKD were on statin therapy. Of the remaining 18 participants with total cholesterol >5mmol/L, only 1 LKD and 2 controls met UK criteria for primary prevention statin therapy (QRISK3 10 year risk >10%) [22].

There was a significant difference in creatinine and eGFR between controls and donors. 3/23 (13%) donors had eGFR consistent with stage 3 CKD while the remainder had eGFR in the range of CKD stage 2. Serum phosphate was significantly lower in LKD. Detectable C reactive peptide (CRP) and median high sensitivity C reactive peptide (hsCRP) were both significantly higher in LKD.

Table 1
Demographic, laboratory and haemodynamic variables.

	Controls (n = 25)	Donors (n = 23)	p value
Demographics			
Age (years)	41 ± 10	46 ± 10	0.098
Male n(%)	18 (72)	16 (70)	0.853
Caucasian n(%)	15 (60)	18 (78)	0.173
BMI (kg/m^2)	25.6 ± 2.3	26.8 ± 4.2	0.230
Smoker n(%) – Current	2 (8)	3 (13)	0.905
Ex	5 (20)	4 (17)	
Never	18 (72)	16 (70)	
Hypertension n(%)	1 (4)	1 (4)	1.0
Hypercholesterolaemia n(%)	8 (32)	13 (57)	0.145
ACE inhibitors n(%)	0 (0)	1 (4)	0.479
Statin therapy n(%)	2 (8)	1 (4)	1.0
Time from donation (months)	n/a	30 (24–67)	n/a
Laboratory data			
Haemoglobin (g/l)	146 ± 11	141 ± 10	0.198
Urea (mmol/l)	5.0 ± 1.3	5.7 ± 1.1	0.061
Creatinine (μmol/l)	80 ± 17	107 ± 15	**<0.001**
eGFR (ml/min/1.73m^2)	99 (91–112)	68 (64–72)	**<0.001**
ACR (mg/mmol)	0.9 (0–2.1)	0.9 (0–1.8)	0.298
Phosphate (mmol/l)	1.13 ± 0.17	1.03 ± 0.17	**0.042**
Corrected calcium (mmol/l)	2.33 ± 0.08	2.36 ± 0.08	0.152
PTH (μmol/l)	5.7 ± 2.1	6.6 ± 2.0	0.237
Total cholesterol (mmol/l)	4.6 (4.0–5.2)	5.1 (4.8–5.6)	0.06
LDL cholesterol (mmol/l)	2.7 ± 1.0	3.2 ± 0.8	0.06
NT-proBNP (ng/l)	40 (22–69)	54 (24–95)	0.391
Detectable CRP n(%)	7 (29)	18 (73)	**0.01**
hsCRP (mg/l)	0.63 (0.41–0.86)	1.31 (0.92–2.0)	**0.006**
Urate (μmol/l)	332 ± 84	366 ± 82	0.158
Renin (mIU/l)	21.2 (16.9–35.6)	17.9 (13.4–35.5)	0.324
Aldosterone (μmol/l)	161 (129–225)	129 (44–222)	0.156
Haemodynamic data			
Systolic BP (mmHg)	116 ± 11	115 ± 12	0.835
Diastolic BP (mmHg)	76 ± 10	76 ± 10	0.816
Heart rate (bpm)	71 ± 12	65 ± 11	0.066

Data are presented as mean ± SD or median (IQR). BMI – body mass index, ACE – angiotensin converting enzyme, eGFR – estimated glomerular filtration rate, ACR – albumin creatinine ratio, PTH – parathyroid hormone, LDL – low density lipoprotein, NT-proBNP – n terminal pro brain natriuretic peptide, CRP – C reactive peptide, hsCRP – high sensitivity C reactive peptide, BP – blood pressure.

There were no significant differences in TTE parameters between controls and LKD - Table 2. One individual had previously undiagnosed severe aortic regurgitation detected on baseline TTE. Markers of systolic and diastolic function were similar between the two groups.

3.2. Doppler coronary flow velocity reserve

Doppler CFVR was not attempted in the subject with severe aortic regurgitation on baseline TTE. The technique was feasible in 46/47 (99%) of subjects in which it was attempted. One subject did not tolerate adenosine and thus no hyperaemic measurements were available. One subject was subsequently excluded from CFVR analysis due to the new finding of thyrotoxicosis on serum biochemistry. Final Doppler TTE CFVR data were available in 22 controls and 23 LKD. SonoVue was used in 31/45 (69%) cases. There was no significant intra-observer variability for offline Doppler CFVR analysis (ICC 0.99 95% confidence interval 0.956–0.998, $p < .001$).

Resting CFV in donors was slightly higher than in controls, although this was not statistically significant [median CFV 19.9 (17.4–22.2) vs 18.1 (15.6–20.4), $p = .114$]. Hyperaemic CFV did not differ (mean CFV 70.2 ± 14.6 vs 70.5 ± 13.8, $p = .944$) – Fig. 1a. CFVR was significantly reduced in LKD compared to controls (mean CFVR 3.4 ± 0.7 vs 3.8 ± 0.6, mean difference 0.4 95% confidence interval 0.03–0.8, $p = .036$) – Fig. 1b. Although no subjects in our study had CFVR<2, 6/23 (26%) LKD had CFVR ≤2.7 (the lowest CFVR value in controls). There was a modest significant correlation between eGFR and CFVR ($r = 0.3$ $p = .034$).

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Table 2
Echocardiographic parameters.

	Controls ($n = 25$)	Donors ($n = 23$)	p value
IVSD (mm)	10 (9–11)	10 (8–11)	0.106
LVIDD (mm)	44 ± 4	44 ± 5	0.946
PWD (mm)	9 (8–10)	9 (8–10)	0.732
LVIDS (mm)	28 ± 3	29 ± 4	0.470
Fractional Shortening (%)	36 (31–38)	32 (31–36)	0.201
LVEDVi (ml/m^2)	46 ± 8	47 ± 10	0.716
LVESVi (ml/m^2)	17 (14–19)	18 (13−22)	0.713
EF (%)	62 (60–65)	61 (57–65)	0.305
TAPSE (mm)	21 ± 3	20 ± 3	0.168
GLS (%)	−19 ± 3	−19 ± 3	0.849
LV mass index (g/m^2)	71 (62–88)	69 (57–76)	0.307
LV geometry n(%) – normal geometry	17 (68)	14 (61)	0.439
Concentric remodelling	6 (24)	9 (39)	
Eccentric hypertrophy	1 (4)		
Concentric hypertrophy	1 (4)		
Left atrial volume index (ml/m^2)	19.3 ± 4.3	20.5 ± 6.8	0.477
E/A ratio	1.2 ± 0.3	1.1 ± 0.2	0.184
E/e'	6 (5–8)	6 (6–7)	0.655

Data are presented as mean ± SD or median (IQR). IVSD – interventricular septal diameter, LVIDD – left ventricular internal diameter diastole, PWD – posterior wall diameter, LVIDS – left ventricular internal diameter systole, LVEDVi – indexed left ventricular end diastolic volume, LVESVi – indexed left ventricular end systolic volume, EF – ejection fraction, TAPSE – tricuspid annular plane systolic excursion, GLS – global longitudinal strain, LV – left ventricular.

3.3. Myocardial contrast echo

No subjects had stress induced wall motion abnormalities or perfusion defects on qualitative MCE. Quantitative MCE was possible in only 14 controls and 19 LKD. Both LAD CFR and global CFR were numerically lower in LKD, although this was not statistically significant – LAD CFR [median CFR 3.4 (2.6–5.0) vs 2.7 (2.2–3.9), $p = .212$] and global CFR [median CFR 3.4 (2.2–3.8) vs 3.0 (2.3–4.2), $p = 1.0$].

3.4. Multiplex immunoassay

The results of the Multiplex immunoassay are shown in Table 3. One control did not provide blood for immunoassay analysis. There were no significant differences between controls and LKD in the assays tested, apart from uromodulin which was significantly lower in LKD.

4. Discussion

This is the first study of CFVR in LKD. Despite only modest reductions in eGFR, LKD had a significantly lower Doppler CFVR than controls. These results suggest that reductions in renal function alone can lead to altered microvascular function. Reassuringly, no subjects in our cohort had CFVR<2, which is known to be a poor prognostic marker [13].

Previous studies using Doppler TTE have shown intra-subject variations in CFVR of 0.3–0.45 [15,23]. Given that the difference in CFVR between controls and LKD in our study was similar to this value, we cannot fully exclude the possibility that this difference was due to chance. However, our sample size was adequate and we would expect similar variability of CFVR measurements in both groups. Furthermore, the magnitude of difference between our controls and LKD is similar to the previously demonstrated difference between controls and subjects with CKD stage 2 – a group that have similar renal function to LKD [10].

The wider variances in CFR by MCE among our subjects suggest that our study may have been underpowered for this secondary endpoint. Adenosine can cause uncomfortable dyspnoea and chest wall movement that compromises the image quality needed for optimal MCE quantification. Previous studies have used intravenous dipyridamole [17], which has fewer respiratory side effects, but was not available in our hospital. Coronary flow reserve by MCE was measurable in 69% of our cohort, which is consistent with previous studies showing that quantitative MCE using adenosine is feasible in only 33–75% of patients [24,25]. Despite these limitations, our MCE data showed a trend towards reduced CFR in LKD, which is consistent with our Doppler CFVR data. We chose Doppler CFVR as our primary endpoint as the technique is feasible and highly reproducible even with limited image quality [15].

The mechanisms of microvascular dysfunction in LKD are not clear but abnormalities of both structure and function may be present. Animal models have demonstrated reduced capillary length and density in the hearts of rats who underwent subtotal nephrectomy and evidence of fibrosis and diastolic dysfunction in rats after uni-nephrectomy [26,27].

The reduced CFVR among LKD in our study was predominantly due to a higher baseline CFV in LKD, with similar maximal hyperaemic values. Elevated resting CFV is seen in CKD and hypertension and has been attributed to increased oxygen demand as a result of hypertension, LVH and diastolic dysfunction [10,28]. Elevated resting CFV may also be related to increased sympathetic nervous system (SNS) activity which causes vasoconstriction of vascular smooth muscle cells, leading to increased coronary vascular resistance and a decrease in coronary perfusion pressure [29]. Increased SNS activity is seen in early CKD but has not been studied in LKD [30]. In addition, the reduced CFVR in LKD also reflects a diminished hyperaemic response to adenosine, indicating impaired vasodilatation in the coronary microcirculation, where adenosine predominantly has its effect [31]. Adenosine-induced vasodilatation is at least partially mediated by nitric oxide release from the endothelium [32], suggesting that endothelial dysfunction may be a contributory mechanism for CMD in LKD. Studies in early CKD have shown that endothelial dysfunction is common and is associated with poor prognosis [33,34]. To date, there are no studies of endothelial function in LKD but the CENS study, which is currently recruiting, will provide a comprehensive assessment of endothelial function in LKD [35].

Chronic inflammation in systemic inflammatory conditions is associated with CMD [36]. Both detectable CRP and mean hsCRP were significantly higher in LKD. An inflammatory response has been shown in the early post-operative period in LKD with an 80-fold increase in CRP in the first week after nephrectomy [37]. Longer term data on chronic inflammation in LKD are conflicting. Huan et al showed no increase in inflammatory markers in LKD at 6 months post donation [38]. However, Moody et al showed an increase in the prevalence of detectable CRP in LKD at 12 months post donation [4]. The elevated hsCRP suggests that a pattern of subclinical chronic inflammation may be present in LKD, as it is in subjects with CKD [39]. Uromodulin, a glycoprotein secreted by the thick ascending limb of the loop of Henle, may play a role in this process. In a normally functioning kidney, uromodulin may have a protective anti-inflammatory role through neutralisation of urinary cytokines. As renal function declines, so does uromodulin. In the presence of tubular damage, as seen in CKD, the reduction in uromodulin may have a pro-inflammatory effect by activating NLRP3 dependent IL-1β secretion and subsequent induction of other pro-inflammatory cytokines [40]. It is possible that the raised CRP, hsCRP and uromodulin in LKD were chance findings due to the large number of variables tested. After adjustment with a Bonferroni correction for multiple endpoints, they fail to reach statistical significance. However, this correction has been subject to criticism [41], and as CKD is characterised by systemic inflammation, there are plausible reasons why subjects with reduced kidney function due to uni-nephrectomy might also exhibit a pro-inflammatory state. The role of inflammation after nephrectomy warrants further research.

