Redefinition of Uraemic Cardiomyopathy with Cardiac Magnetic Resonance Imaging

by

Dr Patrick Mark

MBChB (Hons), MRCP (UK)

Renal Unit, Western Infirmary, Glasgow and

Glasgow Cardiovascular Research Centre,

University of Glasgow, UK

A thesis submitted for the degree of Doctor of Philosophy in the Faculty of Medicine of the University of Glasgow

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List of abbreviations

1^0	Primary
4D	Deutsche Diabetes Dialyse Studie
4S	Scandinavian Simvastatin Survival Study
AAo	ascending aorta
ABPM	ambulatory blood pressure monitoring
ACC/AHA	American College of Cardiology/American Heart Association
ACE	angiotensin converting enzyme
ACEi	angiotensin converting enzyme inhibitor
AD	aortic distensibilty
ALERT	Assessment of LEscol in Renal Transplantation
APKD	adult polycystic kidney disease
ARB	angiotensin receptor blocker
ASE	American Society of Echocardiography
AUC	area under curve
BSA	body surface area
CA	calcium channel antagonist
CAD	coronary artery disease
Ca-P	calcium-phosphate
CD	cardiac death
CHF	chronic heart failure
CI	confidence interval
CKD	chronic kidney disease
CPN	chronic pyelonephritis

CrCl	creatinine clearance
CRF	chronic renal failure
CRP	C-reactive protein
CT	computed tomography
CVA	cerebrovascular accident
CVD	cardiovascular disease
DAo	descending aorta
DBP	diastolic blood pressure
ECF	extra cellular fluid
ECG	electrocardiogram
EDTA	ethylenediamine tetraacetic acid
EDV	end diastolic volume
EF	ejection fraction
ESV	end systolic volume
ETT	exercise tolerance test
FLASH	fast low-angle shot
FoV	field of view
Gd-DTPA-BMA	Gadolinium-diethylentriaminepentaacetate
GFR	glomerular filtration rate
GN	glomerulonephritis
HD	haemodialysis
HDL	high-density lipoprotein
HLA	horizontal long axis
HR	hazard ratio
IDL	intermediate-density lipoprotein

IHD	ischaemic heart disease
IVST	interventricular septal thickness
KDOQI	Kidney Disease Outcomes Quality Initiative
LDL	low-density lipoprotein (LDL)
LGE	late gadolinium enhancement
LV	left ventricle/ventricular
LVEF	left ventricular ejection fraction
LVH	left ventricular hypertrophy
LVID	left ventricular internal diameter
LVMI	left ventricular mass index
LVOT	left ventricular outflow tract
LVSD	left ventricular systolic dysfunction
LVW	left ventricular wall thickness
MET	metabolic equivalent
MI	myocardial infarction
MIA	malnutrition, inflammation, and atherosclerosis
MonoRx	monotherapy
MRI	magnetic resonance imaging
NHS	National Health Service
NPV	negative predictive value
NSF	nephrogenic systemic fibrosis
NT-proBNP	N-terminal-pro Brain Natriuretic peptide
Pap	papillary muscles
PC	personal computer
PD	peritoneal dialysis

PPV	positive predictive value
PTH	parathyroid hormone
PVD	peripheral vascular disease
PWT	posterior wall thickness
PWV	pulse wave velocity
ROC	receiver operator characteristic
ROI	region of interest
RRT	renal replacement therapy
SA	short axis
SBP	systolic blood pressure
SPECT	myocardial scintigraphy
T	Tesla
TE	echo time
TIA	transient ischaemic attack
TR	repetition time
TrueFISP	fast imaging with steady-state precession
Tx	transplant
URR	urea reduction ratio
VAS	volumetric arterial strain
VLA	vertical long axis localiser
VLDL	very low-density lipoprotein

Acknowledgements

I would like to thank my principal supervisor Professor Alan Jardine for his valuable advice, considerable patience and encouragement throughout this project. Throughout this venture I have also been delighted to receive the support and enthusiasm of my co-supervisor Professor Henry Dargie.

I am extremely grateful to Tracey Steedman and Tony Cunningham for their help teaching me the techniques of cardiac magnetic resonance imaging and echocardiography and would also like to thank Dr John Foster for advice with MR physics and Helen MacDonald for secretarial help with patients at the Clinical Research Institute.

This work would not have been possible without the participation of the patients of the Western Infirmary Renal Unit. I am indebted to the Consultant staff of the Renal Unit for encouraging patients to participate: Dr Stuart Rodger, Dr Ellon McGregor, Dr Margaret McMillan, Dr Conal Daly, Dr Neal Padmanabhan, Dr Colin Geddes and Dr Brian Junor. I am particularly grateful to Lynne Thomson, transplant coordinator, who pointed out suitable patients.

Finally, I would like to thank the British Heart Foundation who supported this work through a Junior Research Fellowship.

Declaration

The experimental design of the work presented in this thesis was that of the author and his supervisors, Professor Alan Jardine and Professor Henry Dargie. All experimental work was carried out by the author except acquisition of echocardiographic studies (performed by Tony Cunningham, Clinical Research Initiative, University of Glasgow), acquisition a proportion of cardiac magnetic resonance images (performed by Tracey Steedman, Glasgow Cardiac Magnetic Resonance Unit, University of Glasgow) and performance of pulse wave analysis tracings (Dr Lukas Zimmerli, University of Glasgow). Measurement of brain natriuretic peptide concentrations was performed by Dr JJ Morton (University of Glasgow).

I declare that this thesis has been composed by myself and is a record of work performed by myself. It has not been previously submitted for a higher degree.

Patrick Mark

July 2007

Summary

Patients with end stage renal disease (ESRD) have a 20-100 fold risk of premature cardiovascular death compared to age matched controls from the general population. These patients have many 'conventional' cardiovascular risk factors such as diabetes, ischaemic heart disease, hypertension, cigarette smoking and hyperlipidaemia. However, the relationship between the presence of these risk factors and cardiovascular outcomes is less clear in ESRD than in the general population. In the cases of hyperlipidaemia and hypertension a paradoxical relationship has been demonstrated where lower cholesterol or blood pressure is associated with an increased risk of cardiovascular events. One factor previously demonstrated to be associated with poor prognosis is the presence of uraemic cardiomyopathy, found in approximately 70% of ESRD patients at initiation of dialysis therapy, usually defined echocardiographically as the presence of left ventricular (LV) abnormalities, including left ventricular hypertrophy (LVH), LV dilatation and LV systolic dysfunction. However, echocardiography makes assumptions regarding LV geometry, which is frequently distorted in patients with ESRD. Furthermore any errors in measurements are amplified by the changes in hydration status which occur during the dialysis cycle, leading to changes in LV chamber dimensions. For these reasons, cardiac magnetic resonance imaging (CMR), by providing high fidelity measurements, potentially offers a 'volume independent' method of quantifying LV dimensions. Furthermore, by using gadolinium based contrast agents, tissue abnormalities particularly myocardial fibrosis, indicated by late gadolinium enhancement (LGE) may by identified.

The work contained in this thesis examines the relationship between cardiac dimensions, as defined by CMR and cardiovascular risk factors (both conventional and specific to uraemia). In a study of 145 patients with ESRD using CMR with

gadolinium, two specific pathological processes were demonstrated. First, the presence of subendocardial LGE indicating previous myocardial infarction was associated with the presence of conventional cardiovascular risk factors such as previous ischaemic heart disease and diabetes. Patients with subendocardial LGE frequently had LV systolic dysfunction. Second, diffuse LGE, representing fibrosis throughout the LV wall was identified in patients with LVH. This was an unexpected finding and appears specific to uraemia. Using CMR, isolated LV dilatation was rare. These findings suggest that in uraemia two forms of cardiomyopathy exist- LV systolic dysfunction due to underling myocardial ischaemia and LVH which is a true 'uraemic cardiomyopathy' associated with diffuse myocardial fibrosis. Attempts were made to reassess the relationship between CMR and echocardiographic measures of cardiac dimensions. In keeping with a previous study, it was demonstrated that M-mode echocardiography overestimates LV mass compared to CMR in this population. Thus, CMR may be used to optimise echocardiographic formulae to calculate LV mass. Furthermore, it appeared that either by echocardiography or by CMR the chief determinant of LVH in this population was blood pressure, in particular systolic blood pressure. This has implications for treatment as recent studies aimed at correcting anaemia, previously associated with LVH, either to reduce LV mass or to improve survival, have generally demonstrated increased cardiovascular events with higher haemoglobin. Therefore, if LV mass is a goal of treatment, attempts should be made to reduce blood pressure further in this population.

The patients studied in these investigations were candidates for renal transplantation, the definitive treatment for chronic renal failure. Cardiovascular disease is the leading cause of death both in patients on the renal transplant list, as well as post successful transplantation. There is a great deal of interest in identifying patients at high

cardiovascular risk, to allow strategies to be adopted to minimise this risk, frequently by undertaking invasive investigation such as coronary angiography. In a survival study of 300 potential renal transplant recipients, factors associated with increased risk of mortality were increased age, ischaemic heart disease whilst receipt of a renal transplant was protective. Although the presence of LGE was associated with poorer outcome, this finding was not independent of other variables. One interesting finding was that patients with greater exercise tolerance, measured objectively using the full Bruce exercise test had better outcomes. This observation represents a simple pragmatic method to risk-stratify such patients. A study using the biomarker brain natriuretic peptide (BNP) a peptide released from the LV in response to stretch and hypertrophy, in 114 patients, demonstrated that whilst BNP has potential as a diagnostic tool for the presence of uraemic cardiomyopathy, in particular LVH, this peptide added little prognostic value.

As familiarity with CMR techniques developed, it became clear that vascular function could be investigated with this imaging modality. Previous studies using alternative measures of vascular function have suggested that arterial stiffness is an important predictor of long term outcome in patients with ESRD. A study of 147 uraemic patients using aortic distensibilty and aortic volumetric arterial strain as CMR measures of aortic stiffness demonstrated that both these parameters were associated with an increased risk of cardiovascular events and mortality. To date there do not appear to be any similar outcome studies using these measures, although a number of authors have noted an association between aortic distensibilty and cardiovascular risk factors. These factors may represent potential targets for therapy aimed at reduction of cardiovascular risk in patients with ESRD.

One unfortunate development during the period during which these studies were undertaken, was the emergence of a link between exposure to gadolinium based contrast agents and nephrogenic systemic fibrosis (NSF), a potentially life threatening skin disorder in patients with advanced renal failure. This finding lead to the cessation of contrast CMR studies. A retrospective investigation of factors present in patients in North Glasgow affected by NSF, confirmed that patients with NSF were more likely to have undergone contrast based imaging than unaffected patients, frequently undergoing multiple scans, with high doses of gadodiamide used. Until this issue is clarified, future scans using these agents in this population should be undertaken with caution.

These studies have characterised for the first time the relationship between both uraemic cardiomyopathy and uraemic arterial stiffness and both cardiovascular risk factors and long term outcome. CMR measures of cardiac dimensions and vascular function represent future targets for interventions aimed at reducing cardiovascular risk in patients with advanced renal failure.

Chapter 1

Introduction

1.1 Epidemiology of cardiovascular disease in end stage renal failure

Premature cardiovascular disease is the leading cause of death in patients with end stage renal disease (ESRD) requiring dialysis therapy with approximate risk age adjusted cardiovascular risk and cardiovascular mortality rates around 20 times those of the general population(1;2). Prior to the development of haemodialysis (and subsequently peritoneal dialysis) as treatment for advanced renal failure, pericarditis and pericardial effusions were common and often fatal cardiac manifestations of the syndrome of terminal uraemia. With the advent of dialysis therapy, it became evident that premature cardiovascular disease was reported as cause of death in a disproportionately large number of patients on dialysis programmes, despite the young age of patients selected for dialysis treatment during the early years of this treatment. In 1974, the Seattle group led by Belding Scribner, a pioneer in early haemodialysis treatment, reported the presence of accelerated atherosclerosis in patients treated with dialysis treatment(3). Whilst undeniably a contributory factor to the excess cardiovascular mortality in ESRD patients, this is unlikely to be the sole mechanism, and further analysis of the relationship between conventional cardiovascular risk factors and outcome in this population reveals stark differences between patients with ESRD and the general population.

Using data from the United States Renal Data Services (USRDS), approximately 60% of deaths amongst dialysis patients in the United States can be attributed to myocardial infarction, cardiac arrest, cerebrovascular disease and other cardiac causes(4), with similar figures reported to European renal registries. Whilst the most common mode of cardiovascular death in the general population is myocardial infarction, one striking finding in the ESRD population is the extremely high proportion of deaths attributed to sudden (and therefore presumed arrhythmic) cardiac death (Figure 1.1).

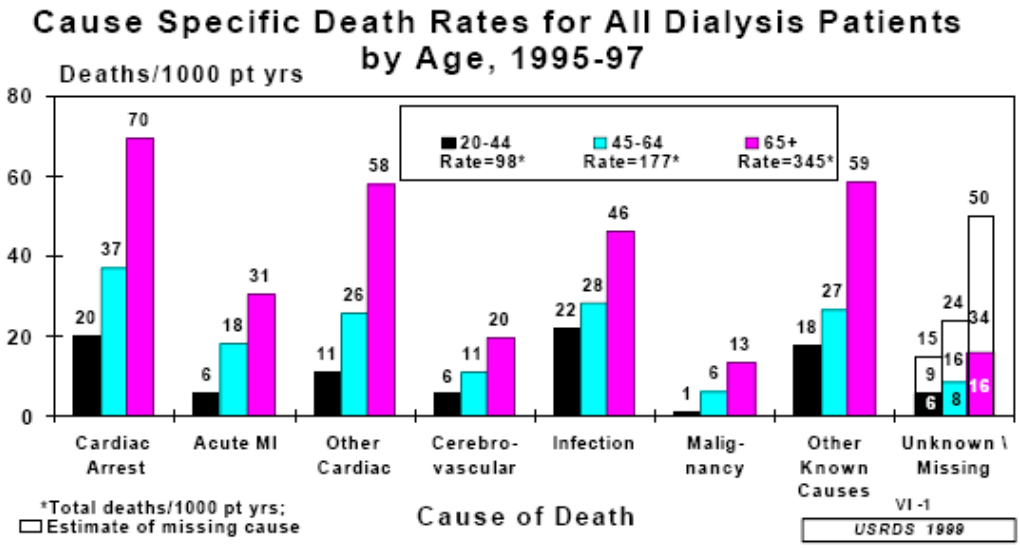


Figure 1.1

Cause specific death rates for all dialysis patients by age, 1995-97 from the USRDS report 1999.

One dilemma here is the difficultly is ascertaining the cause of death in such patients, to then identify reversible risk factors to improve treatment. Nonetheless, as this is a consistent finding, it suggests differences in the pathogenesis of fatal cardiovascular events in patients with ESRD compared to those in the general population. Moreover, although many 'conventional' cardiovascular risk factors are present in patients with ESRD, the relationship between these risk factors and outcome is less clear than in the general population, and additionally there are cardiovascular risk factors either specific to the uraemic milieu which carry a more influential role on future cardiovascular events than in the general population. Table 1.1 lists some of these risk factors which will be discussed in greater detail.

Risk factors for CVD – gen. population	Risk factors for CVD - ESRD
Older age	Haemodynamic and metabolic factors of CRF
Hypertension	Proteinuria
Hyperlipidaemia	↑extracellular fluid (ECF) volume
Diabetes	Electrolyte imbalance
Physical inactivity	Anaemia
Previous MI/CAD/PVD/CVD	Homocysteine
Smoking	↑PTH/calcium-phosphate
Oxidative stress	Arterial calcification
LVH/LVSD	Inflammation

Table 1.1

Risk factors for cardiovascular disease in the general population and in ESRD

1.2 Risk factors for cardiovascular disease in the general population and in ESRD

In the later part of the 20th century it became clear from an abundance of large clinical trials, that by intervention on a specific risk factor, previously identified from observational studies, the risk of future cardiovascular events could be reduced. Focus has directed on which patients derive most benefit from these interventions, frequently dependent on their baseline probability of a certain event occurring. For example, in the case of lipid lowering therapy post myocardial infarction(5), the clinical benefit is now clear and has translated into widespread changes in clinical practice. As the same evidence is not present in the ESRD population, it is tempting to extrapolate these findings from trials in the general population to this group. However, these epidemiological associations from observational studies do not demonstrate the same relationship in ESRD as in the general population and to date interventional trials aimed

at such risk factors in ESRD patients have given conflicting results. It is also clear that some of these risk factors are closely interrelated (e.g. haemodynamic/ metabolic factors of chronic renal failure and extracellular fluid volume). A number of the factors displayed in Table 1.1 are discussed.

1.2.1 Hypertension

In the general population, it has been demonstrated that lowering blood pressure in patients with hypertension reduces cardiovascular risk(6;7). Initially studies suggested that the agent used to treat blood pressure may not be as important as the blood pressure target itself, with certain exceptions. Treatment with diuretics(8), calcium channel antagonists(9), beta blockers(10), angiotensin converting enzyme inhibitors(11) and angiotensin receptor blockers(12) have all been shown to be effective at reducing risk of cardiovascular events in hypertensive individuals. To date, no minimum blood pressure target has been identified and although there is some evidence from observational studies that a 'U' or 'J' shaped curve relationship between diastolic blood pressure and outcome exists(13), suggesting that low diastolic blood pressure may compromise perfusion of vascular beds distal to a stenotic vessel. Nonetheless, this is less of a problem in clinical practice, where frequently achieving target blood pressure is more of an issue. Additionally, it has been clearly demonstrating that reduction of blood pressure is a major modifiable risk factor in patients with chronic kidney disease, both in preventing progression to ESRD and in preventing cardiovascular events(14;15).

The story is more complex in patients with ESRD. Several observational studies have demonstrated that low blood pressure is independently associated with increased mortality in ESRD. Zager *et al* demonstrated that whilst pre-dialysis blood pressure was unrelated to cardiovascular mortality, a clear 'U' shaped curve relationship between post-dialysis blood pressure was seen with post-dialysis systolic blood

pressure>180mmHg and <110mmHg both associated with adverse outcome(16). Similar findings have been demonstrated in other observational studies, with Port *et al* also observing that a low pre-dialysis blood pressure being associated with mortality(17). However, intriguingly Mazzuchi *et al* observed that once incident dialysis patients were followed up for greater than two years, risk of early mortality (after < 2years on dialysis therapy) demonstrated a 'U' shaped relationship with pre-dialysis systolic blood pressure (patients with systolic blood pressure >160mmHg or <110mmHg were most at risk), whilst later mortality (after >2 years on dialysis therapy) demonstrated a linear relationship with increasing blood pressure(18). The inference from this study is that low blood pressure in incident dialysis patients may represent left ventricular systolic dysfunction, hence increasing these patients risk of early death.

There few trials to inform how best to achieve blood pressure targets in ESRD, and observational data supports the use of long-duration dialysis sessions, with slow ultrafiltration as pioneered by Charra *et al* in Tassain, France(19). More recent data have shown similar benefits with nocturnal haemodialysis (allowing for longer treatment sessions)(20). There is a pressing need for large randomised controlled trials in ESRD (both haemodialysis and peritoneal dialysis) to determine both optimal blood pressure targets and strategies to achieve those targets, with either dialysis, pharmacological intervention or a combination of these methods.

1.2.2 Hyperlipidaemia

In the general population, hypercholesterolaemia and dyslipidaemia are interchangeable in terms of prevalence and risk implication. In ESRD, however, this is not the case. ESRD patients typically have either normal or slightly increased low-density lipoprotein (LDL), increased very low-density lipoprotein (VLDL) and intermediate-density

lipoprotein (IDL) leading to elevated triglyceride levels, and decreased levels of high-density lipoprotein (HDL). There are also qualitative changes in dyslipidaemia with a shift to a more atherogenic LDL particle size toward a small, dense apo-B-rich LDL predominance(21). The prevalence of dyslipidaemia in chronic kidney disease patients is very high. In one study of patients of both haemodialysis and peritoneal dialysis patients, the prevalence of dyslipidaemia was approximately 67%(22), where dyslipidaemia was defined as at least one abnormal lipid parameter. In fact, peritoneal dialysis seems to be associated with a relatively more atherogenic lipid profile than haemodialysis(23).

In the general population the relationship between hyperlipidaemia (dyslipidaemia) and cardiovascular disease (predominantly coronary artery disease) is well established and there are proven benefits of lipid-lowering with statins(5;24-28). Only limited epidemiological and even more limited interventional, data exist on the relationship between dyslipidaemia and cardiovascular disease in ESRD. In patients receiving maintenance haemodialysis, reports suggest either no relationship or paradoxical correlations, the so called “reverse epidemiology”, where a lower total cholesterol level has been associated with a higher risk of death (29-31), or conversely, a higher serum cholesterol level has been found in long-term dialysis survivors(32). These studies have often used registry data, and either all cause mortality or unspecified CV mortality. Similar inverse, “J” or “U”-shaped relationships between lipid levels and all cause mortality have been reported in other populations and are thought to reflect a high prevalence of co-morbid disease (specifically malignancy and associated malnutrition) in patients with low cholesterol levels. These observations highlight the need for interventional trials and may have led to under-treatment of dyslipidaemia. Amongst patients starting dialysis, almost 40% are diabetic and 25% have known coronary artery

disease and 30% of dialysis patients have cholesterol levels higher than 5.18mmol/L(33). Despite these observations, and studies showing effective lipid-lowering and safety of statins, less than 10% of patients commencing maintenance dialysis receive statins(34;35). The impetus now lies in clarifying the therapeutic benefit, and unravelling the mechanisms that underlie the cholesterol paradox.

The "4D Trial" (Deutsche Diabetes Dialyse Studie) perhaps unexpectedly, failed to show a significant reduction in cardiovascular events in haemodialysis patients given atorvastatin for a median of four years(36). It was a randomised, placebo-controlled study in 1,255 type II diabetic patients on chronic haemodialysis, who had been on dialysis for less than two years. Atorvastatin lowered mean LDL-cholesterol levels by 42%, to 1.9 mmol/L. However, there was no significant reduction in the primary end point (a composite of cardiac death, nonfatal myocardial infarction, and fatal or nonfatal stroke). The investigators speculated that the negative results could be in part due to a different pathogenesis of vascular events in diabetic patients with ESRD compared to other groups, and that the intervention was too late in the natural history of such complex disease. Atorvastatin therapy did, however, lower the incidence of myocardial infarction. Thus, an alternative explanation is that in the complex mixture of cardiovascular disease that affects patients with ESRD the relationship between cholesterol and myocardial infarction is present (and lipid-lowering reduces this end-point) but that myocardial infarction is not the dominant cardiovascular end-point.

This hypothesis has been explored in patients with ESRD who received renal transplants in the Assessment of LEscol in Renal Transplantation (ALERT) trial. In this related population, all lipid parameters are associated strongly with the development of myocardial infarction but much less so with cardiac death, which is more dependent on

left ventricular hypertrophy (LVH) and hypertension(37). The differences in cardiac outcomes in the 4S study in patients with conventional CVD, the 4D study and the ALERT study are illustrated in Table 1.2. The 4S (Scandinavian Simvastatin Survival Study) study randomised patients to simvastatin 40mg or placebo post myocardial infarction and demonstrated a significant reduction in both myocardial infarction and cardiovascular death(5). In the general population (4S) patients are more likely to have a non-fatal MI than a cardiac death, whereas in patients receiving haemodialysis the relationship is reversed. Additionally it is clear that the cardiac event rate in the 4D exceeds even a very high risk group such as post myocardial infarction patients. In the general population, a minimum threshold for lowering LDL has not yet been defined and safety concerns regarding intensive statin therapy have not been founded. To date only less aggressive statin therapy has been trialled in the ESRD population and although the risk of adverse affects such rhabdomyolysis in this population is higher, so far the safety record in these clinical trials has been good.

	n	LDL-cholesterol			CD+MI annual rate	Non-fatal MI %	CD+MI % reduction	CD % of reduction
		mmol/L baseline	mmol/L change	% change				
4S	4444	4.9	1.7	-35	5.2	16.9	34	42
ALERT	2102	4.1	1.0	-32	2.0	5.2	35	38
4D	1255	3.1	1.2	-42	8.2	11.8	14	19

Table 1.2

Comparison of the 4S study with the 4D and ALERT studies. Abbreviations n= number of patients studied, CD – cardiac death, MI – myocardial infarction

1.2.3 Diabetes mellitus

Diabetic nephropathy is the cause of ESRD in 15-30% of patients with approximately equal proportions of patients having type I and type II diabetes(38). Extra-renal complications of diabetes include macrovascular disease and microvascular disease. The overall risk of death from myocardial infarction in a patient with diabetes is approximately three times that of the general population, with younger patients at higher risk(39). However the development of nephropathy, heralded by the presence of albuminuria, enhances the risks of further macrovascular complications as well as the risk of progressing to ESRD(40). Data from the Diabetes Control and Complications trial has shown that tight diabetic and blood pressure control reduce the risk of progression to ESRD and cardiovascular events in type I diabetics(41;42). However in the United Kingdom Prospective Diabetes Study tight glycaemic control was not shown to reduce risk of myocardial infarction in type II diabetics(43). Better glycaemic control was shown to reduce risk of microvascular complications and better blood pressure control has been demonstrated to reduce risk of cardiovascular events and progression of diabetic nephropathy(44). Once established on dialysis therapy, the survival for diabetic patients is considerably worse than for patients with other causes of ESRD with a median survival on dialysis survival of 2.5 years(45). This is mainly attributable to the excess risk of vascular disease. Studies screening diabetic patients for kidney transplantation have suggested that over half of diabetic renal transplant candidates will have at least one greater than 50% coronary artery stenosis(46).

Once established on dialysis therapy, the options for type I diabetic patients include both simultaneous kidney-pancreas transplantation and kidney only transplantation with or without pancreas after kidney transplantation. Islet cell transplantation has been performed successfully but the long success has to date been relatively disappointing

with <50% of patients insulin independent at 2-3 years(47). Although less widely established than kidney only transplants, kidney-pancreas transplantation in suitable recipients has been demonstrated to have good outcomes for the patient with 85% 1 year graft (kidney and pancreas) survival. Crude analyses have suggested that simultaneous kidney-pancreas transplantation offers a survival advantage compared to cadaveric renal transplantation for type I diabetic patients with ESRD(48).

1.2.4 Ischaemic heart disease

Although sudden cardiac death is a more dominant manifestation of cardiovascular disease in patients with ESRD, there is no question that the prevalence and severity of ischaemic heart disease in the ESRD is a key factor in explaining the dramatically increased risk of cardiovascular death in this population. In some patients, the underlying cause of ESRD will share a common pathogenesis as that of accelerated atherosclerosis (e.g. in diabetic nephropathy or atherosclerotic renal artery stenosis). It is difficult to accurately categorise what proportion of excess cardiovascular morbidity can be attributed to obstructive coronary artery disease in ESRD patients. Some of the sudden cardiac deaths, out of hospital, may be due to acute myocardial infarction, whilst others may be inappropriately labelled as myocardial infarction when primary arrhythmia was the underlying cause. Studies assessing the prevalence of coronary artery disease in patients with advanced renal disease have tended to focus on potential renal transplant candidates who tend to have less co morbidity than the whole ESRD population. Nonetheless, Sharma *et al* reported that in a series of 126 consecutive patients screened with coronary angiography significant coronary artery disease in at least one vessel was found in 30% of patients(49). On the other hand, symptomatic angina is common in patients with ESRD with angiographically normal coronary arteries. Rostand *et al* reported that of 44 ESRD patients with symptomatic angina,

only 53% had obstructive coronary artery disease at angiography(50). Explanations for this phenomenon include subendocardial ischaemia due to left ventricular hypertrophy (LVH), microvascular dysfunction with impaired vasodilator reserve and/or limitations of oxygen delivery.

About 20% of deaths in dialysis patients are attributed to acute myocardial infarction. In patients with ESRD, acute myocardial infarction is associated with extremely poor survival. Herzog *et al* reported a 1-yr 59% mortality and 2-yr 73% mortality in 34,189 dialysis patients with acute myocardial infarction in the US from 1977 to 1995(51). In the 'post thrombolysis' era, 1-yr and 2-yr mortalities of have improved little at 62% and 74% in 1990–95, and 2-yr mortality of 78% in 1991–97(52). Although ESRD is a risk for poor outcome post myocardial infarction, ESRD patients are less likely to receive treatments proven to improve outcome in this setting such as aspirin, thrombolytics and beta blockers(53). Additionally, the presence of advanced kidney failure is frequently an exclusion criterion for clinical trials in myocardial infarction. Therefore, the poor outcome of ESRD patients with myocardial infarction may be partially attributable to undertreatment, either due to fear of potential complications such as haemorrhage, particularly from arteriovenous fistulae used for dialysis, and the unavailability of clinical trial outcome data due to the exclusion of ESRD patients from all AMI trials.

In ESRD patients with critical coronary artery stenosis there is a relative survival advantage associated with coronary artery bypass grafting compared to percutaneous coronary intervention (angioplasty or stent) in dialysis patients. USRDS data reports that for dialysis patients receiving their first coronary revascularisation procedure in 1995–98: 4836 dialysis patients treated with angioplasty, 4280 with angioplasty plus stent, and 6668 coronary artery bypass grafting, the 2-yr all-cause survival was 48% in

both percutaneous coronary intervention groups and 56% after coronary artery bypass grafting(54). In a multivariate analysis, the risk of all-cause death was 20% lower for bypass grafting compared to percutaneous intervention and 6% lower for stenting compared with angioplasty. This must be balanced with the higher than normal in hospital mortality for bypass (4.1% for stenting, 6.4% for angioplasty and 8.6% for bypass grafting). However, the restenosis rates of coronary stents are unknown in dialysis patients, but are likely to be no lower than in the general population, and probably higher. Drug eluting stents (using sirolimus or paclitaxel) have offered promising results with extremely low restenosis rates (0% at 6 months for patients receiving sirolimus- coated stents for native coronary lesions compared to a 26.6% restenosis rate in the Randomized Study with the Sirolimus- Coated Bx Velocity Balloon-Expandable Stent in the Treatment of Patients with De Novo Native Coronary Artery Lesions (RAVEL) trial)(55). Whether these results will translate to benefits in the dialysis population is unclear, particularly in light of some evidence suggesting that late stenosis after one year is more common with both sirolimus-eluting stents and paclitaxel-eluting stents than with bare-metal stents(56).

Whilst in patients with critical coronary disease revascularisation is imperative, the presence of renal failure is a predictor of adverse outcome after percutaneous coronary intervention. Rubenstein *et al* compared the immediate and long-term outcomes of 362 renal failure patients (creatinine >132.6 μmol/L, 27 dialysis patients) with 2972 patients with normal renal function undergoing PCI in 1994–97(57). The in-hospital mortality was significantly higher in the patients with renal dysfunction (10.8% for the renal patients and 1.1% in the controls) with significantly more blood transfusions in the renal patients. The 1-yr actuarial survival was significantly lower at 75% for the renal group compared to 97% for the matched controls. These data suggest that once a patient with

advanced renal disease develops a significant burden of atherosclerotic coronary artery disease, there is paucity of randomised controlled data to inform best practise. Current observational evidence suggests that outcomes are considerably poorer than the general population both in the setting of acute myocardial ischaemia as well as for coronary revascularisation.

1.2.5 Physical inactivity

Evidence from observational studies of the general population suggests that increased physical activity is associated with reduced cardiovascular mortality. Physical activity is frequently limited in ESRD patients. One epidemiological study from the Dialysis Morbidity and Mortality Wave 2 study has demonstrated that limitations in physical activity are common among new patients with ESRD in the United States and correlate highly with increased mortality risk. Frequent exercise of up to 4 to 5 times per week correlate with improved survival. Daily exercise was not associated with any additional protective benefit(58). Although direct benefits of an interventional exercise programme on long term outcome are difficult to demonstrate, in a small single centre study of haemodialysis patients, Mustata *et al* demonstrated that a 3 month exercise programme improved arterial stiffness, a surrogate marker of increased cardiovascular risk in ESRD. A typical class consisted of a 5- to 10-minute warm-up period, 40 to 50 minutes of conditioning exercise (either treadmill running or exercise bicycle), and a 5- 10-minute cool-down(59). It is not clear how this approach would translate to the entire ESRD population.

1.2.6 Cigarette smoking

Of potentially modifiable risk cardiovascular risk factors in ESRD patients, perhaps less attention has been paid to cigarette smoking than other factors. In the USRDS Wave 2

study studying 4024 new dialysis patients smoking at the time of inception of dialysis was associated with a 37% increase in mortality, even after adjustment for co-morbidity. Ex-smokers had event rates similar to non smokers(60). Cigarette smoking also accelerates the progression of chronic renal failure. There are no trials to support which smoking cessation strategy is most effective in ESRD, but meta-analyses suggest that nicotine replacement therapy is part of an effective strategy in the general population(61).

1.2.7 Oxidative stress

Conditions predisposing to atherosclerosis, including hypercholesterolemia, diabetes mellitus, cigarette smoking and chronic kidney disease are associated with increased oxidative stress. The atherogenicity of low-density lipoprotein (LDL) is greatly increased upon oxidative modification(62) as, once oxidised, LDL is taken up by scavenger receptors on monocytes, leading to the conversion of monocytes into foam cells, one of the earliest steps in the development of atherosclerosis. Reactive oxygen species can also directly promote LDL oxidation, stimulate vascular smooth muscle cell proliferation and migration(63). Reactive oxygen species also activate several matrix metalloproteinases, which contribute to atherosclerotic plaque instability and rupture, thereby precipitating acute cardiovascular events(64). Oberg *et al* demonstrated that biomarkers of oxidative stress (plasma protein carbonyl group content, plasma free F2-isoprostane content, plasma protein reduced thiol content) were significantly elevated in patients with chronic renal failure compared to healthy subjects(65). Haemodialysis (HD) may induce oxidative stress, primarily through membrane bio-incompatibility and endotoxin challenge. One study examining the relationship between haemodialysis and cardiovascular disease on serum malondialdehyde levels, a marker of oxidative stress found mean serum malondialdehyde levels were significantly elevated in dialysis

patients with cardiovascular disease(66). However to date no study exists examining the relationship between oxidative stress and long term outcome in ESRD patients.

A number of studies have addressed the issue of whether using antioxidant therapy would reduce the risk of cardiovascular events, both in patients at high risk of cardiovascular events in the general population and in ESRD. The Heart Protection Study randomised 20,536 UK adults (aged 40-80) with coronary disease, other occlusive arterial disease, or diabetes to receive antioxidant vitamin supplementation (600 mg vitamin E, 250 mg vitamin C, and 20 mg beta-carotene daily) or matching placebo. None of these interventions produced any significant reductions in the 5-year mortality from, or incidence of, any type of vascular disease, cancer, or other major outcome(67). Similarly in the Heart Outcomes Prevention Evaluation (HOPE) Study treatment with vitamin E had no apparent effect on cardiovascular outcomes(68). By contrast in the Cambridge Heart Antioxidant Study (CHAOS) in patients with angiographically proven coronary atherosclerosis, alpha-tocopherol (vitamin E) substantially reduced the rate of non-fatal myocardial infarction at one year(69). This study is out of keeping with the larger trials with longer follow up.

Interestingly in ESRD, alpha-tocopherol decreases LDL oxidation in ESRD patients. The benefit appears to be greater in patients on PD, which may be of clinical importance given the greater atherogenicity of lipid abnormalities in peritoneal dialysis(70). Building on this observation, the Secondary Prevention with Antioxidants of Cardiovascular disease in End stage renal disease study (SPACE), assigned haemodialysis patients with cardiovascular disease to high dose (800 IU/day) oral vitamin E or placebo. Vitamin E reduced the risk of the composite cardiovascular disease endpoints (myocardial infarction (fatal and non-fatal), ischaemic stroke,

peripheral vascular disease and unstable angina)(71). A similar large scale trial of antioxidant therapy as primary cardiovascular disease prevention is required in ESRD.

1.2.8 Uraemic cardiomyopathy as a cardiovascular risk factor

Cardiac abnormalities, specifically hypertrophy of the left ventricular wall, were first noted in ESRD by Richard Bright in 1827, where he postulated that it was caused by 'the unusual efforts to which the heart has been impelled'(72). The presence of uraemic cardiomyopathy is strongly associated with adverse outcome in patients will ESRD. Echocardiographic studies report three patterns of cardiomyopathy – left ventricular hypertrophy (LVH) and LV dilatation and left ventricular systolic dysfunction (LVSD) - that affect up to 90% of patients starting dialysis therapy(73). Each is associated with reduced survival compared with unaffected patients; effects that persist even after successful transplantation(74). Parfrey *et al* have demonstrated that the echocardiographic prevalence of LVH is 50-80%, with left ventricular dilatation in 20-40% and LVSD present in approximately 16% of patients in incident ESRD patients(75). When followed up over a 2 year period, these abnormalities were associated with a cumulatively poorer survival compared to those patients with normal ventricles after adjustment for other variables such as age, diabetes and ischaemic heart disease(76). Data from the USRDS registry has demonstrated similar findings with LVH a risk factor for poor outcome in incident dialysis patients(77). A schematic diagram of the potential relationships between ischaemic heart disease, sudden cardiac death and LVH is demonstrated in Figure 1.2.

LVH develops early in the course of chronic kidney disease and is associated with stiffening of the LV wall, a precursor to diastolic heart failure(78;79). The various permissive factors promoting LVH in uraemia will be discussed in detail in Chapter 5

but hypertension, anaemia, hyperparathyroidism and hypoalbuminaemia have all been associated with its development(80). Volume status is a key factor in determining blood pressure in ESRD and may make definition of LV chamber dimensions difficult(81).

Histological analysis of the heart in animal models of experimental renal failure shows that LVH is associated with an increase in cardiomyocyte volume(82). This, in turn, results in increased oxygen diffusion distance and subsequent cardiomyocyte ischaemia. Cardiomyocyte number may increase initially but subsequently is reduced(83). In sub-totally nephrectomised rats, reduced capillary density per volume of myocardium is seen(84). Similar findings have been demonstrated in post mortem specimens of patients with ESRD(85). It is likely that apoptosis is triggered and increased fibrosis follows progressive myocyte death(82). Electrophysiological abnormalities in calcium handling have been demonstrated with abnormal sarcoplasmic calcium uptake, and increased cytosolic calcium concentrations of calcium in diastole(86-88). These electrophysiological changes lower the threshold for arrhythmia and underpin the evolution of diastolic heart failure.

LVH may be considered an adaptive process in response to increased myocardial work due to pressure and volume overload. Pressure overload tends to cause increased wall thickness with an unchanged or reduced LV cavity diameter (concentric hypertrophy), whilst volume overload promotes LV dilatation, with additional changes in wall thickness as an adaptive mechanism to reduce wall stress(89;90). This phenomenon is called eccentric hypertrophy. Both eccentric and concentric remodelling are common in ESRD patients. These remodelling processes are initially beneficial as dilatation allows an increase in stroke volume without increased inotropy, as well as permitting maintenance of stroke volume and cardiac output in the presence of decreased

contractility(91). Physiological hypertrophy may additionally occur in response to increased cardiac work, and the increase in LV mass is appropriate to LV volume such as in highly conditioned athletes(92). However, in dialysis patients the degree of hypertrophy may be inappropriate for the given ventricular stresses, either volume or pressure load, both of which are dramatically elevated(76). This has led to the term 'inadequate hypertrophy', and LVH may be either a precursor to the evolution of cardiac failure or arrhythmia in ESRD patients(93).

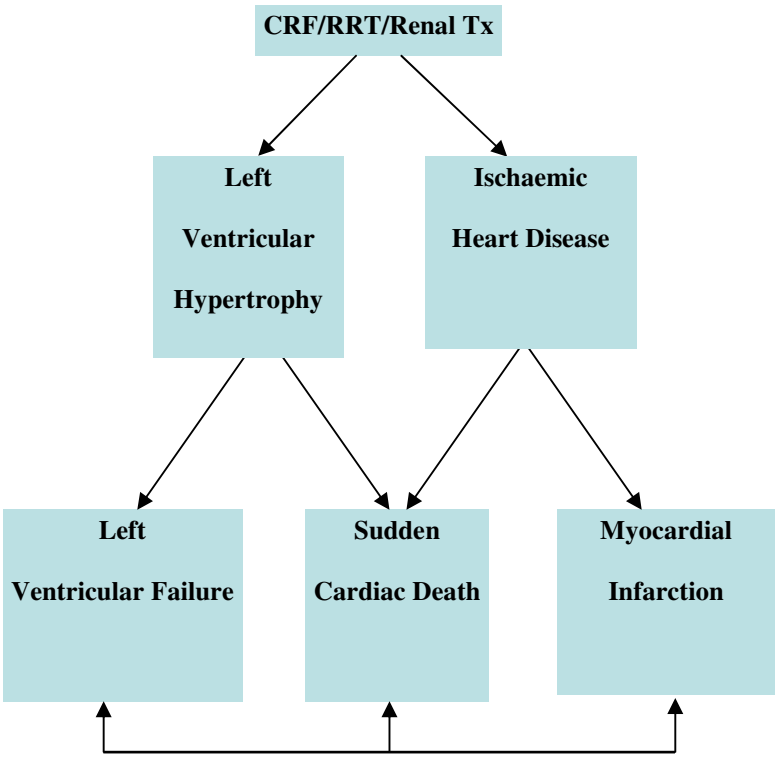


Figure 1.2 Schematic view of the relationship between ischaemic heart disease, LVH and sudden cardiac death in ESRD. Abbreviations CRF- chronic renal failure, RRT – renal replacement therapy, Tx - transplant

1.2.9 Sudden cardiac death

Sudden cardiac death is the cause of up to 60% of cardiovascular deaths in patients with ESRD. The risk of such an event increased with increasing time spent on renal replacement therapy. Although, high risk patients potentially can be identified- patients with LVH or LVSD, previous ischaemic heart disease or potentially using biomarkers such as brain natriuretic peptide (BNP) or troponin T(94), no proven strategy exists to reduce risk of this catastrophic event. Haemodialysis specific risks include pre-dialysis hyperkalaemia and intra-dialytic or post dialysis hypokalaemia. In a large retrospective analysis of dialysis related cardiac arrests (in centre up to 2 hours post dialysis), a low potassium dialysate (0 or 1 mmol/L) was identified as a risk factor for cardiac arrest. More cardiac arrests took place on Mondays, suggestive that fluid overload may also be a risk factor, after the 'long gap' of 2 days since the antecedent dialysis session(95). Evidence is emerging from patients with ischaemic heart disease and cardiac failure that implantable cardiac defibrillators, as a prophylaxis for sudden cardiac death, improve survival(96). Currently these are underused in the ESRD post cardiac arrest despite apparent association with improved survival(97). A prospective trial of implantable defibrillators for reduction of mortality in dialysis patients is warranted.

1.2.10 Haemodynamic and metabolic factors of CRF

Additional to electrolyte shifts related to dialysis, alterations in volume status and hence blood pressure, may cause haemodynamic instability in the dialysis patient. Intermittent haemodialysis leads to rapid changes in fluid and solute status. Hypotension in particular, is a common cardiovascular complication of haemodialysis, which may vary in severity from minor, if troublesome side-effects such as nausea or dizziness, through to more severe cardiac or cerebral ischaemia. The pathogenesis of haemodialysis related hypertension is multi-factorial. The initial trigger is hypotension as the patient is

connected to the dialysis circuit. The main determinants of blood pressure control, which are deranged in ESRD, are intravascular volume preservation, vasoconstriction leading to increased systemic vascular resistance and maintenance of cardiac output by appropriate inotropic and chronotropic response(98).

During haemodialysis, fluid is removed from the intravascular space by ultrafiltration. As intravascular pressure drops, fluid is drawn from the interstitial space in an attempt to maintain intravascular volume. This however is incomplete and limited by the relative increase in extravascular osmotic pressure. Blood volume decreases leading to hypotension(99). Factors influencing this include ultrafiltration rate(100), fluid status of the patient(101), dialysate sodium(102), dialysate buffer(103), temperature(104), venous compliance and food intake which may lead to splanchnic blood pooling(105).

The appropriate response to dialysis related hypovolaemia is normally to increase systemic vascular resistance, by constriction of precapillary vessels. However in ESRD this mechanism is blunted. Although autonomic neuropathy affecting efferent sympathetic pathways may contribute to this disturbance (primarily found in diabetic patients), the dialysis treatment itself is a greater factor for impaired vascular response in this setting. Haemodialysis appears to be associated with impaired vascular reactivity during hypovolaemia. The secretion of vasoconstrictive peptides such as catecholamines, vasopressin and renin is less pronounced with haemodialysis compared to say haemofiltration or isolated ultrafiltration(106). Other factors associated with vasodilatation related to haemodialysis include excess secretion of cytokines such as tumour necrosis factor-α and interleukin-1, perhaps related to the degree of biocompatibility of the dialysis membrane, although this remains controversial(107;108). The temperature of the dialysate in the extracorporeal circuit is

an additional feature influencing haemodynamic stability and control of systemic vascular resistance. Both the blood pressure response and vascular reactivity are improved with the use of cool (35-36^{0}C) compared to standard (37-38^{0}C) dialysate (104;109). This may either be due to reduced cutaneous vasodilatation, which may occur in a bid to reduce 'excessive' heat when warmer standard dialysate is used or the constrictive effect of blood vessels to maintain a constant core temperature when a cooler dialysate is used.

Maintenance of cardiac output in response to hypovolaemia related to dialysis is mainly dependent on pre-existing cardiac function which is discussed elsewhere. Left ventricular diastolic or systolic dysfunction where present impair the heart's ability to maintain cardiac output. Other factors such as autonomic neuropathy and drug therapy with beta-blockade may impair the chronotropic response. Cardiac function may paradoxically improve during dialysis due to a combination of fluid removal reducing after load, the removal of postulated cardiodepressor uraemic toxins (or acidosis) or due to the use of increased dialysate calcium which has a positive inotropic effect(110;111). Factors specific to dialysis which may reduce cardiac output include impaired ventricular filling due to reduced venous return. The presence of the arteriovenous fistula used for dialysis access causes increased cardiac output, promoting LVH and may lead to high output cardiac failure(112).

For the period when the patient is not on dialysis therapy, the relationship between volume status, blood pressure and systemic vascular response is complex with debate about the relative importance of increased peripheral resistance and increased intravascular volume on the development of hypertension in ESRD. In one pilot study from our group, cardiac output was lower in haemodialysis patients than in a small

group of normal subjects, because of a lower stroke volume in the dialysis group(113). As the blood pressure was the same, calculated peripheral resistance was thus higher in the dialysis group compared with controls. In the dialysis group there is a very convincing relationship between end diastolic volume (a reflection of intravascular volume), cardiac output and blood pressure. No such relationship was found between calculated peripheral resistance and any blood pressure variable, suggesting that intravascular volume plays a larger role than increased vascular resistance in the determination of blood pressure in patients on haemodialysis, or is at least the most variable factor. This is consistent with the effects of reduction of intravascular volume ('dry weight') and improved blood pressure control on dialysis(114;115). Although there was no correlation between systemic vascular resistance and blood pressure parameters, systemic vascular resistance was higher in haemodialysis patients. Thus, for a given cardiac output, the achieved blood pressure is substantially greater in haemodialysis patients than in controls. Whether this increase in systemic vascular resistance is a functional consequence of increased vascular tone or of structural changes in the vessel wall is unclear, although it is clearly important as vasodilators are likely to be effective against the former, whereas reversal of strategies structural changes (e.g. associated with calcification) are likely to be more difficult.

1.2.11 Proteinuria

Microalbuminuria (30 to 300mg/day) was first identified as risk factor for cardiovascular disease in patients with diabetes in 1984(116). Subsequently, data from the Framingham studies have shown that proteinuria is a risk factor for cardiovascular mortality in the general population(117). Similar findings have emerged from other large epidemiological studies that either proteinuria or microalbuminuria are not only predictive of progression of renal disease but also of future cardiovascular events(118).

Analyses from the Prevention of Vascular and End Stage Renal Disease (PREVEND) study shows clearly that urinary albumin acts as a continuous risk factor for cardiovascular events with no lower limit(119). Microalbuminuria and proteinuria have been accepted to represent a consequence of renal damage. However, Deckert *et al* proposed in 1989 that albumin leakage is a reflection of widespread vascular damage, with the kidney as a 'window' to the vasculature(120). This hypothesis has been reinforced by studies demonstrating that microalbuminuria is associated with changes in endothelial dysfunction in patients with diabetes(121). The Irbesartan in Patients with Type 2 Diabetes and Microalbuminuria (IRMA-2) study, evaluating the effect of the angiotensin II antagonist irbesartan, demonstrated that reduction of albuminuria in microalbuminuric hypertensive patients with type II diabetes was associated with renal protection and some degree of cardiovascular protection although this study was not powered to address the effect on cardiovascular events(122). From the standpoint of the patient with ESRD, as albuminuria or proteinuria are both markers of cardiovascular risk and of progression of renal disease towards ESRD, it seems likely that proteinuria is a cardiovascular risk factor, which whilst present in some patients in the general population, is grossly overrepresented in patients at the onset of dialysis therapy.

1.2.12 Anaemia

The presence of anaemia, secondary to erythropoietin deficiency is an almost universal finding in patients with ESRD. This is strongly associated with increased mortality and morbidity in patients on maintenance haemodialysis(123). Anaemia has been consistently linked with the development and progression of cardiac structural changes in patients with chronic renal failure. In patients with chronic renal failure, haemoglobin starts to fall as glomerular filtration rate drops to between 25 and 50 ml/min and Levin *et al* demonstrated that in patients with advanced pre-dialysis renal failure each 0.5g/dL

fall in haemoglobin was associated with a increased LV mass(124). However, the outcome of studies directed at haemoglobin correction, either to reduce progression of LVH or to reduce cardiovascular events have generally been associated with increased mortality in patients randomised to achieve a higher haemoglobin with erythropoietin therapy(125-128). Lower haemoglobin is likely to be found in 'sicker' patients with more evidence of inflammation and malnutrition, and therefore it is not surprising that anaemia is associated with increased mortality. What is less clear is what the target haemoglobin should be in dialysis patients, and the optimum time to commence correctional therapy with erythropoietin. The relationship between haemoglobin and uraemic cardiomyopathy and outcome is further discussed in Chapter 5.

1.2.13 Homocysteine

Homocysteine is a sulphur containing amino acid, formed during the conversion of methionine to cysteine. The concept of homocysteine being atherogenic arose from the observation that patients with homocystinuria, an autosomal recessive condition, characterised by the accumulation of homocysteine experienced premature cardiovascular death. Epidemiological studies have demonstrated an association between hyperhomocysteinaemia and atherosclerotic vascular disease. Normal metabolism of homocysteine is either by transsulfuration to cysteine (vitamin B6 dependent) or remethylation to methionine (vitamin B12 and folate dependent). In renal failure it is suggested that remethylation is impaired leading to accumulation of homocysteine. Hyperhomocysteinaemia has been associated with increased risk of cardiovascular events in patients with ESRD in a number of observational studies(129-131).

One large trial of B vitamin (2.5 mg of folic acid, 50 mg of vitamin B6, and 1 mg of vitamin B12) supplementation in the general population to reduce cardiovascular events was disappointing, with no significant reduction in risk(132). In dialysis patients, despite evidence that reduction of serum homocysteine levels can be achieved with folic acid, randomised controlled trials have demonstrated that folic acid supplementation in ESRD is not associated with reduction of cardiovascular events(133) or a reduction in atheroma progression(134). Indeed, further analysis have suggested paradoxically that some patients with higher homocysteine levels are at reduced risk(133;135) forwarding the hypothesis, that homocysteine levels may be a nutritional marker in dialysis patients in the absence of inflammation, a frequent confounder of other markers of interpretation of nutritional status (albumin) in ESRD. This represents a further example of the 'reverse epidemiology' (akin to cholesterol) of cardiovascular risk factors where conventional cardiovascular risk factors in the general population have the reverse relationship with outcome in the ESRD population.

1.2.14 Hyperparathyroidism, calcium-phosphate metabolism and arterial calcification

Hyperparathyroidism is one of the hallmarks of chronic renal failure and is a result of chronic hyperphosphataemia and hypocalcaemia. Secondary and tertiary hyperparathyroidism result in the spectrum of renal bone disease including osteitis fibrosa cystica and mixed renal osteodystrophy. Observational studies demonstrate that as well as being a risk factor for fractures (136), raised serum PTH levels are a risk factor for mortality in dialysis patients(137;138). It is now recognised that parathyroid hormone (PTH) has potentially negative cardiovascular effects. In animal models of uraemia PTH has been demonstrated to promote cardiac interstitial fibrosis and arteriolar thickening(139). PTH has also been shown to stimulate cardiac accumulation

of calcium in myocytes leading to scarring(140). The role of PTH in promoting LVH is further discussed in Chapter 5.

Like hyperparathyroidism, hyperphosphataemia is almost inevitable in patients with ESRD and registry data supports that even mild hyperphosphataemia is an independent predictor of mortality in dialysis patients(137;138;141). Although poor phosphate control may be a reflection of under dialysis, it has become clear that phosphorus retention promotes vascular calcification, perhaps by inducing transformation of vascular smooth muscle cells into an osteoblast-like phenotype(142). Total body calcium or calcium phosphate product as well as PTH have been recognised to be the other key determinants of vascular calcification. Other clinical factors consistently associated with the development of vascular calcification include age, duration on renal replacement therapy, PTH, homocysteine and degree of inflammation(143-147).

Although patients with ESRD, if left untreated, would be calcium deficient due to failure of synthesis of 1,25-dihydroxyvitamin D_3, the combination of treatment with vitamin D analogues to suppress PTH, combined with calcium containing phosphate binders may lead to overall normalised or high body calcium levels. The presence and degree of coronary artery calcification, demonstrated in young adult dialysis patients by electron beam computed tomography, has been associated with ingestion of large amounts of calcium containing phosphate binders and a greater elevation in the calcium-phosphate product(144). Until fairly recently, options for controlling phosphate with phosphate binding medication were limited to compounds containing either calcium or aluminium. The non-calcium containing binder sevelamer hydrochloride has been demonstrated to be associated with a lesser degree of progression of coronary artery calcification in patients new to haemodialysis therapy, compared to those taking

calcium containing phosphate binders(143). It has been suggested that this reduction in vascular calcification using non calcium containing phosphate binders may translate into a survival benefit for these patients(148).

1.2.15 Inflammation and C-Reactive protein

Chronic inflammation is indicated by raised serum C-reactive protein (CRP), a hepatic derived acute phase protein. CRP is raised in response to a number of conditions where inflammation may be present including infection, trauma, infarction and malignancy. The acute phase response is driven by cytokines including interleukin-1, interleukin-6, interferon-gamma, tumour necrosis factor-α, and transforming growth factor-β. In healthy individuals elevated CRP is a risk factor for future coronary events(149;150) as well as in patients with diabetes(151), whilst in patients with acute myocardial infarction, CRP predicts mortality within the first 30 days post infarct (152). Whether CRP is simply an additional marker for established coronary risk factors, such as dyslipidaemia or perhaps the inflammatory nature of the high risk atherosclerotic coronary plaque, or represents a further additive risk factor is less clear. The is no doubt that raised CRP correlates with the cluster of atherogenic risk factors found in the metabolic syndrome(153). Supporting the notion that CRP is directly atherogenic, *in vitro* studies have demonstrated the presence of CRP in atherosclerotic coronary plaque(154), whilst CRP and matrix metalloproteinase co-localise in the endothelial layer and macrophage-rich areas in atherosclerotic plaques suggesting that CRP has a role in rupture of the 'vulnerable' plaque(155). Finally, statins have been demonstrated to lower serum CRP, with greatest clinical benefit derived in patients with a larger magnitude of CRP reduction in response to therapy(156).

Patients with ESRD tend to have higher CRP levels, and this is predictive of cardiovascular outcome in both haemodialysis and peritoneal dialysis patients(157;158)whilst higher levels interleukin-6 have also been associated with increased mortality(159). Possible explanations for elevated CRP in ESRD include direct inflammatory effects of uraemia, chronic infection of vascular access, the presence of indwelling peritoneal or vascular dialysis catheters, bio-incompatibility of haemodialysis membranes or peritoneal dialysis fluids or impurities of water supply used for dialysis.

The association between atherosclerosis and inflammation (together with associated poor nutritional status) in dialysis patients have given rise to the hypothesis of the so called malnutrition, inflammation, and atherosclerosis (MIA) syndrome(160). If truly present this relationship may explain the cholesterol paradox in ESRD patients. One intriguing study addressing this issue performed by Liu and colleagues followed up 823 chronic haemodialysis patients for a median of 2.4 years, and categorised the study population into 2 subgroups based on the presence or absence of inflammation and/or malnutrition (defined as achievement of specified cut-offs for any of serum albumin, CRP and interleukin-6)(35). Overall, 77% of the patients had evidence of inflammation/malnutrition, which was associated with older age, increased co-morbidity, prevalent CV disease, diabetes, and lower total cholesterol levels. A U-shaped association between cholesterol levels and mortality was seen in the overall cohort and in the subgroup with inflammation/malnutrition. However, in the absence of inflammation/malnutrition, total cholesterol was positively associated with all-cause mortality and even more strongly associated with CV mortality. These results give insights into novel methods to address the frequently perplexing issue of the reverse epidemiology of cardiovascular disease in patients with renal failure.

1.3 Assessment of uraemic cardiomyopathy with echocardiography

Echocardiography involves the application of ultrasound in the frequency range of 2 to 10 MHz. The technique requires an ultrasound transducer consisting of a series of piezoelectric crystals, that when electrically activated, emit ultrasound energy. Returning ultrasound energy is converted by the piezoelectric crystal into radiofrequency energy which is processed to digital information by high-speed by digital converters within the ultrasound platform.

The simplest image is the M-mode echocardiogram. An M-mode echocardiogram is acquired by interrogating returning ultrasound signals along a single plane with the images generated recorded on a scrolling video screen. The operator can select this plane to obtain an M-mode image. This plane line can be swept through an area of cardiac anatomy and the image then displayed as a series of concurrent M-mode images. M-mode echocardiography was once the foundation of anatomical diagnosis but has been to an extent abandoned in favour of 90-degree two-dimensional scanning. However, M-mode does confer higher temporal resolution than two-dimensional echocardiography and may provide a higher degree of resolution than two-dimensional scanning. In general, however, its clinical role, as a stand-alone technique, has been largely supplanted by two-dimensional echocardiography. It remains widely used as a method for estimation of LV mass, both for clinical practise as well as in interventional studies. Conventionally “cube” methods, which using M-mode septal, posterior wall, and left ventricular internal dimensions have been used to measure LV mass. These methods assume normal ventricular geometry. More recently, several two-dimensional methods have been shown to provide enhanced accuracy, especially in abnormally shaped ventricles(161).

Echocardiography provides an excellent method for quantification of ventricular function. Linear measurements such as LV wall thickness, internal chamber dimensions, and derived parameters such as fractional shortening traditionally have been obtained from M-mode echocardiography. Normal values for adults are well established(162-164). Global ventricular function and cardiac volumes can be measured with a variety of methods using two-dimensional echocardiography, with Simpson's rule being the most commonly used for quantification of ventricular volume. Determination of left ventricular volume requires manual tracings of the endocardial border in diastole and systole. Once the chamber volumes have been determined, ejection fraction can be calculated. Echocardiographic methods will be discussed in greater detail in Chapters 2 and 4.

Echocardiographic abnormalities have been described in patients with ESRD as early as the 1970s. Initially, as uraemic pericarditis was one of the cardinal features of advanced presentation of ESRD, echocardiography was a key tool in the diagnosis and management of haemorrhagic pericardial effusions. However, abnormal thickening of the LV wall as well as valvular calcification, were soon demonstrated echocardiographically(165) and the prognostic implications of these findings emerged with the expansion of dialysis programmes. Studies from the Canadian dialysis population have demonstrated the importance of LV abnormalities as discussed previously(73;76). However, as echocardiographic measurement of LV mass and function is dependent on LV chamber dimensions, care must be taken in assessing LV structure and performance in patients with ESRD. It is well-established that changes in volume status in these patients during both the inter- and intra-dialytic period result in substantial changes in LV chamber dimensions(81;166), with any potential error in echocardiographic measures being amplified by the assumptions of cubed formulae.

Additionally changes in volume status affect both pre- and after-load. To what extent the changing loading conditions contribute to a change in LV function parameters is difficult to determine. A lower ejection fraction after dialysis may result from a decrease in contractility either *de novo* or in response to myocardial ischaemia invoked by the haemodynamic demands of dialysis, but also from a decreased preload, an increased after load or a combination thereof.

A 'volume-independent' assessment of LV dimensions in ESRD is an extremely attractive concept. Newer echocardiographic techniques such as 3-dimensional echocardiography may be promising but are untested in this population. Whilst LV chamber dimensions remain subject to the hydration status of the patient regardless of imaging method, by using a high resolution method such as cardiac magnetic resonance imaging (CMR) to delineate myocardial dimensions, the presence of structural cardiovascular disease may be more accurately defined in this population.

1.4 Cardiovascular magnetic resonance imaging

Rapid progress has been made with CMR over the past decade, and this has become established as a reliable and clinically important technique for assessment of cardiac structure and function. Furthermore, by using contrast based techniques, myocardial perfusion and viability can be assessed. CMR is non-invasive and avoids the use of ionising radiation. Progress in CMR technology may further widen its clinical application in coronary artery imaging, assessment of valvular heart disease and identification of vulnerable atheromatous coronary plaque.

1.4.1 Principles of cardiac magnetic resonance imaging

CMR (and all magnetic resonance imaging) is based on the principle of nuclear magnetic resonance. Images are generated from signals produced by protons (hydrogen nuclei). The human body consists of mainly of water, an abundant source of hydrogen ions. The proton behaves like a small magnet when placed in a magnetic field, aligning itself with the field and precessing with a frequency that depends on the magnetic field strength. Protons align parallel and anti-parallel to the direction of the primary field, with a small excess of parallel protons that gives rise to a net magnetisation vector. This net vector can be rotated by application of a second temporary radiofrequency (RF) pulse. Once this pulse ceases, the magnetisation vector recovers to its former position, releasing a signal in the form of radio waves. This relaxation of the net vector is attributable to two distinct but simultaneous processes, longitudinal (T1) and the transverse (T2) relaxations(167;168) A pulse sequence consists of a series of RF pulses of varying duration or strength and application of magnetic-field gradients that are adjusted to highlight desired tissue characteristics.

Basic pulse sequences used in CMR are spin-echo and gradient-echo sequences. Spin-echo sequences are often used for assessment of cardiac morphology, and flowing blood normally appears black. Gradient-echo sequences have a lower soft-tissue contrast compared with spin-echo, and flowing blood is represented by high signal intensity and turbulence as areas of signal void. Gradient-echo sequences are used to assess valvular lesions, shunts, and great vessels as well as for measurement of ventricular function and wall-motion characteristics. Flowing blood across a magnetic gradient can be encoded to quantify flow. These phase-encoded velocity maps are based on the principle that magnetic vectors of flowing protons acquire a phase shift that is proportional to flow

velocity. This technique is used to assess flow volumes and velocity profiles across valves and shunts.

Cardiac and respiratory motion artefact can cause difficulty acquiring CMR images. Using breath-hold electrocardiograph-gated techniques to acquire images minimises such artefacts. The presence of cardiac arrhythmias such as atrial fibrillation may interfere with synchronised data acquisition.

1.4.2 Cardiac magnetic resonance imaging for assessment of cardiac function

Unlike echocardiography, where LV mass and dimensions are derived from one- or two-dimensional data, using assumptions of cardiac geometry, with CMR, actual myocardial mass and volumes are obtained. A Simpson's rule algorithm is still used, but acquired using three-dimensional data from a series of thin slices with no geometric assumptions. Therefore, CMR has become established as the 'gold standard' non-invasive reference standard for cardiac function and mass. Data are acquired using bright blood gradient-echo sequences, obtained during a 15- to 20-s breath-hold, are used to cover the entire left ventricle (LV) with short-axis views from the mitral plane and slice thickness of 10 mm. In these studies a TrueFISP (fast imaging with steady-state precession) sequence is used. Individual CMR scanner manufacturers have their own nomenclature for the various imaging sequences, with TrueFISP being that used for this pulsed gradient-echo sequence by Siemens (Erlangan, Germany). The actual CMR protocol used is described in detail in Chapter 2.

Using these techniques, published normal reference ranges for cardiac dimensions and function have been generated(169). As CMR sequences evolve, it is important to use the appropriate reference range for the sequence used to acquire the data as small but

statistically significant differences in LV dimensions have been described between images acquired of the same patients with TrueFISP and FLASH (fast low-angle shot), an alternative gradient-echo sequence(170).

1.4.3 Gadolinium based contrast agents for cardiac magnetic resonance imaging

All the currently approved CMR contrast agents use gadolinium which has paramagnetic properties. Gadolinium based contrast acts as an extracellular contrast agent with a 'fast wash in/slow wash out' characteristics. Gadolinium itself is toxic, and therefore is chelated to a variety of molecules depending on the agent used. This chelate has some effect on the pharmacokinetics of the agent and in the case of Gd-BOPTA (Multihance), further changes the paramagnetic properties of the product. The molecular weights of these agents range between 650 and 2,000 Daltons and their mechanism of action is to shorten T1 relaxation of tissue within the magnetic field.

During the first pass after rapid intravenous injection of contrast, extracellular agents diffuse into the interstitium of the tissues, except in the brain and testes. Approximately 40% of intravenously injected extracellular agents diffuse from the blood into the interstitium during the first pass(171). By tracking a bolus as it transits through a region of interest, time-intensity curves can be generated and used to determine myocardial perfusion. In the presence of altered interstitial tissue architecture, e.g. due to oedema, necrosis, fibrosis or inflammation (or any combination of these factors), passage of the gadolinium chelate will be delayed through the myocardial tissue. This will be indicated by the presence of late gadolinium enhancement (LGE), when this tissue is imaged, typically achieved approximately 5 minutes after bolus injection. Over a period typically lasting several minutes, the agent diffuses back into the blood pool, from which it is excreted by the kidneys.

Gadolinium based agents currently in use for cardiac imaging include Omniscan (gadodiamide, GE Healthcare, UK), Magnevist (gadopentetate dimeglumine, Gadovist, Schering AG, Berlin, Germany), MultiHance (gadobenate dimeglumine, Bracco, Bracco S.p.A., Milan, Italy), Gadovist (gadobutrol, Schering AG, Berlin, Germany), OptiMARK (gadoversetamide, Tyco Healthcare, Hazelwood, Minnesota, United States) and ProHance (gadoteridol, Bracco, Princeton, New Jersey, United States).

1.4.4 Cardiac magnetic imaging for assessment of ischaemic heart disease

CMR is an excellent technique for defining the presence and extent of both acute and chronic myocardial infarction. Like echocardiography, wall motion abnormalities may be present on cine images, suggestive of ischaemic myocardial damage. On first-pass perfusion images with contrast, an area of hypo-enhancement is seen within the infarcted region, correlating with microvascular obstruction inside the infarct zone (172), Figure 1.3. In the presence of chronic myocardial infarction the presence of LGE 5 to 30 min after contrast injection is seen using a turbo FLASH inversion-recovery sequence, Figure 1.4. In acute infarction, LGE reflects myocardial necrosis and abnormal contrast molecule kinetics within infarcts with associated tissue oedema, whereas in chronic infarction, it reflects increased concentration of gadolinium non-viable, infarcted scar tissue(173;174). Combining the extent of LGE, may indicate the presence of viable 'hibernating' myocardium. A myocardial segment with a wall motion abnormality and no LGE may represent myocardial hibernation which may recover with revascularisation, whilst non viable myocardium typically has >75% thickness of tissue demonstrating LGE(175).

Coronary angiography with CMR is an attractive concept and may yet become a non-invasive diagnostic method. Currently, the small size of coronary arteries, their complex

course, and constant motion with cardiac contraction and respiration has made angiography technically difficult. CMR however can evaluate coronary blood flow of myocardial perfusion with first-pass contrast enhanced imaging before and after pharmacologically induced hyperaemia, usually with adenosine. This method has a have high diagnostic accuracy for detection of coronary artery disease with myocardial perfusion reserve having a sensitivity, specificity, and diagnostic accuracy of 90%, 83%, and 87%, respectively, for detection of a >75%, coronary artery stenosis(176). This technique clearly has great potential for non invasive diagnosis of coronary artery disease.

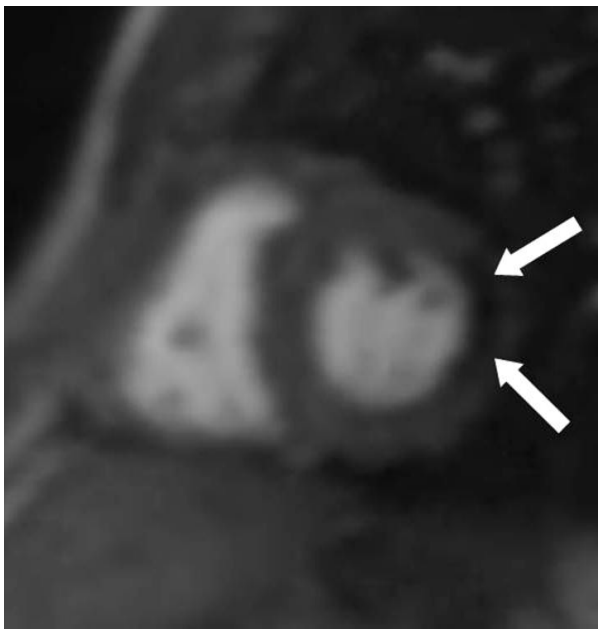


Figure 1.3

First pass imaging demonstrating an area of hypoenhancemnt (arrowed) in keeping with a myocardial perfusion deficit in a patient with a recent lateral myocardial infarction

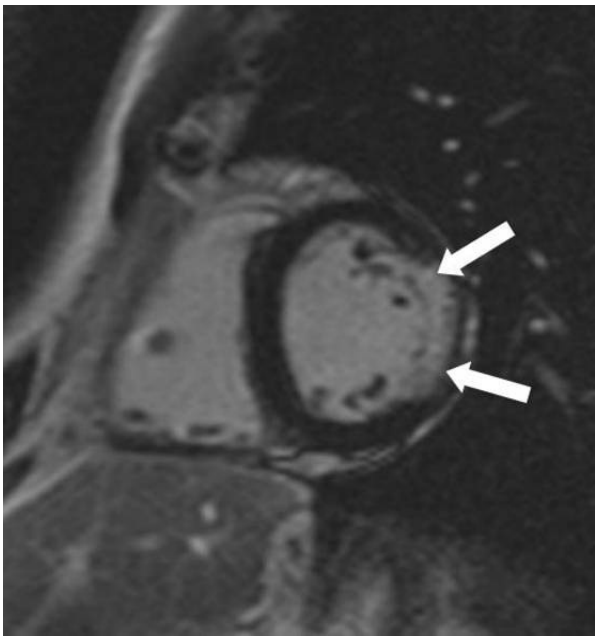


Figure 1.4

Late gadolinium enhancement representing infarcted myocardial tissue in the lateral wall of the left ventricle in the same patient as Figure 1.3

1.4.5 Cardiac magnetic resonance imaging for assessment of cardiomyopathy

CMR allows accurate definition of cardiac structure, function and geometry in the presence of cardiomyopathy. Furthermore, the ability of contrast enhanced CMR to detect areas of tissue fibrosis has allowed detailed study of myocardial composition in a number of cardiomyopathies characterised by the presence of fibrotic tissue (usually collagen) within the myocardium. To date there has been extremely limited study of uraemic cardiomyopathy with CMR, with one small study with CMR from our own group describing LVH morphologically in haemodialysis patients. Other cardiomyopathies have been analysed in more detail. Hypertrophic cardiomyopathy has been intensively studied with CMR (Figure 1.5) and CMR allows both demonstration of the degree of hypertrophy, the phenotypic expression of the pathological fibrosis(177), degree of left ventricular outflow tract obstruction, systolic anterior motion of the mitral valve and can monitor after surgical and pharmacologic interventions(178) or alcohol septal artery ablation(179).

Other cardiomyopathies involving myocardial fibrosis where CMR may be useful in either the diagnosis or long term management include arrhythmogenic right-ventricular cardiomyopathy and dilated cardiomyopathy. In arrhythmogenic right-ventricular cardiomyopathy CMR can demonstrate fatty infiltration of the right ventricle and by detecting characteristic high signal intensity of fat on spin-echo images within the right ventricular myocardium, the presence of fat in the right ventricular wall can be demonstrated and then confirmed with fat suppression techniques. Diffuse and focal thinning and local aneurysms of the right ventricle can be also be detected (180;181). Areas of myocardial fibrosis have been described in dilated cardiomyopathy, and CMR may be a useful method of discriminating dilated from ischaemic cardiomyopathy in patients with heart failure of unknown aetiology(182).

Inflammatory and infiltrative cardiomyopathies have also been described using CMR. In sarcoidosis, infiltrates in myocardium can be are seen as areas of amplified T2 signal intensity (attributable to inflammation, oedema, and granulomas), which enhance after administration of gadolinium. CMR can non-invasively assess response to steroid treatment(183). In acute myocarditis contrast enhancement is present in approximately 90% of patients and correlates with active inflammation defined by histopathology (Figure 1.6)(184). In cardiac amyloidosis, CMR initially allows accurate demonstration of the presence of thickening of the right atrial and right ventricular wall. Following administration of gadolinium in patients with cardiac amyloid, CMR shows a characteristic pattern of global subendocardial late enhancement coupled with abnormal myocardial and blood-pool gadolinium kinetics(185). Additionally, endomyocardial fibrosis (also termed Loeffler's endocarditis), which is characterised by extensive subendocardial fibrosis, apical thrombus formation, and progressive diastolic dysfunction can be quantified using CMR(186).

Recently, the ferromagnetic properties attributable to the deposition of iron in myocardial tissue have been exploited to initially assess the degree of iron overload in the heart of patients with thalassaemia. Myocardial iron loading is assessed with the use of myocardial T2* relaxation characteristics at CMR. This approach has subsequently been used to guide iron chelation therapy in these patients and myocardial T2* has been used as an end point in a clinical trial demonstrating superiority of combination chelation treatment with deferiprone and deferoxamine in comparison to deferoxamine monotherapy, in thalassaemia major patients with mild to moderate cardiac iron loading(187).

1.4.6 Contraindications to cardiac magnetic resonance imaging

Patients with claustrophobia may not tolerate study with CMR. The presence of a cardiac pacemaker or implantable cardiac defibrillator remains an absolute contraindication to study with CMR. There are some reports of patients with pacemakers undergoing CMR without adverse outcome, but this should be only be carried out in a controlled environment, with facilities for both close monitoring and reprogramming of the pacemaker immediately available, and for the purpose of general clinical and research use, a pacemaker remains an absolute contraindication to scanning(188). The presence of intraocular metallic shards (e.g. welders) and intra-cerebral clips or shunts are also contraindications to scanning. Metallic implants can lead to artefacts, which might degrade images. Most cardiac prosthetic valves and coronary stents presently in use are safe for CMR at available clinical field strengths.

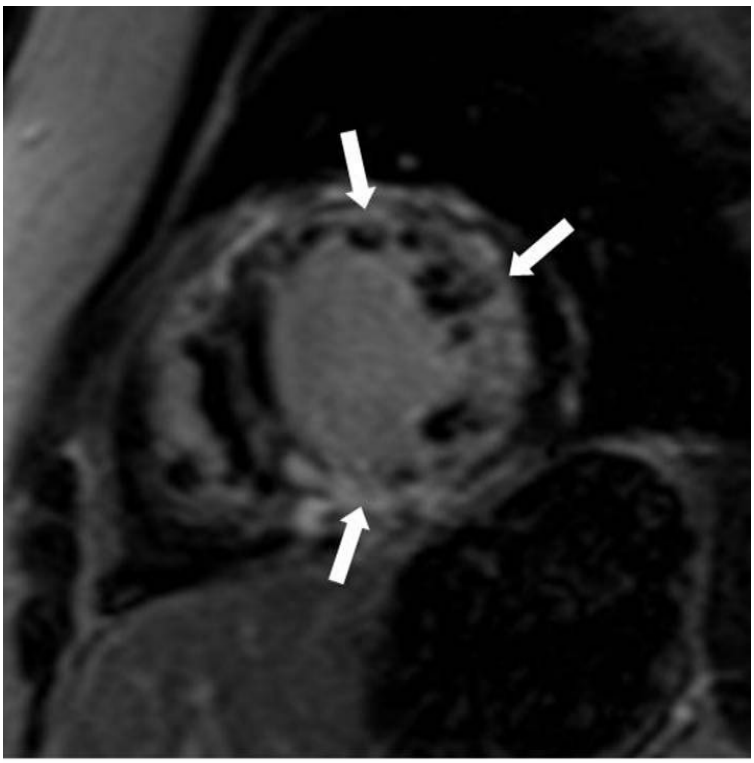


Figure 1.5

Extensive chaotic myocardial collagen deposition indicated by late gadolinium enhancement in a patient with hypertrophic cardiomyopathy

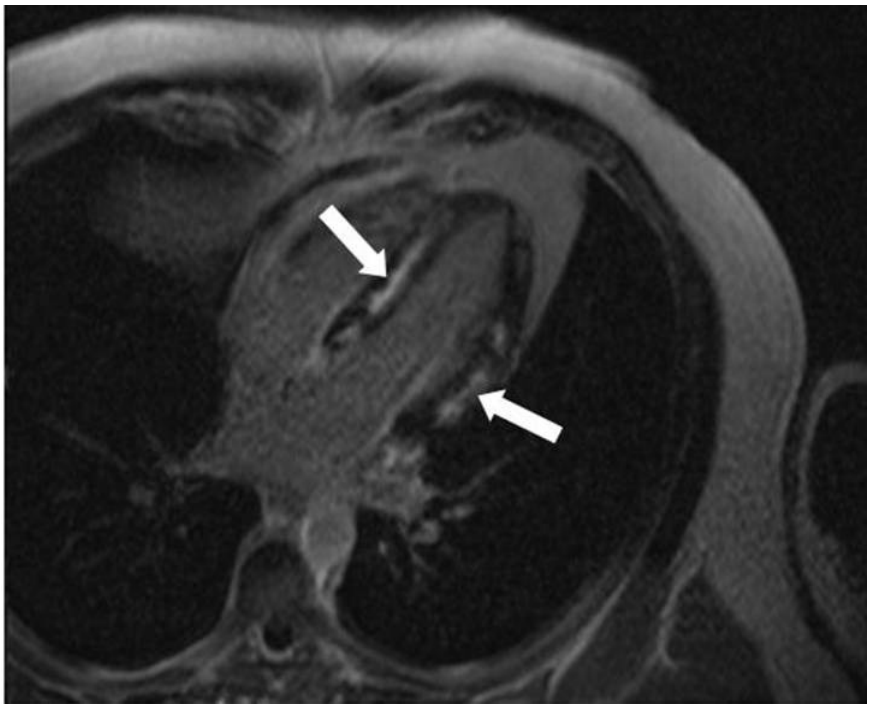


Figure 1.6

Patchy late gadolinium enhancement demon demonstrated in both the septum and lateral walls of the left ventricle in a patient with acute myocarditis

1.5 Redefinition of uraemic cardiomyopathy with cardiovascular magnetic resonance imaging

Despite the dramatically increased cardiovascular risk in ESRD, the relationship between risk factors, interventions and outcome is unclear. One of the few factors to have consistently been demonstrated to indicate poor outcome is uraemic cardiomyopathy, defined by echocardiography. However, as discussed, it is possible that some echocardiographic abnormalities widely present in ESRD, such as LVH may be at best overestimated, as suggested by the one previous report using CMR, or even artefactual(189). Although a variety of myocardial tissue abnormalities have been described either in animal models of uraemia or in post mortem studies of ESRD patients, no method has been able to non invasively describe this histopathological finding *in vivo*. The previous studies of other cardiomyopathies, with some similar histological correlates suggest that CMR has the potential to perform this function. Furthermore, very few CMR studies have been able to assess the long term implications of either morphological ventricular abnormalities or whether the presence of LGE confers increased risk of cardiovascular events or mortality.

All these factors suggest that there would be benefits from studying a cohort of patients with ESRD using CMR. Hopefully, by more accurately characterising cardiac abnormalities in this patient group, novel therapeutic targets for intervention may be identifies. The relationship between ischaemic heart disease and uraemic heart disease is imprecise. If systolic dysfunction, is due primarily to uraemia, then increasing doses of dialysis may be beneficial, on the assumption that myocardial contractility is suppressed by some (potentially undiscovered or least not yet causally linked) ‘uraemic factor’. Renal transplantation is a definitive treatment for uraemia, but unfortunately the current supply of cadaveric organs is outstripped by demand. On the other hand, if as in the

general population, ischaemic heart disease is the leading cause of systolic dysfunction, then evidenced based medical therapy with inhibitors of the renin angiotensin system and beta blockade would be an appropriate strategy. The question of lipid lowering therapy remains unresolved, but requires further study. Many of these agents are currently underused in the ESRD population, and often poorly tolerated.

One further intriguing notion would be to predict the patients at highest risk of sudden cardiovascular death. The recent finding that the presence of myocardial fibrosis demonstrated by CMR was associated with increased risk of ventricular arrhythmia in patients with dilated cardiomyopathy(190), suggests that CMR may have a role in risk stratification for such patients. The same may apply in ESRD, with the highest risk patients being candidates for implantable cardiac defibrillators.

Finally as CMR technology evolves, new methods for imaging and analysis emerge, dependent on the clinical needs of the cohorts of patients examined. Often these techniques may be applicable, across a wider range of patients than previously envisaged. As a unique group of patients at very high cardiovascular risk, patients with ESRD may have distinct differences in both cardiac and vascular function, allowing insights into factors responsible for the pathogenesis of cardiovascular disease in the general population. Abnormalities in these factors which are severely distorted in ESRD, such as arterial stiffness, may be expressed more subtly in the general population. Studying a cohort of ESRD patients may allow novel CMR methods to be developed to investigate cardiac and vascular markers of cardiovascular risk, to the advantage of research into cardiovascular disease in the general population.