The clinical significance of our findings needs further investigation. It is possible that this small reduction in coronary microvascular function in LKD may not have clinical sequelae and is an epi-phenomenon related to persistent low-grade inflammation after uni-nephrectomy. However, there is increasing evidence of a possible role for CMD in the development of heart failure with preserved ejection fraction and

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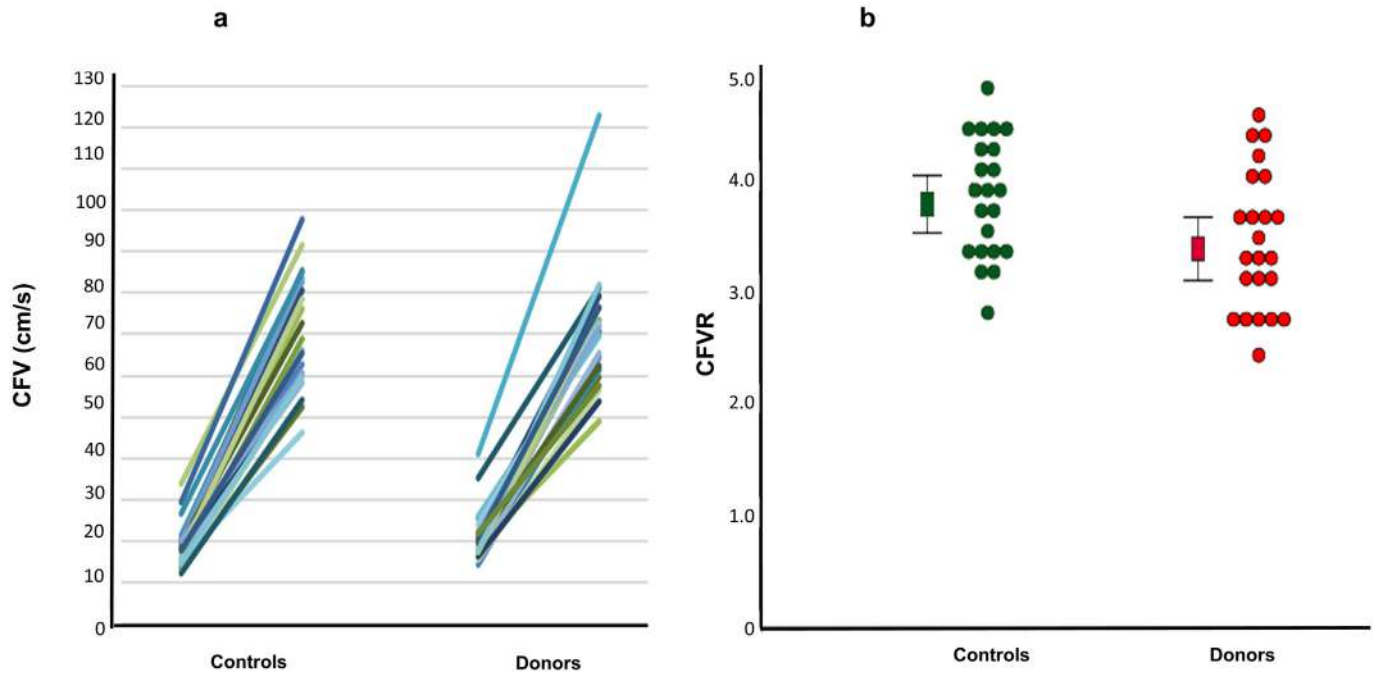

Fig. 1. 1a – Coronary flow velocity at rest and at hyperaemia in controls and living kidney donors. 1b – Doppler coronary flow velocity reserve in controls and living kidney donors. Squares represent mean. Error bars represent 95% confidence intervals. Circles represent individual CFVR measurements. CFV – coronary flow velocity, CFVR – coronary flow velocity reserve.

Table 3
Results of human magnetic luminex assay.

Assay	*Controls (n = 24)*	*Donors (n = 23)*	*p value*
Angiopoetin-2 (pg/ml)	1518 (1260–2006)	1348 (1143–1865)	0.322
Atrial natriuretic peptide (pg/ml)	4730 (3449–6145)	5778 (3653–8248)	0.268
Detectable IL-10 *n*(%)	11 (44)	11 (48)	0.790
Detectable KIM-1 *n*(%)	9 (36)	11 (48)	0.406
Galectin-3 (ng/ml)	0.9 (0.8–1.2)	1.1 (0.8–1.3)	0.317
IL-1ra (pg/ml)	522 (356–655)	503 (340–703)	0.807
IL-6 (pg/ml)	1.26 (0.82–1.86)	1.26 (0.97–1.81)	0.661
IL-8 (pg/ml)	12.3 (8.4–25.5)	11.3 (8–29.1)	0.992
Leptin (ng/ml)	5.7 (3.0–11.1)	4.9 (3.2–8.5)	0.865
MCP-1 (pg/ml)	378 (298–537)	391 (325–480)	0.670
MMP-9 (pg/ml)	9118 (6465–13,292)	9928 (7374–19,628)	0.360
NGAL (ng/ml)	15.5 (14.0–16.6)	16.7 (14.4–18.3)	0.187
ST2 (ng/ml)	12 (9–16)	10 (6–18)	0.444
TNFα (pg/ml)	3.5 (2.53–4.22)	3.37 (2.59–4.28)	0.924
Uromodulin (ng/ml)	98 ± 43	67 ± 35	**0.009**
VEGF (pg/ml)	48 (24–60)	65 (41–93)	0.101

Data are presented as mean ± SD or median (IQR). IL-10 – interleukin-10, KIM-1 – kidney injury molecule 1, IL-1ra – interleukin 1 receptor antagonist, IL-6 – interleukin-6, IL-8 – interleukin-8, MCP-1 – monocyte chemoattractant protein, MMP-9 – matrix metallopeptidase 9, NGAL – neutrophil gelatinase associated lipocalin, TNFα – tumour necrosis factor alpha, VEGF – vascular endothelial growth factor.

uraemic cardiomyopathy [9]. In CKD, the presence of CMD is associated with abnormalities of diastolic function and indices of systolic deformation, as well as adverse cardiovascular outcomes including death, myocardial infarction and heart failure hospitalisation [14]. Thus, a paradigm has been suggested in which risk factors such as inflammation and hypertension lead to CMD, which in turn causes diffuse ischaemia and adverse left ventricular re-modelling, leading eventually to uraemic cardiomyopathy with its adverse prognosis [9]. Our results should stimulate long term studies of LKD to determine their subsequent risk of the development of diastolic dysfunction, adverse left ventricular remodelling and uraemic cardiomyopathy. As long-term cardiovascular risk in LKD remains unclear and CMD carries a poor prognosis, baseline assessment of coronary microvascular function may be worthwhile in potential kidney donors, to help identify individuals who are at increased cardiac risk from kidney donation.

5. Limitations

Similar to other non-invasive studies of CFVR, we could not fully exclude coronary artery disease (CAD) in our cohort without coronary angiography (either computed tomography or invasive). However, all subjects had normal ECG and no coronary distribution perfusion defect or regional wall motion abnormality on vasodilator MCE – a highly sensitive and specific technique for the diagnosis of flow limiting CAD [42]. Thus we have strong evidence that there was no myocardial ischemia due to CAD in our cohort.

Our cohort was predominantly male and Caucasian, limiting the generalisability of our findings to the wider LKD population. However, UK data does show that the majority of LKD are Caucasian [2], and it has previously been shown that there are similar rates of CMD among men and women [43].

Finally, our study was cross-sectional in design, meaning that causation cannot be definitively demonstrated. Future longitudinal work examining CFVR pre- and post-nephrectomy is needed to confirm the observation seen in our study.

6. Conclusions

Our study has shown that Doppler CFVR is reduced in LKD compared to healthy controls, suggesting subclinical impairment of microvascular function. Although current data suggests that living kidney donation remains extremely safe, our study highlights the importance of long-term follow-up and aggressive risk factor management to detect subtle cardiovascular changes and to minimise any future cardiovascular morbidity and mortality in this population. The role of chronic inflammation in LKD also needs further examination.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijcard.2020.08.013.

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Author statement

All authors take responsibility for all aspects of the reliability and freedom from bias of the data presented and their discussed interpretation.

Declaration of Competing Interest

LF has received institutional research grants and non-financial support from European Union, British Heart Foundation, Medical Research Council (UK), DFG and Gilead. LF is listed as inventor on two patents held by University of Birmingham (Atrial Fibrillation Therapy WO 2015140571, Markers for Atrial Fibrillation WO 2016012783). No other authors have any conflict of interest to declare.

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Coronary microvascular dysfunction: a key step in the development of uraemic cardiomyopathy?

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ABSTRACT

The syndrome of uraemic cardiomyopathy, characterised by left ventricular hypertrophy, diffuse fibrosis and systolic and diastolic dysfunction, is common in chronic kidney disease and is associated with an increased risk of cardiovascular morbidity and mortality. The pathophysiological mechanisms leading to uraemic cardiomyopathy are not fully understood. We suggest that coronary microvascular dysfunction may be a key mediator in the development of uraemic cardiomyopathy, a phenomenon that is prevalent in other myocardial diseases that share phenotypical similarities with uraemic cardiomyopathy such as hypertrophic cardiomyopathy and heart failure with preserved ejection fraction. Here, we review the current understanding of uraemic cardiomyopathy, highlight different methods of assessing coronary microvascular function and evaluate the current evidence for coronary microvascular dysfunction in chronic kidney disease.

INTRODUCTION

Chronic kidney disease (CKD) is common, affecting one in seven of Western populations.w1 Usually, it is mild and there is little risk of progression to end-stage renal disease (ESRD), but the risk of adverse cardiovascular events is elevated. There is a well-documented graded inverse relationship between cardiovascular risk and estimated glomerular filtration rate (eGFR) that is independent of age, sex and other risk factors.[1] Patients with CKD have an increased risk of coronary artery disease and an even higher risk of death from heart failure, arrhythmias and sudden death, which rises steeply with more severe CKD.[2] In ESRD, the individual cardiovascular risk is extreme but the public health burden lies in early-stage CKD because of its much higher prevalence.

Pathological structural and functional remodelling occurs in the heart and vascular system in CKD. Left ventricular hypertrophy (LVH) is found in over 70% of patients with ESRD and other manifestations of heart muscle disease such as focal scarring and diffuse interstitial fibrosis (DIF) frequently occur, comprising the phenotype of uraemic cardiomyopathy.[3] These findings are also present to a lesser degree in early-stage disease.[4] Hypertension is near universal. Vascular calcification is common and results from accelerated atherosclerosis (intimal disease) and arteriosclerosis (medial disease).[5] Regardless of the vascular bed affected, these changes confer elevated cardiac risk by increasing arterial stiffness, which can be measured by pulse wave velocity and augmentation index.[5] These arterial changes increase LV afterload which, together with humoral hypertrophic and profibrotic stimuli, lead to the syndrome of uraemic cardiomyopathy.[5] As eGFR declines, the severity of this myocardial disease increases, possibly explaining the very high risk of death due to heart failure and sudden (presumed arrhythmic) cardiac death in ESRD.

URAEMIC CARDIOMYOPATHY

The syndrome of uraemic cardiomyopathy, characterised by LVH, DIF, focal scarring and systolic and diastolic dysfunction, is highly prevalent in ESRD.[2 3] Uraemic cardiomyopathy has been well described in recent years, mainly using cardiac MRI (CMR).[3 6 7] The increased LV mass seen in ESRD is due to both myocyte hypertrophy and an expansion of the interstitial space caused by DIF. Myocardial biopsy studies show that many subjects with ESRD have myocardial appearances resembling the dilated phase of hypertrophic cardiomyopathy (HCM) with severe myocyte hypertrophy, myocyte disarray and extensive DIF.[8] This fibrotic process can be demonstrated non-invasively on CMR by T1 mapping; a technique that quantifies the relaxation time of protons on inversion recovery prepared images (T1 times) by using analytical expression of image-based signal intensities.w2 T1 relaxation times increase with interstitial expansion due to oedema, infarction, infiltration and fibrosis, and thus provide a sensitive, though non-specific marker of different myocardial disease states.w2 Interstitial fibrosis, identified by elevated T1 times, correlates with histological specimens in hypertrophic and dilated cardiomyopathy and valvular heart disease.w3 Patients with ESRD also have increased T1 times, in keeping with these other myocardial disease states.[6 7] The fibrotic process occurs early in CKD, with elevated T1 times documented in patients with stages 2–3 CKD compared with age-matched and sex-matched controls.[4] DIF is probably responsible for reduced systolic function, reflected by reduced markers of deformation,w4 but causes severe diastolic dysfunction as tissue collagen deposition affects viscoelasticity of the myocardium leading to impaired relaxation, diastolic recoil and passive stiffness.w5 It is believed to be a major cause of the clinical syndrome of heart failure and of the increased risk of arrhythmogenesis seen in uraemic cardiomyopathy.[2]