1.6 Aims of this project

The principal aims of this project were:

- To establish the presence and prevalence of left ventricular abnormalities ('uraemic cardiomyopathy'), in a cohort of patients with ESRD using CMR
- To establish the relationship between uraemic cardiomyopathy and conventional cardiovascular risk factors
- Establish the prognostic impact of the presence of uraemic cardiomyopathy described by CMR

Secondary aims:

- To compare conventional methods of defining uraemic cardiomyopathy such as echocardiography with CMR
- To examine whether vascular function could be assessed using CMR
- To determine methods of identifying patients with uraemic cardiomyopathy, in particular left ventricular hypertrophy using the conventional method of echocardiography

1.6.1 Hypothesis

The studies performed are designed to test the hypothesis that specific left ventricular and vascular abnormalities can be identified in patients with ESRD and that these abnormalities have an influence on long term survival of these patients.

1.7 Outline of the studies contained within this thesis

Seven principal studies were performed and are detailed in Chapters 3-9:

Chapter 3 : A study of ventricular dimensions and function in uraemia with contrast enhanced cardiac magnetic resonance imaging.

Chapter 4 : A comparative study of echocardiography and electrocardiography with cardiac magnetic resonance imaging.

Chapter 5 : A study of the determinants of uraemic cardiomyopathy.

Chapter 6 : Survival of patients undergoing cardiovascular screening for renal transplantation.

Chapter 7 : A study of the diagnostic utility of brain natriuretic peptide in end stage renal disease.

Chapter 8 : A study of vascular function with cardiovascular magnetic resonance imaging in end stage renal disease.

Chapter 9 : A study of dermatological complications of contrast enhanced magnetic resonance imaging.

Chapter 2

Materials and Methods

2.1 Introduction

The principal technique used for this thesis was cardiovascular magnetic resonance imaging (CMR). This was supplemented with electrocardiography (ECG), echocardiography and exercise tolerance testing. In this chapter the background, methods, apparatus and protocols used for these techniques will be outlined.

2.2 Ethics Committee approval

The studies performed in this thesis were approved by the North Glasgow Hospitals University NHS Trust Ethics Committee. All subjects gave written informed consent, the forms being approved by the Ethics Committee.

2.2.1 Subjects

Patients with advanced renal failure were recruited from the renal transplant assessment clinic at the Western Infirmary. At inception of the study, patients attending the renal transplant assessment clinic were required to be established on dialysis therapy. The study extended to those patients anticipated to commence dialysis therapy within six months in keeping with changes in British Transplant Society recommendations regarding eligibility for cadaveric renal transplantation. Therefore using KDOQI classification all patients studied would be classified as chronic kidney disease stage 5(191). As cardiovascular disease is the leading cause of both early (with 30 days of surgery) and late patient death with a functioning renal transplant, patients considered to be at increased cardiovascular risk for renal transplantation were referred for cardiovascular screening by the renal transplant co-ordinator. At this point, patients were asked if they were willing to undergo CMR by the investigator (PM). Patients were considered to be at increased cardiovascular risk if any of the following criteria were present, in keeping with published guidelines(192):

- Age ≥50
- Presence of diabetes mellitus
- History of cigarette smoking
- Previous history of ischaemic heart disease
- Previous history of peripheral or cerebrovascular disease
- Strong family history of cardiovascular disease
- Documented history of left ventricular abnormalities
- ECG or previous cardiac stress test suggestive of underlying ischaemic heart disease
- The opinion of the consultant transplant surgeon or consultant nephrologist

All subjects who underwent CMR gave written informed consent and this strategy was approved by the consultant nephrologists and surgeons involved in the transplant assessment process with the protocol approved by the local ethics committee. All patients underwent conventional cardiovascular risk factor assessment including history, clinical examination, ECG and blood sampling. A history of IHD was defined as previous myocardial infarction or angina pectoris, and hypercholesterolaemia was defined as fasting total serum cholesterol >5.0 mmol/L or use of statin therapy. The decision to list a patient for transplantation was not influenced by CMR findings, and was made by an independent transplant surgeon and nephrologist taken on the basis of clinical findings and additional investigations such as exercise testing, myocardial perfusion scanning and coronary angiography as clinically indicated. Due to ethical concerns regarding the potential risks of invasive procedures such as coronary angiography, this was only performed as clinically indicated as judged by an independent cardiologist, and not as part of study protocol.

2.2.2 Exclusion criteria

Exclusion criteria were contraindications to magnetic resonance scanning (permanent pacemaker, implanted ferromagnetic objects, pregnancy, and extreme claustrophobia). Two otherwise eligible patients were also screened and excluded with a documented history or echocardiographic evidence of hypertrophic cardiomyopathy and one further patient with a history of thalassaemia and repeated blood transfusions was excluded; both of which have been associated with characteristic CMR findings(177;187). There was no evidence of other cause of cardiomyopathy (e.g. amyloidosis, Fabry's disease, sarcoidosis, iron overload) in study patients based on clinical (original renal disease, serum ferritin, and clinical review) and echocardiographic findings.

2.3 Cardiovascular Magnetic Resonance Imaging

2.3.1 CMR scan

CMR was performed using a 1.5 Tesla MRI scanner (Siemens Sonata, Erlangan, Germany). As described previously CMR provides a high fidelity, volume independent method for assessing cardiac dimensions and function. To minimise influence of hydration status on ventricular chamber dimensions CMR scanning was performed on the post-dialysis day in patients on haemodialysis, and with patients at their 'dry weight' in patients on peritoneal dialysis, according to clinical charts. All scanning was performed by the investigator (PM) or a cardiac radiographer (Tracey Steedman, Glasgow CMR Unit, Western Infirmary, Glasgow).

2.3.2 Contrast agent

The paramagnetic contrast agent used was gadolinium-diethylentriaminepentaacetate (Gd-DTPA-BMA, Omiscan, Amersham, United Kingdom). The initial dose used was

0.2mmol/kg from September 2003 to March 2004. All studies after March 2004 used 0.1mmol/kg. The change in dose was made to allow for reduction in scan time and to minimise any potential side effects associated with use in patients with advanced renal failure.

2.3.3 CMR protocol- image acquisition

2.3.3.i Patient positioning

Patients were positioned head first and supine in the scanner. Breath hold instructions were relayed via headphones and relaxing music played if desired. A chest coil was employed to optimise image acquisition.

2.3.3.ii Left ventricular mass and function

An ECG gated breath hold fast imaging with steady-state precession (trueFISP) sequence was used to acquire cine images in long axis planes followed by sequential short axis left ventricular cine loops (8mm slice thickness, 2mm gap between slices) from the atrio-ventricular ring to the apex. In full the protocol is as follows:

1. The initial sequence was a multi-slice breath-hold localiser in end expiration. From these images select the best axial image depicting the left ventricle and septum was selected
2. This image was used to plan a vertical long axis localiser (VLA) along the long axis of the ventricle from the mid-point of the mitral valve to the apex. From the resulting VLA scan the horizontal long axis (HLA) localiser was planned, again using the mid-point of the mitral valve and the apex of the ventricle to orientate

3. From this HLA view, three short axis (SA) localiser slices were produced, with the orientation selected as close as possible to parallel to the mitral valve plane. The anatomical landmark of the atrio-ventricular groove was used as a guide
4. The resulting SA images allow the three long axis views to be set. These were the 4-chamber, 2-chamber and left ventricular outflow tract (LVOT) views. Cine studies were acquired in these orientations
5. From the cine 4-chamber study acquired the short axis stack was planned. The first of these cine slices was positioned in an orientation across the mitral valve plane, again using the atrio-ventricular groove as an anatomical land-mark. The slice position would then be incremented and repeated until LV was completely covered

Imaging parameters, which were standardized for all subjects, included repetition time (TR)/echo time (TE)/ flip angle (FA)/ voxel size /field of view (FoV) - 3.14 ms/ 1.6 ms/ 60^0/ 2.2 x 1.3 x 8.0 mm/340 mm.

2.3.3.iii Aortic distensibilty and compliance

Aortic distensibilty was assessed from cine MR images in the transverse plane of the ascending aorta were obtained at the level of the main pulmonary artery utilising a trueFISP sequence (TR=3.2 ms, TE=1.6 ms, FA=60°, FoV 276x340mm, pixel dimensions 2.3x1.3mm, slice thickness=7 mm). The approximately 10 second breath-hold CMR sequence resulted in prospective images with a temporal resolution of at least 22.5ms. During image acquisition, blood pressure was measured using a non-ferromagnetic brachial artery sphygmomanometer cuff (Schiller instruments, Baar, Switzerland).

2.3.3.iv Phase encoded (Flow sensitive) CMR imaging of aortic blood flow

To generate flow measurements along the aorta a fast low angle shot (FLASH) gradient-echo pulse sequence (TR=41 ms, TE=3.2 ms, FA=30°, matrix size=192x256, Pixel Dimensions 1.5x1.5, slice thickness= 5 mm) was applied perpendicular to the aorta at 2 levels: at the level of the crossing of the pulmonary artery through the ascending (in the same plane as the compliance sequence) and descending aorta and at the level of the diaphragm. This resulted in multiphase image pairs of modulus- and velocity encoded images with a temporal resolution of approximately 20.7 ms.

2.3.3.v Contrast enhanced CMR images

Further images were acquired 10 minutes after a manual injection of a peripheral bolus of Gd-DTPA-BMA via a intravenous cannula flushed with 10ml of 0.9% saline. A breath-hold segmented turboFLASH inversion-recovery sequence, using identical slice positions as the cines was used. Standardised settings were used for contrast-enhanced imaging including specific parameters of TR/TE/flip angle/voxel size/FoV/number of segments - 11.6 ms/4.3 ms/20°/2.2 x 1.3 x 8.0 mm/ 23. Inversion time for the turboFLASH sequence was optimized on an individual patient basis. Successful nulling of normal myocardium was deemed to have been achieved once the LV myocardium appeared black and homogenous. Generally, an inversion time of between 150 and 280 ms was required.

2.3.3.vi CMR scan duration

Standard LV mass and function took approximately 20-25 minutes. Measures of vascular function required a further 5 minutes and acquisition of contrast enhanced images added an additional 10 minutes to scan time. Overall, scan time was approximately 30–40 minutes, with a maximum time of 45minutes.

2.4 CMR Analysis

2.4.1 Analysis of left ventricular function

LV function was analysed from the left ventricular short axis cine loops. Epicardial and endocardial borders were traced manually and end-systolic and end-diastolic contours with end-systolic and end-diastolic volumes and LV mass were calculated using conventional analysis software (Argus, Siemens, Erlangen). Volumes and LV mass were also indexed to body surface area with body surface area calculated using the Dubois formula:

Body Surface Area = $0.20247 \times \text{Height(m)}^{0.725} \times \text{Weight(kg)}^{0.425}$

Left ventricular ejection fraction (LVEF) was calculated as:

$$\text{LVEF} = \frac{\text{End diastolic volume} - \text{end systolic volume}}{\text{End diastolic volume}} \quad \text{X } 100$$

2.4.2 Definition of left ventricular abnormalities

LVSD was defined as LV ejection fraction (LVEF) <55%, with LVH defined as left ventricular mass index (LV mass/body surface area; LVMI) greater than 84.1 g m^{-2} (male) or 76.4 g m^{-2} (female), LV dilatation defined as end diastolic volume/body surface area greater than 111.7 ml m^{-2} (male) or 99.3 ml m^{-2} (female) or end systolic volume greater than 92.8 ml (male) or 70.3 ml (female) based on based on published mean normal LV dimensions for healthy volunteers plus two standard deviations(169).

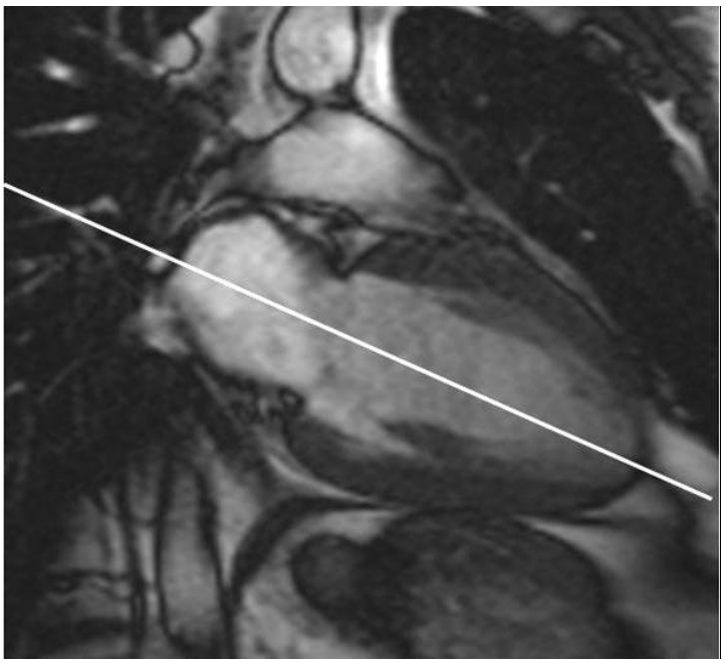


Figure 2.1

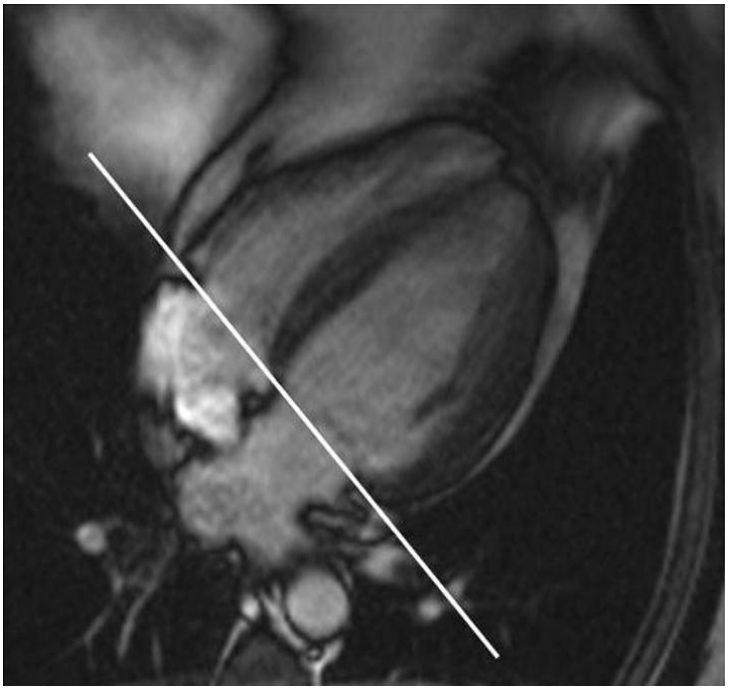


Figure 2.2

Figure 2.1 Vertical long axis (2-chamber) image in end diastole in a patient with left ventricular hypertrophy with line demonstrating plane used to plan horizontal long axis (4-chamber) cine

Figure 2.2 Horizontal long axis (4-chamber) image in end diastole in the same patient with line demonstrating plane used to plan short axis (4-chamber) cine

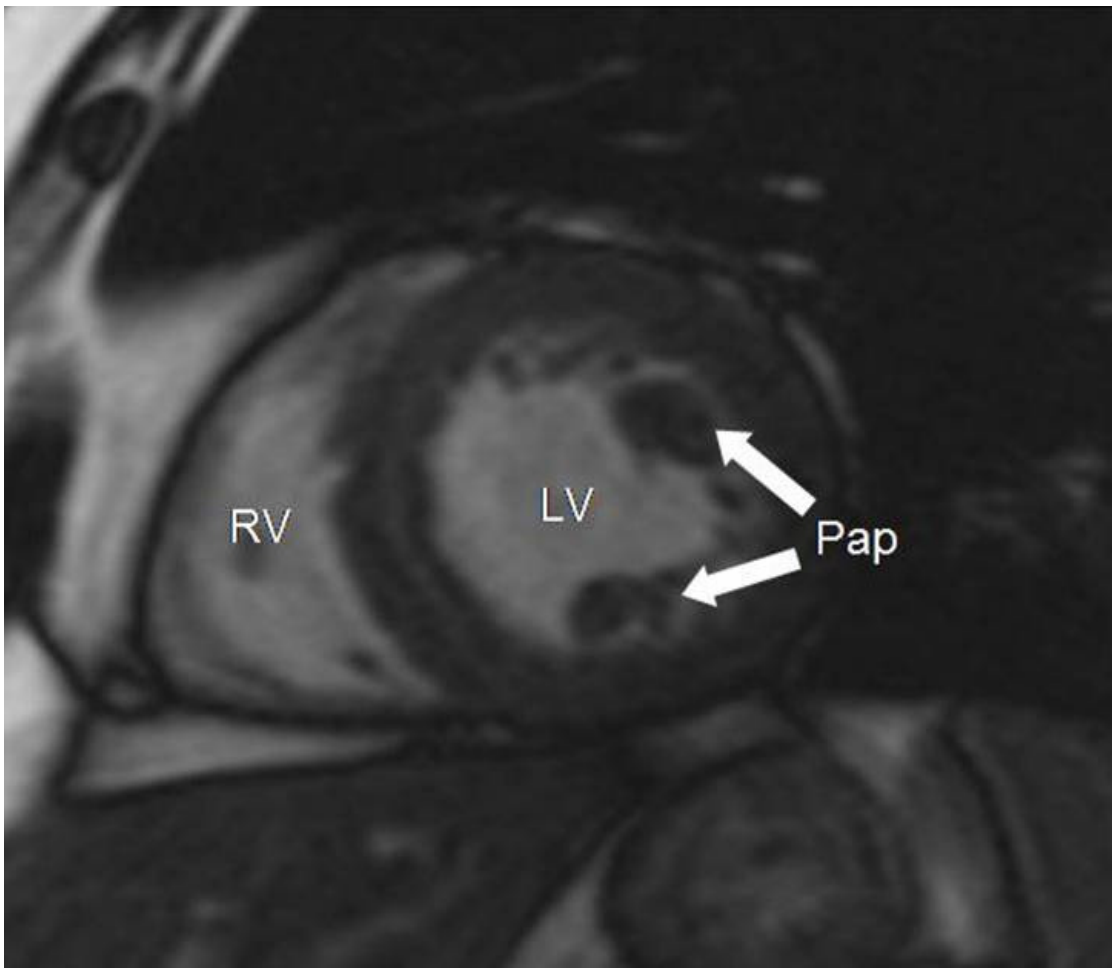


Figure 2.3

Short axis view of the left ventricle in end diastole with the left ventricular (LV) and right ventricular (RV) cavities indicated. The papillary muscles (Pap) are arrowed

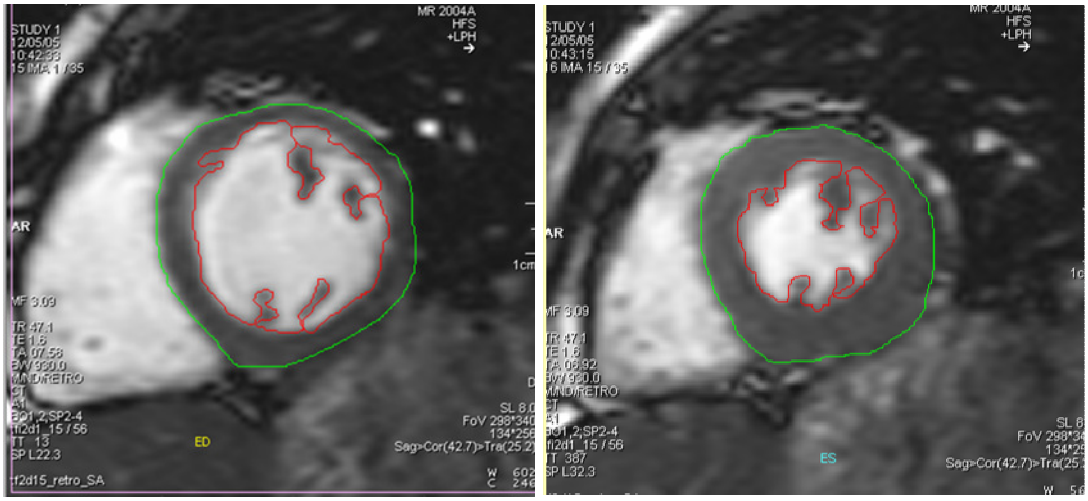


Figure 2.4

End diastolic (left) and end systolic (right) short axis views of the left ventricle with the endocardial (red) and epicardial (green) borders contoured using the Argus analysis package

2.4.3 Contrast-enhanced image analysis

Myocardial fibrosis was documented as indicated by the presence of late gadolinium enhancement (LGE). Images were assessed for the presence, pattern, and volume of gadolinium enhancement. Patients were classed as having positive LGE, if LGE was seen on at least two (of three) views: short axis view, long axis view, and reverse phase sequences, to exclude artefact. Artefact on the contrast-enhanced images was excluded by the acquisition of 'reverse phase' images through slice planes which appeared to demonstrate intra-myocardial contrast enhancement. This technique involves the reversal of the phase encoding and frequency-encoding directions as the phase-encoding direction is particularly prone to artefacts caused by cardiac motion or chest wall movement. Only areas of contrast enhancement that persisted on these reversed phase images were included in subsequent quantification of areas of contrast enhancement. Areas of contrast enhancement were first identified visually and this area was contoured by manual planimetry to calculate volume of enhanced myocardial tissue. Volume of LGE mass was calculated by multiplying LGE volume by myocardial density (1.04 g cm^{-3}) and is used as the absolute measured value of contrast-enhancing tissue seen.

2.4.4 Computerised assessment of areas of late gadolinium enhancement

Mean signal intensities (+/- standard deviation) for areas of contrast enhancement and for an adjacent reference area of non-enhancing myocardium again using the Argus package. LGE was defined as an area of visually identified contrast enhancement with a mean signal intensity that was greater than one standard deviation higher than the mean signal intensity of an adjacent area of reference ventricular myocardium, which although nulled had a mean signal intensity significantly above zero. No manual alteration of image brightness/intensity settings was made during this process to ensure objective visual assessment of LGE. Patterns of gadolinium enhancement were analysed

both subjectively and objectively based on mean signal intensity. LGE volume was determined by manual planimetry of any areas of contrast enhancement meeting these criteria.

2.4.5 Analysis of vascular function

2.4.5.i Aortic distensibilty

To calculate aortic distensibilty, the aortic volumes were measured by manual tracing of the cross sectional area of the vessel lumen using the Argus analysis software, which was multiplied by the slice thickness. Aortic distensibilty was calculated according to the following formula:

$$\text{Aortic distensibilty} = \frac{[(\text{Aortic volume})_{max} - (\text{Aortic volume})_{min}]}{[(\text{Aortic volume})_{min} * \text{pulse pressure}]}$$

where $(\text{Aortic volume})_{max}$ and $(\text{Aortic volume})_{min}$ are the maximal and minimal calculated aortic volumes obtained during the cardiac cycle (Figure 2.5).

Aortic volumetric arterial strain (VAS- non pressure dependent) is calculated from the formula:

$$\text{VAS} = \frac{[(\text{Aortic Volume}_{max} - \text{Aortic Volume}_{min})]}{(\text{Aortic Volume}_{min})}$$

2.4.5.ii Measurement of pulse wave velocity

2.4.5.ii (a) Assessment of aortic length

Aortic length was measured using software developed by Dr. Gang Gao (Department of Computing Science, University of Glasgow). Briefly, a threshold algorithm is used to convert the original image to a binary map with the threshold value selected both manually and automatically. The binary map highlights the aortic region since the aorta appears significantly brighter than other soft tissues around it. To locate the aorta, further manual refinements are required. Once the aortic midline is defined, the aorta is 'skeletonised', to isolate the aortic length to be measured (Figure 2.6).

2.4.5.ii (b) Interpretation of flow velocity curves to calculate time difference between points along the aorta

Initial measurement of blood flow and velocity was made using the Argus CMR analysis software (Figure 2.7). These files were saved for off line plotting, normalisation and interpretation. The time difference of initial flow acceleration between two regions of interest was calculated from normalised velocity curves using off-line software (Origin, OriginLab Corporation, Northampton, MA, USA). The upstroke velocity is approximately linear and usually contains three or more sample points. The location of the best cross-correlation of two partial upstroke velocity curves was used to estimate the time delay (Figure 2.8).

2.4.5.ii (c) Calculation of pulse wave velocity

Pulse wave velocity was then calculated using the equation:

Pulse wave velocity = Distance/Time

where distance is the distance between the points along the aorta and time is the time difference in flow between those points.

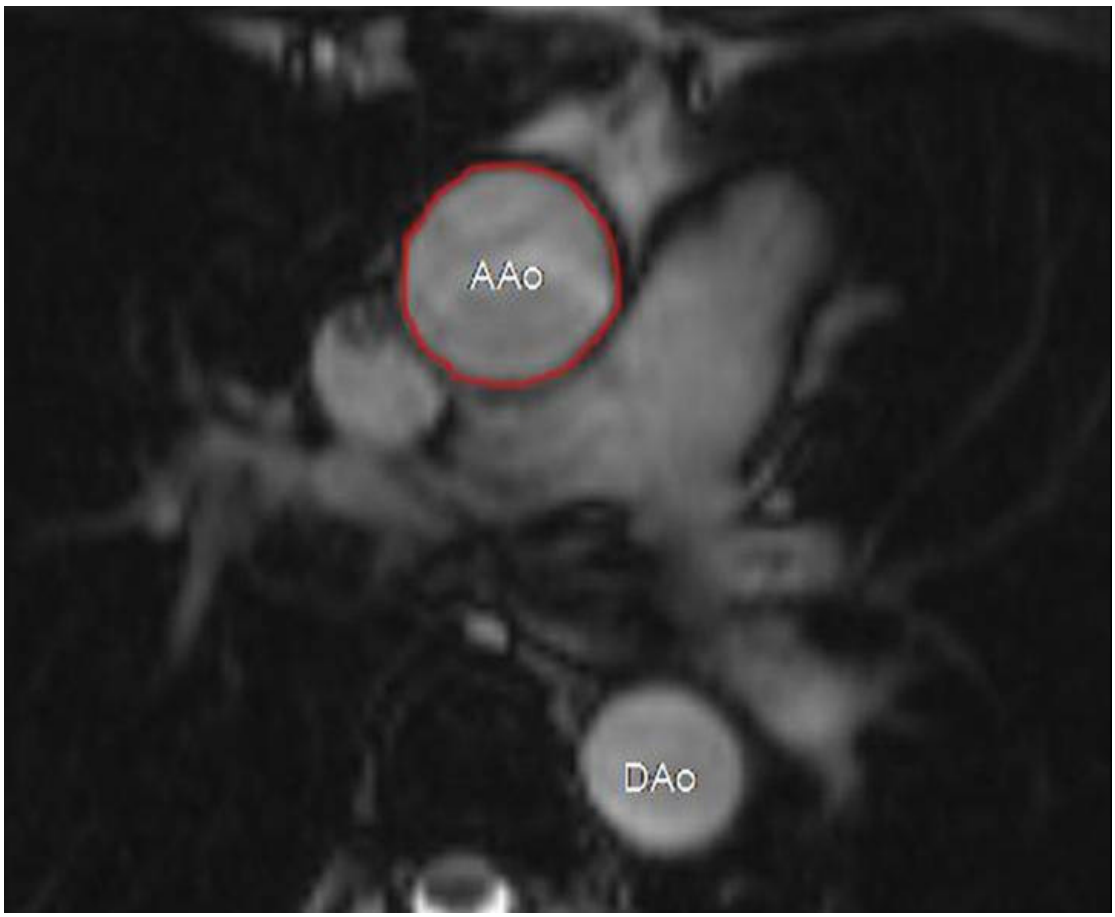


Figure 2.5

Ascending aorta (AAo) and descending aorta (DAo) shown in cross section, with ascending aortic contour traced for distensibilty and volumetric arterial strain measurement

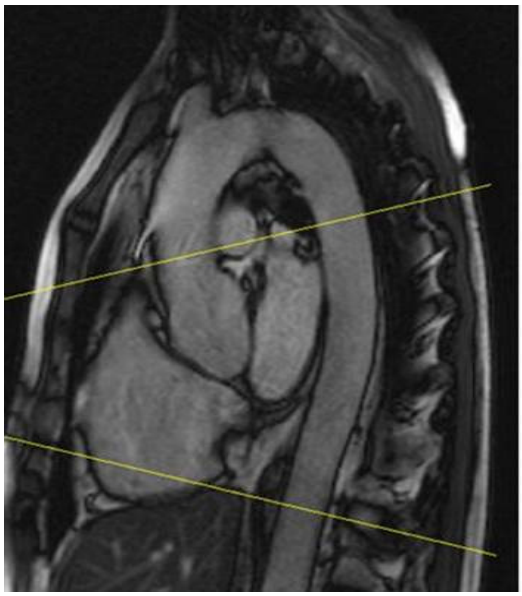


Figure 2.6.1

Figure 2.6.2

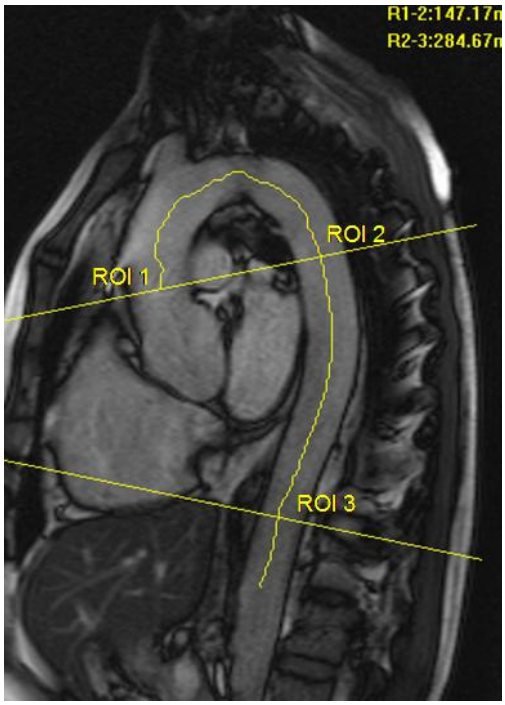


Figure 2.6 Using Cardiowarp to measure aortic length

Figure 2.6.1 Sagittal planning view through the aorta with planes for measurement of blood flow and aortic distensibilty indicated

Figure 2.6.2 The aortic midline has been accurately defined and skeletonised

Figure 2.6.3 The measured aorta with regions of interest (ROI) 1-3 indicated where blood flow velocity will be subsequently measured

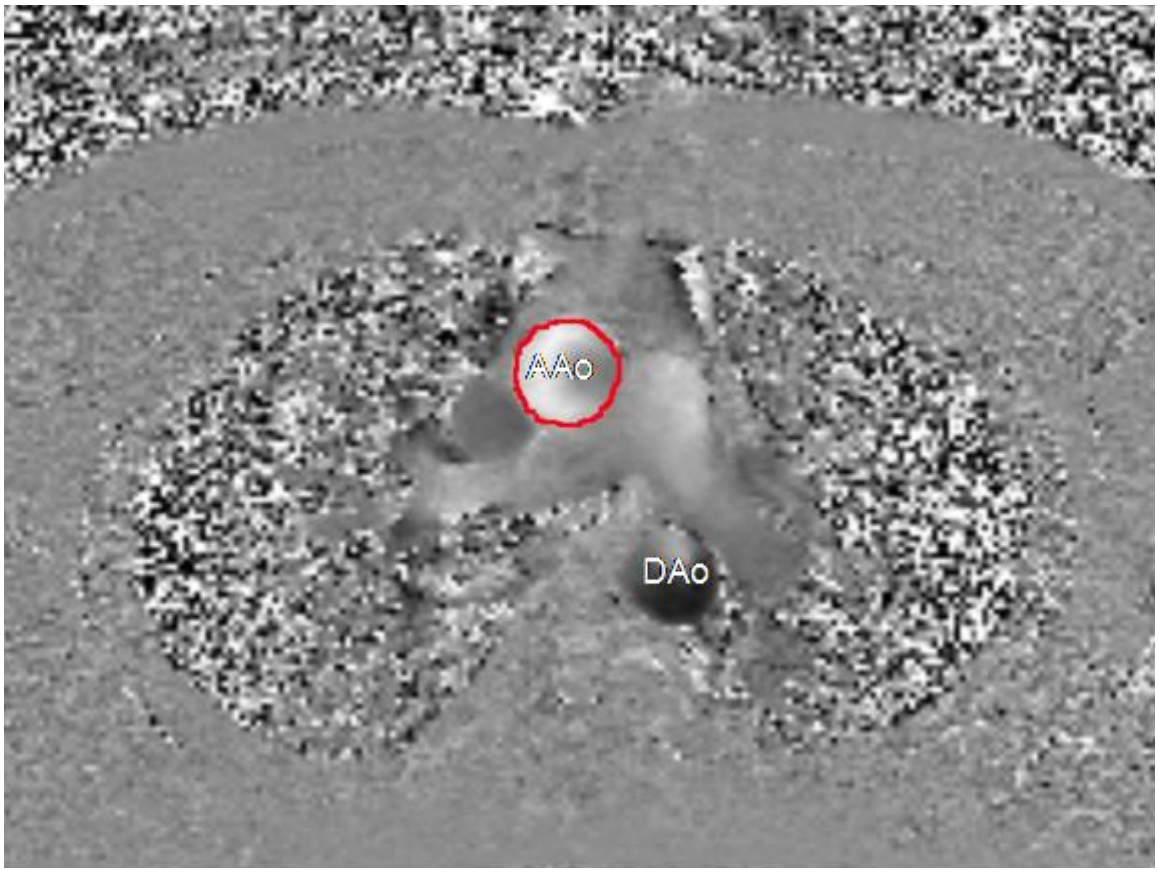


Figure 2.7

Ascending aorta (AAo) and descending aorta (DAo) shown in cross section, with ascending aortic contour traced for aortic blood flow measurement

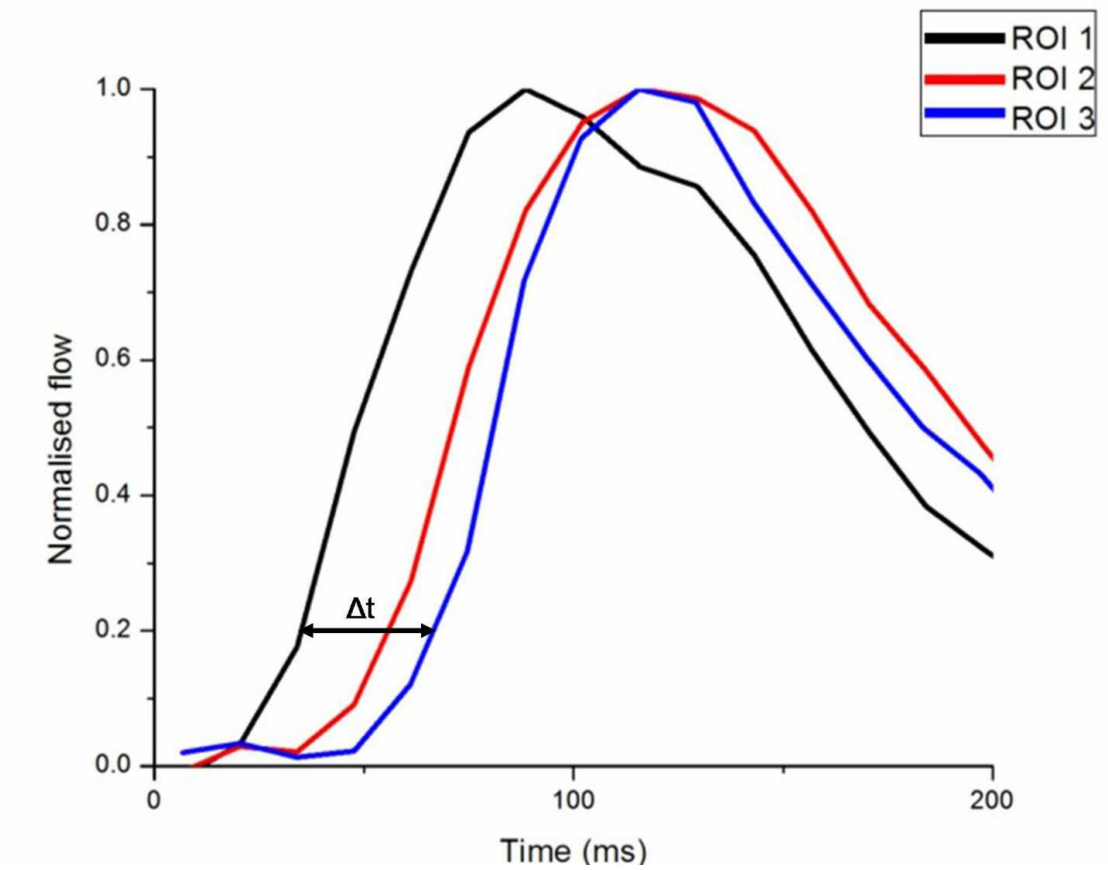


Figure 2.8

Representative plot of normalised blood flow plotted against time. Δt is the time delay subsequently used to calculate pulse wave velocity

2.5 CMR Analysis – duration

Approximate time required analysing left ventricular mass and function was 15 minutes per patient, with a further five minutes required to analyse late gadolinium images. Analysing the measures of vascular function took somewhat longer, with 20 minutes required for all the Argus based analysis and a further 10 minutes per patient to perform the PC based graph construction.

2.6 Coronary angiography

A proportion of patients underwent coronary angiography as part of assessment for renal transplant listing. The decision to perform angiography and interpretation of the angiogram was made by a cardiologist blinded to CMR findings, and was made on clinical grounds, based on symptoms, LVSD, or positive stress test (exercise test or myocardial perfusion scanning). CMR was performed within 3 months (before or after) of angiography. Patients were classified as having normal coronary arteries, mild/moderate coronary artery disease (presence of coronary plaque or up to 70% stenosis of any epicardial coronary artery) or severe coronary artery disease (presence of >70% stenosis of any epicardial coronary artery). Total atheromatous plaque burden was calculated by adding the percentages of the most severe lesion in each artery (thus the range was 0–400% as four vessels- left main stem, left anterior descending, right coronary and circumflex arteries were studied)(193).

2.7 Echocardiography

2.7.1 Echocardiography – general

In all cases echocardiography was carried out on the same day as CMR, either immediately prior to, or after the CMR scan. Echocardiography was performed using an

Acuson 128 machine (Siemens Medical, Bracknell, UK) by a single cardiac technician (Tony Cunningham, Clinical Research Initiative, University of Glasgow) and stored on videotape for off line analysis.

2.7.2 Echocardiography protocol – image acquisition

Patients were placed in the left lateral position and scanned from several different intercostal spaces. Standard trans-thoracic views were recorded from parasternal and apical transducer positions as listed below:

Parasternal Approach

Long-axis plane

Root of aorta—aortic valve, left atrium, left ventricular outflow tract

Body of left ventricle—mitral valve

Left ventricular apex

Right ventricular inflow tract—tricuspid valve

Short-axis plane

Root of the aorta—aortic valve, pulmonary valve, tricuspid valve, right ventricular outflow tract, left atrium, pulmonary artery, coronary arteries

Left ventricle—mitral valve

Left ventricle—papillary muscles

Left ventricle—apex

Apical Approach

Four-chamber plane

Four chamber

Four chamber with aorta

Long-axis plane

Two chamber—left ventricle, left atrium and with aorta

Pulsed wave, continuous wave Doppler, 2-D M-mode, and colour flow mapping were used in each position as appropriate to assess valve flow patterns, diastolic function, anatomy, dimensions and regurgitation respectively. Analysis of LV mass was performed as described in chapter 4. Further characterisation of LVH into concentric and eccentric LVH was dependent on measurements of regional wall thickness (RWT):

$RWT = [(2 \times PWT_d) / LVID_d)$

Concentric hypertrophy was present if the RWT ≥ 0.45 in the presence of LVH with eccentric hypertrophy present if RWT was <0.45 in the presence of LVH.

2.8 Electrocardiogram

A standard 12-lead ECG was recorded at 25 mm s^{-1} and 1 mV cm^{-1} standardisation. The ECG was considered abnormal if any of the following criteria were met in any of the standard limb leads or praecordial leads, except AVR or V1: pathological Q waves, LVH by Sokolow–Lyon criteria or Cornell index, ST depression ≥1 mm, ST elevation ≥1 mm, T wave inversion or bundle branch block (QRS ≥120 ms).

2.9 Exercise tolerance testing

All patients able to perform treadmill exercise underwent treadmill exercise testing according to standard Bruce protocol. The 12 lead ECG was recorded continuously and the following documented: exercise time to limiting symptom, maximal ST segment change, maximal heart rate, maximal systolic blood pressure, limiting symptoms. The test was stopped if any of the following occurred: limiting symptoms (angina, shortness of breath, dizziness, lethargy), ST depression ≥3 mm, ventricular tachycardia, drop in blood pressure ≥30 mmHg, rise in systolic blood pressure ≥230 mmHg. The test was described as inconclusive if stopped before 85% predicted heart rate could be achieved with no cardiac symptoms or significant changes at that stage.

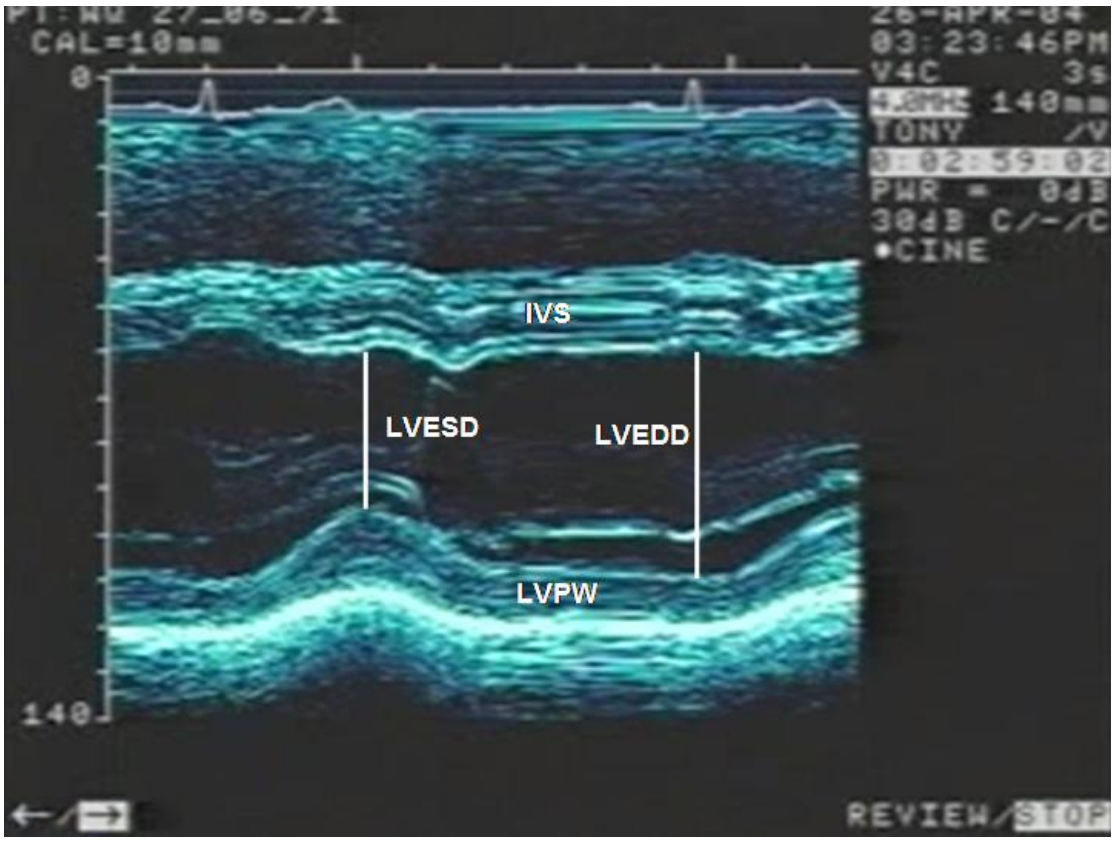


Figure 2.9

M-mode echocardiogram of the left ventricle of a patient with left ventricular hypertrophy. IVS (interventricular septum), LVPW (left ventricular posterior wall), LVESD (left ventricular end systolic diameter), LVEDD (left ventricular end diastolic diameter)

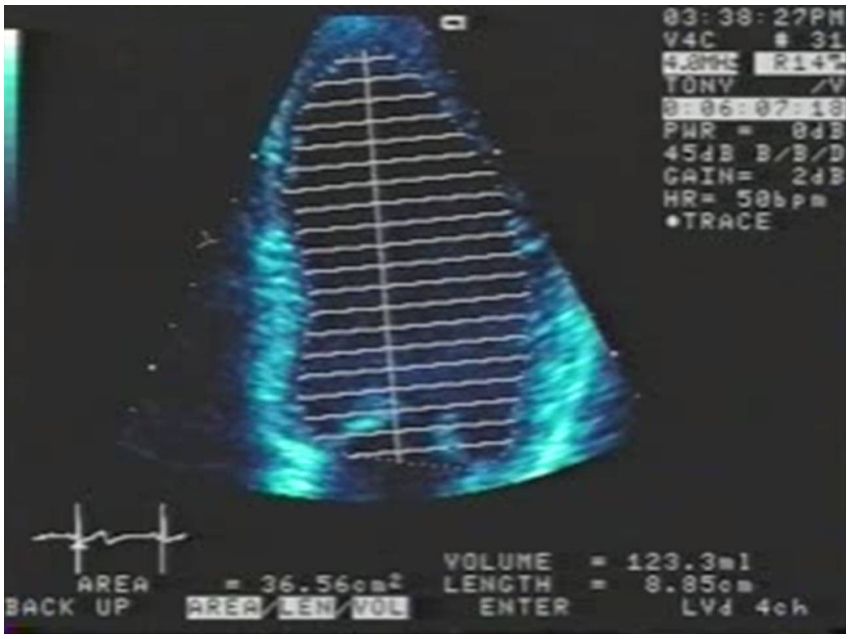


Figure 2.10.1

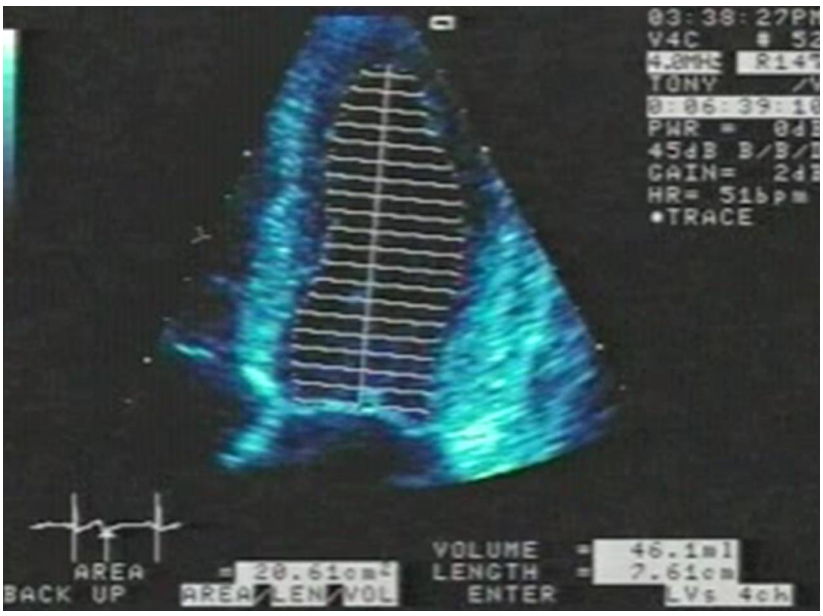


Figure 2.10.2

Figure 2.10.1

End diastolic 4-chamber view of the left ventricle with the endocardial surface contoured

Figure 2.10.2

End systolic 4-chamber view of the left ventricle with the endocardial surface contoured

2.10 Blood sampling

Prior to CMR scanning 30ml of venous blood was drawn and analysed in the hospital biochemistry and haematology laboratories for haemoglobin, electrolytes, urea, creatinine and glucose. A proportion of this blood was centrifuged and frozen within 20 minutes of collection to -70ºC. Serum brain natriuretic peptide was measured using by a one step radioimmunoassay (ShionoRIA, Shinogi, Japan). Plasma total cholesterol, low-density lipoproteins, high-density lipoproteins, triglycerides and high sensitivity C-reactive protein were assessed using standard biochemical methods.

2.11 Follow up

Follow up data were collected from date of the CMR scan to 30th September 2006 using electronic patient records from the Western Infirmary Glasgow and Glasgow Royal Infirmary Renal Units.

Chapter 3

A study of ventricular dimensions and function in uraemia with contrast enhanced cardiac magnetic resonance imaging

3.1 INTRODUCTION

The burden of cardiovascular disease in uraemia has been outlined in Chapter 1 and its relationship with subsequent mortality and morbidity has been highlighted. As discussed, despite the presence of many 'traditional' cardiovascular risk factors for atherosclerosis and subsequent development of coronary artery disease as well as those risk factors specific to uraemia in patients with ESRD; the relationship between these risk factors and outcome is less clear than in the general population. However, based on data from the early 1990s, the presence of uraemic cardiomyopathy, defined echocardiographically by the presence of left ventricular abnormalities, specifically LVH, left ventricular dilatation and left ventricular systolic dysfunction have been shown to be associated with poor outcome in this patient group(76). At the inception of the study, no large scale studies existed to establish whether these relationships between cardiac dimensions and outcome held true using the more accurate method of CMR to assess cardiac dimensions. Moreover data were limited on the use of CMR in patients with ESRD, with only one small pilot study published, from our own group using a 1 Tesla scanner with less sophisticated scanning protocols(189).

This study was designed to accurately characterise left ventricular mass and function in a cohort of patients with ESRD, firstly compared to controls and additionally to explore the relationship between CMR markers of myocardial fibrosis, indicated by late gadolinium enhancement (LGE), and uraemic cardiomyopathy.

3.2 METHODS

3.2.1 CMR technique and analysis

All patients and controls were studied with the CMR technique described in detail in Chapter 2 (2.3.2). Controls did not have contrast enhanced studies with Gd-DTPA-BMA performed. Blood pressure was recorded at the time of scanning.

3.2.2 Statistical methods

Left ventricular dimensions, age and blood pressure were compared between control groups and patients with ESRD by t-test or Mann-Whitney-U as appropriate. In patients with ESRD undergoing contrast enhanced CMR the prevalence of conventional cardiovascular risk factors and LV abnormalities between those patients with, and without, the presence of myocardial fibrosis indicated by CMR were analysed by Chi-squared or Fisher's exact test (as appropriate) for categorical data and paired t-test and Mann–Whitney–U testing, respectively, for continuous data. Correlations between volume of myocardial fibrosis and cardiac dimensions were assessed with Pearson and Spearman correlation co-efficient as appropriate. All analyses were performed using the SPSS 11.5 statistical software package (SPSS Inc., Chicago, IL., USA).

3.3 RESULTS

3.3.1 Patient and control demographics

Patient demographics and drug therapy at the time of scan are shown in Table 3.1. Seven (3.9%) patients consented to be studied but did not complete CMR scanning due to claustrophobia and were not included in the analysis. 172 patients (38 with advanced renal failure not on dialysis therapy and 134 established on dialysis) and 20 control

subjects were studied. The groups of controls and uraemic patients had a similar age and sex distribution. No significant differences in patient demographics or ventricular dimensions or function were exhibited between patients with advanced renal failure not yet on dialysis therapy and those established on renal replacement therapy and therefore these patients were combined as a single 'uraemic' group. Systolic and diastolic blood pressures were higher in uraemic patients than controls as expected.

3.3.2 Left ventricular dimensions

Left ventricular dimensions are shown in Table 3.2 for the uraemic patients and controls. Overall there was no significant difference in left ventricular end diastolic or systolic dimensions between uraemic patients and controls but, as expected, uraemic patients had significantly higher left ventricular mass. As an entire group uraemic patients did not show a reduction in systolic function as indicated by left ventricular ejection fraction compared to controls. Representative CMR images of control and uraemic patients are shown in Figures 3.1 - 3.4.

3.3.3 Reproducibility of measurement of left ventricular dimensions

A proportion of scans (n=20) were reanalysed in a blinded fashion. The intra-observer variability of measurements for ejection fraction, LV mass/BSA, end diastolic volume/BSA and end systolic volume/BSA were 4.2%, 5.3, 5.3% and 8.8% respectively, in keeping with previous studies within our group(189).

	Uraemic		Control		p value
Number	172		20		-
Male (%)	107	(62.2)	12	(60.0)	0.847
Age (years)	51.9	(11.1)	48.8	(9.1)	0.366
Height (m)	168.7	(10.0)	170.4	(9.0)	0.390
Weight (kg)	74.8	(16.2)	74.8	(10.6)	0.984
SBP (mmHg)	140	(25)	116	(10)	<0.001
DBP (mmHg)	82	(13)	75	(8)	0.013
1^0 Renal Disease			-		
Diabetes	47	(27.3)			
APKD	19	(11.0)			
GN	34	(19.8.)			
CPN	19	(11.0)			
Renovascular	7	(4.1)			
Unknown/Other	46	(26.7)			
RRT time (months)	7	(47.8)			
Smoker					
Never	97	(56.4)	13	(65.0)	
Current	51	(29.7)	2	(10.0)	0.123
Ex	24	(14.0)	5	(25.0)	
Previous IHD	30	(17.4)	-		-
TIA/CVA	14	(8.1)	-		-
PVD	12	(7.0)	-		-

Table 3.1

Background demographics for uraemic patients and controls. Results are show as mean with standard deviation in parenthesis or number with percentage in parenthesis as appropriate except for RRT time which is displayed as median and inter quartile range. Tests of significance are t-test and Chi-squared between groups

	Uraemic		Control		p value
LV ejection fraction (%)	66.6	(11.6)	67.4	(4.6)	0.753
LV mass (g)	169.1	(61.4)	123.1	(29.4)	0.001
End diastolic volume (ml)	145.0	(59.5)	137.0	(33.0)	0.556
End systolic volume (ml)	51.7	(36.5)	45.4	(15.3)	0.444
Stroke volume (ml)	91.3	(33.6)	91.7	(20.1)	0.961
LV mass/BSA (g m^{-2})	91.3	(29.4)	65.7	(12.9)	<0.001
End diastolic volume/BSA (ml m^{-2})	78.6	(29.9)	72.7	(14.8)	0.398
End systolic volume/BSA(ml m^{-2})	28.0	(19.1)	24.2	(7.3)	0.391
Stroke volume/BSA (ml m^{-2})	49.6	(16.7)	48.6	(8.9)	0.792

Table 3.2 Left ventricular dimensions in uraemic patients and controls. Results are show as mean with standard deviation in parenthesis. Test of significance is t-test

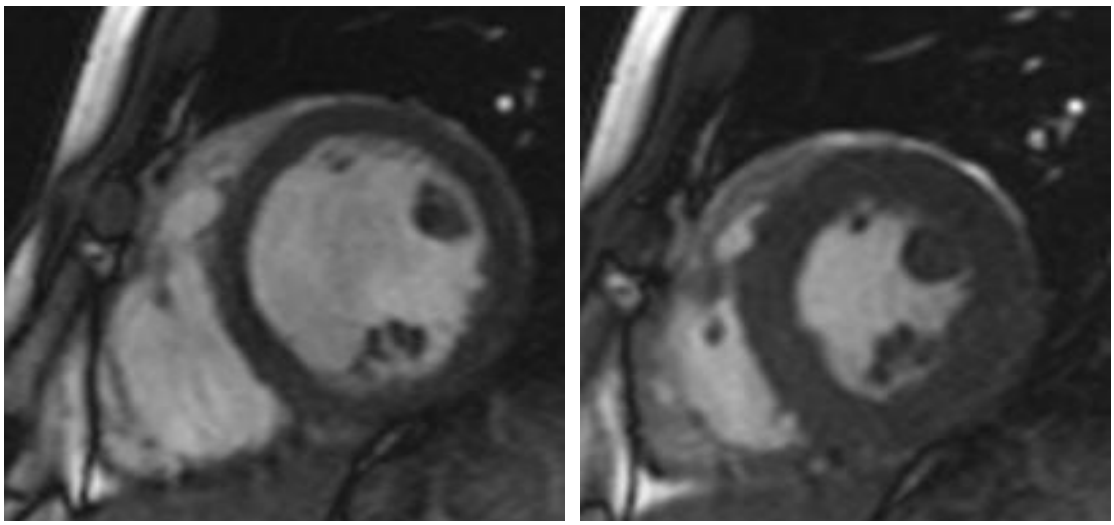


Figure 3.1

Short axis mid left ventricular cavity view of images of the left and right ventricle of a normal control subject in end diastole (left) and end systole (right)

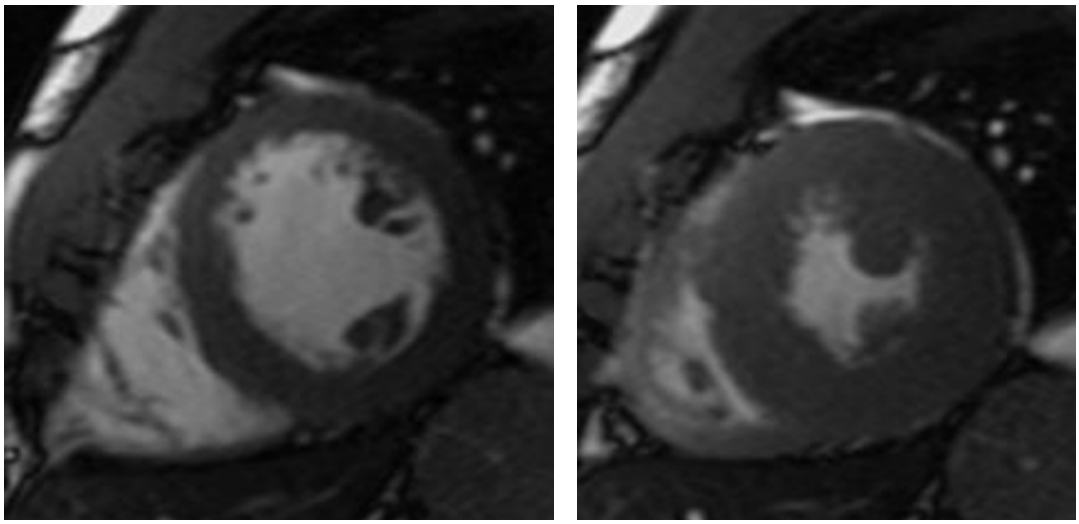


Figure 3.2

Short axis mid left ventricular cavity view of images of the left and right ventricle of a patient with ESRD on haemodialysis with marked left ventricular hypertrophy in end diastole (left) and end systole (right)

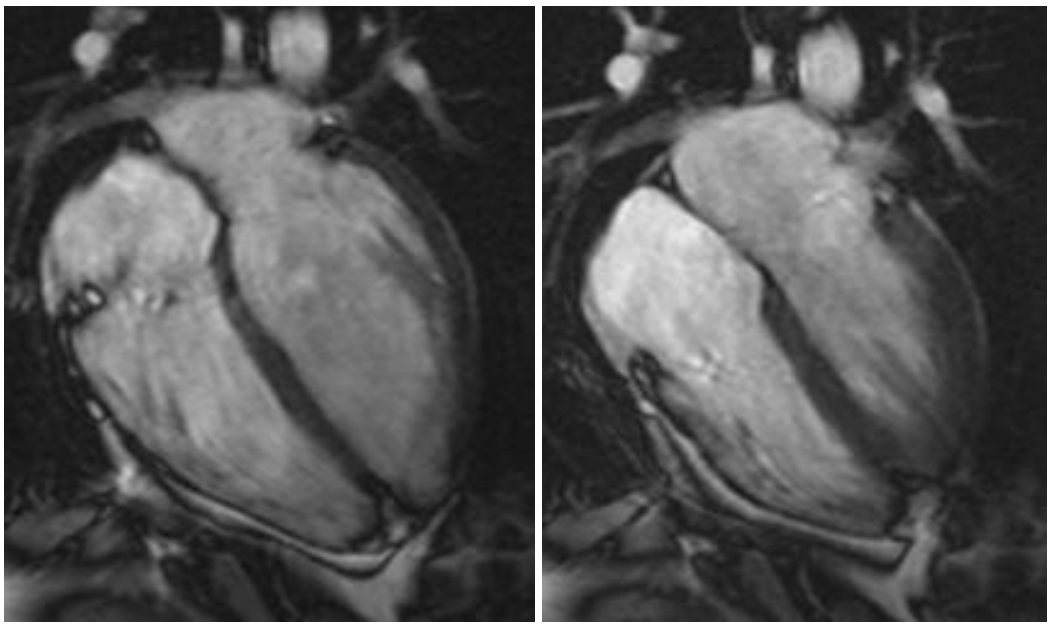


Figure 3.3

Horizontal long axis view of images of the left and right ventricle of a normal control subject in end diastole (left) and end systole (right)

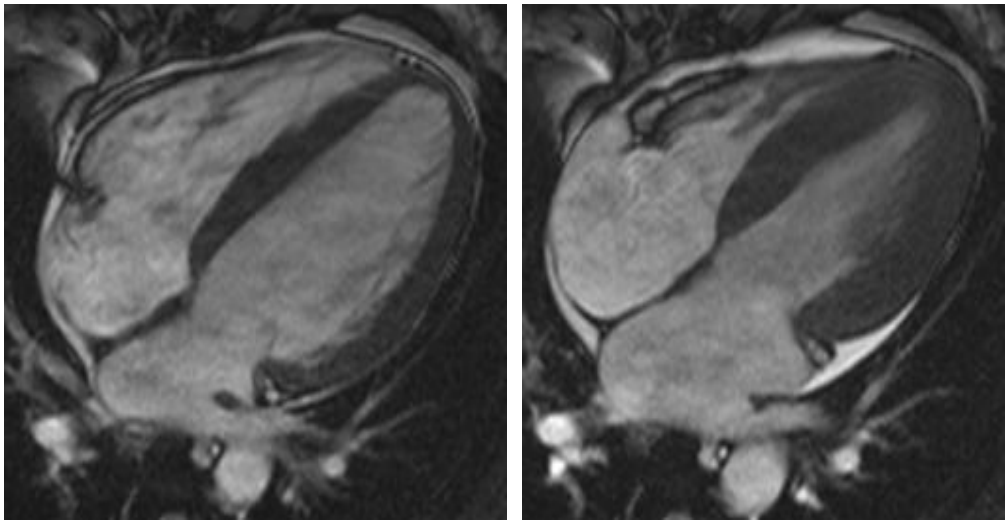


Figure 3.4

Horizontal long axis view of images of the left and right ventricle of the ESRD patient also shown in Figure 3.2 in end diastole (left) and end systole (right)

3.4 Contrast enhanced studies

Contrast enhanced CMR studies were performed in 145 uraemic patients. Contrast was not administered for the CMR scan for the following reasons: concern over precipitating decline in renal function in patient with rapidly deteriorating native renal function (n=17), concern of decline in renal function in rapidly deteriorating renal transplant function (4), patient choice (5) and poor venous access (1). The CMR protocol for image acquisition is described in detail in Chapter 2 (2.3.2.v), with analysis described in 2.4.2. The dose of Gd-DTPA-BMA was 0.2mmol/kg used until March 2005, when it was reduced to 0.1mmol/kg, in light of recognition, that similar scan quality could be achieved with a lower dose (permitting reduced scan time and theoretically safer or less likely to provoke side effects).

3.4.1 Baseline left ventricular dimensions in patients with and without positive myocardial late gadolinium enhancement

Baseline patient demographics of patients undergoing contrast enhanced CMR are displayed in Table 3.3. Results are show as mean with standard deviation in parenthesis or number with percentage in parenthesis as appropriate except for RRT time which is displayed as median and inter quartile range.

There was a high prevalence of conventional cardiovascular risk factors in the overall patient group with 18.6% having a history of ischaemic heart disease (IHD), 31.7% were diabetic (27.6% with diabetic nephropathy), 49.7% had hypercholesterolaemia (39.3% on statin therapy), and 47.6% had a positive smoking history. Overall, 49 (33.8%) patients had evidence of late gadolinium enhancement (LGE) at CMR. Patients who had evidence of LGE (LGE positive) had greater LVMI (LGE positive – mean 100.9 g m^{-2}, LGE negative – mean 86.6g m^{-2}, $p<0.001$), LV dilation (measured as end

diastolic volume: LGE positive – 168.2 ml, LGE negative – 129.2ml, $p<0.001$) and had greater evidence of LVSD (ejection fraction: LGE positive – mean 60.7%, LGE negative – mean 68.8%, $p<0.001$) than patients with no LGE. Representative images of patients with and without LGE (and its subtypes are displayed in Figures 3.5-3.10). As fundamental differences emerged between the functional, clinical and laboratory correlates of different subgroups of LGE further analysis of these factors was performed separately.

Male (%)		88	(60.7)
Age (years)		51.9	(11.1)
Height (m)		168.6	(10.0)
Weight (kg)		74.6	(16.2)
SBP (mmHg)		138.9	(25.3)
DBP (mmHg)		82.4	(13.4)
RRT			
	HD	75	(51.7)
	PD	52	(35.9)
	Pre-Dialysis	18	(12.4)
Duration of RRT (months)		8	(52.5)
1^0 Renal Disease			
	Diabetes	40	(27.6)
	APKD	16	(11.0)
	GN	29	(20.0)
	CPN	15	(10.3)
	Renovascular	6	(4.1)
	Unknown/Other	39	(26.9)
Smoker			
	Never	76	(52.4)
	Current	46	(31.7)
	Ex	23	(15.9)
Diabetes		46	(31.7)
	Type 1	28	(19.3)
	Type 2	18	(12.4)
IHD		27	(18.6)
Hypercholesterolaemia		72	(49.7)
Previous MI		16	(11.0)
TIA/CVA		11	(7.6)
PVD		12	(8.3)

Table 3.3

Background demographics for uraemic patients undergoing contrast enhanced CMR

3.4.2 Description and functional implications of subendocardial late gadolinium enhancement

Further analysis of the pattern of LGE revealed crucial differences between patient groups. Broadly, there were two patterns of LGE. Firstly, subendocardial LGE involving the subendocardium was seen in 24 patients (16.6%). This finding is in keeping with the pattern of LGE previously described in patients with myocardial infarction (Figures 3.6-3.7). Subendocardial LGE was associated with significantly greater degree of ventricular dilatation (end systolic volume/BSA 47.1 vs. 22.9 ml m^{-2}, $p<0.001$) as well as reduced systolic function indicated by left ventricular ejection fraction (54.1 vs. 68.8%, $p<0.001$) compared to patients who were LGE negative, with a corresponding greater proportion of patients with subendocardial LGE having left ventricular systolic dysfunction (54.2% vs. 8.3% of LGE negative, $p<0.001$). These results are presented in Table 3.4. In keeping with this pattern representing old myocardial infarction, the presence of subendocardial LGE was associated with a wall motion abnormality of the corresponding myocardial wall in nearly all cases when the pre-contrast cine images were reviewed (23 of 24 scans exhibited wall motion abnormalities in the areas affected by subendocardial LGE). In patients with subendocardial LGE there was a significant negative correlation between mass of myocardial tissue indicated by LGE and LVEF (Spearman's R= -0.43, p=0.036).

3.4.3 Clinical correlates of subendocardial late gadolinium enhancement

Subendocardial LGE was associated with the presence of conventional cardiovascular risk factors (Table 3.5) and was found in significantly higher proportions in patients with a history of IHD (66.7% of patients with subendocardial LGE had a history of IHD vs. 8.3% of LGE negative patients, Chi- squared $p<0.001$), hypercholesterolaemia (79.2% of patients with subendocardial LGE had a history of hypercholesterolaemia vs.

48.3% of LGE negative patients, p=0.002), and a positive smoking history (79.2% of subendocardial LGE vs. 41.7% LGE negative patients had ever smoked, p=0.001). Diabetes was present in a significantly greater proportion of patients with subendocardial LGE compared to LGE negative patients (54.2% vs. 30.2, Chi-squared p=0.028). The presence of subendocardial LGE was not associated with significant differences in gender, dialysis modality or duration, or a difference in blood pressure compared to those patients who were LGE negative.

3.4.4 Laboratory correlates of subendocardial late gadolinium enhancement

There were no significant differences in haemoglobin, dialysis adequacy in haemodialysis patients, calcium, phosphate or albumin between patients with subendocardial LGE and LGE negative patients (Table 3.6). Although LDL cholesterol was non-significantly lower in these patients (2.2 vs. 2.9 mmol/L, p=0.054), a higher proportion of these patients were treated with statin therapy that LGE negative patients (60.9%% vs. 38.7%, p= 0.055). Serum parathyroid hormone was significantly higher in these patients compared to LGE negative patients (median 32.5 vs. 12.5 pg/mL, p = 0.003). Unexpectedly in the small number of patients on peritoneal dialysis (n=5) with subendocardial LGE dialysis adequacy, as indicated by total weekly creatinine clearance, was greater in patients with subendocardial LGE (112.1 vs. 81.0 L/week/1.73m^2, p=0.009). This was primarily accounted for by greater residual renal function in these patients (64.7 vs. 31.1 L/week/1.73m^2, p=0.010). The converse was true in patients on haemodialysis with patients with subendocardial LGE having numerically poorer dialysis adequacy, as indicated by higher urea reduction ratio compared to LGE negative patients (66.5 vs. 70.8%, p=0.084).

3.4.5 Description and functional implications of diffuse late gadolinium enhancement

The second pattern, diffuse LGE, was less intense, without subendocardial dominance (Figures 3.8-3.10). Diffuse LGE was seen in 25 patients (17.2%). While this pattern was still 'patchy' and was therefore regional, this appears to represent regional areas of diffuse fibrosis within the left ventricle. These two patterns were not mutually exclusive and one patient, with severe LVH, diabetes, and coronary artery disease had both patterns of LGE. By contrast to subendocardial LGE, patients with diffuse LGE had no or minimal impairment of systolic function, compared to those without LGE (Table 3.4). However, diffuse LGE was associated with significantly greater LV mass compared to patients without LGE (105.7 vs. 100.9 g m^{-2}, p=0.003), and whilst the presence of LGE was also associated with a greater LV dilatation indicated by increased end diastolic volume (156.9 vs. 129.2 ml, p=0.023), this did not appear to have a negative impact on systolic function, with no overall reduction in ejection fraction (67.0 vs. 68.8%, p=0.389). There were no significant differences between measures of LV dilatation between patients with diffuse LGE and LGE negative patients when ventricular dimensions were normalised to body surface area. Unlike subendocardial LGE, the correlation between the mass of diffuse LGE and LV dimensions or function was less clear with no significant correlation between mass of myocardial tissue indicated by diffuse LGE and any ventricular dimension. Additionally, no wall motion abnormalities were observed in any ventricular segments affected by diffuse LGE.

3.4.6 Clinical correlates of diffuse late gadolinium enhancement

There was no association between the presence of diffuse LGE and the presence of cardiovascular risk factors such as blood pressure, smoking history, gender or history of previous IHD, nor was this associated dialysis modality (Table 3.5). Patients with

diffuse LGE had been receiving treatment with renal replacement therapy for a significantly longer duration than LGE negative patients (median time 36.0 vs. 7.0 months, p=0.036).

3.4.7 Laboratory correlates of diffuse late gadolinium enhancement

There were no significant differences in haemoglobin, dialysis adequacy or albumin between patients with diffuse LGE and LGE negative patients (Table 3.6). Systemic inflammation, as indicated by high sensitivity C-reactive protein was numerically higher in these patients (9.3 vs. 4.5 mg mL^{-1}, p=0.087). Despite proportionally lower numbers of these patients being treated with statin therapy (29.2 vs. 38.7 of LGE negative patients, p=0.387), patients with diffuse LGE had significantly lower total cholesterol than LGE negative patients (4.2 vs. 5.0 mmol L^{-1}, p=0.013) and LDL cholesterol (2.2 vs. 2.9 mmol L^{-1}, p=0.033). Additionally these patients had greater biochemical evidence of hyperparathyroidism than LGE negative patients (median PTH 30.5 vs. 12.5 pg mL^{-1}, p=0.029). Conversely, the overall calcium-phosphate product was significantly lower in patients with diffuse LGE (3.6 vs. 4.2 mmol L^{-1}, p=0.044).

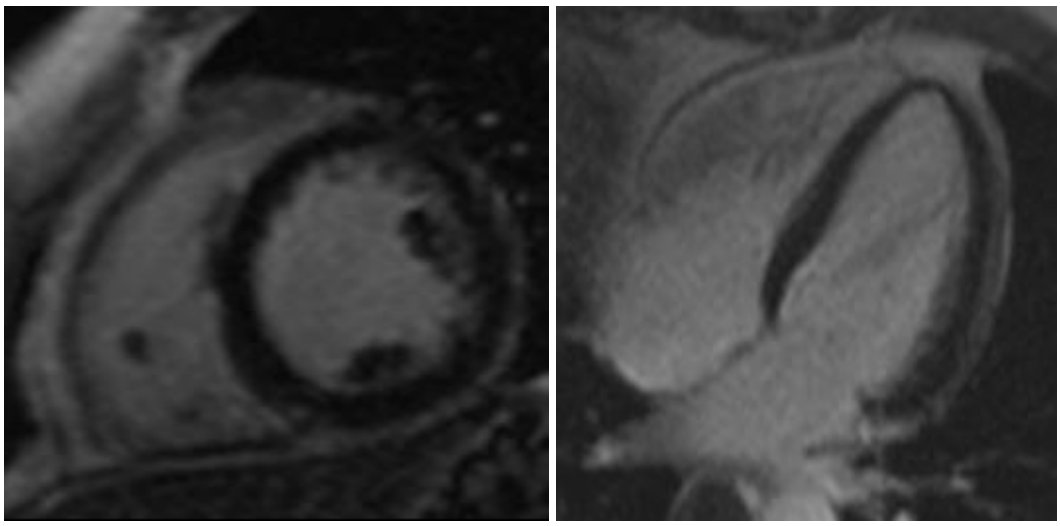


Figure 3.5

Contrast enhanced images of the left ventricle in a patient with ESRD with no evidence of LGE. A short axis view (left) and a horizontal long axis view are shown (right). The myocardium has been successfully nulled (black) and in this patient ventricular dimensions and function are normal

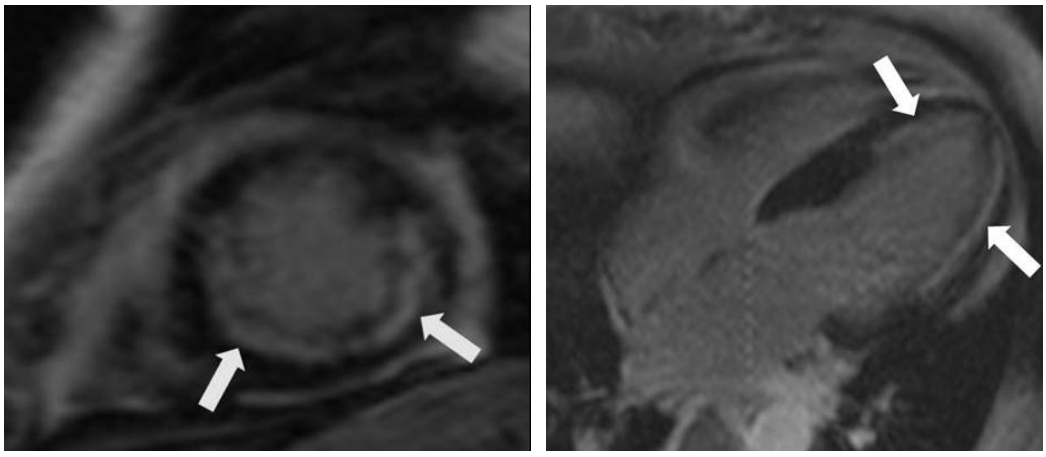


Figure 3.6

Contrast enhanced images of the left ventricle in a patient with ESRD with evidence of subendocardial LGE indicating a large old inferior myocardial infarction (arrowed). A short axis view (left) and a horizontal long axis view are shown (right)

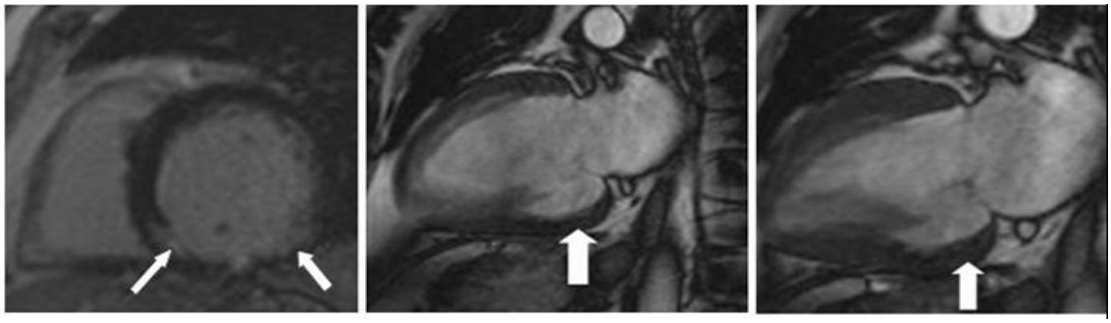


Figure 3.7

Patient with an inferior myocardial infarction indicated by subendocardial LGE (arrowed left panel) and a corresponding basal inferior wall motion abnormality demonstrated by a dyskinetic basal inferior segment arrowed in end diastole (centre panel) and end systole (right panel)

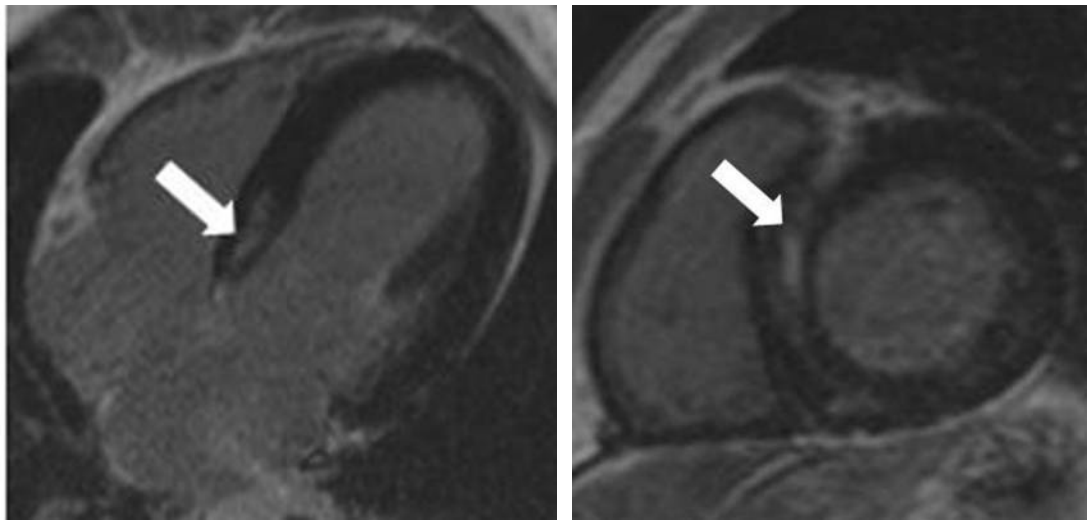


Figure 3.8

Horizontal long axis (left) and short axis (right) view of the heart of a patient with severe left ventricular hypertrophy and septal diffuse LGE (arrowed). Coronary angiography in this patient was normal with large unobstructed coronary arteries

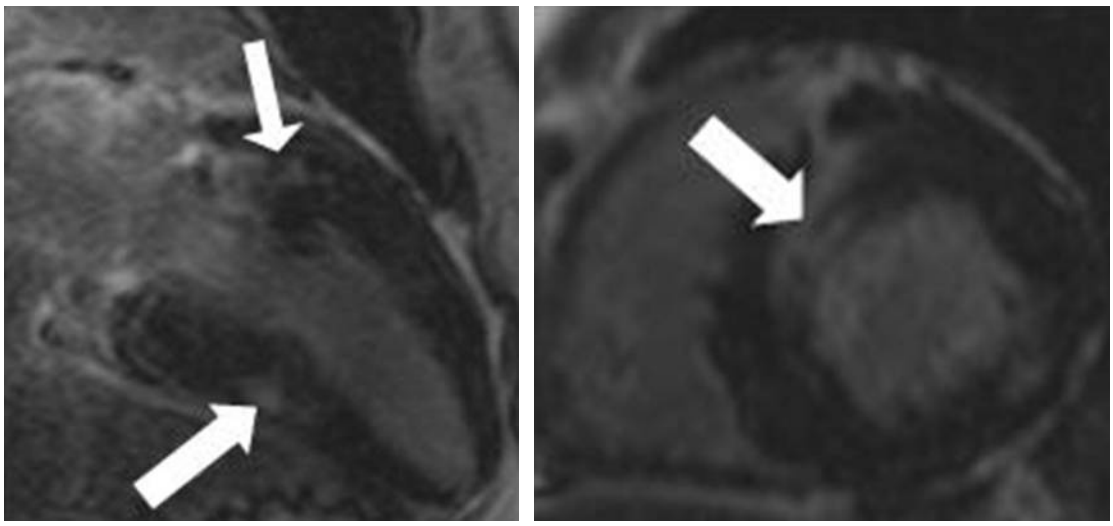


Figure 3.9 Vertical long axis (left) and short axis (right) view of the left ventricle in a further patient with severe left ventricular hypertrophy and septal and lateral wall diffuse LGE (arrowed). Coronary angiography in this patient was normal with large unobstructed coronary arteries and additionally the patient performed 11:30 minutes of a Bruce protocol exercise tolerance test with no ECG changes

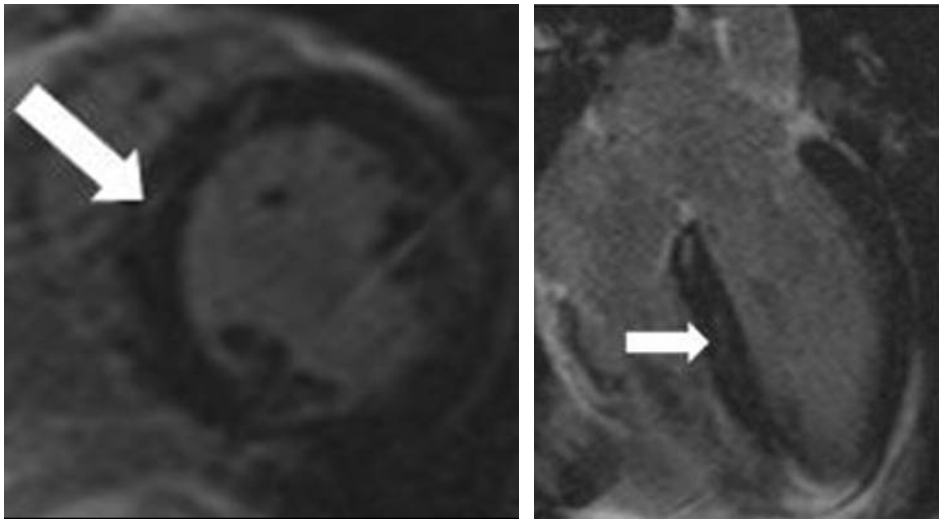


Figure 3.10 Short axis (left) Horizontal long axis (left) and view of a patient with moderate left ventricular hypertrophy and a lesser degree of septal diffuse LGE (arrowed). There is a minor degree of movement artefact on the left image. The patient was 30 years old with no angina and performed 12 minutes of a Bruce protocol exercise tolerance test with no ECG changes

3.4.8 Objective computerised analysis of identification and classification of late gadolinium enhancement

Owing to the subjective nature of classification of pattern of gadolinium enhancement, objective measurement was made to compare signal intensity (or pixel intensity on the analysed images), of the region of interest of LGE compared to that of nulled myocardium. The mean signal intensity of nulled myocardium was 6.4 units compared to a mean value 25.5 for all LGE positive tissue ($p<0.001$, Table 3.5). Subendocardial LGE had significantly higher mean signal intensity than that of diffuse LGE (32.6 vs. 18.1, $p<0.001$) demonstrating the distinct presence of diffuse LGE separate from any artefact in the acquisition of images, as well as potentially indicating the different tissue properties of subendocardial and diffuse LGE (Figure 3.11).