MEDIATORS OF ADVERSE CARDIAC REMODELLING IN CKD

The development of uraemic cardiomyopathy is likely to be multifactorial. Haemodynamic factors include increased afterload due to hypertension and arterial stiffness, and increased preload due to anaemia and sodium overload.[2] A wide range of humoral and local factors are involved. Activation of the renin–angiotensin–aldosterone system, hyperuricaemia, uraemic toxins such as asymmetric dimethylarginine,

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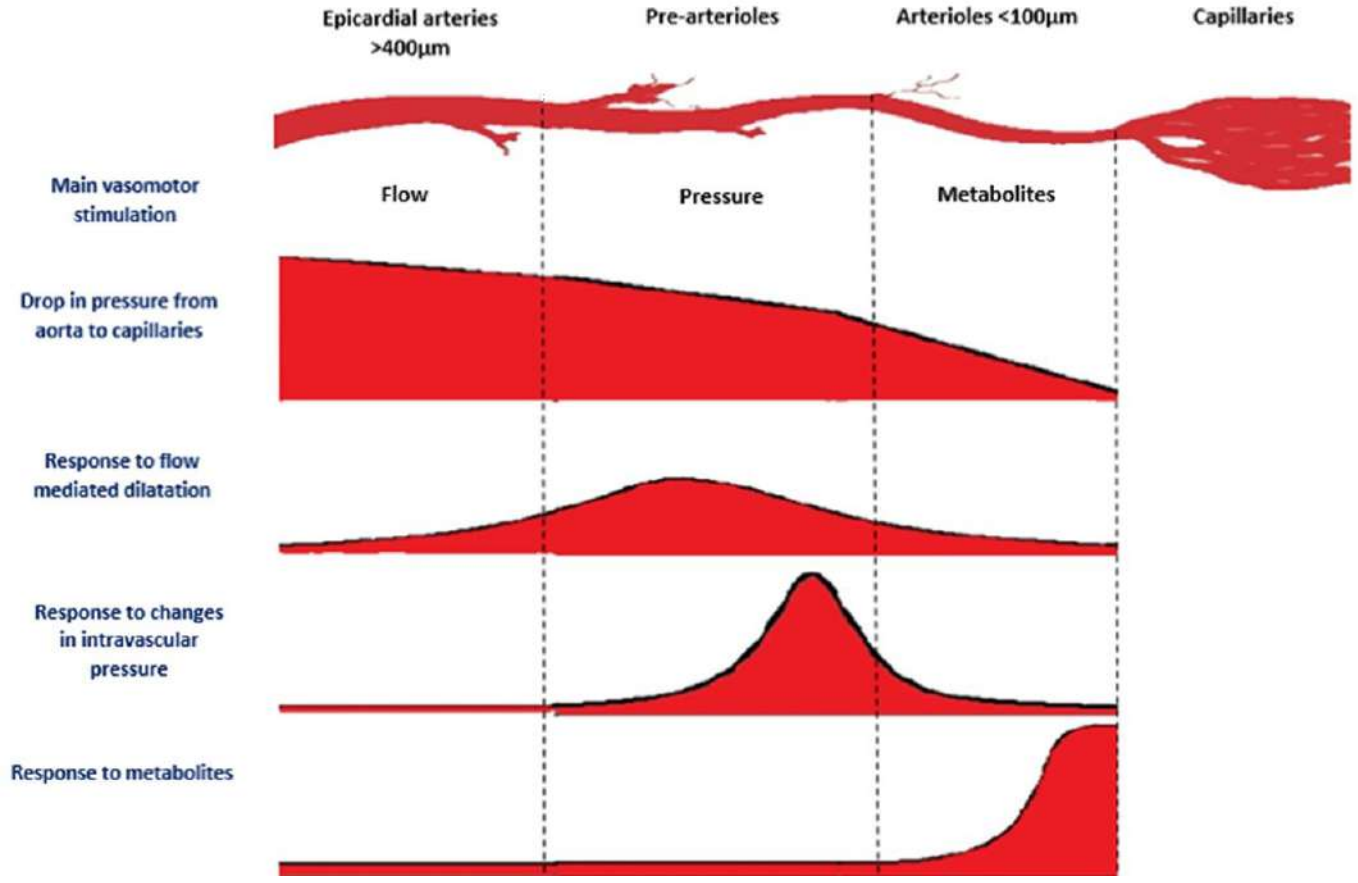

Figure 1 Functional anatomy of the coronary circulation. Adapted from De Bruyne *et al.w6*

hyperphosphataemia, abnormal bone mineral metabolism, elevated levels of hormones that regulate phosphate (parathyroid hormone and fibroblast growth factor-23 (FGF-23)), oxidative stress and chronic low-grade inflammation have all been implicated in the development of myocardial hypertrophy, fibrosis and increased cardiovascular mortality.[2]

A common consequence of these disparate mediators may be the development of pathological changes in the coronary microcirculation, a phenomenon that is evident in other myocardial disease states,w6 and requires further investigation.

THE CORONARY MICROCIRCULATION AND MYOCARDIAL DISEASE

Chilian proposed an elegant model of the coronary circulation consisting of three anatomically distinct but functionally interlinked compartments (figure 1).w7

The proximal compartment consists of large epicardial coronary arteries that function as capacitance vessels and respond to shear forces by endothelial mediated dilatation. The middle compartment consists of pre-arterioles that are characterised by a measurable pressure drop along their length. The distal compartment consists of the intramural arterioles that have diameters <100 µm, have high resting tone and are responsible for the majority of coronary vascular resistance.[9] They dilate in response to changes in myocardial oxygen consumption. Vasoactive mediators such as adenosine and hydrogen peroxide act directly on these vessels to produce vasodilatation.w8 Endothelium-dependent mechanisms involving nitric oxide and endothelium derived relaxing factors are also important, with animal studies showing attenuated vasodilatation of the coronary microvasculature when nitric oxide synthesis is inhibited.w8 Finally, the capillary bed delivers oxygen and substrates to the myocytes. Thus, the coronary circulation matches myocardial oxygen demand with supply via a complex interplay between myogenic tone, metabolic signals, circulating hormones and the intrinsic properties of the endothelium.w7

Abnormalities of all of these coronary vessels are seen in uraemia with atherosclerosis and medial thickening and calcification of the epicardial vessels, and medial hypertrophy and a reduction in the cross-sectional surface area of the pre-arterioles.[5] Myocyte–capillary mismatch and reduced LV capillary density have also been demonstrated in uraemic hearts in both animal models and postmortem human studies.w9 w10

Abnormalities of coronary microvascular function are evident in myocardial disease states such as HCM and heart failure with preserved ejection fraction (HFpEF) that, like uraemic cardiomyopathy, are characterised by hypertrophy and fibrosis. In HCM, studies using positron emission tomography (PET) have documented impaired microvascular function.w11 This predicts clinical consequences including reduced LV systolic function, adverse ventricular remodelling, ventricular arrhythmias, clinical heart failure and cardiovascular death.[9]w11 Similarly in HFpEF, coronary microvascular dysfunction (CMD) is common with a recent multicentre study identifying CMD in 75% of patients. This was associated with kidney damage, as measured by albuminuria, as well as a higher N-terminal pro-brain natriuretic peptide and systemic arterial dysfunction.w6

Although not fully understood, a paradigm is emerging which holds that risk factors such as obesity, hypertension, hyperglycaemia and we suggest kidney dysfunction cause CMD, probably as a result of inflammation and oxidative stress. The consequent

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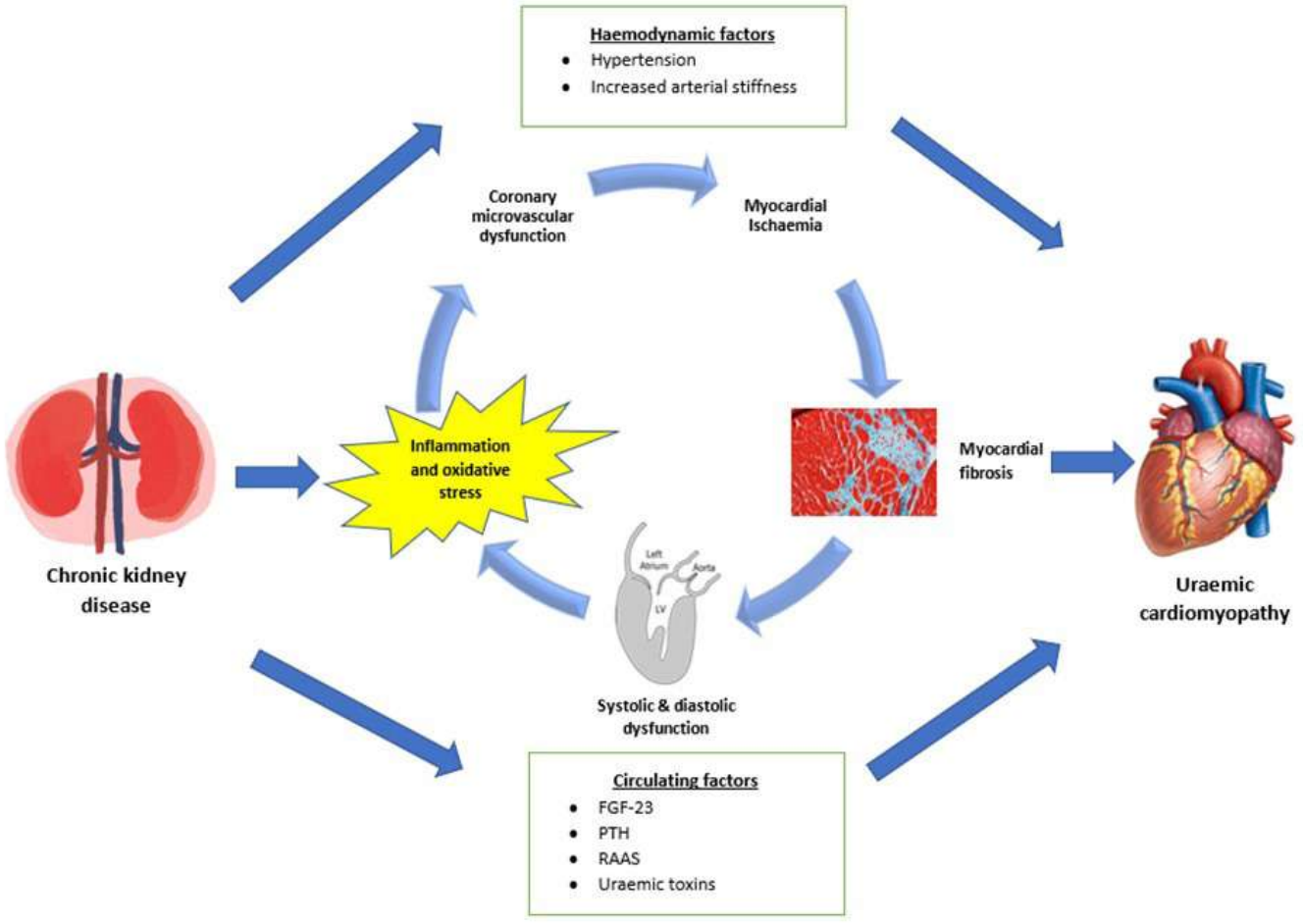

Figure 2 Proposed mechanism of uraemic cardiomyopathy. FGF-23, fibroblast growth factor-23; PTH, parathyroid hormone; RAAS, renin-angiotensin-aldosterone system.

failure to match myocardial blood flow (MBF) with demand results in widespread ischaemia, DIF, ventricular remodelling and systolic and diastolic dysfunction.w12 In CKD, the effect is likely to be exacerbated by hypertension, increased arterial stiffness and humoral factors such as FGF-23 and aldosterone leading to the clinical syndrome of uraemic cardiomyopathy.[5] It is not clear if CMD is the cause or consequence of myocardial disease in uraemic cardiomyopathy. However, it is plausible that the relationship between myocardial fibrosis and CMD is reciprocal and a vicious circle is initiated in which both factors exacerbate each other causing progressive ischaemia and myocardial dysfunction leading to heart failure, arrhythmia and death (figure 2).w12

METHODS OF ASSESSING CORONARY MICROVASCULAR FUNCTION

The coronary microcirculation cannot be directly visualised in vivo. All assessments depend on indirect measures of microvascular function. Coronary flow reserve (CFR) is the most widely reported parameter and has been measured using many different techniques (figure 3), which are summarised below and in table 1. To calculate CFR, hyperaemia is induced, usually with a pharmacological vasodilator, and CFR is measured as the ratio of maximal hyperaemic to resting flow. Adenosine is the most commonly used agent, as it is safe with a rapid onset and offset of action.w13 In normal subjects, coronary flow can increase up to fivefold and should at least double with hyperaemic stimuli. Thus, a CFR <2 is considered abnormal.w13 w14 CFR reflects both epicardial coronary artery disease as well as microvascular function. Therefore, exclusion of significant coronary artery disease is required before reduced CFR can be attributed to CMD.[9] This is often difficult without angiography and is a limitation of many studies. A diagnostic algorithm for CMD in uraemic cardiomyopathy is suggested in figure 4.

Invasive coronary angiography

CFR can be assessed during invasive coronary angiography. Two different methods exist but both expose patients to infrequent but significant risks including vascular injury, contrast nephropathy and death.

Doppler guidewire

An angioplasty wire tipped with a high frequency piezoelectric Doppler transducer can be used to measure flow velocities in a coronary artery at rest and at hyperaemia. CFR is calculated as the ratio of hyperaemic/resting flow.w14

Intracoronary thermodilution

CFR can be assessed via thermodilution. A pressure wire is positioned in the distal third of a target vessel. The shaft of the pressure wire acts as a proximal thermistor while the sensor at its tip acts as a distal thermistor. Normal saline at

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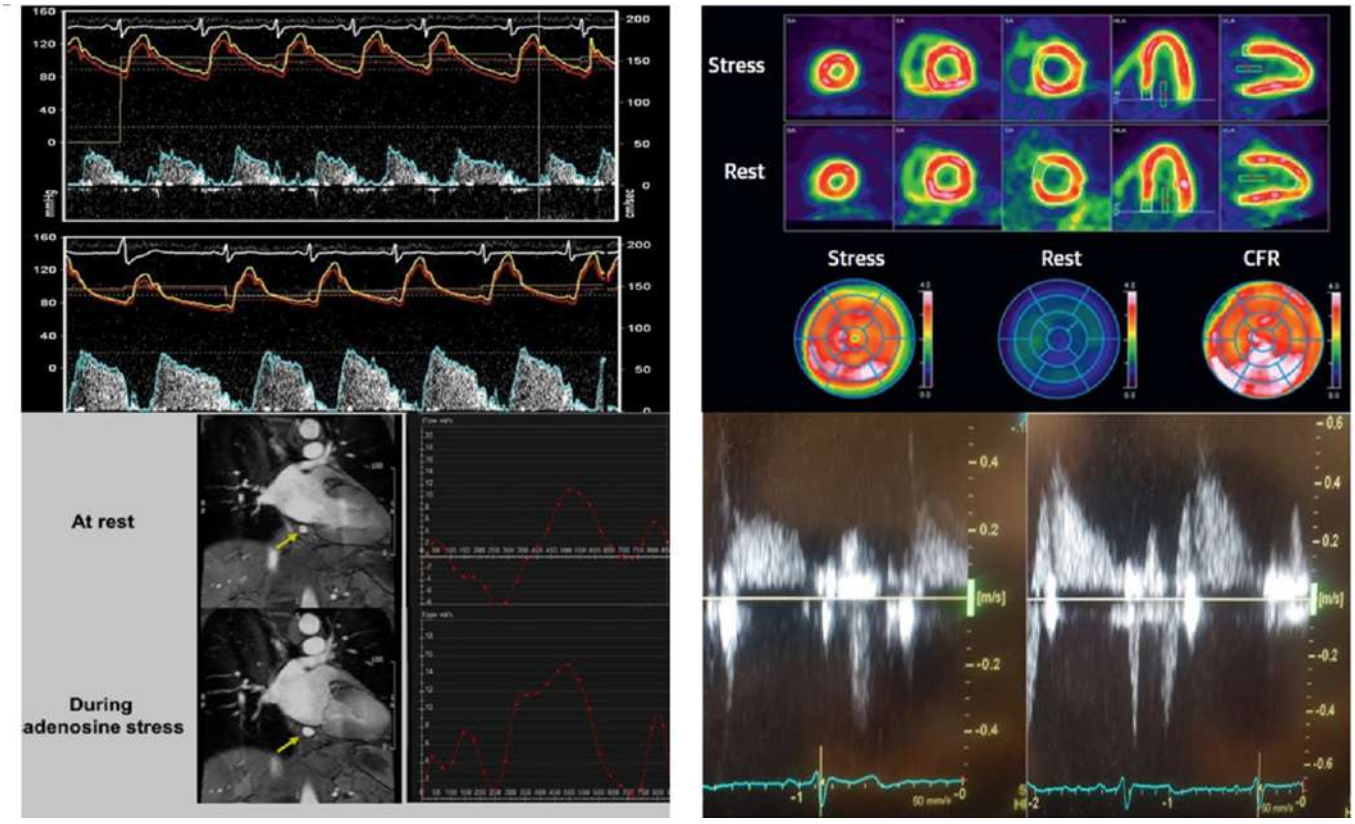

Figure 3 Different methods of assessing CFR: intracoronary Doppler angiography (top right) showing CFR of 1.8 in a patient with coronary artery disease, PET (top left), MRI coronary sinus flow (bottom left) and Doppler transthoracic echocardiogram (bottom right) showing CFR of 2.12 in a patient with chronic kidney disease stage 4. Adapted from Amier *et al*, w16 Feher *et al w17* and Nakamori *et al*.w18 CFR, coronary flow reserve; PET, positron emission tomography.

room temperature is injected down the coronary artery and its transit time is measured by thermodilution. CFR is the ratio of hyperaemic transit time/baseline transit time. This technique correlates well with Doppler flow derived CFR.w15

Positron emission tomography

The non-invasive 'gold-standard' method of assessing CFR is quantitative PET. Absolute values of MBF at rest and during hyperaemia can be calculated. Advantages of PET include

Table 1 Summary of advantages and disadvantages of different modalities used to assess coronary flow reserve

Modality	Advantages	Disadvantages
Invasive angiography (Doppler and thermodilution)	1. Definitive exclusion of epicardial coronary artery disease 2. Widely available	1. Invasive procedure 2. Ionising radiation
Positron emission tomography	1. Non-invasive 2. Can assess myocardial ischaemia and scar 3. Allows calculation of regional and global myocardial blood flow	1. Ionising radiation 2. Not widely available in UK
Coronary sinus flow	1. Non-invasive 2. Sequences and analysis are quick to perform	1. Contraindications to MRI limit its widespread use
First pass perfusion	1. Non-invasive 2. Can assess myocardial ischaemia and scar 3. Myocardial viability can be ascertained	1. Requires gadolinium limiting its utility in chronic kidney disease 2. Scan sequences can be lengthy to perform and analyse 3. Contraindications to MRI limit its widespread use
Stress T1 mapping	1. Non-invasive 2. Provides additional myocardial tissue characterisation	1. Contraindications to MRI limit its widespread use 2. Not well validated
Doppler transthoracic echo	1. Non-invasive 2. Cheap 3. Portable	1. Only assesses left anterior descending artery territory
Myocardial contrast echo	1. Non-invasive 2. Cheap 3. Portable 4. Allows calculation of regional and global myocardial blood flow	1. Requires good acoustic windows

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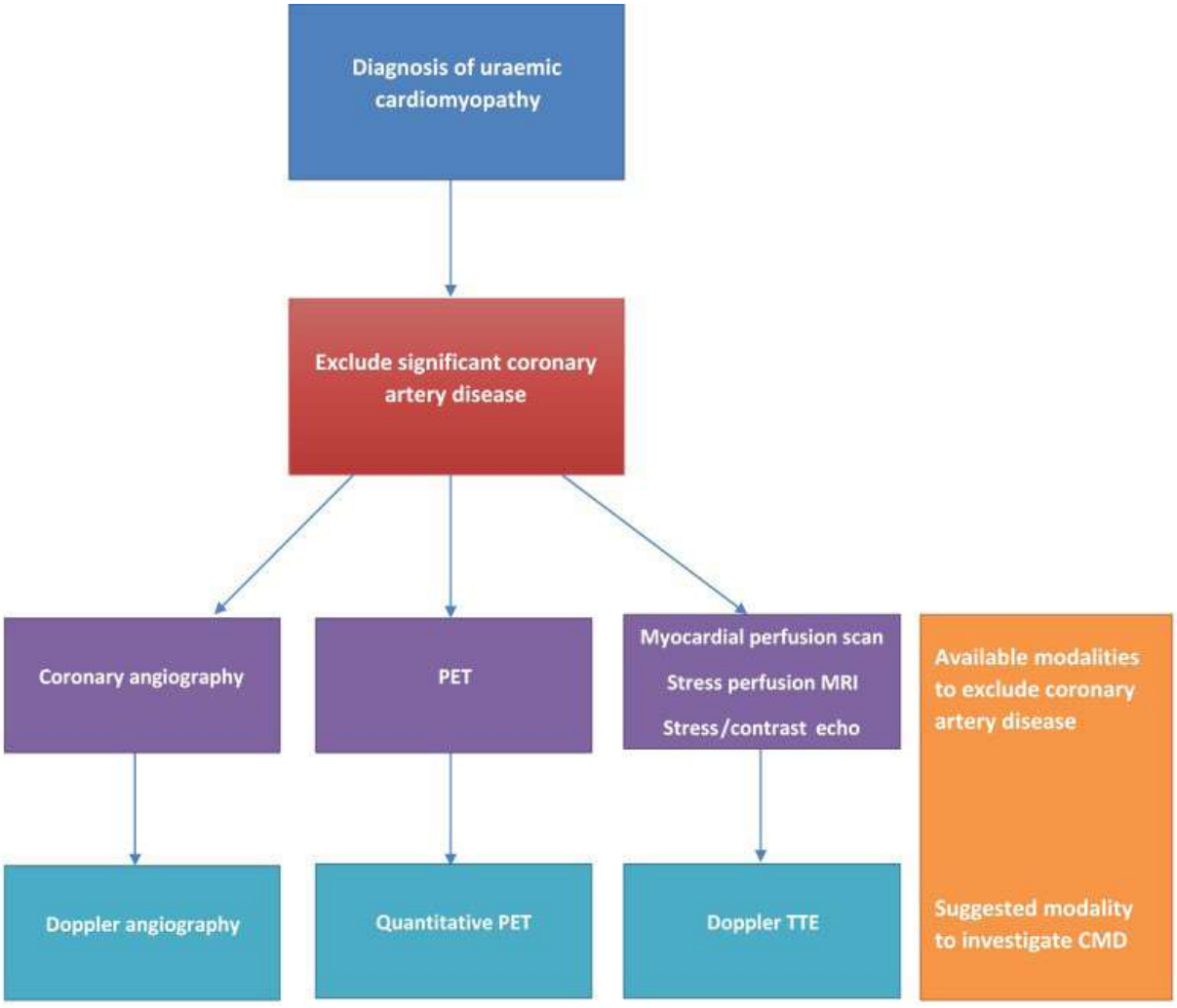

Figure 4 Proposed diagnostic algorithm for coronary microvascular dysfunction (CMD) in uraemic cardiomyopathy. PET, positron emission tomography; TTE, transthoracic echocardiography.

its ability to assess regional blood flow, myocardial scar and myocardial ischaemia as well as CFR.[10] Disadvantages include exposure to ionising radiation, high cost, difficulties in accessing radio-isotopes and the relative unavailability of the technique.

MRI

MRI is emerging as a useful tool for the non-invasive assessment of CFR, although it remains less validated than other imaging modalities. Methods include:

Coronary sinus flow

The majority of blood from epicardial ventricular veins drains into the coronary sinus, which can be visualised on MRI using velocity encoded cine sequences. CFR is the ratio of blood in the coronary sinus after hyperaemia compared with baseline.w16

First pass perfusion

Myocardial perfusion is recorded in dedicated basal, mid-ventricular and apical short axis slices at rest and during stress. The ratio of the maximal up-slopes of signal intensity during vasodilatation over resting condition is defined as myocardial perfusion reserve.[10] Perfusion defects can be identified to help localise coronary artery lesions and assessments of viability can be made using late gadolinium enhancement. However, the need for gadolinium limits its utility in CKD.