3.4.9 Relationship between results of contrast enhanced studies and coronary angiography

In total, 64 patients underwent coronary angiography (Table 3.6). Overall 39.1% had normal coronary arteries, with 28.1% having mild/moderate coronary artery disease (CAD) and 32.8% having severe CAD. A significantly greater proportion of patients with subendocardial LGE had severe CAD compared to LGE negative patients (82.4 vs. 12.9%, $p<0.001$) and additionally had a significantly atheromatous disease burden score than either patients with diffuse LGE or LGE negative patients (Mann-Whitney-U test, $p<0.001$ for comparison with either group). By contrast, the majority of patients with diffuse LGE had either normal coronary arteries or mild/moderate CAD with only 18.8% of these patients having severe CAD.

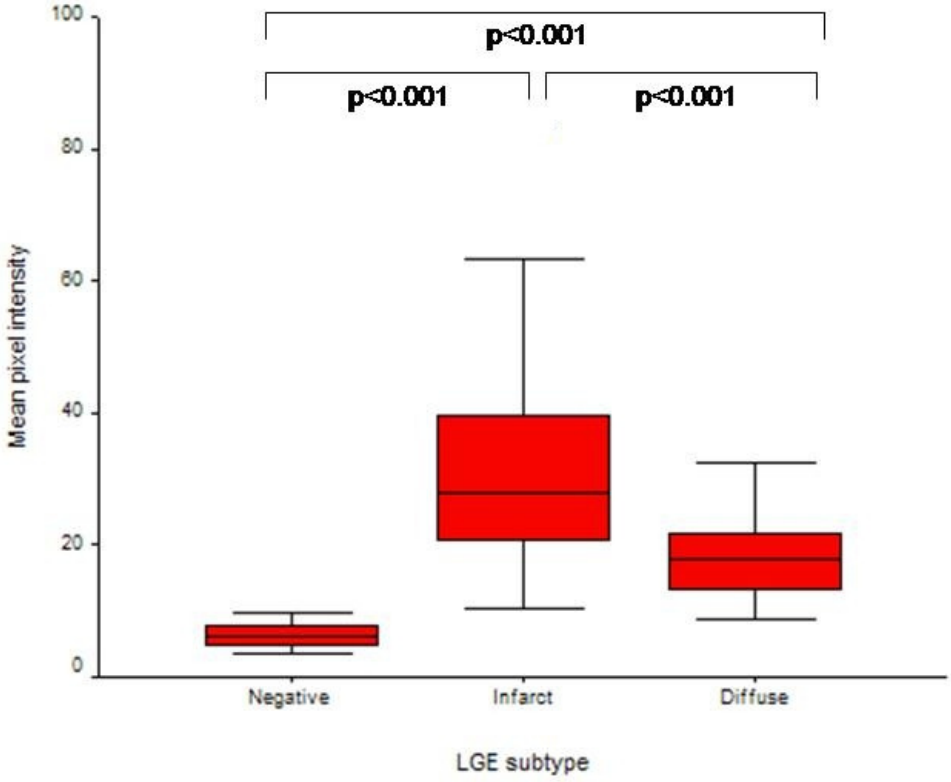


Figure 3.11 Box plot showing mean pixel intensity for subtypes of LGE pattern. Each box shows the median, quartiles, and extreme values within the LGE category

Variable	All patients	Gadolinium negative	Gadolinium positive	Subendocardial Gadolinium	Diffuse Gadolinium
Number (%)	145	96 (66.2)	49 (33.8)	24 (16.6)	25 (17.2)
LVH (%)	80 (55.2)	46 (47.9)	34 (69.4) *	14 (58.3)	20 (80.0) †
LV dilatation (%)	24 (16.6)	12 (12.5)	12 (24.5)	10 (41.7) †	2 (8.0)
LVSD (%)	25 (17.2)	8 (8.3)	17 (34.7) ‡	13 (54.2) ‡	4 (16.0)
EF (%) (SD)	66.1 (11.8)	68.8 (8.5)	60.7 (15.1) ‡	54.1 (14.9) ‡	67.0 (12.7)
LV mass (g)	168.3 (60.5)	157. 7 (60.6)	189.0 (55.4) †	177.3 (53.9)	200.2 (55.5) †
LV mass/BSA(g m^{-2})	91.4 (28.6)	100.9 (27.1)	86.6 (28.2) †	95.4 (28.5)	105.7 (25.5) †
ESV(ml)	51.3 (35.4)	41.6 (25.4)	70.4 (43.9) ‡	87.8 (47.7) ‡	53.6 (32.8)
ESV/BSA (ml m^{-2})	27.7(18.3)	22.9 (13.3)	37.2 (22.8) ‡	47.1 (24.4) ‡	28.4 (17.1)
EDV (ml)	142.4 (58.0)	129.2 (53.9)	168.2 (57.5) ‡	180 (61.5) ‡	156.9 (52.2) *
EDV/BSA(ml m^{-2})	77.2 (28.6)	71.3 (27.1)	89.1 (28.2) ‡	96.2 (30.1) ‡	82.8 (25.5)
Mass of LGE (g)	-	-	8.2 (5.4)	9.3 (6.4)	7.2 (4.3)
Myocardial Signal Intensity		6.4 (1.8)	25.5 (14.5) ‡	32.6 (16.7) ‡	18.1 (5.8) ‡

Table 3.4 Left ventricular dimensions displayed by subtype of LGE. Data are expressed as number with percent of specific subtype of late gadolinium enhancement (LGE) in parenthesis; all data are mean and standard deviation in parentheses or number and percentage are shown as appropriate. Percentages shown are as a proportion of that subtype of LGE. Tests of significance compared to patients with negative LGE: *- $p<0.05$, †$p<0.01$, ‡$p<0.001$

Variable	All patients	Gadolinium negative	Gadolinium positive	Subendocardial Gadolinium	Diffuse Gadolinium
Number (%)	145	96 (66.2)	49 (33.8)	24 (16.6)	25 (17.2)
Age (years)	51.9 (11.1)	51.1 (11.6)	53.4 (10.1)	54.4 (9.7)	52.4 (10.6)
Sex (% male)	88 (60.7)	56 (58.3)	32 (65.3)	16 (66.7)	16 (64.0)
Haemodialysis (%)	75 (51.7)	45 (46.9)	30 (61.2)	13 (54.2)	17 (68.0)
Duration of RRT	8.0 (52.5)	7 (22.0)	25 (100.5)	12.0 (97.0)	36.0 (98.5) *
History of IHD (%)	27 (18.6)	8 (8.3)	19 (38.8) ‡	16 (66.7) ‡	3 (12.0)
History of MI (%)	16 (11.0)	2 (2.1)	14 (28.6) ‡	14 (58.3) ‡	0 (0)
Diabetes (%)	46 (31.7)	29 (30.2)	17 (34.7)	13 (54.2) *	4 (16.0)
Hypercholesterolaemia (%)	72 (49.7)	42 (43.8)	30 (61.2)*	19 (79.2) †	11 (44.0)
Positive smoking history (%)	69 (47.6)	40 (41.7)	29 (59.2)*	19 (79.2) †	11 (44.0)
SBP (mmHg)	138.9 (25.3)	137.3 (22.9)	141.9 (29.5)	144.6 (26.8)	139.2 (32.2)
DBP (mmHg)	82.4 (13.4)	82.5 (12.1)	82.3 (15.7)	85.7 (14.7)	79.0 (16.2)

Table 3.5 Clinical characteristics of patients as grouped by presence and subtype of LGE. Numbers displayed are absolute number with percentage of group of LGE subtype or as mean and standard deviation as appropriate except for RRT time which is displayed as median and inter quartile range. Tests of significance are Chi-squared or t-test/Mann-Whitney-U as appropriate with significance indicated as * - $p<0.05$, † - $p<0.01$, ‡ - $p<0.001$ compared to LGE negative group

Variable	All patients	Gadolinium negative	Gadolinium positive	Subendocardial Gadolinium	Diffuse Gadolinium
Number (%)	145	96 (66.2)	49 (33.8)	24 (16.6)	25 (17.2)
Haemoglobin (g dL^{-1})	11.6 (1.7)	11.6 (1.8)	11.3 (1.4)	11.5 (1.6)	11.8 (1.6)
Cholesterol (mmol L^{-1})	4.8 (1.2)	5.0 (1.2)	4.3 (0.9) *	4.5 (1.1)	4.2 (0.7) *
Triglycerides (mmol L^{-1})	2.3 (1.4	2.3 (1.2)	2.4 (1.9)	2.7 (2.6)	2.1 (1.0)
VLDL (mmol L^{-1})	1.1 (0.6)	1.1 (0.5)	1.1 (0.8)	1.2 (1.1)	1.0 (0.5)
LDL (mmol L^{-1})	2.7 (1.1)	2.9 (1.0)	2.2 (1.0) †	2.2 (1.2)	2.2 (0.9) *
HDL(mmol L^{-1})	1.1 (0.4)	1.1 (0.4)	1.0 (0.5)	1.0 (0.4)	1.0 (0.5)
CRP (mg L^{-1})	4.8 (10.7)	4.5 (8.2)	9.2 (29.0)	7.8 (31.5)	9.3 (28.5)
PTH (pg mL^{-1})	16.7 (35.0)	12.5 (27.0)	31.0 (51.6) †	32.5 (57.6) †	30.5 (53.8) *
Corrected Calcium (mmol L^{-1})	2.4 (0.2)	2.4 (0.2)	2.3 (0.2)	2.3 (0.1)	2.3 (0.2)
Phosphate (mmol L^{-1})	1.7 (0.5)	1.8 (0.5)	1.7 (0.5)	1.8 (0.5)	1.6 (0.4)
Ca-P product	4.1 (1.2)	4.2 (1.2)	3.9 (1.3)	4.3 (1.5)	3.6 (1.0) *
Albumin (g L^{-1})	39.2 (4.5)	39.1 (4.4)	39.5 (4.8)	39.9 (5.4)	40.1 (4.1)
URR (%)	70.5 (7.5)	70.8 (7.5)	70.0 (7.7)	66.5 (8.4)	73.0 (5.6)
CrCl (L/week/1.73m^2)	85.5 (25.1)	81.0 (21.7)	97.8 (30.4)	112.1 (28.4) †	83.6 (27.6)

Table 3.6 Laboratory characteristics of patients grouped by presence and subtype of LGE. Numbers displayed are absolute number with percentage of LGE group subtype or as mean and standard deviation as appropriate. CRP and PTH are displayed as median and inter quartile range. URR is for HD patients. Weekly creatinine clearance is for patients on peritoneal dialysis. Tests of significance are Chi-squared or t-test/Mann-Whitney-U as appropriate with significance indicated as * - $p<0.05$, † - $p<0.01$, ‡ - $p<0.001$ compared to LGE negative group

Variable	All patients	LGE negative	Subendocardial LGE	Diffuse LGE
Number (%)	64	31 (48.4)	17 (26.6)	16 (25.0)
Normal angiogram (%)	25 (39.1)	18 (58.1)	0 (0)	7 (43.8)
Mild/moderate CAD (%)	18 (28.1)	9 (29.0)	3 (17.6)	6 (37.5)
Severe CAD (%)	21 (32.8)	4 (12.9)	14 (82.4) ‡	3 (18.8)
Atheroma score	65 (164.3)	48 (100.0)	195 (155.5) ‡	36.5 (134.0)

Table 3.7

Coronary angiography results by presence and subtype of LGE. Numbers displayed are absolute number with percentage of LGE group subtype. Median and inter quartile range are shown for atheroma score. Tests of significance are Chi-squared or Mann-Whitney-U as appropriate with significance indicated as ‡ - $p<0.001$ compared to LGE negative group

3.5 DISCUSSION

3.5.1 Uraemic cardiomyopathy defined by CMR

Left ventricular abnormalities, previously described echocardiographically as 'uraemic cardiomyopathy' have been demonstrated to convey important prognostic implications in patients with ESRD. Epidemiological studies of patients with ESRD suggest that at inception of renal replacement therapy the majority of patients display some abnormality in left ventricular dimensions, geometry or function. This study was designed to reassess the prevalence of LV disorders using CMR, and furthermore explore the relationship between LV abnormalities and myocardial fibrosis indicated by late gadolinium enhancement.

Firstly, as expected patients with ESRD had significantly higher left ventricular mass compared to controls. Using published reference ranges for LV dimensions with CMR, 41.3% of this cohort of uraemic patients had normal LV dimensions, higher than reported echocardiographically. Whether this lower prevalence of LV disorders is due to a 'healthier' cohort of patients, as these patients were fit enough to be considered for renal transplantation, better treatment of hypertension and hence left ventricular hypertrophy, or due to genuine differences between measurements made with CMR versus echocardiography requires further study of direct comparison between CMR and echocardiography. Only 16.2% of patients in the uraemic cohort displayed evidence of left ventricular systolic dysfunction, which is in keeping with reported prevalence in echocardiographic studies of patients with ESRD as well as those few pilot studies that exist with CMR(75;113;189). Blinded analysis demonstrated that CMR measures of LV dimensions are more reproducible than has been reported with echocardiography(194).

This indicates that CMR may allow substantial reduction in numbers for interventional studies where LV mass or ejection fraction is a clinical end point(195).

At the time of performing this study contrast enhanced CMR using gadolinium based contrast agents had become an established clinical tool in the management of patients with heart failure, cardiomyopathies and coronary artery disease. However, this represents the first published study using this technique in patients with ESRD. Other centres have performed smaller pilot studies, reported in abstract form, whose results appear similar to our own. The published literature has categorised uraemic cardiomyopathy defined by echocardiography into three subtypes – LVH, ventricular dilatation or systolic dysfunction (or a combination of these patterns) with each pattern associated with cumulatively worse survival(75;76). By contrast, the results of this study based on CMR findings, suggest that there are only two types of uremic cardiomyopathy. The first is due to underlying ischaemic heart disease where LV dilatation and systolic dysfunction are due primarily to previous myocardial infarction revealed by CMR. The second form, diffuse LGE found in patients with more severe LVH, is more specific to uraemia is consistent with fibrosis in the hypertrophied ventricle.

Gd-DTPA-BMA is an extracellular contrast agent that diffuses into the interstitial space between cells and exhibits its effect by shortening the T_1 relaxation of tissue in a magnetic field. There is greater space for Gd-DTPA-BMA accumulation both in areas of myocardial fibrosis and oedema. In areas of ischaemia or infarction, gadolinium leaks into the surrounding fibrotic or oedematous, tissue resulting in late gadolinium enhancement on CMR imaging(174). However, the presence of LGE is not specific and further subjective assessment of the pattern of LGE is required. LGE has also been

described in patients with myocarditis, dilated cardiomyopathy and other inflammatory cardiomyopathies(182-184). Importantly, LGE has been shown to represent collagen deposition in hypertrophic cardiomyopathy(177).

3.5.2 Clinical implications of subendocardial LGE

Subendocardial LGE, consistent with that described in myocardial infarction, follows a primarily subendocardial distribution. This reflects the perfusion of the subendocardium by end-arteries. Therefore, a high prevalence of myocardial infarction in this cohort of patients has been demonstrated indicated by subendocardial LGE. Patients with subendocardial LGE had greater evidence of LV systolic dysfunction, clearly highlighting the importance of underlying ischaemic heart disease as the cause for ventricular impairment in these patients. While this was associated with a previous history of ischaemic heart disease, eight patients (4.7% of the whole cohort or one third or all patients with CMR evidence of infarction indicated by subendocardial LGE) had CMR evidence of myocardial infarction, despite no preceding history, in keeping with studies suggesting that ESRD patients are more likely to have silent myocardial ischaemia(53). Subendocardial LGE was associated with many 'classical' risk factors for ischaemic heart disease, present in the general population such as diabetes and positive smoking history. Although a significantly higher number of these patients were labelled as hypercholesterolaemic, this may be due to the factor that a higher numerically proportion of patients were on statin therapy than LGE negative patients (60.9 vs. 38.7%, p=0.055). This however did not in turn lead to a significantly lowered total or LDL cholesterol compared to LGE negative patients, and consequently one may speculate that patients with subendocardial LGE may have had higher pre-treatment serum cholesterol than LGE negative patients.

The only risk factors specific to uraemia associated with subendocardial LGE were raised serum PTH levels and greater dialysis adequacy in patients on peritoneal dialysis. This second finding was unexpected and is unlikely to be of clinical significance given the extremely small numbers of peritoneal dialysis patients with subendocardial LGE. This finding may be explained by higher numbers of diabetic patients on peritoneal dialysis. These patients, at greatest cardiovascular risk are also likely to be commenced on renal replacement therapy at a higher residual GFR due to excessive symptoms of uraemia. Higher dialysis adequacy has not been shown to be associated with improved outcomes in peritoneal dialysis patients(196). By contrast, the presence of hyperparathyroidism contributes to accelerated cardiovascular disease in uraemia by two mechanisms. Firstly, it is likely that high levels of PTH are associated with arterial calcification, which in turn may be associated with atherosclerosis (particularly intimal calcification)(197). Of interest, in the current study, this was not associated with significant abnormalities in serum calcium, phosphate or calcium-phosphate product. Additionally, it has been shown that parathyroid hormone as a permissive factor promoting cardiac fibroblast activation and intercardiomyocytic fibrosis therefore promote LVH(139). This is likely to be important in the pathogenesis of diffuse LGE, which will be discussed later in this section.

The data on coronary angiography explore further the relationship between results of contrast enhanced CMR, LGE and cardiomyopathy. First, in the absence of LGE patients were relatively unlikely to have significant coronary artery disease requiring intervention. Only 12.9% of these patients had severe coronary artery disease. As this technique represents a static scan, a negative CMR for LGE does not exclude the presence of critical coronary disease, but makes it less likely. Conversely, no patients with subendocardial LGE had a normal angiogram and the majority of these patients

had critical coronary stenosis, where coronary intervention with angioplasty or bypass surgery may be appropriate. Similarly, these patients have a significantly higher total atheroma burden than either LGE negative patients or those with diffuse LGE.

The discovery of this high prevalence of myocardial infarction in ESRD patients is important given the poor long-term survival of these patients post-infarction and general trend towards under treatment in this patient group(53). Thus, the diagnosis of sub-clinical myocardial infarction with CMR is likely to have prognostic and therapeutic implications and may be an indication for further assessment with angiography, particularly if the patient is being considered for renal transplantation. Furthermore, in patients with coronary artery disease requiring intervention, CMR can assess myocardial viability prior to revascularisation, allowing optimal identification of patients likely to benefit from coronary intervention(175).

3.5.3 Other studies displaying myocardial ischaemia with subendocardial LGE at CMR

Recent studies using similar CMR protocols to detect the presence of myocardial infarction, represented by the presence of subendocardial LGE, have focused on various goals such as early detection of myocardial infarction, detection of silent infarction, accurately characterising the location of infarcted tissue and most promisingly prediction of myocardial viability post revascularisation in patients with critical coronary artery lesions:

- Kim *et al* used CMR to identify reversible myocardial dysfunction can be identified by contrast-enhanced MRI before coronary revascularisation (either surgical or by percutaneous intervention). Their results suggest that in areas of abnormal ventricular contraction, improvement in contractility can be predicted

by the percentage of that myocardial segment that is composed of tissue indicted by LGE. For instance, contractility increased in 256 of 329 segments (78 percent) with no LGE before revascularisation, but in only 1 of 58 segments with LGE occupying more than 75 percent of tissue. The percentage of the left ventricle that was not enhanced before revascularisation was strongly related to the degree of improvement in the ejection fraction after revascularisation(175)

- In a study of patients with dilated cardiomyopathy of unknown aetiology CMR differentiated between patients with ischaemic cardiomyopathy and idiopathic dilated cardiomyopathy. This has important implications for both secondary prevention in ischaemic heart disease and genetic screening for patients with idiopathic dilated cardiomyopathy which may have an underlying genetic basis(182)

3.5.4 Clinical implications of diffuse LGE

The second pattern of gadolinium enhancement demonstrated was a pattern of diffuse, less intense, LGE. This was an unexpected finding. This diffuse gadolinium enhancement is less striking than that seen in, for example, myocardial infarction or hypertrophic cardiomyopathy. However, it was reproducible and not due to artefact in the imaging process, and was associated with features of uraemic cardiomyopathy, most notably LVH. Coronary angiography revealed that that many patients with diffuse LGE had either no or minimal coronary artery disease. This was perhaps unexpected but suggests that diffuse LGE is not due to large vessel coronary artery disease. This does not exclude that it may be due to low grade ischaemia leading to fibrosis or to permissive factors in uraemia as discussed previously.

Diffuse gadolinium enhancement implies an additional pathological process, other than large vessel myocardial infarction. It may reflect diminished capillary blood supply with associated fibrosis, which is a prominent histopathological finding in the uraemic heart, or low-grade ischaemia. It may be that small vessel disease is also present in patients in the absence of epicardial atherosclerosis. Alternatively, this pattern of enhancement may most likely represent fibrosis throughout the hypertrophic ventricle, being evident in severe hypertrophy. Overall, the presence of gadolinium enhancement may not be due to a single process. To address whether uraemia or ventricular hypertrophy is a dominant factor in its pathogenesis, a larger study of gadolinium enhancement in patients with LVH due to hypertension and normal renal function is required.

The clinical and laboratory correlates of diffuse LGE to an extent support the notion that LVH represents a form of cardiomyopathy more specific to uraemia than systolic dysfunction, which is primarily due to underlying ischaemic heart disease. Diffuse LGE was in particular associated with a significantly greater duration of renal replacement therapy than LGE negative patients. There were no associations with conventional risk factors for coronary artery disease such as smoking history, age, diabetes, blood pressure or hypercholesterolaemia. Indeed, despite lower proportions of patients with diffuse LGE being on statin therapy (29.2%), these patients had significantly lower serum total and LDL-cholesterol than LGE negative patients as well as higher (although non-significantly) C-Reactive protein. This suggests that these patients are at greater risk of the malnutrition-inflammation complex syndrome, which has been postulated in patients on dialysis therapy(160). Further support for this concept is illustrated by significantly lower serum phosphate levels in these patients which may suggest poor nutritional intake. Conversely lower phosphate levels are usually a marker of good

dialysis and have been previously associated with better outcome, therefore this result may be paradoxical(137).

3.5.5 Other studies displaying diffuse LGE with contrast enhanced CMR

Although no directly comparable reports of use of CMR in patients with ESRD exist, a number of authors have performed similar studies in other patient groups:

- Recent data have emerged suggesting that areas of scarring similar to diffuse LGE are present in patients with adaptive LVH secondary to aortic stenosis. Although the terminology used by the authors for the description of the pattern of LGE is different in the study of aortic stenosis the images are remarkably similar(198)
- Our own group have demonstrated areas of diffuse LGE in the interventricular septum in patients with right ventricular pressure overload due to primary pulmonary hypertension(199)
- Patchy foci of midwall LGE have been described in patients with idiopathic dilated cardiomyopathy. In this study the authors speculate that these foci of midwall LGE probably reflect focal segmental fibrosis found at autopsy in patients with dilated cardiomyopathy(182)
- A form of diffuse LGE has been described in patients with systemic amyloidosis and associated infiltrative cardiomyopathy(185)
- LGE has also been reported in two storage diseases with myocardial involvement, namely glycogenosis and Anderson-Fabry disease(200;201). In Anderson-Fabry disease diffuse LGE was mainly localised to the basal inferolateral LV wall and was not involving the subendocardium

3.5.6 Other studies assessing myocardial tissue composition in ESRD

A number of studies have reported tissue abnormalities in patients with ESRD:

- Aoki *et al* performed endomyocardial biopsies in patients with no evidence of coronary artery disease and cardiac dysfunction and found evidence of myocardial fibrosis(202)
- Post mortem studies have shown evidence of tissue fibrosis in patients with ESRD(85)
- Animal models have demonstrated similar findings and explored factors which may promote tissue fibrosis in renal failure(203)
- Using CMR spectroscopy it has been demonstrated that myocardial metabolism is altered in patients with ESRD. This abnormality is accentuated by diabetes and may be ameliorated by renal transplantation(204). Whether these metabolic abnormalities are related to myocardial fibrosis is unknown

3.5.7 Limitations

This study has some weaknesses. The patients studied were being considered for renal transplantation, who are fitter than the majority of patients with ESRD, and hence found a lower prevalence of systolic dysfunction than might be expected. To fully appreciate the burden of cardiovascular disease in patients with ESRD, it would be necessary to study the entire dialysis population. Therefore, selection bias cannot be excluded, and a higher prevalence of LVSD may be expected (but also an even higher prevalence of IHD) in an unselected cohort of dialysis patients. Coronary angiography (and endomyocardial biopsy) in all patients would provide additional information regarding the relationship between LGE and coronary artery disease, but is invasive, and therefore not without risk. This was not deemed ethically justifiable by the consultants responsible for the patients' overall care. Hence correlation between angiographic and

CMR findings should be interpreted with caution. In analysis of LGE, using signal intensity, greater distinction between nulled myocardium and areas of LGE (using a signal intensity of LGE >2 standard deviations higher the mean intensity of an area of reference myocardium), would give greater distinction in image analysis, but may exclude definite areas of LGE. Future use of computer software to automatically detect areas of LGE will optimise detection of enhancement.

3.5.8 Conclusions

In this study, systolic dysfunction and ventricular dilatation are not present in the absence of late gadolinium enhancement and it is only patients with focal gadolinium enhancement that have significant ventricular dilatation and systolic dysfunction. This suggests that LVH is the only cardiomyopathy truly associated with uraemia (and presumably is a consequence of hypertension and other factors associated with renal failure) whilst systolic dysfunction is not due to uraemia, but to underlying ischemic heart disease. Therefore CMR represents an advance for cardiovascular investigation to guide invasive assessment, particularly in asymptomatic patients, undergoing evaluation for renal transplantation. The implication is that patients with systolic dysfunction require investigation of coronary heart disease. The prognostic implications of both subendocardial and diffuse LGE require further study.

Chapter 4

A comparative study of echocardiography and electrocardiography with cardiac magnetic resonance imaging

4.1 INTRODUCTION

Left ventricular function, dimensions and geometry have conventionally been assessed using echocardiography. As previously discussed, this technique is convenient and reproducible and conveys important prognostic and diagnostic information for patients with ESRD. However M-mode echocardiography, a one-dimensional technique, overestimates left ventricular mass in this population compared to CMR(189). The disparities between the two methods have also been demonstrated in other patient populations. The geometrical assumptions used by M-mode echocardiography have primarily been validated in normal hearts or in small studies compared to post mortem specimens(205;206). Echocardiography is operator dependent and is limited by the acoustic window which depends on cardiac geometry, body habitus and co-morbidity such as respiratory disease or obesity. No attempt has been made to derive a correction factor to optimise M-mode echocardiographic measures of left ventricular mass in this population.

The electrocardiogram (ECG) is readily available, easy and inexpensive to acquire and is widely used in both research and clinical practise to diagnose left ventricular hypertrophy (LVH). Like echocardiography, ECG measures of LVH have prognostic value in patients with ESRD(207). However, the criteria used to classify LVH were derived from echocardiographic measures of left ventricular mass which may have limitations, particularly in groups where there is a high prevalence of LVH. These studies were designed to:

- Compare echocardiographic measures of left ventricular dimensions in patients in patients with ESRD with CMR
- Use CMR to optimise echocardiographic formula to derive left ventricular mass

- Evaluate the Sokolow-Lyon voltage, Cornell sex-specific voltage, Sokolow-Lyon product and Cornell product criteria against left ventricular mass index as measured by CMR in a cohort of patients with ESRD
- Use CMR to derive optimised ECG partition values for LVH in the ESRD population

4.2 METHODS

4.2.1 CMR and echocardiography

All patients were studied with CMR technique and echocardiography performed on the same day described in detail in Chapter 2 (2.3.2). As some of the disparity between echocardiographic and CMR measures of LV dimensions may be due to changes in hydration status related to the dialysis cycle, only patients with ESRD established on dialysis therapy on with both echocardiographic and CMR data were studied.

4.2.2 Echocardiogram- analysis

Left ventricular (LV) dimensions were determined from two-dimensional guided M-mode images, using American Society of Echocardiography leading edge recommendations. The mean of three measurements was taken. As the most widely used method the Penn (Devereux-Reichek) was used for the majority of analyses of LV mass. The utility of the following alternative formulae for calculation of left ventricular mass was also assessed(205;206;208;209):

Penn: = $1.04 \times [(IVST + PWT + LVID)^3 - LVID^3] - 13.6$

ASE: = $0.80 \times [(IVST + PWT + LVID)^3 - LVID^3] + 0.6$

Troy = $1.05 \times [(IVST + PWT + LVID)^3 - LVID^3] - 13.6$

Teichholz: = $1.04 \times \{[7/(2.4 + IVST + PWT + LVID)] \times (IVST + PWT + LVID)^3 - [7/(2.4 + LVID)] \times (LVID)^3\}$

where LVID - left ventricular internal diameter, IVST - interventricular septal thickness and PWT - posterior wall thickness. LVH was defined by conventional criteria (LVMI $\geq$131 or 110 g/m^2 in men and women, respectively). Systolic function was assessed by measuring LV ejection fraction (LVEF), calculated from end-systolic and end-diastolic volumes determined from the mean of three measurements in different cardiac cycles using modified biplane disc summation (Simpson's rule).

4.2.3 Electrocardiogram

This was performed prior to echocardiography. ECG criteria were analysed in a blinded fashion. The following ECG criteria were tested: Sokolow-Lyon voltage(210), Cornell sex-specific voltage(211), Cornell product, and Sokolow-Lyon product(212). LVH was defined as a Sokolow-Lyon voltage amplitude of (SV1+RV5 or RV6)≥35 mV, a Cornell voltage of (RaVL+SV3)≥28 mV for men and ≥20 mV for women, and a Cornell product of [(RaVL+SV3)*QRS duration] ≥2440 mV ms. There is no recognised partition value for the Sokolow-Lyon product [(SV1+RV5 or RV6)*QRS duration].

4.2.4 Statistical methods

Correlations between cardiac dimensions by the various methods were assessed with Pearson and Spearman correlation co-efficient as appropriate, with comparisons made using Bland-Altman plots. Receiver operator characteristic (ROC) curves were plotted

to assess diagnostic utility of tests for categorical variables. All analyses were performed using the SPSS 13.0 statistical software package (SPSS Inc., Chicago, IL., USA), except Bland-Altman plots and ROC curves which were performed with the MedCalc software package (MedCalc 8.1, MedCalc Software, Belgium). Derivation of revised echocardiographic formula was performed with OriginPro 7.0 software (OriginLab Corporation, Northampton, MA, USA).

4.3 RESULTS OF ECHOCARDIOGRAPHIC STUDIES

4.3.1 Patient demographics

Echocardiography combined with CMR were available on 128 patients, of whom 28 were excluded as not established on long term dialysis therapy (25 advanced renal failure not yet started dialysis, 3 failing renal transplant). Patient demographics for the 100 patients studied at the time of scan are shown in Table 4.1. 15 (15.0%) patients had insufficient M-mode echocardiographic images to calculate LVM and 30 (30.0%) had inadequate images for calculation of LVEF by Simpson's bi-plane method.

4.3.2 Echocardiographic compared to CMR descriptions of uraemic cardiomyopathy

Subdividing the cohort by conventional definitions of uraemic cardiomyopathy using the Penn formula to calculate LVM with echocardiography; 16.5% had normal left ventricular dimensions and function, 81.2% had LVH, 34.9% had left ventricular dilatation and 18.7% had left ventricular systolic dysfunction. This compares to CMR measures for the entire cohort of patients on dialysis therapy of 54.0% with LVH, 18.0% LV dilatation and 13.0% systolic dysfunction. 39.0% of patients had normal LV dimensions by CMR. For both imaging modalities these patterns were not mutually

exclusive and by echocardiography, 50.6% had isolated LVH, with 15.3% of patients having combined LVH and LV dilatation and 7.1% of patients having combined LVH, dilatation and LVSD. By CMR, 39.0% had isolated LVH, 9.0% had combined LV dilatation and LVH, and 6.0% having combined LVH, dilation and LVSD (Figures 4.1-4.2). LVM was greater in patients on haemodialysis compared to peritoneal dialysis (LVMI by CMR 102.9 vs. 78.7g m^{-2}, $p<0.001$).

4.3.3 Echocardiographic measures of left ventricular geometry

Using the criteria described in Chapter 2 to describe ventricular remodelling, 45.2% had normal ventricular geometry and 54.8% had eccentric remodelling. Of the patients with LVH, 44.9% had concentric LVH and 55.1% had eccentric LVH. There were no significant differences in remodelling patterns in between patients on haemodialysis compared to peritoneal dialysis (44.2% of haemodialysis patients had normal geometry compared to 46.9% of peritoneal dialysis, Chi-squared p=0.813), nor between patients with a past history of ischaemic heart disease (41.2% of patients with IHD had normal geometry compared to 46.3% with no history of IHD, Chi-squared p=0.706). Patients with eccentric remodelling had a significantly greater degree of left ventricular dilatation than those with normal or concentrically remodelled ventricles (117.3 vs. 89.7 ml m^{-2}, p=0.001; Figure 4.3).

4.3.4 Comparison of CMR with echocardiography for calculation of left ventricular mass and dimensions

Results and correlation between the echocardiographic dimensions using the Simpson's bi-plane method and Penn formula for LV mass and CMR studies are shown in Table 4.2 The correlations between CMR and echocardiographic measures of LV mass and LVMI measures using the Penn, ASE, Troy and Teichholz formulae are shown in Table

4.3 and Figure 4.4. A Bland-Altman plot (Figures 4.5 and 4.6) comparing the two methods indicates that using echocardiographic formula the Penn formula overestimates LVM by a mean of 141.9g and the ASE formula overestimates a mean of 89.9g compared to CMR. Echocardiography overestimates LVMI by 77.6g m^{-2} and 49.4g m^{-2} with the Penn and ASE formulae respectively compared to CMR. The Teichholz formula overestimated LV mass by and LVMI by 15.5g and 9.4g m^{-2}. There was no significant difference in over estimation of LV mass when patients were compared by pattern of ventricular remodelling (overestimation for eccentric remodelling and hypertrophy 140.6 vs. normal geometry and concentric LVH 143.6 g, p=0.87). Using the Simpson's bi-plane method to calculate end diastolic and end systolic volumes, echocardiography underestimates end diastolic volume by a mean of 46.9ml and end systolic volume by 7.2ml compared to CMR (Figures 4.7 and 4.8).