Stress T1 mapping

T1 relaxation times of tissues are prolonged by increased water content. Coronary vasodilatation, by increased myocardial blood volume, would be expected to prolong T1 times.w17 Using this principle, measurement of rest and stress T1 times provide an indirect indication of increased MBF and myocardial perfusion reserve.w17

Doppler transthoracic echocardiography

CFR can be measured using Doppler transthoracic echocardiography (TTE) and correlates well with invasive Doppler and PET.w6 w14 The mid to distal left anterior descending artery (LAD) can be identified in a modified apical two-chamber view using a high frequency transducer and appropriate machine settings to identify low velocity flow. Pulse wave Doppler signals can be measured in the LAD at rest and during hyperaemia to calculate CFR.w14 This technique is feasible in most patients, including those who are obese, as it is less reliant on good acoustic windows due to the superficial location of the LAD.w6

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Table 2 Summary of studies on coronary microvascular dysfunction in chronic kidney disease (CKD)

Study	Year	Country	Population	Modality	Findings
Ragosta *et al*[18]	2004	USA	Controls (n=32) Patients with diabetes with no kidney disease (n=11) Patients with diabetic nephropathy (n=21)	Doppler angiography	Significantly lower CFR in patients with diabetic nephropathy compared with other two groups.
Tok *et al*[25]	2005	Turkey	Controls (n=14) Patients on HD (n=10)	Doppler TTE	Significantly lower CFR in HD patients.
Chade *et al*[17]	2006	USA	GFR >60 mL/min (n=481) GFR <60 mL/min (n=124)	Doppler angiography	Non-significant trend towards reduced CFR as eGFR falls.
Viganò *et al*[26]	2007	Italy	Controls (n=17) Renal transplant recipients (n=25)	Doppler TTE	CFR impaired in half of cases.
Niizuma *et al*[24]	2008	Japan	Controls (n=20) Patients on HD (n=21)	Doppler TTE	Significantly lower CFR in HD patients.
Caliskan *et al*[12]	2008	Turkey	Controls (n=39) HD (n=48) Renal transplant recipients (n=27)	Doppler TTE	Significantly lower CFR in ESRD and in renal transplant recipients. Lower CFR in ESRD than renal transplant recipients.
Bezante *et al*[11]	2009	Italy	Patients with hypertension and normal renal function (n=64) Patients with hypertension and renal impairment (n=12)	Doppler TTE	Significantly lower CFR in patients with hypertension and renal impairment.
Koivuviita *et al*[20]	2009	Finland	Controls (n=10) CKD stages 3–5 (n=22)	PET	Non-significant trend towards reduced CFR as eGFR falls.
Turiel *et al*[27]	2009	Italy	Controls (n=25) Renal transplant recipients (n=25)	Doppler TTE	Significantly lower CFR in renal transplant recipients compared with controls.
Bozbas *et al*[13]	2009	Turkey	Controls (n=26) ESRD (n=30) Renal transplant recipients (n=30)	Doppler TTE	Significantly lower CFR in ESRD and in renal transplant recipients. Lower CFR in ESRD than renal transplant recipients.
Charytan *et al*[21]	2010	USA	CKD stages 1–3 (n=435)	PET	Non-significant trend towards reduced CFR as eGFR falls
Akagun *et al*[28]	2011	Turkey	Renal transplant recipients (n=20)	Doppler TTE	CFR <2 in 65%
Murthy *et al*[29]	2012	USA	eGFR <60 mL/min (n=866)	PET	CFR <1.5 associated with increased risk of cardiac mortality.
Imamura *et al*[23]	2014	Japan	Controls (n=15) CKD stages 1–5 (n=175)	Doppler TTE	Significant decrease in CFR as eGFR falls. Incremental reduction in CFR with albuminuria.
Shah *et al*[14]	2016	USA	Dialysis-dependent patients (n=168)	PET	CFR <1.5 associated with increased risk of cardiac mortality.
Nakanishi *et al*[15]	2013	Japan	eGFR <60 mL/min (n=139)	Doppler TTE	CFR <2 associated with worse cardiovascular outcomes.
Tona *et al*[30]	2016	Italy	Simultaneous kidney pancreas transplant recipients (n=48)	Doppler TTE	Lower CFR associated with worse cardiovascular outcomes.
Paz *et al*[16]	2017	USA	ESRD awaiting transplant (n=131)	PET	CFR <2 in 58.8% of patients with ESRD.
Charytan *et al*[22]	2018	USA	Controls (n=198) CKD stages 1–5 (n=3748)	PET	Significant decrease in CFR as CKD stage increases.
Nelson *et al*[19]	2019	USA	Controls (n=15) ESRD (n=15)	Doppler angiography	Significantly reduced CFR in ESRD compared with controls.

CFR, Coronary flow reserve; eGFR, estimated glomerular filtration rate; ESRD, end-stage renal disease; HD, haemodialysis; PET, positron emission tomography; TTE, transthoracic echocardiography.

Myocardial contrast echo

Myocardial contrast echocardiography uses protein microbubbles that have a lower diameter than the red blood cell, resist arterial pressure and remain intravascular in the intact circulation. These qualities enable direct quantification of microvascular perfusion and allow absolute MBF as well as CFR to be calculated.[10]

CMD IN CKD: THE EVIDENCE SO FAR

A structured PubMed database search was carried out for articles between 1966 and 2019 using the keywords 'coronary flow reserve', 'myocardial perfusion reserve' or 'coronary microvascular dysfunction' combined with 'chronic kidney disease', 'end-stage kidney disease', 'end-stage renal disease' or 'uraemic cardiomyopathy'. A total of 396 articles were identified. After removal of duplicates, 20 studies were considered relevant to this topic. Included studies are summarised in table 2.

There are limited conflicting data on coronary microvascular function in CKD. CMD appears common with prevalence rates of 24%–90%.[11–16] The largest angiographic study examined CFR in 605 patients stratified as normal or reduced kidney function, using an arbitrary cut-off eGFR of 60 mL/min. Crude analysis indicated a reduced CFR in patients with CKD but this was not statistically significant after correction for age, gender and comorbidities including hypertension and diabetes.[17] By contrast, smaller angiographic studies have shown a significant decrease in CFR in patients with diabetic nephropathy and in ESRD, compared with healthy controls.[18 19]

Using PET, a study of 10 controls and 22 patients with CKD stages 3–5 showed a trend towards reduced CFR with increasing stage of CKD, but this did not reach statistical significance.[20] Similarly, retrospective calculation of CFR using PET in 435 patients with stages 1–3 CKD also found there was no significant difference in CFR after correction for cardiovascular risk factors

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such as age, sex and blood pressure.[21] The largest PET study to date is a retrospective analysis of 3946 patients who underwent stress PET at a single US institution. This study demonstrated a significant decrease in CFR as renal function declined, with the largest drop being in patients with CKD stage 4 and no significant further drop in stage 5 or those on dialysis.[22] In patients undergoing cardiovascular assessment for renal transplant by dipyridamole PET perfusion imaging, 59% of patients had a CFR <2 and even in those patients without any feature of infarction or ischaemia, 63% had abnormal CFR.[16]

Several studies have been performed using TTE. The relationship between albuminuria and CMD was investigated in a prospective Japanese study of 175 patients with CKD. Significant reductions in CFR with increasing stages of CKD were evident and patients with albuminuria, had significantly lower CFR at each stage of CKD.[23] In patients with essential hypertension, the presence of CKD was associated with a 10-fold increase in the risk of CMD.[11] Small studies of dialysis patients and controls have also shown reduced CFR measured by TTE in patients on haemodialysis compared with controls.[24 25]

There has been limited investigation into the effects of kidney transplantation on CFR. As kidney function is partially restored, one would expect an improvement in coronary microvascular function after kidney transplantation. This is suggested in cross-sectional data showing that CFR may be higher in transplant recipients compared with patients with ESRD.[12 13] Possible explanations are that some of the microvascular changes seen in ESRD are reversible or alternatively that repeated haemodialysis causes microvascular dysfunction. Despite this, rates of CMD remain high after kidney transplant, with 8%–65% of renal transplant recipients having a CFR <2.[26–28]

The mechanisms of CMD in CKD are not fully understood. Patients with CKD demonstrate increased resting coronary flow but an impaired response to vasodilator stimuli, leading to reduced CFR.[11 14 18] The reduced response to adenosine and other vasodilatory stimuli is likely to be due to factors such as reduced cross-sectional area of the microcirculation, increased coronary sympathetic arteriolar tone, endothelial dysfunction and a reduced smooth muscle response which may be due to defects at both receptor and post receptor levels.w9 w18 w19 Impaired myocardial vascular reserve and MBF autoregulation has been demonstrated in animal models of CKD.w20

THE PROGNOSTIC ROLE OF REDUCED CFR IN CKD

Several studies have investigated the relationship between reduced CFR and prognosis in CKD. These are mainly retrospective and included patients with comorbidities such as diabetes and hypertension that are known to affect CFR. In a retrospective analysis of 866 patients with moderate to severe CKD who underwent stress PET, even after adjustment for clinical risk factors, LV systolic function, extent of ischaemia and scar, a CFR below the median (<1.5) was associated with a 2.1-fold increased risk of cardiovascular mortality.[29] The same investigators also retrospectively examined a cohort of 186 patients with dialysis dependent ESRD and again found that CFR below the median (1.4) was associated with a significant increased risk of all-cause and cardiovascular mortality over a median follow-up period of 3 years. Log transformed CFR was independently associated with all-cause and cardiovascular mortality.[14] A prospective study using Doppler TTE assessed 139 patients with CKD, and identified that patients with CFR <2 had significantly higher rates of cardiac events and all-cause mortality even after adjustment for cardiovascular risk factors.[15]

There is very limited information on the role of CFR in predicting prognosis in renal transplant recipients. Data are conflicting with a small Turkish study (n=20) showing no prognostic role for CFR measurement in kidney transplant patients.[28] By contrast, an Italian study of 48 patients who had undergone simultaneous kidney/pancreas transplant showed that a CFR <2 was associated with increased risk of major adverse cardiovascular events.[30] However, it is difficult to draw any meaningful conclusions from these studies given the small numbers involved.

CONCLUSIONS

CMD provides a plausible mechanism by which factors associated with impaired kidney function, including oxidative stress and inflammation, might result in myocardial damage and dysfunction leading to the syndrome of uraemic cardiomyopathy. Current data on CMD in uraemic cardiomyopathy are limited and conflicting, hampered by the retrospective design of most studies. Consequently, there is a need for well-designed prospective studies of CMD in CKD, to identify whether CMD might be a key mediator in the development of uraemic cardiomyopathy. Future studies will need to investigate the utility of strategies to prevent or reduce CMD and thus fibrosis and ventricular dysfunction in CKD. Possible agents that may be effective in in this regard might include new biological agents acting on inflammatory cytokines, antioxidants and, further down the pathway, specific antifibrotic drugs. Given the prevalence of CKD in the general population, a better understanding of the mechanisms behind uraemic cardiomyopathy is a vital step towards improving the significant cardiovascular morbidity and mortality seen in CKD.

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APPENDIX III: LIST OF OTHER PUBLICATIONS RELATED TO THIS WORK

- Pickup LC, Law JP, **Radhakrishnan A**, Price AM, Loutradis C, Smith TO, Edwards NC, Steeds RP, 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;8:2045-2057.

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APPENDIX IV: CRIB-FLOW STUDY PROTOCOL

Coronary Microvascular Dysfunction in Chronic Kidney Disease: The Chronic Renal Impairment in Birmingham Coronary Flow Reserve (CRIB-FLOW) study

CRIB-FLOW trial protocol

Version 8

5th June 2019

Principal Investigator: Professor Jonathan Townend

1: Background

Cardiovascular risk in chronic kidney disease

It is well established that patients with chronic kidney disease (CKD) have increased cardiovascular morbidity and mortality. There is a linear graded inverse relationship between glomerular filtration rate (GFR) and cardiovascular risk, that is independent of age, sex and other risk factors.[1] Although patients with CKD do have increased rates of coronary artery disease compared to the general population, this is not the main driver of their increased cardiovascular risk. Data from the United States Renal Data System indicates that cardiovascular disease accounts for 40% of the mortality in end stage renal disease (ESRD). Of this cardiovascular mortality, myocardial infarction only accounts for a small proportion (approximately 5%).[2] Patients with CKD are at increased risk of heart failure, arrhythmia and sudden death, which cannot be explained purely by their increased incidence of coronary artery disease.

Coronary microvascular dysfunction

There is increasing understanding that abnormalities of the microvasculature play an important role in a variety of different conditions. The normal function of the coronary microvasculature is a complex interplay between the vasculature, the endothelium and vasoactive mediators such as nitric oxide and prostaglandins.[3] Camici et al propose an elegant model of the coronary arterial circulation as consisting of 3 interlinked compartments.[4] The proximal compartment consists of the large epicardial coronary vessels that function as capacitance vessels and respond to shearing forces by endothelial mediated dilatation. The middle compartment consists of the pre-arterioles that are involved in the auto-regulation of coronary blood flow. The distal compartment consists of the intramural arterioles that supply the myocardium. These distal vessels are responsible for the majority of the vascular resistance in the coronary supply and are most sensitive to vasoactive mediators.[3] There is evidence from pathological specimens that disease states such hypertension can alter the structure of these arterioles with medial hypertrophy and a reduction in the cross sectional surface area, leading to increased vascular resistance which may contribute to microvascular dysfunction.[5] This has also been shown in uraemic hearts with both animal models and post mortem studies demonstrating reduced left ventricular capillary density.[6,7]

Coronary microvascular dysfunction (CMD) has been studied in many different populations including stable angina, hypertrophic cardiomyopathy, heart failure with preserved ejection fraction, diabetes and aortic stenosis.[8] These conditions share features with CKD including left ventricular hypertrophy, myocardial fibrosis and diastolic dysfunction and so may explain the underlying pathophysiology behind the condition in CKD. Across conditions, reduced CFR is associated with worse prognosis, although the exact mechanism of this increased mortality is not clear.[8]

Assessing coronary microvascular function

There is currently no method to directly visualise the coronary microcirculation. Therefore, all assessments depend on indirect measures of microvascular function. Coronary flow reserve (CFR) is one such measure. There are many methods of calculating CFR, all of which have advantages and disadvantages. A common principle in all techniques is the use of a pharmacological vasodilator to induce hyperaemia and the comparison of blood flow at rest and at hyperaemia to calculate CFR. In the absence of obstructive epicardial coronary artery disease, reduced CFR signifies impaired microvascular function.

The gold standard method of assessing CFR is with intravascular Doppler ultrasound at the time of invasive coronary angiography. However, this is an invasive procedure with its inherent risks. Furthermore, coronary angiography is associated with a risk of contrast nephropathy and worsening renal function, which precludes its role for research purposes in patients with CKD.

Doppler Transthoracic echo (TTE)

Doppler TTE is a well-established, non-invasive and cheap technique for the assessment of CFR. With this technique, blood flow velocity in the left anterior descending artery (LAD)is assessed using pulse wave doppler. The mid to distal LAD can be identified in a modified apical 2 chamber view. CFR is determined by the ratio of hyperaemic to basal peak coronary flow.

Advantages of TTE are its widespread availability, low cost and lack of ionising radiation. No special patient preparation is required. It has been shown to correlate extremely well with both invasive doppler and PET, which is the non-invasive gold standard of measuring CFR.[9,10] Disadvantages are the reliance on good acoustic windows. However, in a recent large multicentre trial of patients with HEFpEF, CFR was successfully measured by TTE in 87% of patients.[11]

Coronary flow reserve in CKD

There are conflicting data on the effect of CKD on CFR. Studies of CFR in patients with CKD are complicated due to the heterogeneity of populations in different studies and the high level of confounding comorbidities such as hypertension and diabetes that also affect microvascular function.

Charytan et al investigated coronary flow reserve using PET in 435 patients with stage 1-3 CKD and found there was no significant difference in coronary flow reserve after correction for risk factors such as age, sex and bp although there was trend towards reduced peak myocardial blood flow as renal function declined. [12] Chade et al used coronary angiography and intravascular Doppler on 605 patients with normal or mild coronary artery disease, stratified based on normal or reduced kidney function, using eGFR of 60ml/min as a cut off. There was reduced CFR in patients with reduced renal function. However after correction for age, gender and comorbidities including hypertension and diabetes, this was not statistically significant. [13]

By contrast, a large retrospective study of 3946 patients undergoing stress PET demonstrated a significant decrease in CFR as renal function declined, with the largest drop being in patients with CKD stage 4 and no significant further drop in stage 5 or those on dialysis.[14] Imamura et al prospectively studied patients with CKD using TTE and found significant reductions in CFR with increasing stages of CKD. Patients with albuminuria, implying worse renal impairment, had significantly lower CFR at each stage of CKD.[15] A small Japanese study of dialysis patients and controls with no obstructive LAD disease also measured CFR using TTE. Patients on haemodialysis had reduced CFR compared to controls.[16] However, patients in this study had significant lesions in other coronary arteries, so their CFR did not reflect purely microvascular function.[16] A small Turkish study measured CFR using TTE in healthy controls, patients with end stage renal disease (ESRD), and patients with renal transplant. Patients with ESRD and renal transplant had significantly lower CFR than healthy controls. Interestingly, patients with renal transplant had higher CFR than ESRD,[17] suggesting that some of the microvascular changes seen in ESRD may be reversible. These findings were also replicated by Caliskan et al.[18] To date, there are no prospective longitudinal studies that measure CFR in patients before and after kidney transplant. Similarly, kidney donors are a group of patients with reduced renal function who have not specifically been studied in the literature.

It is often difficult to distinguish between coronary microvascular dysfunction purely due to CKD and the effect of contributing comorbidities such as diabetes and hypertension. Bezante et al demonstrated that in patients with essential hypertension, having impaired renal function was associated with a 10 fold increase in the risk of having a CFR <2.[19] Similarly an angiographic doppler study by Ragosta et al demonstrated that patients with diabetic nephropathy had significantly lower CFR than matched patients with diabetes and no renal disease.[20] Thus the reduced CFR in patients with impaired renal function in these studies may simply reflect more aggressive primary disease.

Prognostic role of CFR in CKD

Several studies have assessed the link between reduced CFR and prognosis in CKD. These are mainly retrospective and included patients with comorbidities that are known to affect CFR. Murthy et al carried out a retrospective analysis of 866 patients with moderate to severe renal impairment who underwent stress PET. Even after adjustment for clinical risk factors, left ventricular systolic function, extent of ischaemia and scar and stress induced LVEF augmentation, CFR <1.5 was a significant predictor of mortality.[21] Shah et al also retrospectively examined a cohort of 186 patients with dialysis dependent ESRD and found that CFR <1.5 was again associated with a significant increase risk of cardiac mortality.[22] To date, the only prospective study assessing the prognostic impact of reduced CFR in CKD has been carried out by Nakanishi et al. This study involved 139 patients with CKD and assessed CFR using TTE. Mean eGFR in the study was 46ml/min. Patients with CFR<2 had significantly higher rates of cardiac events and all-cause mortality. The increased risk of cardiac events in patients with CFR<2 remained significant even after correction for cardiovascular risk factors.[23]

Only small studies have been carried out in renal transplant patients regarding the role of CFR in predicting prognosis. Data are conflicting with a small Turkish study showing no prognostic role for CFR measurement in kidney transplant patients.[24] However, an Italian study of patients who had undergone simultaneous kidney/pancreas transplant showed that a CFR <2 was associated with increased risk of major adverse cardiovascular events.[25]

Rationale for the study

CFR assessment using TTE and myocardial contrast echo are well described in the literature but are not widely practiced in the UK. Initial funding was secured to study 30 patients in the pilot phase of this study with the aim of establishing these techniques in Birmingham and gaining pilot data on values of CFR in healthy controls and in patients with CKD. Further funding has now been obtained to carry out a larger cross-sectional study of CFR in Birmingham, to build on this initial data.

2: Trial design

This is a single centre cross sectional observational study of healthy controls, kidney donors and patients with chronic kidney disease.

3. Trial objectives

Hypotheses

1. Coronary flow reserve is impaired in kidney donors compared to controls
2. Coronary flow reserve is impaired in patients with CKD 5 compared to controls
3. There are differences in coronary flow reserve between pre-dialysis patients with CKD 5 and patients on peritoneal dialysis.

Primary endpoint

1. Difference in mean CFR (calculated by Doppler TTE) between groups

Secondary endpoints

1. Difference in myocardial blood flow
2. Difference in LV ejection fraction
3. Arterial stiffness
4. Markers of inflammation, myocardial stretch and injury

4. Selection of participants

Where possible, participants in this study will be recruited from 2 other ongoing British Heart Foundation funded studies already taking place at the Queen Elizabeth Hospital (CRIB DONOR II and RETRACT). The CRIB DONOR II study is a 5 year follow-up study of the original CRIB DONOR study that examined the cardiovascular effects of uni-nephrectomy by studying living kidney donors and matched controls using cardiac MRI.[26] The RETRACT study is a prospective longitudinal controlled study that will examine the cardiovascular impact of kidney transplantation on kidney transplant recipients, again using cardiac MRI. It started recruiting in December 2018. Participants from these two studies have been chosen as they will already undergo extensive cardiovascular phenotyping.

If unable to recruit from these 2 studies, then new controls, kidney donors and patients with CKD will be approached.

Participants who potentially fulfil the inclusion criteria for this trial will have their eligibility confirmed by a research fellow who will have access to and a full understanding of the potential participant's medical history. If eligibility has been assessed and documented by the research fellow, then the process of informed consent will take place.

Participants who potentially fulfil the inclusion criteria for this trial will have their eligibility confirmed by the research fellow who will have access to and a full understanding of the potential participant's medical history. If eligibility has been assessed and documented by the research fellow, then the process of informed consent will take place.

Inclusion criteria

- Age over 18
- Able to provide written informed consent
- Healthy control, kidney donor or CKD stage 5

Exclusion criteria

- Pregnancy
- Diabetes mellitus
- Uncontrolled hypertension
- Evidence of 2nd or 3rd degree AV block or sick sinus syndrome in absence of a pacemaker
- History of allergic/adverse reaction to adenosine or Sonovue
- History of long QT syndrome
- Severe hypotension
- Significant valvular heart disease
- Significant COPD or asthma with bronchospasm
- Unstable angina not controlled with medication
- Concurrent use of dipyridamole

- Decompensated heart failure
- Poor echo acoustic windows

5. Recruitment

Healthy controls and kidney donors will predominantly be recruited from patients enrolled in previous BCRG studies. Patients with CKD 5 will be predominantly recruited from patients enrolled in the RETRACT study or from renal clinics at the Queen Elizabeth Hospital, Birmingham.

All patients will initially be contacted in person at a clinic appointment or by telephone/email/post with a letter of invitation, patient information sheet (PIS), pre-paid envelope and reply slip. If a patient expresses interest in participating in the study, they will be invited to attend for a screening visit. If confirmed to be eligible and they wish to take part in the study, they will be asked to sign a written consent form.

Patients who have not replied after 7 days of receiving the patient information sheet will be contacted again by post, phone, email or directly in clinic.

6. Screening, Consent and Withdrawal

6.1 Screening

The renal team will inform patients in clinic that they may be approached regarding participation in the study. Participants in other BCRG studies will be asked for consent to be approached regarding this study. The research fellow will then approach potentially eligible participants in person or by telephone, email or post to inform them of the study. Participants who express an interest will be invited to take part.

The research fellow will ensure that the participant is eligible for the trial as per the inclusion and exclusion criteria. We will use information that is routinely collected as part of clinical care to ensure eligibility. If no recent UEs are available, then a sample will be taken at the study visit. It is not anticipated that any other specific additional investigations will be required as part of the screening process.

6.2 Consent

Informed consent will be taken after eligibility is confirmed. This will be carried out by the research fellow who has undergone Good Clinical Practice (GCP) training. The research fellow will explain that there is no obligation for a patient to enter the trial, and that they can withdraw at any time during the trial, without having to give a reason. A copy of the signed informed consent form will be given to the participant. The original signed form will be retained at the study site in the Investigator Site File and a copy placed in the medical notes. With the participants prior consent their General Practitioner (GP) will also be informed.

6.3 Withdrawal

Participants may withdraw at any time during the trial if they choose not to continue or if their clinical team feel that continued participation in the trial is inappropriate.

7. Study Visit

As part of the CRIB-FLOW study, participants will undergo the following assessments:

1. Clinical history and examination
2. Observations including blood pressure, height and weight.
3. Electrocardiogram to ensure no resting conduction disease.
4. Urine albumin creatinine ratio.
5. Arterial stiffness using Sphygmocor
6. Biomarkers: Blood will be assayed for markers of inflammation and myocardial stretch and injury. The assays will include hsCRP, IL-6, hsTroponin, NT-proBNP and ST2 (a member of the IL-1 receptor family and a novel biomarker of mechanical stress).[27]
7. Echocardiogram:
    a. Left ventricular volumes and ejection fraction will be measured using Simpson's biplane method. Diastolic function will be quantified using multiple parameters according to current guidelines.[28]
    b. Strain imaging will be performed using TDI and speckle tracking and analysed offline using proprietary software (Phillips Q lab).
    c. CFR assessment – The LAD will be identified using high resolution colour Doppler in the interventricular sulcus in a modified apical 2 chamber view. PW Doppler will be used to sample flow velocity signals at rest and during maximal hyperaemia using a standard adenosine protocol (140mcg/kg/min over 5-10 minutes). CFR will be calculated as the ratio of hyperaemic/resting velocity.
    d. Myocardial blood flow will be determined using very low mechanical index myocardial contrast echo. Using Philips Q lab, selected regions of interest in multiple myocardial segments will be chosen and results averaged. Background-subtracted plots of peak myocardial contrast intensity will be constructed, representing capillary blood volume vs. pulsing intervals, from which the slope of the replenishment curve depicting mean microbubble velocity will be derived. Resting and hyperaemic myocardial blood flow (MBF), assessed as (peak contrast intensity × myocardial blood velocity) will be derived and CFR (MBF at stress/MBF at rest) will then be calculated.[29]
    e. Absence of pharmacological stress induced regional wall motion abnormalities or perfusion defects will be used as a surrogate measure to exclude significant obstructive coronary artery disease.

Adenosine will be infused at rate of 140micrograms/kg/min for 3-5 minutes to produce a vasodilatory effect. 2 doctors with ALS training will be present at all times while adenosine is being infused.