Number	100	
Male (%)	65	(65.0)
Age (years)	52.3	(11.0)
Height (m)	168.5	(10.2)
Weight (kg)	74.7	(16.7)
SBP (mmHg)	138.8	(26.1)
DBP (mmHg)	81.7	(18.3)
Haemodialysis (%)	59	(59)
1^0 Renal Disease (%)		
Diabetes	22	(22.0)
APKD	9	(9.0)
GN	25	(25.0)
CPN	10	(10.0)
Renovascular	5	(5.0)
Unknown/Other	29	(29.0)
RRT time (months)	9	(59.3)
Previous IHD (%)	21	(21.0)

Table 4.1

Background demographics for patients studied with CMR and echocardiography. Results are show as mean with standard deviation in parenthesis or number with percentage in parenthesis as appropriate except for RRT time which is displayed as median and inter quartile range

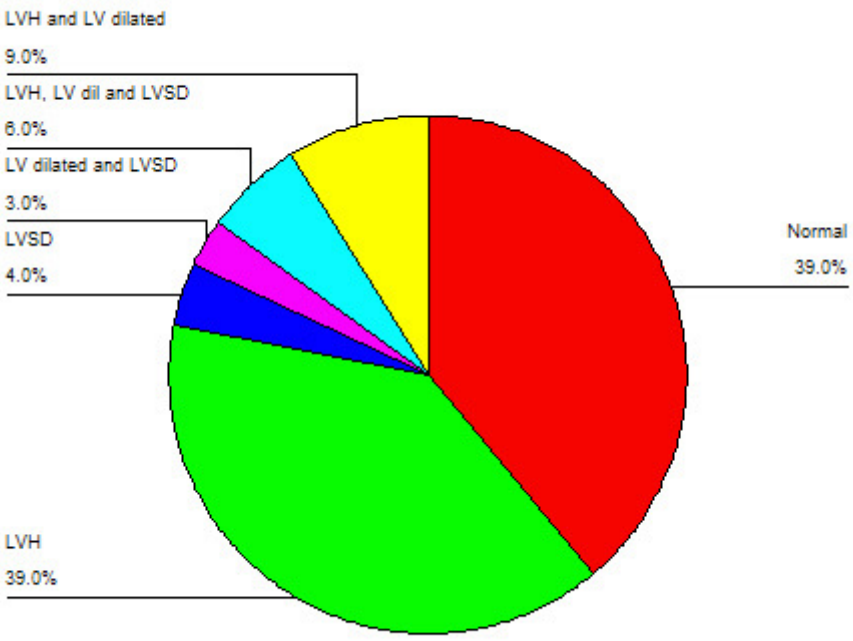


Figure 4.1 Prevalence of left ventricular abnormalities as detected by CMR

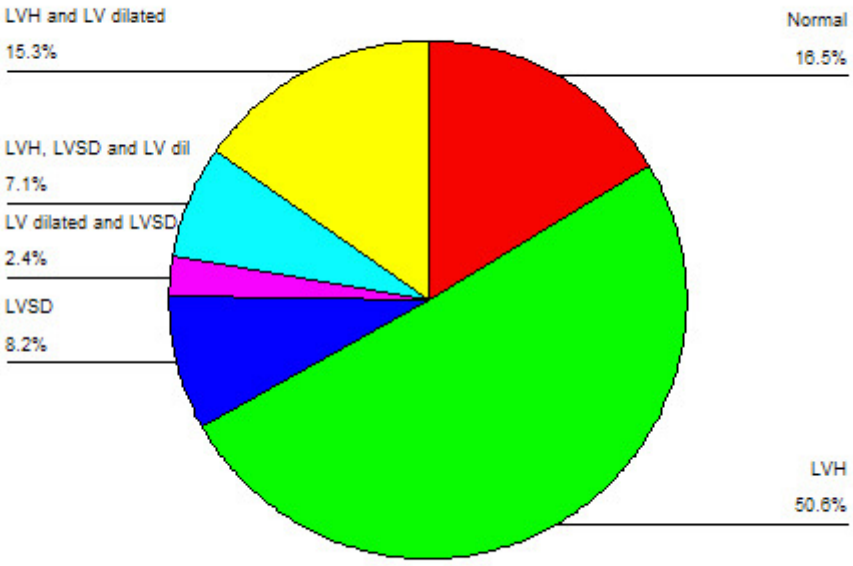


Figure 4.2

Prevalence of left ventricular abnormalities as detected by echocardiography

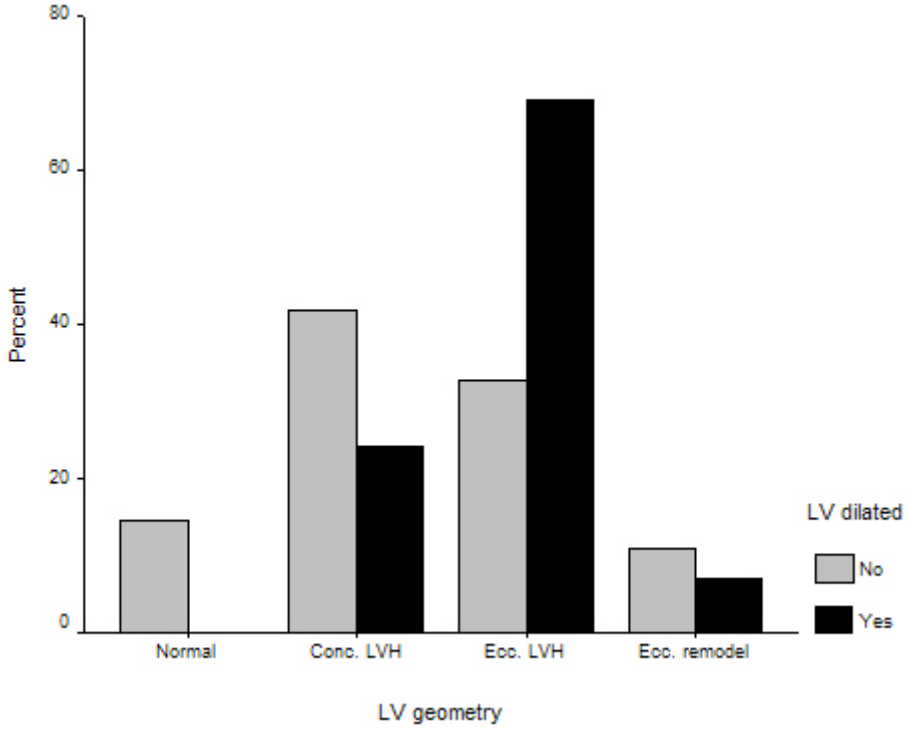


Figure 4.3

Proportion of patients with left ventricular dilatation as classed by echocardiographic pattern of remodelling

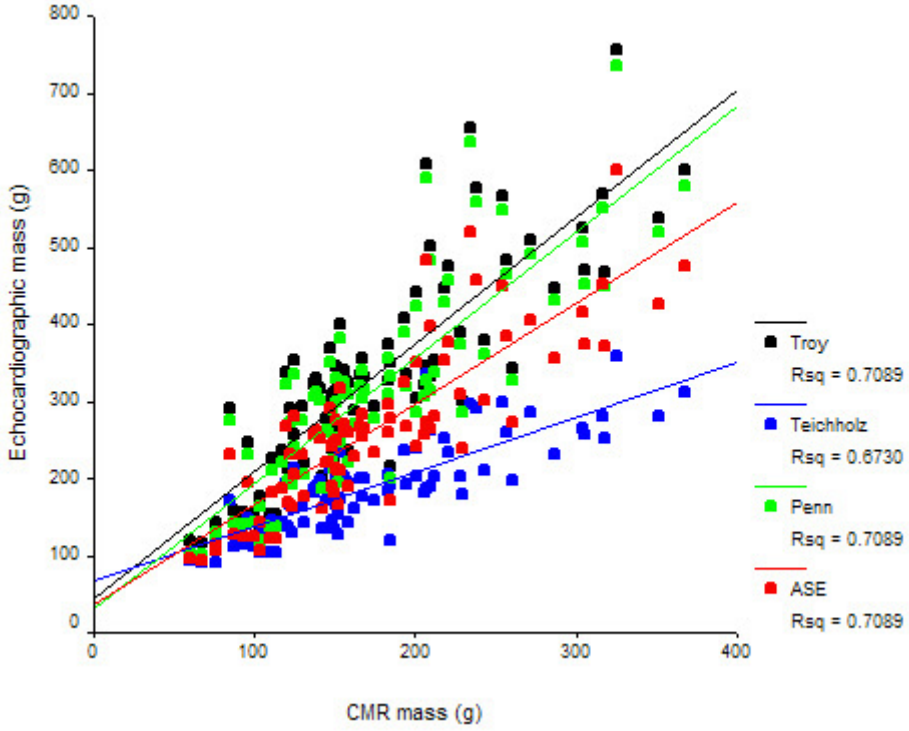


Figure 4.4

Scatter plots of echocardiographic measures versus CMR measures of LV mass for various M-mode echocardiographic formulae to calculate LV mass

	Echocardiography		CMR		R	p
EF (%)	58.4	(7.4)	67.6	(10.5)	0.51	<0.001
EDV (mL)	105.7	(34.9)	142.8	(59.1)	0.86	<0.001
ESV (mL)	45.0	(20.0)	48.5	(31.4)	0.82	<0.001
EDV/BSA (mLm^{-2})	57.8	(17.0)	77.1	(28.9)	0.86	<0.001
ESV/BSA (mLm^{-2})	24.6	(10.3)	26.1	(16.1)	0.82	<0.001
Mass (g)	315.2	(130.1)	173.1	(65.2)	0.84	<0.001
Mass/BSA ($g\ m^{-2}$)	171.5	(63.7)	93.2	(30.8)	0.81	<0.001

Table 4.2 Comparison of echocardiographic and CMR measures of left ventricular dimensions. Values displayed are mean with standard deviation in parentheses. Correlation between variables (R) is by Spearman co-efficient

Method	Mean LV mass (g)		R	p	Mean LVMI ($g\ m^{-2}$)		R	p
ASE	263.6	(104.1)	0.84	<0.001	143.5	(50.8)	0.81	<0.001
Penn	315.2	(130.1)	0.84	<0.001	171.5	(63.7)	0.81	<0.001
Troy	332.0	(131.4)	0.84	<0.001	180.7	(64.1)	0.81	<0.001
Teichholz	190.1	(58.2)	0.82	<0.001	104.0	(28.7)	0.76	<0.001

Table 4.3

Comparison of M-mode methods of calculating left ventricular mass. Values displayed are mean with standard deviation in parentheses. Correlation is by Spearman co-efficient (R) compared to CMR measures of LV mass

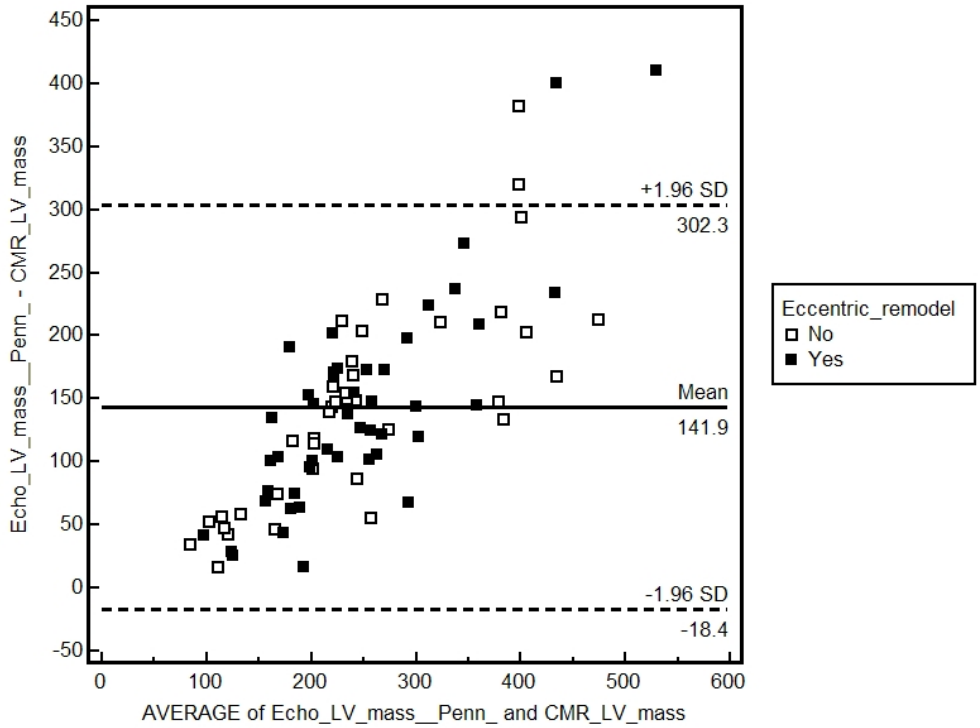
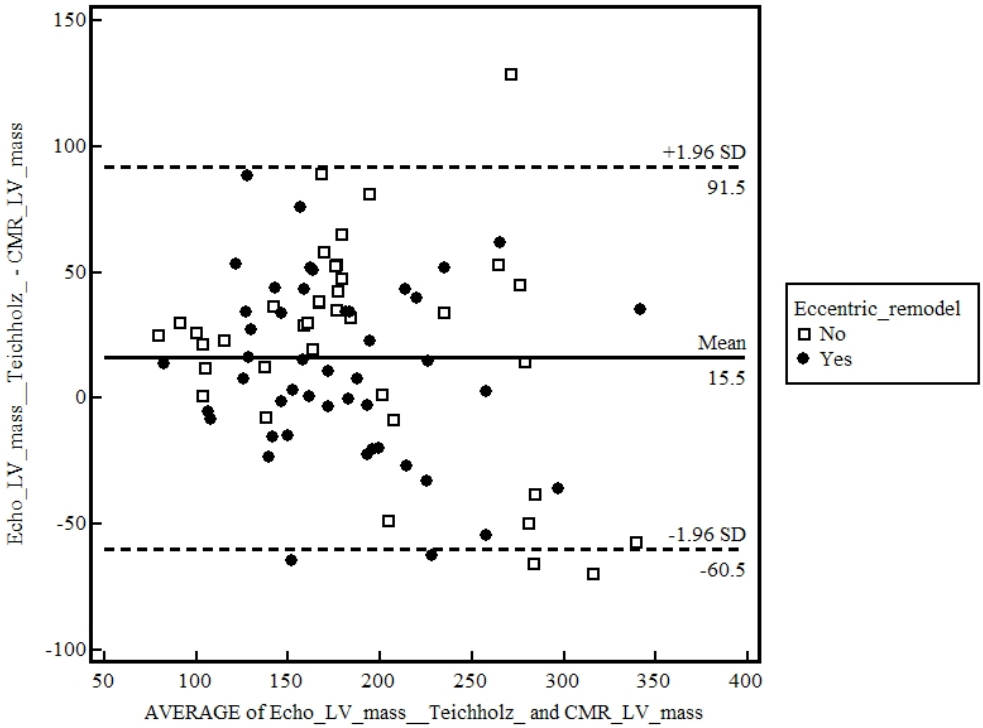


Figure 4.5



Figures 4.5 and 4.6. Bland-Altman plots of Echocardiographic LV mass minus CMR LV mass against mean of echocardiographic and CMR LV mass for the Penn (Figure 4.5) and Teichholz formulae (Figure 4.6)

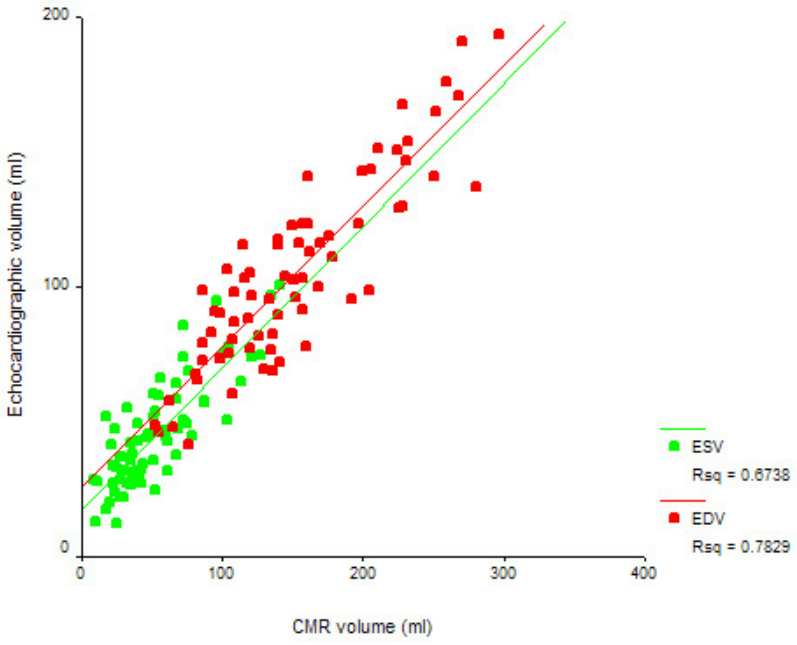
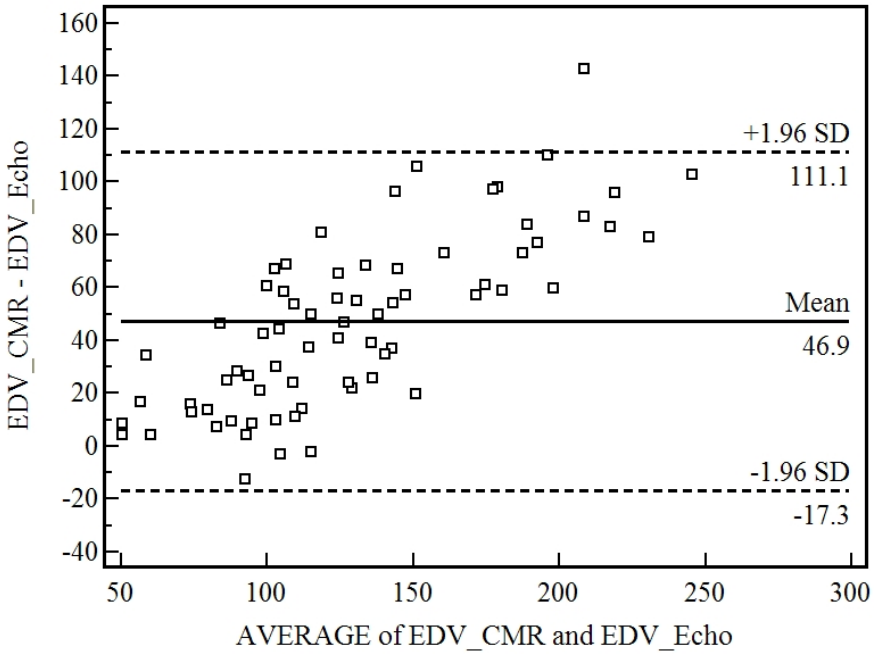


Figure 4.7 Scatter plots of echocardiographic measures versus CMR measures of LV volume for end diastolic and end systolic volume. End diastolic and end systolic volume are calculated by Simpson's bi-plane method



Figures 4.8

Bland-Altman plot of CMR LV mass minus Echocardiographic LV mass minus against mean of echocardiographic and CMR LV for end diastolic volume

4.3.5 Derivation of revised echocardiographic formula for use in ESRD patients

The highest correlation between CMR and echocardiographic MV mass is using the cubed based formulae (Penn, ASE, Troy). As the best fit for a relationship between cubed based formulae is linear (Figure 4.4), a linear fit equation can be derived to estimate LV mass calculated from CMR, using M-mode echocardiographic recordings continuing with cubic assumptions of ventricular geometry. Derivation is shown from Figure 4.9 and subsequently the final formula is plotted against CMR LV mass in Figure 4.10.

Therefore: CMR LVM (Y) = A + B *x* X

If the LV is assumed to be cubic then :

$$\mathbf{X = (LVID + PWT + IVST)^3 - (LVID)^3}$$

Then deriving from linear fitting of this graph: **A = 31.3** **B = 0.45**

A potentially re-derived cubed formula for use in ESRD would therefore be:

$$\mathbf{CMR\ LV\ Mass = 31.3 + 0.45\ \{(LVID + PWT + IVST)^3 - (LVID)^3\}}$$

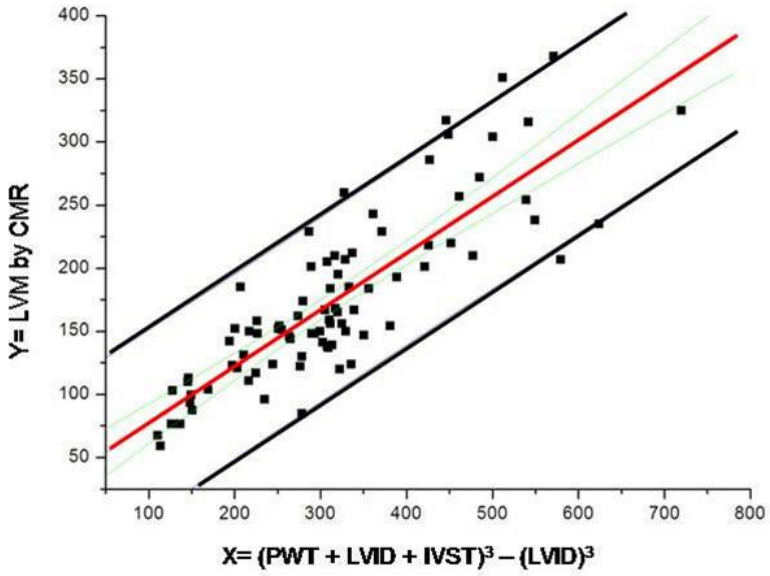


Figure 4.9

LV mass by CMR plotted against the LV dimensions cubed to calculate volume of LV myocardial tissue with 95% confidence intervals shown

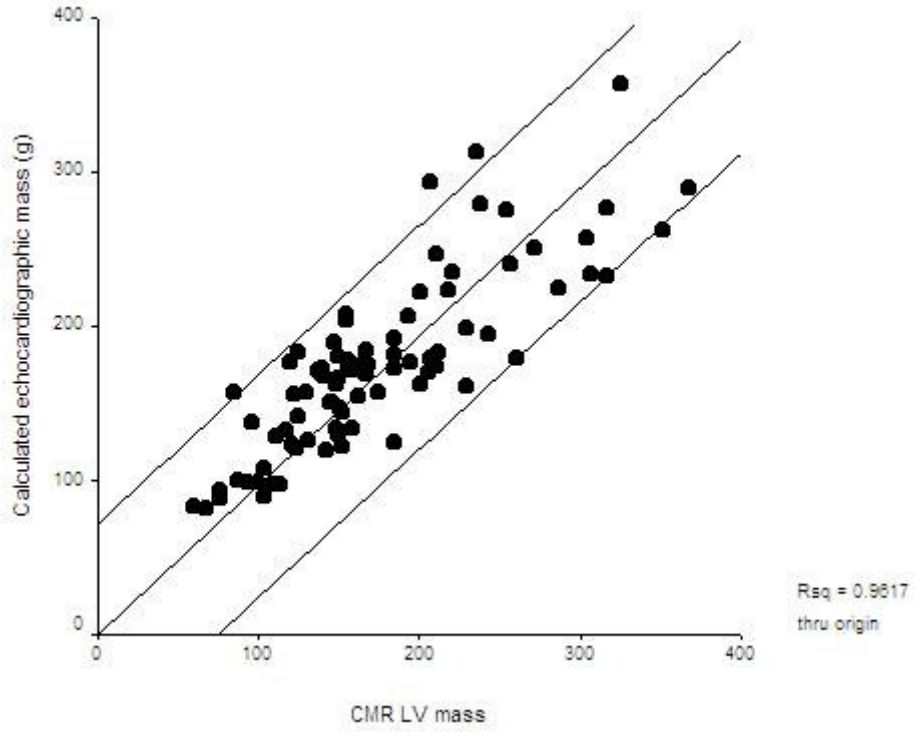


Figure 4.10

Calculated LV Mass using formula LVM= 31.3 + 0.45 {(LVID + PWT + IVST)^3 – (LVID)^3} derived above plotted against CMR LV mass with 95% confidence intervals shown

4.4 RESULTS OF ELECTROCARDIOGRAPHIC STUDIES

4.4.1 Patient demographics

Electrocardiogram (ECG) combined with CMR data were available on 139 patients (61.9%) male), of whom 30 (21.5%) were not yet established on long term dialysis therapy (28 advanced renal failure not yet started dialysis, 2 failing renal transplant), 61 (43.9%) on haemodialysis and 48 (34.5%) on peritoneal dialysis. 92.1% of patients had a previous history of hypertension and 16.5% had a past history of ischaemic heart disease.

4.4.2 Prevalence of left ventricular hypertrophy by CMR

The mean CMR LVMI for the cohort was 91.6 g m^{-2} (S.D. 29.4). LVMI was significantly higher in males than females (101.3 g m^{-2} vs. 76.3 g m^{-2}, $p<0.001$). Based on published normal ranges of LV mass, significantly higher numbers of males had LVH as defined by CMR compared to females 64.0% of males and 45.3% (Chi-squared $p=0.031$). Significantly higher numbers of patients on haemodialysis (72.1%) had LVH compared to those on peritoneal dialysis (41.7%) or those yet to start dialysis therapy (50.0%), $p=0.003$.

4.4.3 Prevalence of left ventricular hypertrophy by ECG

Using the Cornell criteria for LVH 20.1% of patients had LVH, with 23.7% by Sokolow-Lyon criteria and 15.8% by Cornell product. To examine need for revised partitions values for LVH in ESRD patients, the cohort were divided by the presence of LVH on CMR (Table 4.4) with gender specific QRS voltages and QRS voltage-duration products according to LVH status calculated. Patients with CMR LVH had greater Sokolow-Lyon voltage, Sokolow-Lyon product, Cornell and Cornell product than those

without LVH ($p<0.001$ for all criteria). As voltage criteria for LVH are gender specific, criteria were compared between males and females, with significantly greater Sokolow-Lyon product in males with LVH than females with LVH ($p=0.022$). In patients without LVH males had significantly greater Cornell product ($p=0.046$), Sokolow voltage ($p=0.023$) and Sokolow-Lyon product ($p=0.013$) than females.

4.4.4 Optimisation of ECG criteria for left ventricular hypertrophy

The sensitivity and specificity of the four criteria were tested using established partition values: Sokolow-Lyon ≥35 mV; Cornell gender specific, male ≥28 mV, female ≥20 mV and Cornell product ≥2440 mV ms. There is no current Sokolow-Lyon product value defined as a cut point for diagnosing LVH and Sokolow-Lyon product ≥2940 mV ms was used. This was also tested in males and females to determine revised gender specific partition values for the various criteria. Using currently defined criteria for Sokolow-Lyon product had the highest overall sensitivity (37.9%). Sokolow-Lyon voltage and Sokolow-Lyon product were more sensitive in males while Cornell voltage and Cornell product were more sensitive in females (Table 4.5). Receiver operator characteristic (ROC) curves (Figures 4.11-4.12) were constructed to test sensitivity and specificity of the four criteria and derive new ECG partition values for the diagnosis of LVH compared to CMR. Area under a ROC curve was similar for all criteria in the whole cohort. When compared by gender, area under the ROC curve was greater in females for all criteria, with the maximum area under the curve of 0.828 for Cornell voltage in female subjects (Table 4.6). To optimise clinical applicability of partition values, the respective sensitivities of the four criteria were calculated at a specificity of 95% (Table 4.7).

ECG criteria	LVH on CMR		No LVH on CMR	
	Male	Female	Male	Female
Cornell (mV)	18.8 (8.1)	21.5 (8.1)	15.3 (6.5)	12.45 (5.1)
Cornell product (mV.ms)	1974 (828)	1828 (854)	1403.2 (644)*	1097.5(500)
Sokolow (mV)	32.0 (13.6)	26.4 (8.3)	23.7 (6.9)*	19.4 (7.4)
Sokolow product (mV ms)	3089* (1386)	2382 (777)	2164.90(684)*	1698.4 (719)

Table 4.4

Mean values for QRS voltages and QRS voltage-duration products displayed by gender and CMR LVH status. Standard deviations are in parenthesis. * - $p<0.05$, male compared to female

	Sensitivity	Specificity
All patients		
Cornell voltage	30.3 %	93.3 %
Cornell product	24.1 %	95.0 %
Sokolow-Lyon voltage	36.9 %	93.3 %
Sokolow Lyon product	37.9 %	90.0 %

	Sensitivity	**Specificity**	**Positive predictive value**	**Negative predictive value**
	% (95% CI)			
Male				
Cornell voltage	16.4 (7.8- 28.8)	93.5 (78.5- 99.0)	81.8	38.6
Cornell product	20.0 (10.4- 33.0)	93.5 (78.5- 99.0)	84.6	39.7
Sokolow-Lyon voltage	34.5 (22.2- 48.6)	93.5 (78.5- 99.0)	90.5	44.6
Sokolow Lyon product	43.6 (30.3- 57.7)	83.9 (66.3- 94.5)	82.8	45.6
Female				
Cornell voltage	50.0 (29.1- 70.9)	93.1 (77.2- 99.0)	85.7	69.2
Cornell product	33.3 (15.7- 55.3)	96.6 (82.2- 99.4)	88.9	63.6
Sokolow-Lyon voltage	20.8 (7.2- 42.2)	100.0 (87.9- 100.0)	100.0	60.4
Sokolow Lyon product	20.8 (7.2- 42.2)	96.6 (82.2- 99.4)	83.4	59.5

Table 4.5 Sensitivities and specificities of the established ECG criteria for LVH compared to CMR with gender specific sensitivities and specificities

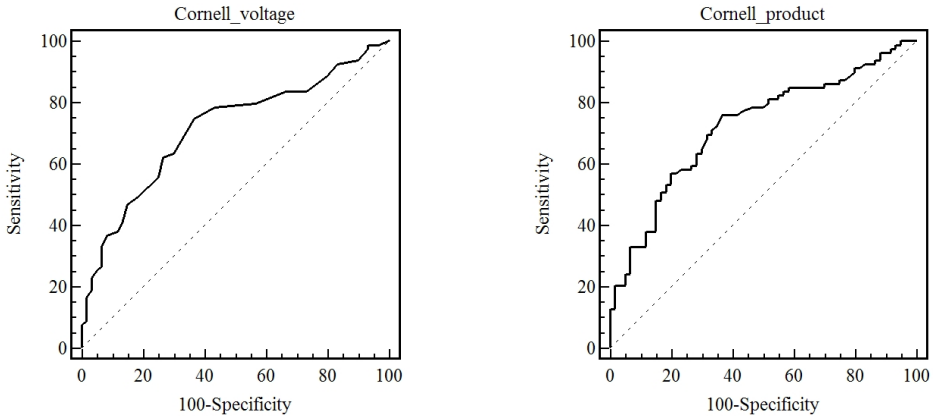


Figure 4.11 Receiver operator characteristic curves for Cornell voltage and Cornell product for diagnosis of LVH by CMR

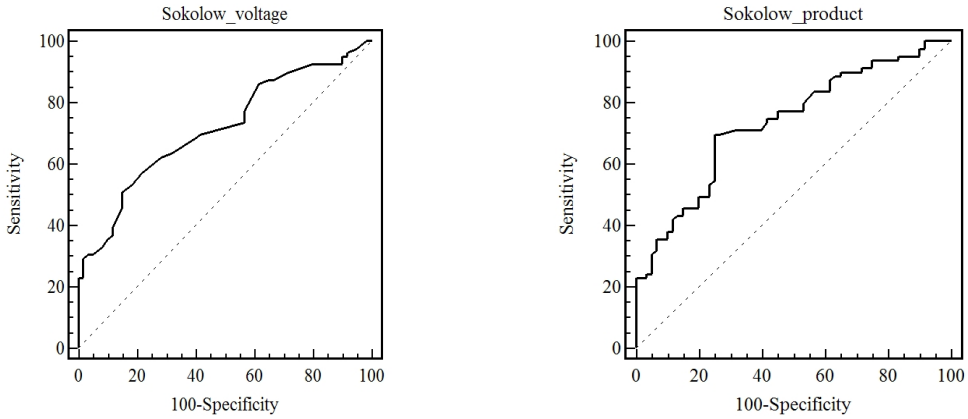


Figure 4.12 Receiver operator characteristic curves for Sokolow-Lyon voltage and Sokolow-Lyon product for diagnosis of LVH by CMR

	Area under ROC curve (95% CI)	p	Best cut point	Sensitivity % (95% CI)	Specificity % (95% CI)	Positive predictive value	Negative predictive value
All patients							
Cornell Voltage	0.714 (0.631- 0.787)	< 0.0001	14	74.7 (63.6- 83.8)	63.3 (49.9- 75.4)	72.8	65.5
Cornell product	0.722 (0.639- 0.794)	< 0.0001	1224	75.9 (65.0- 84.9)	63.3 (49.9- 75.4)	73.1	66.7
Sokolow-Lyon voltage	0.715 (0.632- 0.788)	< 0.0001	28	50.6 (39.1- 62.1)	85.0 (73.4- 92.9)	81.6	56.7
Sokolow Lyon product	0.734 (0.653- 0.805)	< 0.0001	2184	69.6 (58.2- 79.5)	75.0 (62.1- 85.3)	78.5	65.2
Male							
Cornell Voltage	0.631 (0.520- 0.732)	0.0318	14	70.9 (57.1- 82.4)	51.6 (33.1- 69.8)	72.3	49.9
Cornell product	0.645 (0.534- 0.745)	0.0160	1350	69.1 (55.2- 80.8)	58.1 (39.1- 75.4)	74.5	51.4
Sokolow-Lyon voltage	0.696 (0.588- 0.791)	0.0005	26	61.8 (47.7- 74.6)	71.0 (52.0- 85.7)	79.1	51.1
Sokolow Lyon product	0.703 (0.595- 0.797)	0.0003	2184	70.9 (57.1- 82.4)	64.5 (45.4- 80.8)	78.0	55.5
Female							
Cornell Voltage	0.828 (0.700- 0.918)	< 0.0001	14	83.3 (62.6- 95.2)	75.9 (56.5- 89.7)	74.1	84.6
Cornell product	0.826 (0.697- 0.916)	< 0.0001	1152	87.5 (67.6- 97.2)	69.0 (49.2- 84.7)	70.0	86.9
Sokolow-Lyon voltage	0.731 (0.591- 0.843)	0.0011	28	45.8 (25.6- 67.2)	93.1 (77.2- 99.0)	84.6	67.5
Sokolow Lyon product	0.756 (0.618- 0.863)	0.0002	2160	66.7 (44.7- 84.3)	86.2 (68.3- 96.0)	80.0	75.7

Table 4.6 Area under receiver operator curves for ECG diagnostic criteria for LVH, with best cut point, sensitivities, specificities, positive and negative predictive values defined for the cohort and subdivided by gender

	Sensitivity %	Male Sensitivity %	Female Sensitivity %	Male partition value	Female partition value
Cornell voltage	22.8	14.5	37.5	>29	>25
Cornell product	25.3	18.2	41.7	> 2668	>2132
Sokolow-Lyon voltage	31.6	34.5	25.0	> 36	> 30
Sokolow-Lyon product	29.1	30.9	25.0	> 3588	> 2760

Table 4.7 Sensitivities of the four ECG criteria and the male/female partition values at 95% specificity

4.5 DISCUSSION

4.5.1 Results of echocardiographic studies

As discussed previously, left ventricular disorders, namely LVH, LV dilatation and LVSD, so called 'uraemic cardiomyopathy', have survival implications for patients with ESRD. Previous studies in both ESRD and other populations have suggested that echocardiography overestimates LV mass compared to CMR. However in ESRD this finding was based on a small pilot study, using a 1.0 Tesla scanner and a fast low angle shot (FLASH) imaging sequence(189). The current study was performed using a 1.5 Tesla scanner using a trueFISP sequence, allowing better contrast between the ventricular myocardium and blood pool. Echocardiography is well established, portable and widely available and therefore will likely remain a vital clinical tool but has drawbacks, including operator dependence and difficulty in obtaining satisfactory acoustic windows. The major limitation of echocardiography is the geometric assumptions required to calculate LV mass and function. As ventricular dimensions are measured in the minor axis and then volumes obtained by cubing these values, any error in these initial errors is cubed. Moreover, these techniques assume the left ventricle to be cubic and while these methods have been validated in normal hearts, patients with ESRD have a high prevalence of LV abnormalities, particularly eccentric remodelling(213). As LVH in ESRD is due to a combination of pressure and volume overload, both concentric and eccentric remodelling ensues. The high prevalence of eccentric remodelling in this study (54.8%) essentially invalidates these formulae to calculate LV mass in this population.

Previous studies have suggested that in incident ESRD patients, the echocardiographic prevalence of LVH is 50-80%, with left ventricular dilatation in 20-40% and LVSD present

in 16% of patients(75). Echocardiographic prevalence of LV disorders from the current study is similar to these historical data. However, the CMR data suggests that this may represent an overestimation of the prevalence of these disorders, due to inherent inaccuracies in the use of echocardiography in the ESRD patient population. Overall, by CMR, 39.0% of patients had normal left ventricular dimensions and whilst LVH was the most common abnormality, the prevalence was lower than by echocardiographic studies at 54%. The majority of these cases had isolated LVH, rather than a combination of LVH with LV dilatation and LVSD. This suggests that as LV chamber dimension is dependent on hydration status which varies during the interdialytic period in patients on haemodialysis (or is chronically overloaded in patients on peritoneal dialysis), any error in calculation of LV mass due to changes in chamber dimension is greatly magnified, when echocardiography is used. Some changes in LV mass do occur though the dialysis cycle due to changes in the water content of the interstitial tissues of the heart in volume overload, but these have been shown by CMR to be of the order of 10g(166).

4.5.2 Echocardiographic studies used to derive measures of left ventricular mass

The first calculations used to estimate LV mass from M-mode echocardiography were described in 1972 by Troy *et al*, based on the assumption that the LV is cubic(209). Subsequent attempts have been made to refine these:

- The Penn convention equation was derived in later from a modified regression equation, based on echocardiographic studies, validated from subsequent post mortem studies in 34 patients. Although this study was pivotal, the patient group was somewhat heterogeneous, with ten patients with normal hearts, two had congestive cardiac failure, previous myocardial infarction was present in twelve patients and a variety of underlying conditions, both cardiac and non cardiac, were

the cause of death. The time between echocardiography and death was variable ranging from 1-120 days(205)

- A new adjusted formulae (the ASE convention) was derived and validated by the same group of authors on a further 52 individuals. This refined formula is widely used, but as the authors acknowledge LV shape was relatively normal in almost all patients in this study and therefore the authors recommend that their formulae must be restricted to patients without gross distortion of LV chamber shape(206)
- The Teichholz formula was developed at approximately the same time as the cubed formulae and has been shown to be effective in correcting systematic errors in LV volumes calculated by the cube function but has not been adopted widely for use in clinical studies, perhaps due to its complexity(208)

4.5.3 CMR as a gold standard for calculation of left ventricular dimensions

These studies to both compare and improve echocardiography and electrocardiography methods is dependent on the fundamental premise that CMR does truly represent a 'gold standard' for measurement of LV dimensions, i.e. a reproducible, accurate method, independent of geometric assumptions and ideally validated with post mortem specimens.

A number of studies have shown CMR to be reproducible with low inter- and intra-observer variability, both in normal volunteers as well as in patients with left ventricular hypertrophy, heart failure(194), and dilated cardiomyopathy(214). Post mortem validation comparing *in vivo* CMR images with post mortem specimens has not been performed, although animal studies and *ex vivo* imaging studies in humans show close agreement between CMR measured ventricular mass and true LV mass(215;216).

4.5.4 Feasibility of deriving novel regression formula for calculation of left ventricular mass in end stage renal disease

Derivation of a revised echocardiographic formula for estimation of LV mass in ESRD is an attractive concept, and the formula derived displayed good correlation with CMR LV mass. However this formula requires prospective validation in a separate cohort of ESRD patients. Other potential limitations of this formula exist. Critically, it is still derived from the cube method. This is subject to the same assumptions and limitations regarding ventricular geometry as other cube formula. As these measures were developed, conventionally the papillary muscles are included in drawing endocardial borders to perform CMR analysis of LV mass(169). With echocardiography, the papillary muscles are avoided for M-mode measurements and excluded from the endocardial border for bi-plane measurements. This would appear to be a crucial issue but perhaps as both methods have evolved essentially separately and been validated against post mortem specimens, animal models and latex models, this issue is underplayed in the literature.

Despite there being a high prevalence of eccentric remodelling in this study, the pattern of ventricular remodelling did not influence the degree of overestimation of LV mass (and associated underestimation of end diastolic volume) by echocardiography. The finding that end diastolic volume was under-estimated while LV mass was overestimated are consistent, but different methods were used (M-mode and Simpson's bi-plane) to calculate these parameters. These combined findings suggest that fundamentally the disparities between CMR and echocardiographic measures are due to difficulty in accurately defining endocardial borders and subsequent errors being magnified by calculations to calculate dimensions. These errors are accentuated in the ESRD population due to high prevalence of both hypertrophied and dilated ventricles.

4.5.5 Studies comparing echocardiographic and CMR measures of left ventricular mass in other populations

A number of studies have compared CMR with M-mode echocardiographic measures of LV mass. The correlation between CMR and echocardiography and the degree of any systematic discrepancy between the methods have to an extent been dependent on the population studied.

- In a study of 212 healthy army recruits echocardiography (ASE formula) consistently *underestimated* LV mass compared to CMR by a mean of 14.3g(217)
- Echocardiography (Penn formula) overestimated LV mass in 39 patients with LVH due to aortic stenosis scheduled for aortic valve replacement by a mean of 37g(218)
- In one study of hypertensive patients using echocardiography and CMR, echocardiography overestimated LV mass by 41g(219) whilst in another small study (n=20) of patients with LVH echocardiography overestimated LV mass by 27.6g(220)

Overall these studies, in keeping with the current study and others in ESRD, suggest that in patient groups with a high prevalence of LVH (and therefore distorted LV geometry) echocardiography tends to overestimate LV mass, but the opposite is true when large population with normal hearts are studied.

4.5.6 Alternative echocardiographic techniques to assess left ventricular mass compared to CMR

Although less widely used to estimate LV mass than the M-mode method, two-dimensional (2D) echocardiography is thought to be more accurate and reproducible than M-mode

methods. However one study using these methods in patients with hypertension suggests that this technique still suffers from intra-observer variability as well as wide limits of agreement compared to CMR(221). Using intravenous contrast combined with harmonic Doppler imaging has been shown to improve the accuracy of LV mass measurements with echocardiography but using this method the convenience of echocardiography as a clinical tool is diminished(222). Finally three-dimensional echocardiography measures of LV mass appear to show close correlation with CMR measures, although few comparative studies exist to date(223). Three-dimensional echocardiography is currently constrained by limited availability compared to conventional echocardiography.

4.5.7 Reduction in numbers required to power studies using CMR compared to echocardiography

As CMR is highly reproducible, it offers the potential advantage of reducing the number of patients required in clinical trials to show the benefit of a therapeutic intervention on cardiac dimensions. Using the data generated from these studies a clinical trial aimed at regression of LV mass index of 10g m^{-2} with 80% power would require 76 patients at 5% significance if performed with CMR but 328 patients with echocardiography(224).

4.5.8 Discussion of ECG studies

Using current ECG criteria, in this population with a high prevalence of LVH, all the current criteria in use have a high sensitivity for diagnosing LVH compared to when used in the general population (Table 4.5). However, the performance of the various criteria varied by gender with the Cornell voltage and Cornell product being superior in females, with the Sokolow-Lyon voltage and product being more sensitive indicators of LVH in the males. This anomaly in performance when compared by gender is similar to previous

studies of ECG versus CMR for diagnosis of LVH in the general population. Similarly, when area under a ROC curve was used to compare the criteria (Table 4.6), it is apparent that, whilst overall performances of the criteria are similar, the Cornell criteria provide considerably superior diagnostic measures in females. Although use of the QT interval to calculate Cornell and Sokolow-Lyon product would be expected to further improve sensitivity and specificity, in reality this was not the case, perhaps due to differing sensitivities of these criteria in this patient group due to baseline prevalence of LV disorders, but also as QT dispersion in this population is highly variable.

4.5.9 Feasibility of redefinition of partition values for left ventricular hypertrophy in end stage renal disease

Due the high prevalence of LVH in ESRD, the diagnostic utility of the ECG for diagnosing LVH is much higher in the general population and therefore this study confirms it is a useful screening test in this group of patients, particularly in light of its low cost and ready availability. Other authors have used CMR to optimise partition values in patients with hypertension and LVH(225). The high prevalence of LVH theoretically permits further increases in the sensitivity of the ECG for diagnosis of LVH by lowering the partition values. Although, as expected specificity is lost, it may be that the ECG could be used as a screening tool using this method to identify patients for clinical trials. Whilst gender was taken into account in analyses to identify optimise partition values for diagnosis of LVH, there appears to be very little difference in gender specific optimised cut points (Table 4.7). If however values are selected to maintain 95% specificity, it can be seen that female partition values for all four criteria are lower than male. Even at 95% sensitivity, the diagnostic utility of the ECG for diagnosing LVH is higher than in the non ESRD population.