After observation and once side effects have subsided, the patient will be allowed to depart.

8. Sample Size and Statistical Considerations

After kidney donation, all donors will have a GFR consistent with stage 2 CKD and in over a third of donors the final GFR is <60 ml/min putting them into the category of stage 3 CKD. Based on previous work by Imamura et al[15] [mean CFR for controls with normal renal function (3.8 ± 0.4), mean CFR for CKD stage 2 (3.2 ± 0.7), mean CFR for CKD stage 3 (3.0 ± 0.6)], we estimate that 25 patients in each group will provide 80% power with an alpha value of 0.05 to demonstrate a difference in mean CFR of 0.6 between donors and controls.

There are some data that patients on dialysis have higher CFR than pre-dialysis CKD 5.[14] However, there have been no studies looking specifically at PD patients. We aim to study 25 patients who are pre-dialysis CKD 5 and 25 patients who are on dialysis to look for any difference in CFR between the two groups.

In total we will seek to recruit 100 patients (25 controls, 25 kidney donors and 50 patients with CKD).

Data will be presented as means +/- standard deviation. CFR between groups will be compared using a one-way ANOVA. Non-parametric data will be log-transformed. Correlation between CFR and eGFR will be assessed using Pearson's correlation for parametric data or Spearman's correlation for non-parametric data.

9. Data management and quality assurance

Anonymised data will be stored for analysis. Data will be stored on trust computers which are password protected. Paper CRFs will be stored in a locked office which is accessible only by the study team. Data may occasionally be analysed on university computers but only anonymised data will be transferred out of trust premises.

The study will be deemed to be over when the last patient has undergone the study investigations.

10. Ethics and Regulatory Approval

The investigators will ensure that the study has appropriate approval from a Research Ethics Committee, the HRA as well as trust R&D approval prior to commencement of study. The protocol and all relevant study documents will be submitted for regulatory review prior to commencement of study.

11. Statement of Compliance

The CRIB FLOW study will be conducted in compliance with the approved protocol, EU GCP and the applicable regulatory requirements.

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APPENDIX V: RETRACT STUDY PROTOCOL

A Prospective Study of the Effects of Renal Transplantation on Uraemic Cardiomyopathy using Magnetic Resonance Imaging – (RETRACT study)

Abstract

Chronic kidney disease is associated with a high risk of death and morbidity due to cardiovascular disease. Much of this is caused by left ventricular disease characterized by hypertrophy and myocardial fibrosis. This process appears to start early in the course of CKD and causes heart failure and dangerous arrhythmias. Previous work suggests that the process may be reversible by kidney transplantation but almost all of the studies are small, not controlled, not rigorously designed and not analysed blindly. Furthermore, they almost all use echocardiography, which is known to be inaccurate in patients with CKD. We plan to perform the first large, prospective, controlled, blind-analysed study using cardiac magnetic resonance imaging to determine whether CKD associated cardiomyopathy is reversed by kidney transplantation and if so, whether factors such as blood pressure and mediators of metabolic bone disease/myocardial fibrosis are important in effecting this change. Greater understanding of the possible reversibility of CKD associated cardiomyopathy and insight into potential mechanisms will help to inform the design of future interventional studies. The ultimate aim would be to develop treatments aimed at reducing the very high cardiovascular mortality associated with CKD, especially in the majority of patients on dialysis that will never receive a kidney transplant.

Cardiovascular Disease in Chronic Kidney Disease

Chronic kidney disease (CKD) is a major but poorly recognized and under-treated risk factor for cardiovascular disease. There is a graded inverse relationship between cardiovascular risk and glomerular filtration rate (GFR), which is independent of other risk factors.[1,2] This risk is already present at minor levels of renal impairment with most studies showing an increased risk at a GFR below 60-90 ml/min/1.73m2.[3,4] The risk, however, is extreme in patients with end-stage renal disease (ESRD) requiring dialysis treatment.[5] In the UK, the one year risk of death for a dialysis patient is 19 times that of the general population at age 35-39 years.[6] Mortality of patients with ESRD is twice as high as the mortality of patients with congestive heart failure and four times the mortality of patients with diabetes mellitus.[5] In all studies to date, cardiovascular disease is the predominant cause accounting for over 50% of all deaths.[1,2,5,6] In ESRD most of this cardiovascular mortality is attributed to sudden cardiac death and heart failure rather than myocardial infarction.[3,5]

Although the relationship between CKD and cardiovascular risk is well established, the mechanisms are less clear. It is therefore, uncertain what measures should be implemented to reduce cardiovascular risk. With previous support from the BHF (PG97/162, PG02/153, PG04/109/17796) the Birmingham Cardio-Renal Group (http://www.birmingham.ac.uk/bcrg) have performed and published a series of studies which have clarified cardiovascular risk factors in CKD[7] examined the relationship between GFR and endothelial function,[8,9] demonstrated abnormalities of arterial and left ventricular (LV) function in early stage CKD[10] and shown conclusively that arterial stiffness and LV dysfunction can be improved by mineralocorticoid receptor antagonism.[11] Most recently, Dr William Moody in his BHF Clinical Research Fellowship (FS/11/17/28700) investigated the impact of uni-nephrectomy on cardiovascular structure and function in healthy kidney donors. Compared to controls, at 12 months after nephrectomy there was an increase in LV mass, reduced LV strain, reduced aortic

distensibility, increased hsCRP and a rise in the prevalence of detectable troponin and albuminuria but

no change in blood pressure.[12] These changes were accompanied by the activation of mediators of bone mineral disease such as parathyroid hormone (PTH) and fibroblast growth factor-23 (FGF23), both of which are potential disease mediators with adverse, hypertrophic and possibly fibrotic effects upon LV myocardial tissue.[13–15] We now seek to investigate this problem from the opposite point of view, namely to see whether or not established LV damage in CKD can be reversed, in this case by kidney transplantation. The ultimate challenge is to develop treatment to prevent or ameliorate the development of CKD associated cardiomyopathy (also known as uraemic cardiomyopathy) in patients with CKD at all stages, whether they are receiving a transplant or not.

The pathophysiology of myocardial and vascular disease in chronic kidney disease

Patients with CKD exhibit:

1. Increased LV mass with a high prevalence of LV hypertrophy (LVH). Criteria for LVH, often accompanied by systolic and diastolic dysfunction, are present in over 70% of patients with ESRD.[16,17]

2. Myocardial fibrosis has been shown to be very common in ESRD in post-mortem studies and this has recently been confirmed in 2 studies using T1 mapping cardiac magnetic resonance (CMR).[18,19] We have previously shown that it can be detected even in early stage CKD.[20]

3. Arterial wall thickening, stiffening and calcification (arteriosclerosis).[3,4]

4. Coronary and peripheral artery endothelial dysfunction and atherosclerosis.[21,22]

The causes of these pathologies are still unclear but may include hypertension, fluid and salt overload, oxidative stress, inflammation and activation of the renin angiotensin aldosteronesystem (RAAS).[3,4] More recently, evidence has accumulated to suggest that phosphaturic hormones such as PTH and FGF23 exert powerful effects upon the myocardium leading to LVH and fibrosis.[15] In a large CKD cohort, elevated FGF23 levels were independently associated with LVH.[23] In isolated rat myocytes, FGF23 caused pathological hypertrophy via activation of the calcineurin-NFAT signalling pathway, an effect that was independent of Klotho, the co-receptor for FGF23 in the kidney and parathyroid glands.[15] Chronically elevated FGF23 levels have been postulated to contribute directly to high rates of LVH and mortality in individuals with CKD.[24,25] Many of these changes may also lead to hypertrophy and fibrosis of the arterial media including the aorta, which together with calcification due to disordered calcium phosphate metabolism, leads to arterial 'stiffening'.[3,4][3, 4] In addition, endothelial dysfunction occurs early in the course of CKD leading to both atherosclerosis and also promoting arterial stiffening.[22]

Cardiovascular disease in kidney transplant recipients

Successful kidney transplantation is associated with improved survival, improved quality of life and healthcare cost savings compared to dialysis.[26,27] However, despite recipients having undergone rigorous cardiovascular investigations before transplantation, cardiovascular mortality remain very high accounting for approximately 50% of all deaths.[28] The rate is 20 times that of age- and sex-matched members of the general population.[29] Death with a functioning graft accounts for half of all transplant loss.[27]

What is known about cardiovascular changes after kidney transplantation?

A systematic review published in 2016 identified 7 studies examining endothelial function before and after transplantation with all of them showing at least some improvement.[30] Seven studies examined arterial stiffness using aortic pulse wave velocity (PWV) with 4 showing improvement, 2 no change and 1 showing an increase after kidney transplantation. To-date there are 13 English language published studies of adults, which have examined changes in LV mass after transplantation with ten showing a significant reduction. (See Table). These studies however were of low methodological quality according to the Newcastle-Ottowa scale.[31] The majority were small and opportunistic in design and therefore open to significant bias. Only one study was fully blinded and only two were controlled with a contemporaneous non-transplanted group of patients with ESRD. All except one were done using echocardiography which has limited accuracy in ESRD patients due to large inter-dialytic variation in intraventricular volumes[32] and its reliance on geometric assumptions to calculate volume which is a major problem in ESRD because of eccentric LV remodelling.[33]

Study/year of publication	Transplant subjects	Control group (N)	Blinded assessment	Imaging modality	Change in LV mass
Ikaheimo 1982 [35]	13	No	No	Echo	Y
Cueto 1983 [36]	18	No	No	Echo	Y
Huting 1992 [37]	24	No	No	Echo	N
Parfrey 1995 [38]	102	No	No	Echo	Y
Himelman 1998 [39]	36	No	No	Echo	Y
Riggatto 2000 [40]	143	No	No	Echo	Y
McGregor 2000 [41]	67	No	No	Echo	N
Ferreira 2002 [42]	24	No	No	Echo	Y
Dudziak 2005 [43]	43	No	No	Echo	Y
Patel 2008 [44]	25	Yes (25)	Yes	CMR	N
Keven 2008 [45]	28	Yes (23)	No	Echo	Y
Vaidya 2012 [46]	105	No	No	Echo	Y
An 2015 [47]	767	No	No	Echo	Y

Cardiac magnetic resonance (CMR) is considered the "gold-standard" method for assessing LV mass and dimensions, including for subjects with ESRD.[34,35] A single CMR study has examined the effect of kidney transplantation on LV structure and function in 25 patients at a mean interval of 2 years.[36] This opportunistic study demonstrated no regression of LV mass although it would not have been sufficiently powered to detect small changes and did not examine myocardial fibrosis.[36]

The role of coronary microvascular dysfunction in uraemic cardiomyopathy

It is increasingly recognised that coronary microvascular dysfunction may play a role in the cardiovascular changes seen in uraemic cardiomyopathy. Recent work has demonstrated that CMD is a feature of myocardial disease states characterised by interstitial fibrosis and increased ventricular stiffness such as hypertrophic cardiomyopathy and heart failure with preserved ejection fraction (HFpEF). In hypertrophic cardiomyopathy (in which the diffuse fibrosis resembles uraemic cardiomyopathy) a number of studies using positron emission tomography (PET) have documented impaired microvascular function. This is predictive of the development of a number of clinical

consequences including reduced LV systolic function, adverse ventricular remodelling, ventricular arrhythmias, clinical heart failure and cardiovascular death.[37–39] Heart failure with preserved ejection fraction in particular has many features in common with uraemic cardiomyopathy including ventricular hypertrophy and fibrosis, diastolic dysfunction and reduced systolic strain, increased ventricular and arterial elastances and a predisposition to sudden cardiac death. In the PROMIS HFpEF trial, 75% of patients with HFpEF had evidence of CMD and this was associated with kidney damage, as measured by albuminuria, as well as a higher NT-proBNP and systemic arterial dysfunction.[40]

Coronary microvascular dysfunction provides a plausible mechanism by which factors associated with impaired kidney function, including oxidative stress and inflammation, might result in myocardial damage and dysfunction leading to the syndrome of uraemic cardiomyopathy.

Coronary microvascular function can be indirectly assessed by measuring coronary flow reserve (CFR) - the magnitude of increase in coronary flow produced by maximal vasodilatation in the absence of obstructive epicardial coronary artery disease. This can be done cheaply and non-invasively using echo-Doppler and contrast echo techniques.[41] CFR is the ratio of hyperaemic/rest flow. Pharmacological vasodilators such as adenosine or dipyridamole are used to induce hyperaemia.

Previous studies have demonstrated that CFR has a linear relationship with estimated glomerular filtration rate[42] and is reduced in transplant recipients compared to controls.[43,44] A reduced CFR has also been shown to be of prognostic significance in large retrospective studies using PET.[45,46] However, there are no studies to date investigating the direct impact of kidney transplantation on CFR.

Mineral bone disorders in CKD (CKD-MBD) and cardiovascular remodelling

Most of the cardiovascular risk factors associated with the increased mortality found in ESRD patients including fluid overload, severe anaemia, inflammation and activation of the renin-angiotensin-aldosterone system rapidly improve after successful kidney transplantation.[47–50] Although successful renal transplantation corrects the metabolic abnormalities responsible for the pathogenesis of secondary hyperparathyroidism, regression of parathyroid gland hyperplasia is uncommon and inappropriately high PTH persists in up to 50% of patients at 1 year.[51,52] Analogous to the persistence of hyperparathyroidism, high levels of FGF-23 can persist post-transplantation.[53] This variable response to transplantation allows examination of the association of these factors with changes in cardiovascular structure and function.

Key questions

Whether CKD associated cardiomyopathy is a reversible process is unclear. If so, it may be amenable to treatment, and possibly prevention, by early use of appropriate interventions. By investigating the association between changes in cardiac structure after renal transplantation and changes in microvascular function, blood pressure and circulating potential mediators including FGF23, this fellowship work will provide evidence to evaluate current mechanistic concepts and a rationale for future interventions.

Hypotheses

1. The restoration of GFR after successful renal transplantation is associated with beneficial cardiac and vascular effects, which include:

i. Decreased LV mass

ii. Regression of myocardial interstitial fibrosis

iii. Improvement in left ventricular systolic and diastolic function

iv. Improvement in coronary flow reserve

v. Reduction in arterial stiffness

vi. Improved endothelial function

2. There is no significant association between the size of the effects on cardiac and vascular parameters after kidney transplantation and the size of effects on systolic blood pressure control.

3. Changes in PTH and FGF23 after kidney transplantation are directly associated with the magnitude in changes in cardiac and vascular parameters.

Experimental details and design of proposed investigations

This is a prospective, controlled, observational, non-randomised, blinded end-point study. In addition, a selection of participants will be invited for an echocardiographic sub study assessing coronary flow reserve.

Subjects: In our unit, patients scheduled to receive a kidney transplant from a live donor are given an operation date 6 weeks in advance. Patients will be approached and studied in that window. This approach proved very successful in our study examining the vascular effects of kidney donation.[12] Patients admitted to receive a cadaveric donor transplant will be approached on the day of transplantation. A review of the last 100 cadaveric transplants show that patients are admitted an average of 18.5 hours before being taken to theatre with an average of 7.5 hours between 9am-5pm. The recent opening of a dedicated research MRI scanner makes scanning on the day of admission feasible. (http://www.birminghamhealthpartners.co.uk/exciting-vision-for-newly-opened-itm-imagingcentre/) We will aim to recruit 60 such subjects with the intention of being able to restudy 54 (allowing for a 10% drop-out) recipients who have undergone successful kidney transplantation without major complication at 12 months. Age and sex-matched controls will be recruited (n=40) from subjects listed for cadaveric kidney transplantation without the prospect of a live donor. This will allow for 25 to be restudied at 1 year (25% transplanted and 10% dropout). There are currently 600 patients on the transplant waiting list at our institution with approximately 200 newly listed every year. Those few subjects recruited as controls who are fortunate enough to receive a kidney transplant within one year of recruitment (current median waiting time is 3 years) will also be re-studied at 12 months and the data used to supplement the recipient group. This plan for recruitment has been discussed with the Renal Patient and Public Participation Involvement in Research Group set up by Prof Ferro as part of his NIHR Fellowship in 2013. Support for the study was generally enthusiastic. Main concern was that participating in the study would delay the timing of transplantation. Assurances will be given and the study abandoned should there be any risk of this happening.

Echo substudy

A selection of participants in the RETRACT study will be invited to take part in an echo sub study to assess the impact of kidney transplantation on coronary flow reserve. Both kidney transplant recipients and non-transplanted controls will be included in the sub study.

Exclusion criteria for main study: Non-standard anti-rejection treatment. Patients transplanted at QEHB are maintained on triple anti-rejection treatment (prednisolone, tacrolimus and mycophenolic acid).

Exclusion criteria for echo sub study: Known coronary artery disease, significant valvular heart disease, diabetes, uncontrolled hypertension, atrial fibrillation, previous renal transplant, any contraindication to adenosine, poor echo acoustic windows.

Endpoints: Measurements will be performed before and 12 months after transplantation for both the main study and the echo sub-study.

Left Ventricular Mass: Up to 70% of patients have LVH at the start of dialysis.[54] As with other patient groups, LVH is a powerful independent predictor of mortality in CKD and regression of LVH is associated with improved cardiovascular outcomes.[55]

LV interstitial fibrosis (Native T1 mapping): Is increased in patients with CKD[20] and ESRD[18,56] and has been shown to be reproducible in dialysis patients without being affected by fluid changes.[56] Measures of LV interstitial fibrosis are emerging as important predictors of mortality across a range of cardiovascular disorders[57].

Coronary flow reserve: Reduced CFR has been demonstrated in kidney transplant recipients compared to controls.[44]

Arterial stiffness: Carotid-femoral pulse wave velocity (PWV) is currently considered the gold standard measure of arterial stiffness and is positively associated with cardiovascular mortality in a number of conditions including CKD and ESRD.[3]

Biochemical Markers of CKD-MBD: Serum Calcium, phosphate, PTH and FGF23.[3,4]

Biomarkers of cardiac fibrosis: One of the secondary aims of this study is to identify circulating biomarkers that may reflect the potential change in measured myocardial fibrosis. Biomarker assays to be performed include markers of: cardiac function (N-terminal B natriuretic peptide), inflammation (hsCRP, IL-6) oxidation and endothelial injury (isoprostanes and asymmetric dimethylarginine) and fibrosis (PICP, PIINP, matrix metalloproteinase-1). There are currently several novel biomarkers that reflect dysregulated organ-remodeling under development.These are specific fragments of different ECM proteins generated by protease-mediated degradation or formation of new molecules; reflecting fibrolysis and fibrogenesis respectively. Serum and plasma will be stored for future potential analysis of validated markers of fibrosis at a later stage. The cost of this has not been included in this application but will be funded separately.

Methods: Blood pressure will be measured according to BHS guidelines and by 24 hour ambulatory monitoring. Clinical details, co-morbidity, medication, smoking status, height and weight will be recorded. Cardiovascular phenotyping will be performed as follows:

1. Cardiac Magnetic Resonance Imaging (Siemens Skyra 3T): will be performed using protocols and techniques already in use in our group.[11] All CMR scan derived parameters will be analysed with the investigator blinded to treatment allocation as in previous studies.[12]

a. Ventricular structure and function*:* SSFP imaging will be used for quantification of ventricular volumes, ejection fraction and mass.[58] Intra-observer and interstudy variability for LV mass in our unit is low (0.99 (95%CI 0.98-1.00) and 0.98 (0.91-1.00) respectively).[12]

b. Myocardial tissue characterisation: T1 (MOLLI) and T2 (T_2-prepared single-shot SSFP technique) mapping will be performed to generate parametric maps (MyoMaps, Siemens HealthCare) allowing tissue characterisation including myocardial interstitial fibrosis and myocardial oedema. Increased T1 times to correlate closely with histological myocardial fibrosis and increased T2 times with oedema.[59] These sequences are in current use by the BCRG. We have our own control data for T1 and T2 mapping at 1.5 and 3T, and are collaborating in an international programme to confirm stability of signal using phantoms for T1 mapping. We will give gadolinium contrast during the post-transplantation studies (if eGFR > 30ml/min/1.73m$_2$) to assess the degree of replacement fibrosis with late gadolinium enhancement. Comparison will be made with pre-transplant T1 map to assess how much irreversible fibrosis might have been present.

c. Myocardial deformation: Changes in regional systolic deformation will be assessed using tissue tracking (CVi 42®) on standard cine images. Deformation is the most accurate measure of LV global function and correlates with the extent of myocardial fibrosis. Data for normal ranges and reproducibility using this software from our centre has been accepted for publication.[60]

2. Echocardiogram: All echo studies will be performed on a dedicated Philips iE33 machine equipped with contrast cardiology software.

2.1 LV volumes and ejection fraction will be measured using Simpson's biplane method. Diastolic function will be quantified using multiple parameters according to current guidelines.[61]

2.2 Strain imaging will be performed using TDI and speckle tracking and analysed offline using proprietary software (Phillips Q lab).

2.3 CFR assessment – The LAD will be identified using high resolution colour Doppler in the interventricular sulcus in a modified apical 2 chamber view. PW Doppler will be used to sample flow velocity signals at rest and during maximal hyperaemia using a standard adenosine protocol (140mcg/kg/min over 5-10 minutes). CFR will be calculated as the ratio of hyperaemic/resting velocity.

2.4 Myocardial blood flow will be determined using very low mechanical index MCE. Using Philips Q lab, selected regions of interest in multiple myocardial segments will be chosen and results averaged. Background-subtracted plots of peak myocardial contrast intensity will be constructed, representing capillary blood volume vs. pulsing intervals, from which the slope of the replenishment curve depicting mean microbubble velocity will be derived. Resting and hyperaemic myocardial blood flow (MBF) (peak contrast intensity × myocardial blood velocity) will be derived and CFR (MBF at stress/MBF at rest) will then be calculated.[62]

3. Arterial stiffness: will be assessed by carotid-femoral PWV using the SphygmoCor system (AtCor Medical. Sydney, Australia).[12] This technique has been widely used by our group and overcomes several of the methodological problems associated with measuring PWV.

4. Endothelial Function: will be assessed non-invasively by digital pulse amplitude tonometry using the Endo-PAT system (Itamar-Medical, Israel). Measurements are analysed with a computerized automated algorithm to reduce intra- and inter-observer variability. Previous studies have shown that this system has excellent reproducibility in a number of conditions A recent study in haemodialysis patients found this method to be acceptable to patients, reproducible over time and positively associated with troponins, a marker of myocardial damage.

5. Biomarkers of cardiac fibrosis: These will be measured at a future date in the fibrosis research laboratories within the Institute of Cardiovascular Sciences at the University of Birmingham.

6. Markers of CKD-MBD: – calcium, phosphate, alkaline phosphatase and PTH will be analyzed in serum by mass spectroscopy and FGF-23 will be analyzed by ELISA at 0 and 12 months.

Sample Size and Statistical Considerations

The primary end point will be change in LV mass. Using the effect sizes and variances from our previous work (change in LV mass 7g, SD of change 10g) we calculate that by studying 50 transplanted subjects and 25 non transplanted controls, we will have 80% power to detect a reduction in LV mass of 7 g with an alpha value of 0.05.[11, 12] This effect is clinically important; a fall in LV mass index of one SD has been shown to be associated with a 38% reduction in cardiovascular mortality.[63] 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 transplanted) over two years.

Echo substudy:

For paired analyses of CFR pre and post-transplant, 40 patients will provide 99% power to detect a difference of 0.5 in CFR as a result of transplantation.

The primary analysis will test differences between groups at 12-months using repeated measures ANOVA. Analysis of change in LV mass and other continuous variables will be performed using a general linear model, comparing primarily changes from baseline in recipients to those in non transplanted controls and secondarily changes within each group between baseline and 12 month studies. The study is observational in design but as both recipients and non-transplanted controls will be recruited from the same patient group it is highly unlikely that the two groups will differ in any major characteristic. Studies will be anonymised and stored on the QEHB research imaging PACS server. Observers blinded to treatment will perform all CMR analyses. Feasibility: The Queen Elizabeth Hospital Birmingham has one of the largest kidney transplant programs in the UK, performing 200

transplants per year. Recruiting 60 recipients from a pool of 400 (15%) and 40 listed patients from a pool of 600 (5%) into an observational study over 2 years is very feasible. Indeed, the BCRG has a strong track record of successfully recruiting renal patients to interventional studies (mainly BHF funded). Recent Studies include: Dr Edwards PhD 2012: 'MR Blockade in Early Stage CKD Effects on Conduit Arteries and the Left Ventricle'. -112 patients; Dr Chue PhD 2013: 'Does phosphate binding with sevelamer carbonate improve cardiovascular structure and function in patients with early CKD?'- 120 patients and Dr Moody PhD 2015 'The Effects of a Modest Reduction in Renal Function on Cardiovascular Structure and Function: A Study of Kidney Donors - 124 patients. This success has been due both to the large size of the renal unit and to the strong collaboration and involvement from senior investigators and clinical research fellows.

Expected Value of Results

This study will provide insights into the associations between structural change and important potential mechanisms such as **microvascular dysfunction,** blood pressure, PTH and FGF-23, thus paving the way for future mechanistic and interventional clinical studies, to show causation and reveal new therapeutic approaches. The ultimate goal is to improve cardiovascular outcomes in all patients with CKD, especially the majority of patients with ESRD who will never receive a transplant.

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