4.5.10 Limitations of ECG for diagnosis of LVH in ESRD

The majority of the patients in this study had a history of hypertension in common with the broader ESRD population. Although patients with previous myocardial infarction were included, this study did not take into account the influence of previous infarction and subsequent myocardial remodelling on the ECG. The chief influence on LV mass is likely to be blood pressure but valvular heart disease (particularly due to calcific aortic stenosis) is a significant promoter of LVH(226). No attempt to delineate between valvular and hypertensive LVH was made and it may be that these features are associated with different remodelling processes and direct diagnostic use in ESRD patients with purely valvular remodelling is not appropriate. Similarly, although LVH has prognostic implications, both for patients with ESRD as well as the general population(227), the presence of secondary repolarisation abnormalities or 'LVH with strain' has much greater influence on subsequent cardiac outcome in patients with ESRD(207). Whilst long term study of this cohort of patients can identify if this effect persists in this group, it is difficult to derive a simple algorithm or cut point for diagnosis of LVH that takes into account the presence of these repolarisation abnormalities. Finally, as QT dispersion in this group of patients is variable and dependent on time point in the dialysis cycle(228), these revised partition values can only be applied at the same point that these patients were studied. As a heterogeneous group (haemodialysis, peritoneal dialysis and advanced chronic renal failure) this may not be appropriate.

4.5.11 ECG as inclusion point for studies

The Losartan Intervention For Endpoint reduction in hypertension (LIFE) study used ECG criteria for LVH to identify patients at higher cardiovascular risk who were subsequently

randomised to therapy with atenolol or losartan based antihypertensive therapy(12). As well as demonstrating a reduction in cardiovascular morbidity with losartan, this study was also able to demonstrate electrocardiographic regression of LVH with both interventions but to a greater extent with losartan. Use of the ECG as an inclusion or end point for studies is attractive due to its low cost and availability, but is unlikely to be a successful strategy in ESRD, due to the large numbers of patients required to use this strategy. Moreover it remains unclear to what extent LVH can genuinely be regressed in this population, as opposed to artefactual improvements in LV mass primarily due to better volume control, such as that seen post renal transplantation, leading to reduced overestimation of LV mass with echocardiography.

4.5.12 Conclusions on ECG studies

Using current criteria for ECG diagnosis of LVH, it is possible to identify patients with elevated LV mass index. Therefore, when calibrated against the gold standard of CMR, ECG remains a useful tool in identifying patients with LVH. Further refinement of these partition values is possible, but has to be interpreted in the context of reduced specificity. Using CMR to optimise more readily available investigations such as ECG and echocardiography may represent one way of utilising an expensive and highly specialised cardiovascular investigation.

Chapter 5

A study of the determinants of uraemic cardiomyopathy

5.1 INTRODUCTION

As discussed in the introduction, previous reports suggest that the presence of uraemic cardiomyopathy is associated with poor long term outcome in patients with ESRD. LVH, LV dilatation and LVSD cumulatively confer a worse prognosis. Risk factors previously associated with the presence of LVH include hypertension(229), anaemia(124;230), hyperparathyroidism(231) and arterial stiffness(232) whilst the presence of ischaemic heart disease is the predominant risk factor for LVSD(233). Studies performed in chapter 3 confirm that the presence of conventional cardiovascular risk factors such as diabetes, cigarette smoking and a past history of ischaemic heart disease are strongly associated with CMR findings in keeping with previous myocardial infarction and therefore likely to correlate with the presence of LVSD. All previous studies describing the determinants of uraemic cardiomyopathy have been performed using echocardiography.

It is not clear if the same factors are responsible for the evolution of LVH (or other forms of uraemic cardiomyopathy), when defined by the more accurate method of CMR. For example, anaemia has previously been associated with LVH, but it is possible that anaemia is secondary to haemodilution in ESRD in fluid overloaded patients and in turn this leads to hypertension(113). By using a volume independent measure of LV mass, the following studies will attempt to assess how much of these relationships are artefactual interactions between systematic overestimation of LV mass in volume overloaded patients and whether these relationships are confirmed with CMR. These studies were performed to assess the clinical determinants of LVH assessed both by CMR and echocardiography in ESRD.

5.2 METHODS

5.2.1 CMR and echocardiography

Patients were studied with CMR and echocardiography on the same day (described in detail in Section 2.3.2). Only patients with ESRD established on dialysis therapy on with both echocardiographic and CMR data were studied. CMR and echocardiograms were analysed as previously described.

5.2.2 Dialysis data and blood sampling

Pre- and post-haemodialysis blood pressures were recorded from the dialysis data recorded on the electronic patient record. The mean of readings from the three dialysis sessions preceding the CMR scan were used. Haemoglobin, albumin, corrected calcium, phosphate and urea reduction ratio were collected from the mean of values from the three months preceding the scan. All these results were collected as routine part of monitoring for audit and clinical care of patients on haemodialysis. The value for the three months preceding the scan was used for parathyroid hormone levels, which are checked less often. In patients on peritoneal dialysis blood pressure readings were the mean of the three clinic blood pressures preceding the scan. The blood results as before were averaged for these three clinic visits, with weekly urinary, peritoneal and total creatinine clearances assessed from the recording during the three months prior to the scan.

5.2.3 Statistical methods

To assess the determinants of cardiac dimensions correlations were sought between these and continuous clinical variables by the various methods were assessed with Pearson and Spearman correlation co-efficient as appropriate. Independent determinants of cardiac

dimensions were assessed using a linear regression model. All analyses were performed using the SPSS 13.0 statistical software package (SPSS Inc., Chicago, IL., USA)

5.3 RESULTS

5.3.1 Patient demographics

Only patients established on dialysis therapy were studied. The cohort consisted of 148 patients of whom 93 (62.8%) were male. 88 (59.5%) patients were on haemodialysis with 60 (40.5%) on peritoneal dialysis. Baseline clinical and laboratory data for haemodialysis and peritoneal dialysis patients are shown in Table 5.1. Cardiac dimensions are in Table 5.1 are as measured by CMR. Tests of significance are t-test or Mann-Whitney-U as appropriate.

5.3.2 Determinants of left ventricular mass in ESRD using CMR

Overall cardiac dimensions (LVM, end diastolic and end systolic volume) were significantly elevated in HD patients compared to those on PD (Table 5.1). Significant correlations were displayed between haemoglobin (Pearson's R= -0.23, p=0.006), parathyroid hormone (Spearman's R= 0.24, p=0.006) and serum phosphate levels (Spearman's R= 0.17, p=0.044) and LVMI. In patients on haemodialysis both pre-dialysis (Pearson's R- systolic blood pressure= 0.66, p<0.001, diastolic blood pressure= 0.59, p<0.001) and post-dialysis blood pressure (Pearson's R- systolic blood pressure= 0.76, p<0.001, diastolic blood pressure= 0.60, p<0.001) correlated strongly with LVMI. Similarly in PD patients LVMI was significantly correlated with clinic blood pressure (Pearson's R- systolic blood pressure= 0.55, p<0.001, diastolic blood pressure= 0.32, p=0.023). The overall correlation between blood pressure and LVMI is show in Figure 5.1.

Although in HD patients there was a significant negative correlation between dialysis adequacy as measured by urea reduction ratio and LVMI (Pearson's R= -0.33, p=0.004), no significant relationship existed between dialysis adequacy measured by total weekly creatinine clearance and LVMI in patients on PD.

Table 5.2 shows the results of multivariate linear regression analysis on the determinants of LVMI in patients. In these analyses, post-dialysis blood pressure measures were used in HD patients with clinic blood pressures used in PD patients. Due to their close interdependence systolic and diastolic blood pressure were entered separately into the model. The table shown uses systolic blood pressure. The major, significant determinants of LVMI were systolic or diastolic blood pressure and cholesterol.

The analysis was repeated using the same multivariate linear regression model for only HD patients to assess the impact of HD adequacy on LVMI. From this analysis urea reduction ratio was not an independent determinant of LVMI.

5.3.3 Determinants of left ventricular mass in ESRD using echocardiography

Echocardiographic data were available in 88 patients. Again LVMI (181.9 vs. 148.7 g m^{-2}, p=0.013), end diastolic volume (Simpson's biplane end diastolic volume- 111.4 vs. 92.3 ml, p=0.017) and end systolic volume (48.8 vs. 37.5 ml, p=0.014) were significantly greater in patients on haemo- compared to peritoneal dialysis. Significant correlations were demonstrated between LVMI and duration of renal replacement therapy (Spearman's R= 0.27, p=0.010), blood pressure (post-dialysis blood pressure measures in HD patients combined with clinic blood pressures in PD patients - Pearson's R- systolic blood pressure= 0.53, p=0.001, diastolic blood pressure= 0.39, p=0.001), parathyroid hormone levels

(Spearman's R= 0.33, p=0.002) and haemoglobin (Pearson's R= -0.24, p=0.029). Additionally aortic valve gradient, not easily measured by CMR, displayed a significant correlation with LVMI (Spearman's R= 0.40, p<0.001). Echocardiographic aortic jet velocity and aortic valve pressure gradient were assessed in 93 patients. Severe aortic stenosis was not present in the patients studied although 5 (5.4%) patients had moderate aortic stenosis (aortic jet velocity 3.0-4.0 m s^{-1}).

As before, multivariate linear regression analysis was performed to assess the determinants of LVMI in these patients as measured by echocardiography. Only nine variables were entered in the model as fewer patients were available to analyse. The following variables were not predictive in initial exploration of the data and were not entered in the model- age, gender, diabetes, C - reactive protein and smoking history. Blood pressure variables were treated as previously. The major, significant determinants of LVMI were systolic or diastolic blood pressure, duration of renal replacement therapy, cholesterol, phosphate, aortic valve gradient and serum corrected calcium.

	Haemodialysis		Peritoneal dialysis		p value
Number	88	(59.5)	60	(40.5)	
Male	62	(70.5)	31	(51.7)	0.02
Age (years)	52.5	(11.0)	53.0	(10.5)	0.757
Height (m)	170.1	(10.5)	166.2	(9.3)	0.026
Weight (kg)	75.2	(17.5)	73.4	(14.1)	0.522
RRT time (months)	12.5	(93.0)	5.0	(11.0)	<0.001
1^0 Renal Disease					
Diabetes	15	(17.0)	23	(38.3)	0.06
APKD	12	(13.6)	5	(8.3)	
GN	21	(23.9)	10	(16.7)	
CPN	11	(12.5)	4	(6.7)	
Renovascular	3	(3.4)	4	(6.7)	
Unknown/Other	26	(29.5)	23	(38.3)	
Pre-d SBP (mmHg)	149.9	(24.9)	-		
Pre-d DBP (mmHg)	82.6	(13.1)	-		
Post-d SBP (mmHg)	136.0	(25.7)	-		
Post-d DBP (mmHg)	75.9	(14.2)	-		
Clinic SBP (mmHg)	-		139.7	(22.5)	
Clinic DBP (mmHg)	-		82.3	(11.5)	
Haemoglobin (g dL^{-1})	11.7	(1.8)	11.5	(1.6)	0.375
Albumin (g L^{-1})	40.0	(4.2)	37.9	(4.6)	0.005
Corrected Calcium (mmol L^{-1})	2.4	(0.2)	2.4	(0.2)	0.929
Phosphate (mmol L^{-1})	1.8	(0.5)	1.7	(0.4)	0.734
Ca-P product	4.1	(1.3)	4.2	(1.0)	0.852
PTH (pmol L^{-1})	20.0	(34.0)	17.15	(39)	
URR (%)	70.7	(8.2)	-		
CrCl (L/week/1.73m^2)	-		86.6	(25.8)	0.871
LV mass (g)	184.2	(65.5)	153.5	(49.7)	0.003
LV mass/BSA(g m^{-2})	99.3	(31.7)	84.3	(25.8)	0.003
Ejection fraction (%)	65.8	(11.9)	66.9	(12.2)	0.590
ESV(ml)	57.8	(40.4)	42.6	(26.2)	0.011
ESV/BSA (ml m^{-2})	31.2	(21.3)	22.9	(13.0)	0.009
EDV (ml)	157.6	(62.7)	123.9	(43.7)	<0.001
EDV/BSA(ml m^{-2})	85.1	(31.3)	67.5	(21.2)	<0.001

Table 5.1 Baseline characteristics of patients. Data are number or mean with percentage or standard deviation in parentheses expect for RRT time and PTH where median and interquartile range are shown

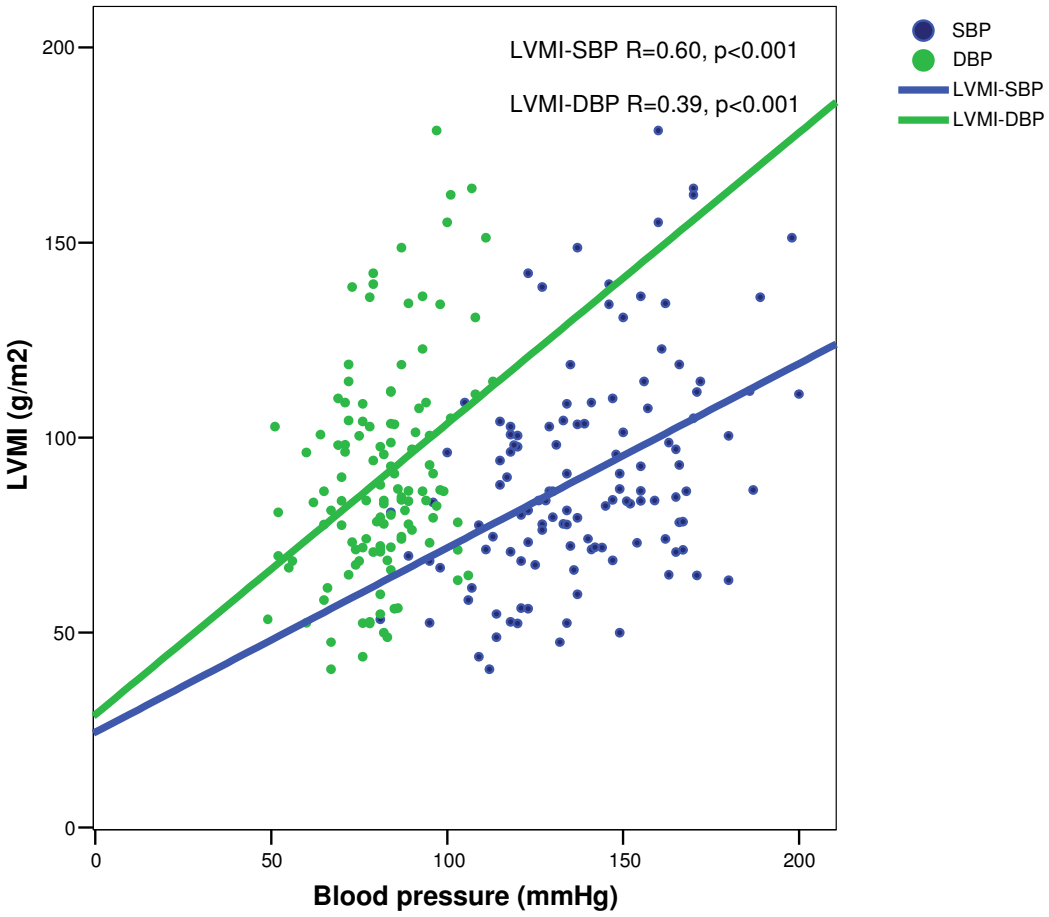


Figure 5.1

Scatter plot of systolic and diastolic blood pressures plotted against LVMI assessed by CMR. Post-dialysis blood pressure measures were used in HD patients with clinic blood pressures used in PD patients

	Standardised coefficient (β)	Significance (p)	95% CI for β	
LVMI (CMR)				
R^2= 0.62				
(Constant)	89.0	0.175	(-40.68,	218.72)
SBP	0.54	<0.001	(0.40,	0.92)
DBP	0.40	0.001	(0.23,	1.40)
Cholesterol	-0.30	0.008	(-13.37,	-2.11)
Diabetes	-2.00	0.080	(-28.25,	1.63)
Haemoglobin	-0.18	0.129	(-7.18,	0.93)
Albumin	0.12	0.278	(-0.67,	2.29)
PTH	0.06	0.600	(-0.15,	0.26)
Calcium	-0.07	0.518	(-45.18,	22.99)
Phosphate	0.06	0.593	(-9.18,	15.94)
RRT time	0.16	0.158	(-0.03,	0.15)
CRP	-0.07	0.546	(-0.51,	0.27)

Table 5.2

Determinants of LVMI as measured by CMR in a multivariate linear regression model

	Standardised coefficient (β)	Significance (p)	95% CI for β	
LVMI (echo)				
R^2= 0.63				
(Constant)	-24.64	0. 813	(-232.34,	183.07)
RRT time	0.31	<0.001	(0.18,	0.45)
SBP	1.54	<0.001	(1.06,	2.02)
DBP	1.93	<0.001	(0.97,	2.88)
Cholesterol	-14.25	0.002	(-23.03,	-5.46)
Phosphate	27.25	0.010	(6.89,	47.61)
Calcium	-64.71	0.026	(-121.22,	-8.20)
Aortic gradient	1.19	0.045	(0.03,	2.35)
Albumin	2.40	0.050	(-0.002,	4.807)
PTH	0.18	0.293	(-0.157,	.51)
Haemoglobin	2.40	0.493	(-4.56	9.34)

Table 5.3

Determinants of LVMI as measured by echocardiography in a multivariate linear regression model

5.4 DISCUSSION

5.4.1 Overall determinants of left ventricular mass

Multiple factors were associated with raised LVMI in this cohort of patients. When CMR was used as the method of measuring LVMI elevated blood pressure, hyperphosphataemia, hyperparathyroidism and anaemia were all associated with greater LVMI. Improved dialysis adequacy was associated with lower LVMI in haemodialysis patients but not in peritoneal dialysis patients. Haemodialysis patients had greater LVMI compared to those on peritoneal dialysis. Other groups have observed this and a commonly cited mechanism for this is the presence of an arterio-venous fistula for dialysis access leading to increased cardiac output(112). Additionally work from our group has observed that for a given rise in cardiac output (e.g. due to the arterio-venous fistula) patients with ESRD have an augmented blood pressure response which in turn promotes LVH(113). In any case, patients on haemodialysis are exposed to greater systolic blood pressures particularly pre-dialysis as shown in Table 5.1, in turn promoting LVH.

Factors which correlated with echocardiographic measures of LVH included blood pressure, time on renal replacement therapy, hyperparathyroidism, anaemia and peak aortic valve pressure gradient. When subjected to multivariate analysis the chief determinant of elevated LVMI measured by either CMR or echocardiography was blood pressure, chiefly systolic blood pressure. One other variable independently associated with higher LVMI measured by either method was hypocholesterolaemia. Male gender was the only other independent predictor of increased LVMI measured by CMR, but by echocardiography time on renal replacement therapy, hyperphosphataemia, hypocalcaemia and peak aortic valve pressure gradient were additional independent predictors of raised LVMI.

5.4.2 Blood pressure as a determinant of LVH in ESRD

Overall in this study hypertension was the major determinant of LVH and represents a potential target for treatment to reduce LVH. The nature of this study does not however demonstrate the natural history of evolution of LVH in earlier CKD and by the time the patient has established ESRD, intervention may be less likely to succeed. Previous work form our group has demonstrated that LVMI is elevated even in early CKD, when serum creatinine is normal(78). This study only analyses blood pressure trends over the preceding three months and whilst this may represent a continuing insult to the uraemic heart, may not reflect the long history of exposure to hypertension prior to commencing dialysis. Similarly extracellular fluid volume, the chief determinant of changes in blood pressure was not assessed in this study. Clinical assessment is notoriously inaccurate for precise measurement of extracellular fluid volume, and bioimpedance is required to assess this exactly(234). Many other groups have reported the association between hypertension and LVH(235;236). There is some debate as to the optimum time to measure blood pressure particularly in haemodialysis patients where blood pressure is dependent on timing with respect to the dialysis cycle(237). In a similar sized cohort of patients Fagugli *et al* used bioimpedance analysis to demonstrate a close correlation between extracellular water volume, blood pressure and LVMI(235).

A number of studies have suggested that 24 hour ambulatory blood pressure monitoring (ABPM) is superior for diagnosis and monitoring of hypertension in dialysis patients. Cannella *et al* have demonstrated using ABPM that many haemodialysis patients with borderline treated hypertension have significantly higher systolic and diastolic blood pressures over a 24 hour period than the pressures measured in the dialysis centre(238).

Again blood pressure correlated strongly with LVMI in this study. Similarly, Agarwal *et al* found systolic blood pressure 24 hour ABPM to be a much stronger predictor of LVMI than any dialysis centre reading.(237)

Multiple sources of inaccuracy exist in measurement of blood pressure in dialysis patients. Appropriate measurement of blood pressure should be after 5 minutes in quiet room without talking or active listening. This is difficult to achieve in the dialysis unit. Other non-uraemia specific sources of inaccuracy include the white coat effect, cuff size, and obesity or vascular disease making obtaining the brachial pulse difficult. Haemodialysis specific inaccuracies may be due to difficulty measuring brachial blood pressure in an arm with previous haemodialysis access surgery. Immediately post dialysis there is a rapid reduction in extracellular fluid volume leading to hypotension. However, the reduction in serum potassium leads to vasoconstriction in skeletal muscle and subsequent hypertension. Therefore, for accuracy post-dialysis blood pressure should normally be measured at least 20 minutes post dialysis, but this is frequently inconvenient for patients. 24 hour ABPM is likely to demonstrate the true blood pressure more accurately but is time consuming, expensive and additionally as dialysis patients frequently have high systolic blood pressures and a wide pulse pressure, the high cuff pressures required for measurement lead to discomfort(239).

5.4.3 Comparison of determinants of left ventricular mass as assessed by CMR compared to echocardiography

Variables that were predictors for LVH by echocardiography, but not by CMR, included duration of renal replacement therapy, hyperphosphataemia, hypocalcaemia and aortic valve gradient. As discussed previously, severity of aortic stenosis is not readily assessed

within the context of an uncomplicated CMR protocol and CMR sequences to measure this were not performed in this study. Perhaps unexpectedly duration of renal replacement therapy was not predictive of LVH by CMR. As patients have been on dialysis for longer, they become more chronically volume overloaded leading to LV chamber dilatation and ventricular remodelling may ensue. It may be that inaccuracies in measurement of LVM with echocardiography are augmented in patients with a long history of renal replacement therapy. As CMR measurements are less volume dependent and volume dependent inaccuracy is minimised. It is difficult to explain why hyperphosphataemia and hypocalcaemia are associated with greater LVM as measured by echocardiography but not CMR.

5.4.4 Hyperparathyroidism, hyperphosphataemia and vascular calcification as determinants of LVH

In this study serum PTH and phosphate correlated with LVMI. Both of these factors are likely to increase LVMI by a direct effect on the myocardium and indirect vascular effect. Animal models of uraemia have demonstrated both PTH and phosphate to have a permissive effect on development of myocardial fibrosis therefore demonstrating a close interaction between these circulating factors and development of LVH(240). These animal studies are confirmed by a number of clinical cohort studies(73;241). Moreover hyperphosphataemia and hyperparathyroidism are key drivers in development of arterial calcification in patients in ESRD. Arterial calcification increases cardiac after load hence further promoting LVH(232). One dilemma is that patients with the longest history of receiving renal replacement therapy are those who have more complications of ESRD such as secondary hyperparathyroidism making it difficult to assess the interdependence of the relationships between duration of ESRD and the various complications of ESRD such as

LVH and secondary hyperparathyroidism. Analysis of the determinants of LVH in patients with ESRD from the Dialysis Morbidity and Mortality Study Wave 2, a study of 2,584 new ESRD patients commencing dialysis therapy demonstrated that PTH was an independent risk factor for LVH(77).

A number of studies have demonstrated that increased arterial calcification, measured either by pulse wave velocity or by electron beam CT scanning has been associated with LVH in ESRD patients:

- One study in children with stages 2-4 CKD and ESRD has demonstrated that these alterations in arterial calcification and LVM occur early in CKD and are dependent on hyperphosphataemia(241)
- One study has reported improvements in LVM in a case series of 12 dialysis patients following parathyroidectomy(242)
- Finally, in patients with primary hyperparathyroidism and normal renal function cardiomyopathy and cardiac calcification has been reported presumably due to a direct effect of parathyroid hormone on the myocardium(243)

5.4.5 Other potential determinants of LVH in ESRD

Hypocholesterolaemia appears in this study to be directly linked to the presence of LVH. Although a direct mechanistic link between low serum cholesterol and increased LVMI is unlikely this can be interpreted in two ways. First, patients with LVH are at higher cardiovascular risk and the clinician responsible for the patient's care may have elected to treat these patients with statins leading to a reduction in serum cholesterol. Second, although not demonstrated in this study, other authors have demonstrated a weak correlation between hypoalbuminaemia and increased LVM(244). The same study reported a close independent correlation between C - reactive protein and LVM. It is therefore

plausible that low serum cholesterol represents either malnutrition (represented by hypoalbuminaemia) or inflammation (high C - reactive protein) and in conjunction with LVH may represent the presence of the malnutrition-atherosclerosis-inflammation syndrome, thereby explaining why low cholesterol offers little cardiovascular protection in ESRD.

Although in this current study, patients on haemodialysis had greater LVMI than those on peritoneal dialysis, other authors have reported the reverse(245). The explanation given by the authors was that patients on peritoneal dialysis are more volume expanded. However, in that study pre-dialysis blood pressure and blood pressure in the peritoneal dialysis patients were similar and therefore it is possible that peritoneal dialysis patients in this study were exposed in total to a greater blood pressure load(245).

5.4.6 Aortic stenosis as a risk for LVH in ESRD

Aortic valve peak pressure gradient at echocardiography was identified as an independent determinant of LVMI. In the general population aortic stenosis classically causes pressure induced concentric remodelling, followed by the development of ventricular hypertrophy. The presence of aortic valvular calcification has been previously shown to be associated with higher LVMI. The severity of aortic stenosis, measured either by peak flow velocity or pressure gradient has been demonstrated to correlate with LVMI(226). Therefore prevention of valvular calcification may be a therapeutic target for reduction of LVH. Once established, aortic stenosis has been demonstrated to progress rapidly in patients with ESRD, thereby increasing the necessity for early valve replacement(246).

5.4.7 Anaemia as a risk factor for LVH in ESRD- fact or fiction?

In this study, as well as others from our group, anaemia correlated with greater LVMI, whether measured with echocardiography or CMR. When subjected to multivariate analysis, however this was not an independent predictor of LVMI. In the anephric patient anaemia, volume status, blood pressure and haemodilution are closely related and it is difficult to tease out whether the relationship between low haemoglobin and increased LVMI is a direct effect or indirectly due the action of hypervolaemia on both haemodilution and increased blood pressure. A number of studies have demonstrated either a correlation between the presence of anaemia and the presence of LVH or showing anaemia to be a risk factor for progression of LVH. Most of these progression studies are in patients established on dialysis therapy:

- In a study of 48 dialysis patients, prior to availability of recombinant erythropoietin for treatment of renal anaemia, an increase in LVMI increased significantly in parallel with the decrease in haemoglobin(247)
- In 175 patients initiated on dialysis therapy, followed up with echocardiograms at an 18 month interval, anaemia and systolic blood pressure were independent risk factors for LVH(75)
- In 246 dialysis patients with echocardiography performed at a 12 month interval, even after adjusting for baseline LVMI, haemoglobin level and systolic blood pressure remain independently important predictors of left ventricular growth (124)
- By contrast, in the era of established treatment with erythropoietin from the Dialysis Morbidity and Mortality Study Wave 2, a studying of 2,584 new ESRD patients commencing dialysis therapy, anaemia was not identified as an independent risk factor for LVH at commencement of renal replacement therapy(77)

However despite the correlation between the presence of anaemia and LVH and the comparable comparison between persisting low haemoglobin levels and increasing LVMI, evidence from randomised controlled trials using various forms of erythropoietin does not support a treatment strategy of full correction of anaemia in patients with either early chronic kidney disease or ESRD:

- Roger *et al* showed that treatment with erythropoietin alpha to maintain haemoglobin at 12-13g/dL did not affect progression of LVH compared to maintaining haemoglobin at 11-12g/dL (female) or 11-13g/dL (male) in patients with stage 3-4 chronic kidney disease(127)
- Besarbab *et al* demonstrated in randomised controlled trial of 1233 haemodialysis patients with clinical evidence of congestive heart failure or ischaemic heart disease, the use of increasing doses of erythropoietin to achieve and maintain a haematocrit of 42 percent, was associated with excess mortality compared to maintain a haematocrit of 30 percent(126)
- More recently, in the Correction of Haemoglobin and Outcomes in Renal Insufficiency (CHOIR) trial, the use of a target haemoglobin level of 13.5 g/dL (as compared with 11.3 g/dL) was associated with increased risk of cardiac events and no incremental improvement in the quality of life in patients with GFR 15-50ml/min(128), whilst the Cardiovascular Risk Reduction by Early Anaemia Treatment with Epoetin Beta (CREATE) study showed no significant difference in LVM in patients randomised to a haemoglobin of 10.5-11.5 g /dL compared to a normal haemoglobin of 13.0-15.0 g/dL in patients with GFR of 15-35.0 ml/min(125)

Therefore whilst there is no doubt that historically anaemia and LVH have been interrelated, it is now unclear in the era of treatment of with erythropoietin, what the

optimum target haemoglobin should be, either to prevent LVH or improve quality of life, whilst minimising risks from non-physiological overcorrection.

5.4.8 Reinventing the wheel -treatment strategies to reduce LVH in ESRD

Based on this work, focus should once again be redirected at treating blood pressure, chiefly systolic blood pressure, to prevent development and progression of LVH. Regression of LVH is likely to be more difficult once the patient has established ESRD and focus should be directed at aggressive antihypertensive treatment earlier in the course of chronic kidney disease, as part of standard management of patients with progressive renal disease and hypertension.

Once established on dialysis therapy the options for treatment of hypertension primarily are maintenance of euvolaemia ('dry weight'), antihypertensive medication or a combination of these strategies. A number of studies have addressed these issues using changes in echocardiographic LVMI as an end point. One confounding factor is that any intervention that reduces extracellular fluid will reduce ventricular dilatation and consequently the degree of artefactual overestimation of LVMI. Therefore while this strategy is likely to be beneficial due to its effect on blood pressure, the positive outcome reported on LVMI may partly be due to optimisation on intra-ventricular fluid volume. A number of examples of such studies illustrate this point:

- One uncontrolled Turkish study of 19 haemodialysis has demonstrated excellent blood pressure control with rigorous salt control and fluid restriction. LVMI reduced by a mean of 43g m^{-2} over a 6 month period. Although this is an excellent outcome for these patients, this reduction in LVMI is so dramatic that it is more likely that some of this improvement is artefactual(248)

- A number have studies have demonstrated improvements in LVMI following conversion to nocturnal haemodialysis, allowing slower ultrafiltration over a greater dialysis period. Chan *et al* showed that nocturnal haemodialysis allowed reduction in the need for antihypertensive therapy, improved blood pressure and a reduction in LVMI over a follow up period of 1 year in 28 patients switched to nocturnal haemodialysis compared to their counterparts on conventional haemodialysis(20)
- Changing patients from thrice weekly to short daily on-line haemodiafiltration as dialysis modality has been demonstrated to significantly reduce LVMI in one small study (eight patients). Interestingly this study used CMR to measure LVMI and found similar magnitudes of reduction as the echocardiographic studies quoted above. This intriguing finding needs explored in larger controlled studies. Whether this dialysis modality confers additional benefits via actions on uraemic toxins is unknown(249)
- Similar findings have been reported in peritoneal patients on both blood pressure and LVMI in an open-label randomised controlled trial using icodextrin compared to conventional glucose peritoneal dialysis for the night time dwell. Again it is likely that the main beneficial effect on LVMI was mediated via improved volume status and blood pressure(250)

Limited trial data exist on the use of pharmacological agents to reduce blood pressure or LVMI in ESRD. Two small studies have demonstrated a benefit of using angiotensin receptor blockers in haemodialysis patients either as monotherapy. In an non-blinded three treatment arm study of 33 patients, Suzuki *et al* reported increased regression of LVMI with combined losartan and enalapril either agent alone independent of blood pressure(251), whilst losartan was shown to have a beneficial effect as monotherapy on LVMI compared to amlodipine or enalapril in a similar study(252).

Out with treating blood pressure and revisiting the dilemma of treatment targets in renal anaemia, alternative strategies to treat LVMI may revolve around addressing calcium phosphate balance and secondary hyperparathyroidism. Sevelamer hydrochloride has already been shown to produce less coronary artery calcification (as measured by electron beam CT scanning) compared to calcium containing phosphate binders(143). Reduction of arterial (and potentially valvular) calcification may reduce cardiac after load and minimise LVH. This end point has not been studied using this agent. Similarly vitamin D analogues such as calcitriol have been demonstrated in small studies to reduce progression of LVH(253). The calcimimetic agent, cinacalcet, currently used for treatment of secondary and tertiary hyperparathyroidism may yet prove to have beneficial outcomes on either arterial calcification or LVH. Whilst CMR is not able to directly demonstrate calcium deposition, techniques developed for assessing arterial stiffness may prove useful end points for such studies and may yet provide surrogate equivalent measures of vascular function, at the same time as providing definitive LV assessment.

Chapter 6

Survival of patients undergoing cardiovascular screening for renal transplantation

6.1 INTRODUCTION

Cardiovascular disease (CVD) is the leading cause of death after renal transplantation as well as the leading cause of death of patients on the renal transplant waiting list(254). Various guidelines have been developed to assess peri-operative risk in the general population(255) as well as for renal transplant assessment(192;256). Further studies using a variety of clinical scoring criteria or screening tests have been performed in an attempt to identify patients at increased risk of early post transplant death(257). Many of the guidelines used in the general population use conventional cardiovascular risk factors to classify those patients at increased peri-operative risk. However some of these risk factors, such as diabetes, hypertension, ECG abnormalities (or even renal dysfunction itself) are either ubiquitous or have a much higher prevalence in the ESRD population. Other risk factors such as hypercholesterolaemia have different relationship with outcome in the ESRD population. No study to date has used CMR to generate long term follow up data in the ESRD/renal transplant candidate population, including those patient undergoing assessment who are not transplant listed, and there are still relatively few outcome studies for any conditions using CMR.

Identifying patients at high risk is beneficial for a number of reasons. Firstly, some patients will be at such high peri-operative risk or have such a short anticipated survival that renal transplantation is unlikely to offer any improvement in the quality or longevity of life for that patient. As cadaveric organs are in short supply, it is unlikely to be an appropriate use of a valuable resource to allocate kidneys to such patients. Other patients may be at increased risk of dying, but their risk may potentially be lowered, either by medical therapy or surgical intervention. In these cases where evidence may not exist for intervention these patients may be identified as suitable for clinical trials of interventions to reduce risk. Some

patients who are at lower cardiovascular risk may not benefit from screening, although in these cases results may be reassuring and provide a baseline assessment as patients frequently face an uncertain wait for a cadaveric kidney. For this reason, the screening tests should ideally be non invasive and carry a minimal risk of harm. At the time of study, CMR was ideally placed to offer such a test.

The aims of these studies were to assess the utility of CMR in identifying the renal transplant candidate at high risk of premature death. Specific hypotheses to be addressed included:

- Does the presence of uraemic cardiomyopathy, as defined by cardiac dimensions at CMR predict outcome?
- Would the presence of LGE on CMR scanning predict poorer patient prognosis?
- Can CMR provide additional information above standard clinical information to inform likely patient outcomes?

6.2 METHODS

6.2.1 Patient demographics

Patients were recruited from the renal transplant assessment clinic at the Western Infirmary as described in Chapter 2. To achieve greater statistical power a larger cohort was created by addition of patients assessed for transplantation during the period February 2002 – March 2006, including patients studied as part of a previous British Heart Foundation Fellowship to Dr Nicola Johnston, as well as the current studies.

6.2.2 Cardiovascular assessment

All patients studied underwent CMR to assess cardiac dimensions and function as described previously. Contrast-enhanced CMR was performed in the majority of patients scanned with reasons for not administering Gd-DTPA-DMA as before (precipitating decline in renal function, patient choice, scan duration and poor venous access). A proportion of patients underwent other tests including clinical history and examination, electrocardiography, exercise tolerance testing and angiography as clinically appropriate. Methods described in Chapter 2 were used for these tests. All tests were performed within the Department of Cardiology at the Western Infirmary, Glasgow.

6.2.3 Coronary angiography

A subset of patients underwent coronary angiography as part of assessment for renal transplant listing. Criteria used to select patients for angiography were as described in Chapter 3.2.3 with angiography performed within three months (before or after) of CMR. Decision to perform angiography was by an independent cardiologist blinded to the results of these studies. Angiograms were classified for severity of coronary artery disease as previously described.

6.2.4 Follow up

Follow up data was collected from date of the CMR scan to 9th November 2006 using electronic patient records from the Western Infirmary, Glasgow and Glasgow Royal Infirmary Renal Units. With this cohort, death was the only end point used. All patient deaths were included in the patient survival analysis. From these data the influence of various parameters on patient survival were determined.

6.2.5 Statistical methods

Measures of cardiac function were compared between patients who died during the follow up period, with t-test and Chi-squared/Fisher's exact test used to compare groups. Unadjusted survival analysis was performed using the Kaplan–Meier method. Statistical significance was determined by the log-rank test. The data were then analysed by Cox survival analysis to assess the influence of multiple variables on outcome. Variables identified as possibly influential on outcome by univariate analysis were then entered into a forward stepwise regression model. All analyses were performed using the SPSS 13.0 statistical software package (SPSS Inc., Chicago, IL., USA).

6.3 RESULTS

6.3.1 Patient demographics and survival

Baseline patient demographics are shown in Table 6.1. A total of 300 patients were studied (84 with advanced renal failure not on dialysis therapy, two patients with failing renal transplants 157 patients on haemodialysis and 57 on peritoneal dialysis). The median follow period was 32.5 months. There were 60 patient deaths during the follow up period. Survivors were significantly younger than those who died during the follow up period. A significantly higher proportion of patients with a past history of diabetes mellitus, ischaemic heart disease (Figure 6.1), cerebrovascular disease and peripheral vascular disease died. Gender, dialysis modality at the time of scanning, duration of renal replacement therapy, smoking history and blood pressure at the time of scanning were not associated with patient mortality.

6.3.2 Laboratory data and patient outcome

Certain laboratory parameters were associated with better patient outcome (Table 6.2). Patients who died during the follow up period were more likely to be anaemic than survivors and additionally were more likely to be malnourished with lower serum albumin. Evidence of greater systemic inflammation indicated by higher C- reactive protein was non-significantly higher in patients who died during the follow up period. There were no differences in corrected calcium, phosphate, calcium phosphate product or cholesterol levels between patients alive and dead at the end of the follow up period.

6.3.3 Transplant list status, renal transplant listing and outcome

78 (26.0%) patients were not transplant listed following independent assessment. 222 patients (74.0%) were transplant listed. 80 (26.7%) patients went on to receive a renal transplant of which five diabetic patients received combined cadaveric kidney-pancreas transplants. Patients who were not transplant listed were significantly likely to be older, more likely to be diabetic or have a history of peripheral vascular disease. There were a non significantly lower proportion of patients with ischaemic heart disease in the transplant listed group. The outcome of the patients divided by transplant outcome is shown in Figure 6.2. There were only seven post transplant deaths during the follow up period with a 1 year post transplant patient survival of 95.3%. As the post transplant numbers are relatively small with a short post transplant follow up data were not censored at the point of transplantation and post transplant outcomes were not studied in detail.

	Alive		Dead		p value
Number	240	(80.0)	60	(80.0)	
Male	154	(64.2)	37	(61.7)	0.719
Age (years)	50.3	(10.8)	56.5	(9.6)	<0.001
SBP (mmHg)	136.2	(24.5)	140.1	(24.0)	0.283
DBP (mmHg)	81.3	(12.8)	80.1	(12.0)	0.525
1^0 Renal Disease					
Diabetes	49	(20.4)	17	(28.3)	0.294
APKD	30	(12.5)	6	(10.0)	
GN	47	(19.6)	8	(13.3)	
CPN	25	(10.4)	2	(3.3)	
Renovascular	12	(5.0)	4	(6.7)	
Unknown/Other	77	(32.1)	23	(38.3)	
RRT time (months)	12.0	(39.0)	20.0	(41.0)	0.139
Smoker					
Never	126	(52.5)	27	(45.0)	0.359
Current	62	(25.8)	21	(35.0)	
Ex	52	(21.7)	12	(20.0)	
Diabetes	52	(21.7)	21	(35.0)	0.031
Previous IHD	36	(15.0)	20	(33.3)	0.001
TIA/CVA	13	(5.4)	9	(15.0)	0.011
PVD	10	(4.2)	7	(11.7)	0.025

Table 6.1

Baseline demographic data for patients alive and dead at the end of the follow up period. Data are number with percentage in parentheses or mean with standard deviation in parentheses except for RRT time where median and intra-quartile are shown. Tests of significance are t-test and Chi-square except for renal replacement therapy time where Mann-Whitney is used

	Alive		Dead		p value
Haemoglobin	11.6	(1.8)	11.0	(1.4)	0.017
Albumin	39.7	(4.5)	36.0	(5.1)	0.001
Corrected calcium	2.36	(0.2)	2.38	(0.2)	0.654
Phosphate	1.75	(0.5)	1.75	(0.6)	0.988
Ca-P product	4.1	(1.1)	4.3	(1.5)	0.402
PTH	20.0	(31.3)	20.0	(37.5)	0.876
Cholesterol	5.4	(1.8)	5.1	(1.4)	0.262
CRP	7.0	(11.0)	16.0	(34.0)	0.074

Table 6.2

Laboratory parameters for patients alive and dead at the end of the follow up period. Data are mean with standard deviation in parentheses except for parathyroid hormone and C - reactive protein where median and intra-quartile are shown. Tests of significance are t-test except for parathyroid hormone and C - reactive protein where Mann-Whitney is used

	Not transplant listed		Transplant listed but not transplanted		Transplanted		p value
Number	78	(26.0)	142	(47.3)	80	(26.7)	
Male	49	(62.8)	84	(59.2)	58	(72.5)	0.137
Age (years)	54.3	(11.3)	52.3	(10.0)	47.5	(11.1)	<0.001
1^0 Renal Disease							
Diabetes	24	(30.8)	26	(18.3)	16	(20.0)	0.412
APKD	5	(6.4)	24	(16.9)	7	(8.8)	
GN	13	(16.7)	26	(18.3)	16	(20.0)	
CPN	6	(7.7)	14	(9.9)	7	(8.8)	
Renovascular	4	(5.1)	7	(4.9)	5	(6.3)	
Unknown/Other	26	(33.3)	45	(31.7)	29	(36.3)	
RRT time (months)	12.0	(40.0)	10.0	(43.0)	24.0	(39.0)	0.739
Diabetes	30	(38.5)	26	(18.3)	17	(21.3)	0.003
Smoker							
Never	38	(48.7)	81	(57.0)	34	(42.5)	0.250
Current	25	(32.1)	33	(23.2)	25	(31.3)	
Ex	15	(19.2)	28	(19.7)	21	(26.3)	
Previous IHD	20	(25.6)	25	(17.6)	11	(13.8)	0.144
TIA/CVA	10	(12.8)	8	(5.6)	4	(5.0)	0.095
PVD	12	(15.4)	3	(2.1)	2	(2.5)	

Table 6.3 Baseline demographic data for patients subdivided by the transplant status at the end of the follow up period. Data are number with percentage in parentheses or mean with standard deviation in parentheses except for RRT time where median and intra-quartile are shown. Tests of significance are Chi-square except for age and renal replacement therapy time where one way analysis of variance is used

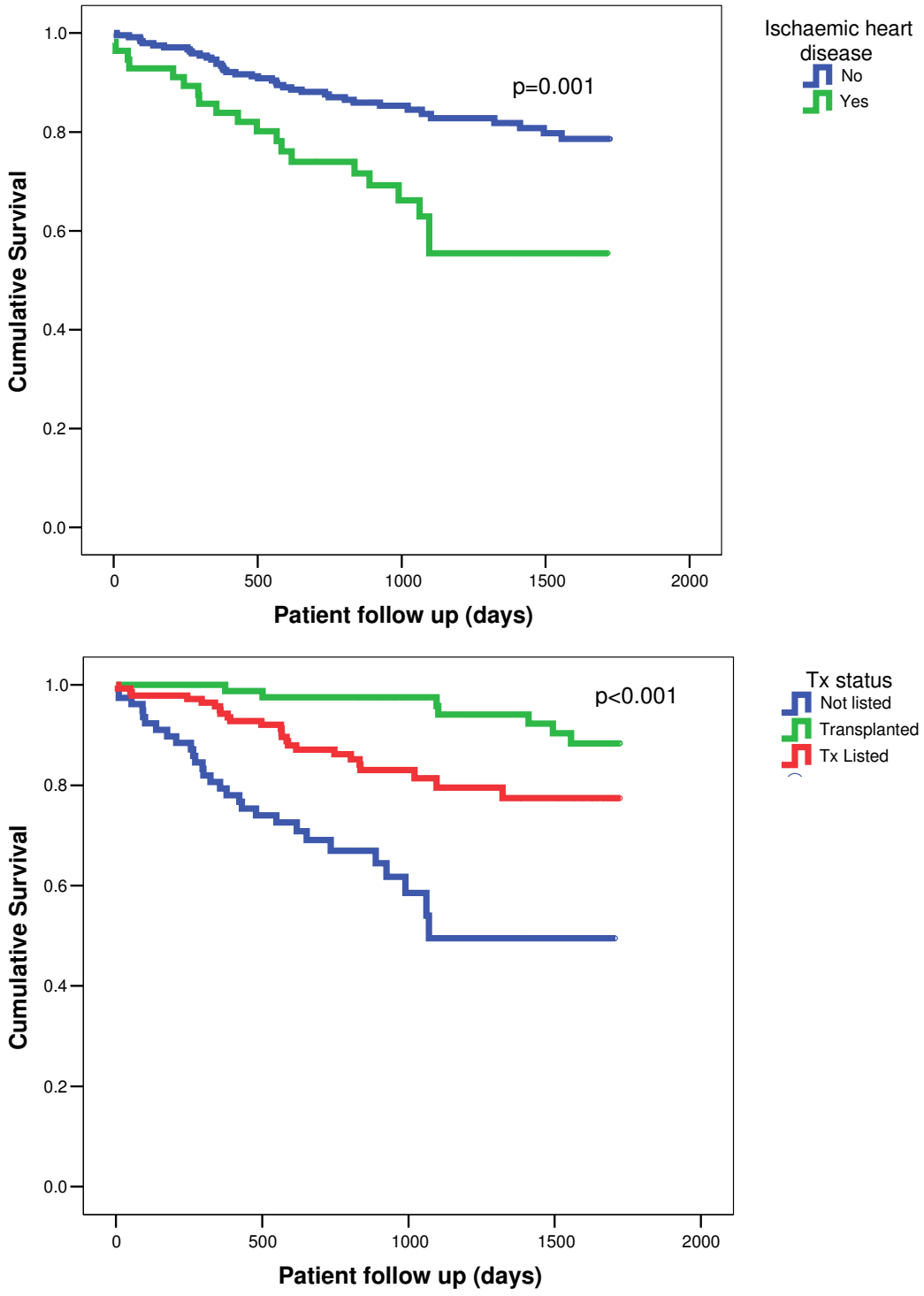


Figure 6.1 (above) and 6.2 (below) Kaplan-Meier survival curves of patients stratified by the presence of ischaemic heart disease and transplant status respectively

6.3.4 Uraemic cardiomyopathy as assessed by CMR and outcome

In the entire cohort of patients there were no significant differences in cardiac dimensions or ejection fraction between patients who died and survivors (Table 6.4). There was however a trend towards poorer survival in patients with systolic dysfunction compared to those with isolated LVH (Figure 6.3). In those patients undergoing contrast enhanced CMR, a significantly greater number of patients who died displayed evidence of myocardial fibrosis indicated by LGE, in particular subendocardial LGE demonstrating previous myocardial infarction (Figure 6.4).

6.3.5 Resting and exercise electrocardiogram and outcome

263 patients had a resting 12-lead ECG taken at the time of assessment available for analysis. Of these 143 (54.4%) were normal. 82 (31.2%) showed LVH by Sokolow-Lyon criteria. A total of 79 (30.0%) showing ischaemic changes (either ST abnormalities or the presence of Q-waves) of which 39 (14.8%) showed LVH with additional ischaemic changes ('LVH plus strain'). Patients who died during the follow up period demonstrated a significantly greater prevalence of presence of ischaemic changes on the ECG (Figure 6.5). The presences of LVH or LVH plus strain in isolation were not associated with poorer survival (Table 6.5).

212 (70.7%) patients were able to attempt a Bruce protocol exercise tolerance test (Table 6.5). Survival was significantly poorer in those patients unable to exercise. Of those patients who could exercise, the presence of electrocardiographic changes at exercise testing was not predictive of long term outcome. However, exercise time was a predictor of outcome, with lower median exercise times for patients who died during follow up and with

those patients unable to exercise for 6 minutes (as an arbitrary but clinically pragmatic cut point) having a greater mortality (Figure 6.6).

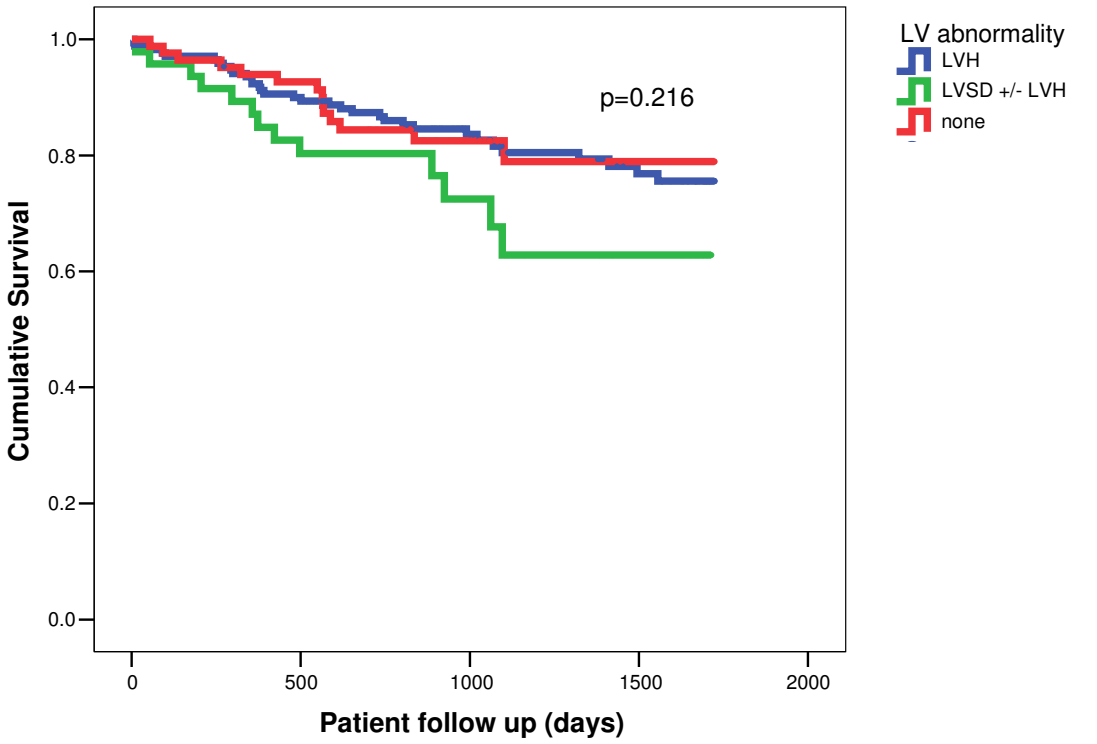
6.3.6 Coronary angiography and outcome

96 (32.0%) patients underwent coronary angiography as selected by an independent cardiologist. Overall survival in patients who underwent angiography was no different compared to those who did not have this investigation performed (p=0.633). A greater burden of coronary artery disease at angiography was associated with poorer outcome, with those patients with a total atheromatous plaque burden greater than 74 (equating to one coronary artery stenosis >74% or an accumulation of lesser plaque disease) having significantly poorer survival (Figure 6.7).

	Alive		Dead		p value
	240	(80.0)	60	(20.0)	
LV ejection fraction (%)	66.5	(11.7)	65.0	(13.5)	0.378
LV mass (g)	175.1	(64.9)	184.3	(64.4)	0.328
End diastolic volume (ml)	141.2	(58.8)	142.0	(62.7)	0.930
End systolic volume (ml)	50.4	(37.9)	54.4	(44.7)	0.479
LV mass/BSA (g m^{-2})	94.7	(31.8)	100.0	(31.2)	0.259
End diastolic volume/BSA (ml m^{-2})	75.7	(29.4)	77.1	(32.7)	0.765
End systolic volume/BSA(ml m^{-2})	27.3	(20.6)	29.0	(23.6)	0.605
LGE given	168	(82.8)	35	(17.2)	0.804
LGE positive	45	(26.8)	16	(45.7)	0.026
Subendocardial LGE	23	(13.7)	11	(31.4)	0.008
Diffuse LGE	21	(12.5)	5	(14.3)	0.540

Table 6.4

Left ventricular dimensions in patients who died and those who were still alive at the end of the follow up period. Data are expressed as mean with standard deviation in parentheses for cardiac dimensions and number with percentage in for LGE results. Tests of significance are t-test and Chi-square/Fisher's exact test as appropriate



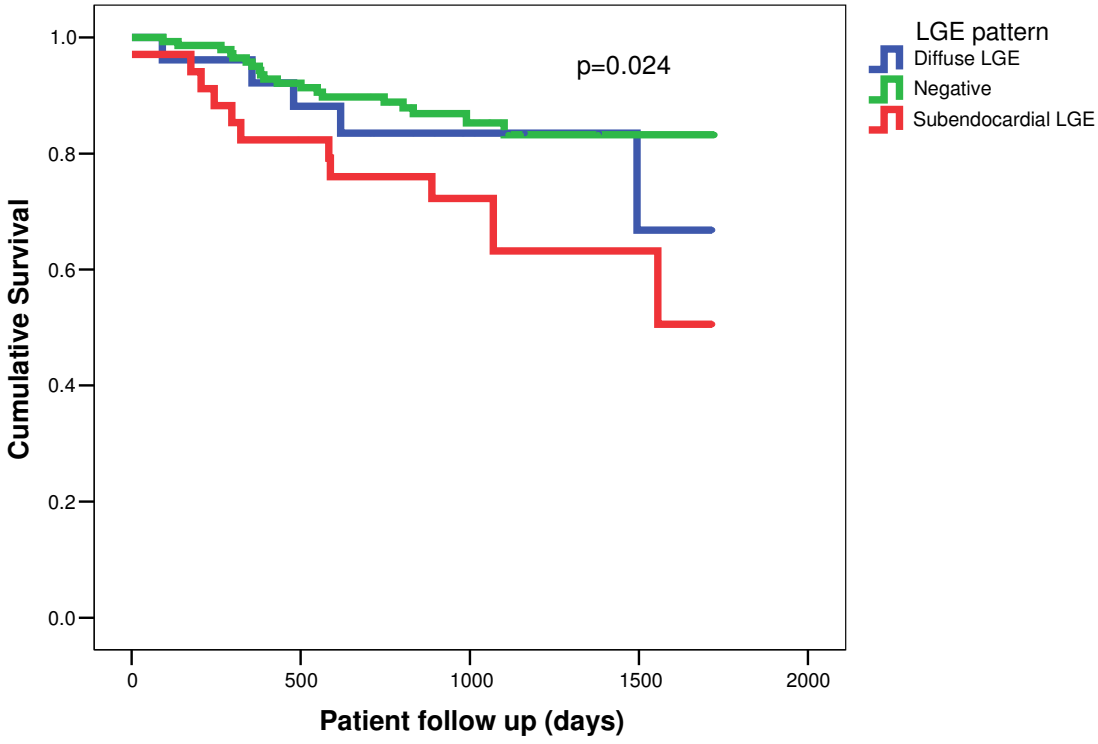


Figure 6.3 (above) and 6.4 (below) Kaplan-Meier survival curves of patients stratified by the presence of left ventricular abnormalities and presence and subtype of LGE respectively

6.3.7 Independent predictors of survival in all patients

In the Cox multivariate model based on factors identified during the earlier analyses; age, previous ischaemic heart disease, receiving a renal transplant and ability to attempt an exercise tolerance test were identified as independent predictors of mortality (Table 6.6). As there may be an inherent degree of bias in patient selection to receive a renal transplant (e.g. patients on the transplant list were younger than those not listed), the analysis was performed again leaving this factor out of the model. The results were no different with age, previous ischaemic heart disease, and ability to attempt an exercise tolerance test identified as independent predictors of mortality in this model. When a similar model was used in patients who underwent contrast enhanced CMR, the presence of LGE was not a predictor of patient survival independent of other variables (Table 6.7). When only patients studied with contrast enhanced CMR age were analysed, age, diabetes, and ability to attempt an exercise tolerance test were independent predictors of mortality.

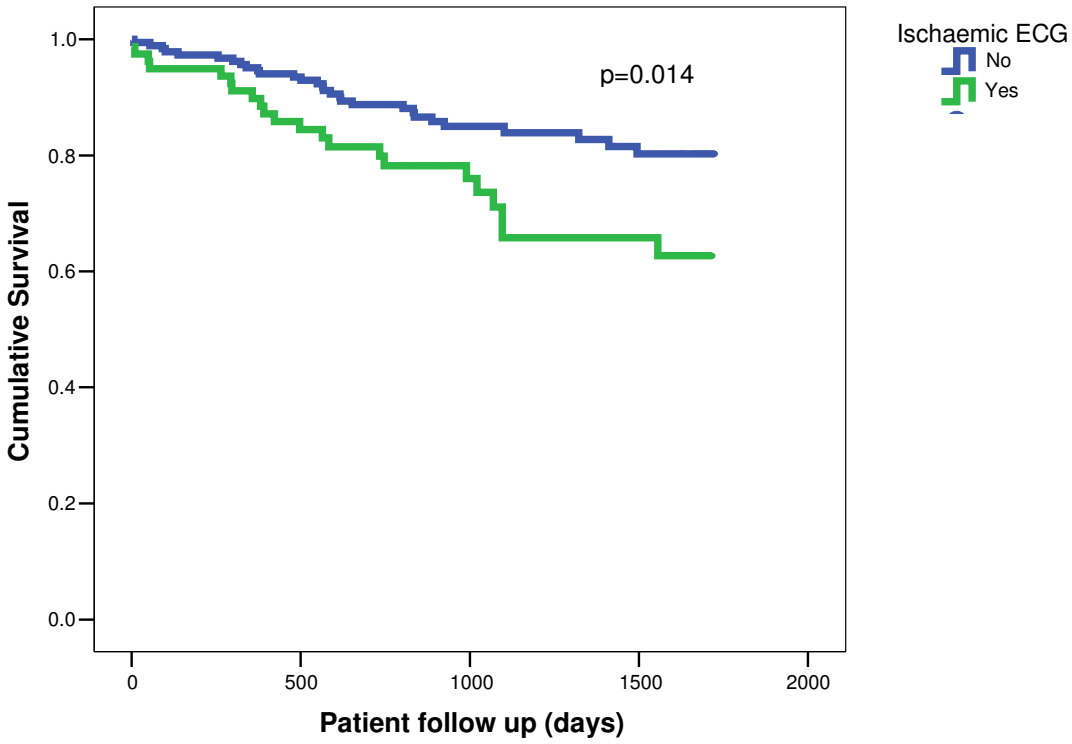
6.3.8 Independent predictors of patients listed for renal transplantation

As identifying patients at high risk of mortality on the renal transplant waiting list or early post renal transplantation may optimise both patient selection and post transplant outcomes, a similar Cox multivariate model was performed on the cohort of patients on the renal transplant list (Table 6.8). In these patients predictors of mortality were age, history of ischaemic heart disease and female gender. When a similar model was used in patients on the transplant list who underwent contrast enhanced CMR, the presence of LGE was not a predictor of patient survival independent of other variables.

	Alive		Dead		p value
Normal ECG	120	(56.6)	23	(45.1)	0.139
LVH	66	(31.1)	16	(31.4)	0.973
LVH plus strain	30	(14.2)	9	(17.6)	0.528
ST changes	50	(23.6)	19	(37.3)	0.046
Q waves	16	(7.5)	7	(13.7)	0.161
Any ischaemic changes	57	(26.9)	22	(43.1)	0.023
Performed exercise tolerance test	178	(74.2)	31	(51.7)	0.001
ECG changes	30	(16.6)	7	(22.6)	0.416
Median exercise time (minutes)	7.0	(3.25)	5.0	(4.0)	<0.001

Table 6.5

Electrocardiographic data for patients who died and those who were still alive at the end of the follow up period. Data are number with percentage in parentheses except for exercise time where median and intra-quartile are shown. Tests of significance are Chi-square except for exercise time where Mann-Whitney-U is used



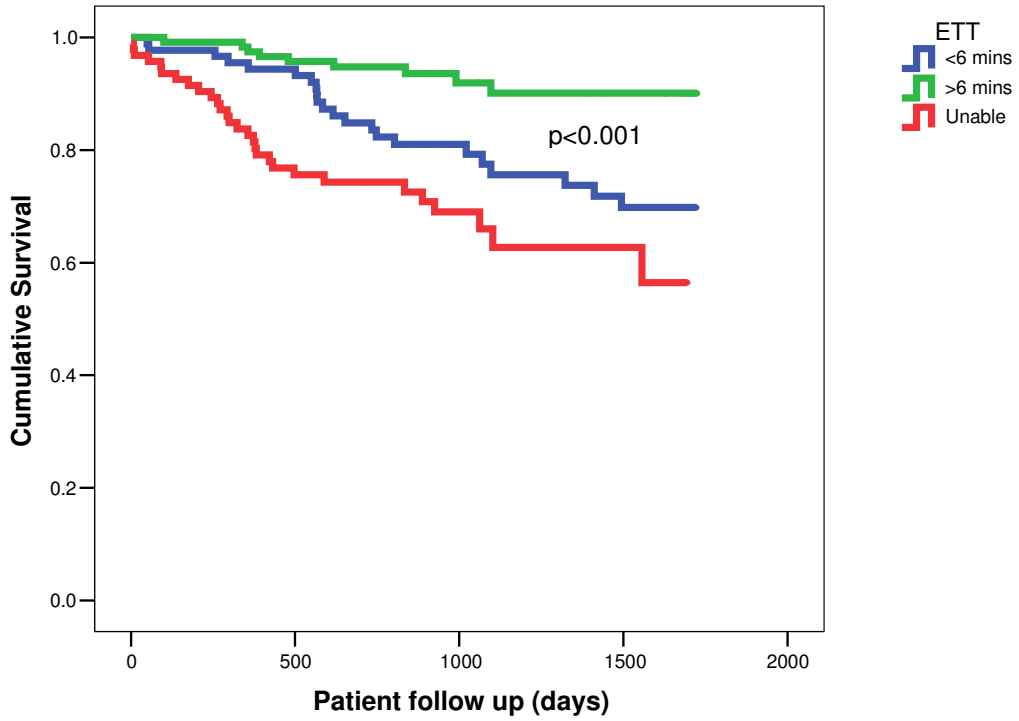


Figure 6.5 (above) and 6.6 (below)

Kaplan-Meier survival curves of patients stratified by the presence of an ischaemic ECG and ability to perform an exercise tolerance test

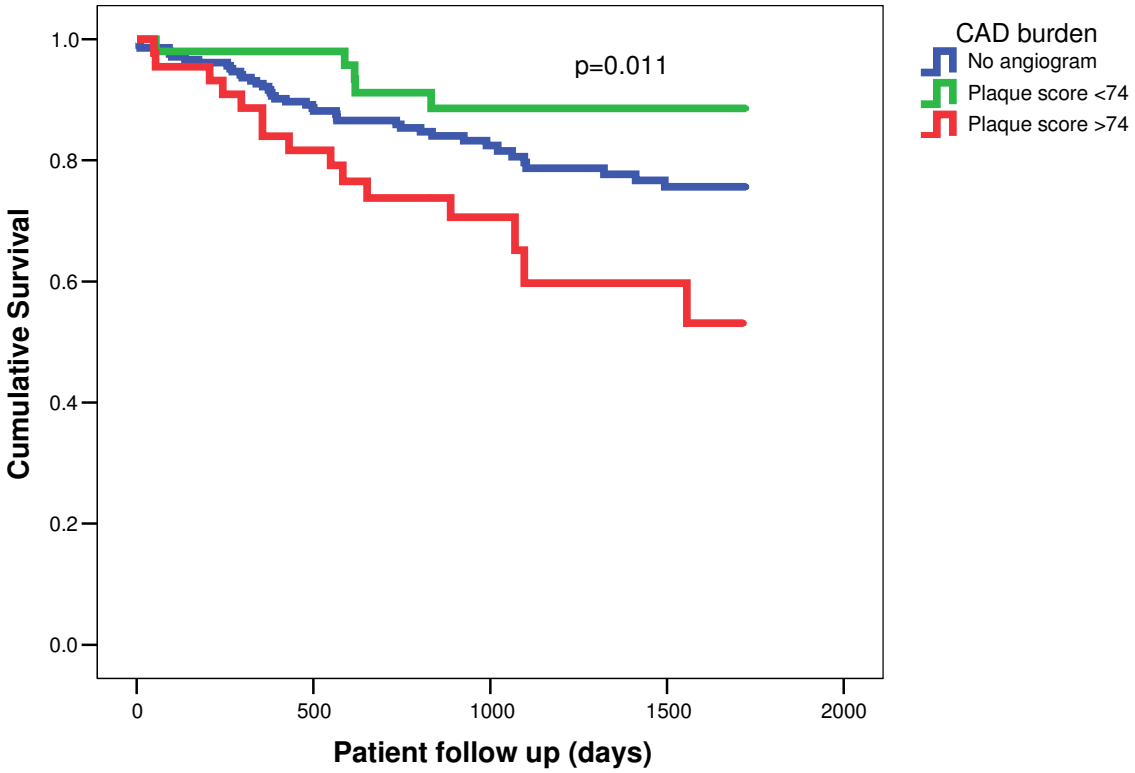


Figure 6.7

Kaplan-Meier survival curves of patients stratified by coronary angiography status

	Patient survival							
	Univariate analysis				Multivariate analysis			
Variable	**HR**	**(95.0% CI)**		**p**	**HR**	**(95.0% CI)**		**p**
Age	1.064	(1.025,	1.104)	0.001	1.063	(1.025,	1.102)	0.001
IHD	2.786	(1.333,	5.824)	0.006	2.828	(1.488,	5.374)	0.002
Transplanted	0.339	(0.145,	0.790)	0.012	0.361	(0.157,	0.830)	0.016
Able to attempt ETT	0.550	(0.278,	1.087)	0.085	0.510	(0.273,	0.954)	0.035
Haemoglobin	0.882	(0.751,	1.035)	0.123				
Diabetes	1.800	(0.834,	3.882)	0.134				
SBP	0.989	(0.975,	1.004)	0.158				
Gender (ref female)	0.629	(0.308,	1.282)	0.201				
Ischaemic ECG	1.566	(0.769,	3.189)	0.217				
RRT time (months)	0.995	(0.987,	1.004)	0.263				
LVMI	1.006	(0.994,	1.017)	0.354				
Ejection fraction	1.012	(0.987,	1.037)	0.365				
Ever smoked	1.321	(0.667,	2.616)	0.425				

Table 6.6

Results of the Cox regression survival analysis for all patients

	Patient survival							
	Univariate analysis				Multivariate analysis			
Variable	**HR**	**(95.0% CI)**		**p**	**HR**	**(95.0% CI)**		**p**
Diabetes	3.386	(1.162	9.866	0.025	2.777	(1.095,	7.044)	0.032
Age	1.051	(0.993,	1.112)	0.083	1.057	(1.004,	1.113)	0.036
Ischaemic ECG	2.095	(0.784,	5.594)	0.140	2.486	(1.063,	5.814)	0.036
SBP	0.982	(0.961,	1.004)	0.108				
Transplanted	0.406	(0.117,	1.403)	0.154				
Gender (ref female)	0.447	(0.147,	1.361)	0.156				
Able to attempt ETT	0.534	(0.199,	1.435)	0.214				
Ever smoked	1.741	(0.680,	4.460)	0.248				
Presence of LGE	1.744	(0.554,	5.497)	0.342				
Ejection fraction	1.024	(0.971,	1.080)	0.373				
IHD	1.870	(0.428,	8.168)	0.405				
RRT time	0.997	(0.987,	1.007)	0.509				
LVMI	1.007	(0.985,	1.029)	0.541				
Haemoglobin	0.981	(0.779,	1.237)	0.874				

Table 6.7

Results of the Cox regression survival analysis for patients who underwent contrast enhanced CMR

	Patient survival							
	Univariate analysis				Multivariate analysis			
Variable	**HR**	**(95.0% CI)**		**p**	**HR**	**(95.0% CI)**		**p**
Age	1.114	(1.055,	1.177)	0.000	1.111	(1.056,	1.168)	<0.001
IHD	5.597	(1.953,	16.036)	0.001	6.154	(2.616,	14.476)	<0.001
Gender (ref female)	0.273	(0.102,	0.729)	0.010	0.301	(0.131,	0.692)	0.005
Transplanted	0.314	(0.107,	0.922)	0.035				
Haemoglobin	0.836	(0.668,	1.046)	0.117				
Diabetes	2.605	(0.751,	9.030)	0.131				
Able to attempt ETT	0.513	(0.204,	1.286)	0.154				
Ejection fraction	0.975	(0.934,	1.018)	0.245				
Ever smoked	1.659	(0.667,	4.127)	0.277				
Ischaemic ECG	1.426	(0.470,	4.321)	0.531				
RRT time	0.998	(0.985,	1.010)	0.701				
LVMI	0.997	(0.978,	1.018)	0.804				
SBP	0.998	(0.976,	1.021)	0.869				

Table 6.8

Results of the Cox regression survival analysis for patients on the renal transplant list

6.4 DISCUSSION

6.4.1 Predictors of survival in a cohort of patients screened for renal transplantation

In this cohort of all patients undergoing cardiovascular screening as part of assessment for renal transplantation, the independent predictors of patient survival were younger age, absence of ischaemic heart disease, receipt of a renal transplant and ability to attempt an exercise tolerance test. Similarly in patients on the transplant list, age, ischaemic heart disease and female gender were predictors of mortality. The original hypothesis that factors consistent with uraemic cardiomyopathy would be associated with adverse outcome was not confirmed as patients who died had neither significantly greater LVMI nor lower LVEF. However, with contrast enhanced CMR, the presence of LGE, in particular subendocardial LGE, indicating myocardial fibrosis, was associated with adverse outcome. Cardiovascular risk factors either associated with, or indicating the presence of ischaemic heart disease, such as ischaemic changes on the ECG or diabetes were also associated with mortality.

Although the presence of LGE at contrast enhanced CMR conferred a worse prognosis, this finding was not independent of other variables. It is not clear if the lack of independent prognostic information provided by CMR is due to lack of statistical power, with fewer patients studied with this method or due to the close association between the findings at contrast enhanced CMR and cardiovascular risk factors leading to a lack of any independent association. Nonetheless, the follow up data support the notion that CMR may identify those patients at greater risk of premature death. Other studies have looked at the prognostic value of LGE in different patient groups at high cardiovascular risk:

- In a CMR study of 101 patients with idiopathic dilated cardiomyopathy the presence of midwall myocardial fibrosis indicated by LGE was demonstrated by multivariate analysis to be the sole significant predictor of death or hospitalisation(190)
- A study of 195 patients with classical ischaemic heart disease but with not documented previous myocardial infarction the presence of LGE provided further prognostic value for major adverse cardiac events and cardiac mortality beyond common clinical, angiographic, and functional predictors(258)

Both these studies have shown that in other patient groups at high risk of cardiovascular disease LGE identifies patients at elevated risk of major adverse cardiac events, hospitalisation or death. As patients with ESRD are at least as high risk as these patient groups it seems plausible that contrast enhanced CMR may provide further useful information of long term outcome in patients which may yet inform either transplant workup, or the decision to proceed to invasive investigations.

6.4.2 The resting and exercise ECG and outcome in potential renal transplant candidates

Although many of these findings demonstrating the relationship with cardiovascular risk factors and outcome in patients screened for renal transplantation have been reported previously(207;259); the finding that objective assessment of functional capacity with the Bruce protocol exercise tolerance test predicts outcome is novel. This simple, widely available test has been used to risk stratify patients with suspected or confirmed ischaemic heart disease for many years. Conventional logic suggests that this test is unlikely to be useful in cohorts of patients with renal failure due the high prevalence of ECG

abnormalities and the lower functional capacity due to issues such as fluid status, arthropathy associated with renal bone disease and peripheral vascular disease. As a tool for indicating which patients have underlying severe coronary artery disease, the exercise ECG is unlikely to be a useful tool and perhaps paradoxically the resting ECG has been demonstrated to a better predictor of the presence of coronary artery disease(49). However, this study has shown that the exercise test is an excellent test to assess functional capacity, irrespective of the presence of ECG changes. Furthermore the American College of Cardiology/American Heart Association (ACC/AHA) guidelines for perioperative cardiovascular evaluation for noncardiac surgery suggest that functional assessment in metabolic equivalent (MET) levels should form part of evaluation for surgery(255). Stage 1 of the exercise test is equivalent to 4.8 MET and therefore is a reassuring finding prior to non/semi-urgent surgery such as renal transplantation.

6.4.3 Guidelines for screening for cardiovascular disease in potential renal transplant recipients

Various guidelines for screening for cardiovascular disease in potential renal transplant recipients have been generated under the auspices of organisations such as the American Society of Transplantation and the European Renal Association(192;256). Interestingly, at the time of writing the previous draft guidelines from the British Transplant Society have been withdrawn for revision and the draft Australian guidelines (Caring for Australians with Renal Impairment) have been withdrawn and not replaced. Further British guidelines may appear from the Renal Association at some point. It is recognised that cardiovascular disease is the leading cause of death after renal transplantation. However in Western populations cardiovascular disease is often the leading cause of death in the general population as well as in the dialysis population. Therefore, the focus should be on reducing

the risk of post transplant cardiovascular disease, and in particular perioperative and early post transplant death. Hence the reasons for screening potential renal transplant recipients for cardiovascular disease are threefold. First, identifying transplant candidates with ischaemic heart disease allows patients and clinicians to better understand the risk of transplantation. Second, it would be hoped that pre-transplant revascularisation that may reduce the risk of transplantation. Finally intensive risk factor modification may lead to better patient and graft outcomes(37).

The most detailed guidelines from the American Society of Transplantation suggest the following strategy. First, assess ischaemic heart disease risk factors: a prior history of IHD, men ≥45 or women ≥55 years, (IHD in a first degree relative, current cigarette smoking, diabetes, hypertension, hypercholesterolaemic and left ventricular hypertrophy). Next aggressive risk factor modification should be pursued. Patients at high risk, diabetic nephropathy, prior history of IHD, or ≥2 risk factors, should have a cardiac stress test and those with a positive cardiac stress test should undergo coronary angiography for possible revascularisation prior to transplantation(192). The current European Renal Association guidelines are less detailed but suggest that as cardiovascular disease is the main cause of mortality after transplantation careful evaluation is mandatory to detect and treat symptomatic coronary disease, valve disease, cardiomyopathy and pericardial disease(256). The ACC/AHA guidelines for perioperative cardiovascular evaluation for noncardiac surgery are broadly similar suggesting that the risk of the surgical procedure, risk factor assessment, functional capacity and non invasive testing should guide patient selection for coronary angiography(255).

The dilemma exists in patients with ESRD that the evidence for cardiovascular risk factor reduction with statins or with blood pressure management is not present. Many of the cardiac risk factors have accumulated over a long period of renal insufficiency and are potentially irreversible (such as LVH, family history and diabetes). Most of these recommendations for assessment are based on observational studies either with radionuclide myocardial perfusion imaging or stress echocardiography rather than on any specific intervention.

Reviewing the studies of perfusion imaging, a meta analysis by Rabbat *et al* of the 12 studies using myocardial perfusion scanning in potential renal transplant recipients concluded that both reversible and fixed perfusion defects were associated with a significantly increased risk of cardiac death and myocardial infarction(260). As there was no difference between fixed and reversible defects, it is difficult to know whether these studies identify patients who would have benefited from coronary revascularisation. However, a negative scan is reassuring. Similarly, in a comparative study between myocardial scintigraphy (SPECT), dobutamine stress echocardiography, and coronary angiography in 126 renal transplant candidates showed a sensitivity and negative predictive value of <75% for the presence of coronary disease suggesting that whilst these scans may well carry prognostic information they are of limited value at guiding the need for coronary angiography(261).

6.4.4 Coronary angiography, ischaemic heart disease and renal transplantation

In the current study, survival was not significantly different between those patients undergoing angiography and those who did not. However patients with a greater burden of coronary atheroma, fared significantly worse over the follow up period. The implication is

that irrespective of the original indication for performing angiography, the degree of coronary atherosclerosis predicts outcome. In this cohort of 96 patients undergoing angiography, very few went on to have coronary revascularisation (eight undergoing percutaneous intervention and two undergoing coronary artery bypass grafting).The reasons for this are unclear but may reflect perceived high procedure risk, absence of symptoms leading to reluctance to perform revascularisation, or the technical challenge of performing revascularisation in ESRD where the vessels are typically heavily calcified. With such a small cohort it is not possible to analyse any impact coronary revascularisation has on survival.

There are few studies to inform the decision to revascularise asymptomatic patients prior to major surgery. One large study, the Coronary Artery Revascularisation Prophylaxis (CARP) trial, assessed the long-term benefit of preoperative coronary-artery revascularisation among 510 patients with stable coronary artery disease scheduled for elective vascular surgery (aneurysm repair or peripheral vascular surgery(262). At 30 days after the vascular operation, a postoperative myocardial infarction, defined by elevated troponin levels, occurred in 12 percent of the revascularisation group and 14 percent of the no-revascularisation group ($p=0.37$) indicating no significant difference between the groups, suggesting that in patients on optimal medical management, a strategy of coronary-artery revascularisation before elective vascular surgery among patients with stable cardiac symptoms cannot be recommended. These patients did not have significant renal impairment with only one patient in each group requiring post operative dialysis therapy.

In patients with ESRD assessed for transplantation undergoing coronary revascularisation, only one study exists of 26 asymptomatic diabetic patients who were found to have

stenoses of greater than 75%(263). Patients randomised to revascularisation (either percutaneous intervention or bypass grafting) prior to transplantation had fewer post transplant cardiovascular disease events than patients managed with medical therapy. However this study could be criticised as patients in the medical treatment arm received short acting nifedipine, which has been suggested to cause an increase in ischaemic heart disease events, where current optimal medical management would include a beta-blocker. The small sample size makes it possible that the observed differences were due to chance and additionally as this study was only performed in diabetics it is not clear whether the findings can be extrapolated to non diabetic patients.

A more practical strategy may be to use perioperative beta blockade which has been shown in retrospective analysis of larger United States patient databases to be associated with lower risk of perioperative death in patients at high (but not low) cardiac risk(264). This strategy has not been trialled in ESRD, but would appear to be a more pragmatic approach than undertaking a programme of coronary revascularisation, particularly where the evidence from the general population does not support such a course of action.

6.4.5 Renal transplantation and survival compared to dialysis as treatment for end stage renal disease

Until fairly recently it was difficult to be certain whether renal transplantation offered a survival advantage compared to long term dialysis therapy. Inevitably selection bias exists between patients on the transplant list who tend to be younger, fitter and have less co morbidity, and those patients who are not transplant listed. Wolfe *et al* studied outcome in 228,552 patients on dialysis using data from the United States Renal Data System 1991-1996. Only 46,164 patients were on the transplant list, of whom, 23,275 received a first

cadaveric renal transplant(265). Patients on the transplant list were younger and healthier than those who were not, but long-term mortality rate was 48 to 82 percent lower among transplant recipients than patients on the waiting list with relatively larger benefits among patients who were 20 to 39 years old, white patients, and younger patients with diabetes. Similar benefits were shown in a much smaller cohort of patients comparing wait listed patients to transplanted patients in Ontario, Canada(266). However it still remains unclear whether patients at greatly increased cardiovascular risk, at much higher perioperative mortality would still benefit from renal transplantation, which becomes a greater dilemma when one considers that the demand for cadaveric kidneys greatly outstrips supply.

Oniscu *et al* studied patients listed for transplantation in Scotland and have suggested that even in elderly patients (>60 years old) transplantation offers significant survival advantage as a treatment for ESRD than dialysis(267). Again in this study, patients who were transplanted had significantly less co morbidity. In another United States Renal Data System analysis, Meier-Kriesche *et al* observed that rates of cardiovascular disease peaked in the first three months post operatively and thereafter continued to fall, whilst in wait listed patients the risk of cardiovascular events continued to rise inexorably, and hence the authors proposed that transplantation halts the progression of cardiovascular disease in ESRD(268). An attractive but implausible interpretation of this study would be to suggest that patients at the very highest risk of cardiovascular disease should be offered cadaveric kidneys as a priority, as long as they are prepared to accept the increased perioperative risk of death. One dilemma in patients with greater risk of cardiovascular disease is that they may require further cardiac investigations, which dependent on healthcare resources, may be time consuming. This may result on further time spent on the renal transplant waiting list (or delay access to the waiting list). The long term consequences of this may result in

poorer graft outcome post transplantation as it is well documented that increased waiting time on dialysis is a powerful predictor of poorer graft survival(269).

6.4.5 Limitations of the current study of outcome in patients undergoing cardiovascular screening for renal transplantation

As a single centre study, this analysis is too small to truly assess the impact of transplantation on patient survival. The very low revascularisation rate has made it impossible to contribute further to debate on impact of this intervention in potential renal transplant recipients. Furthermore, as not all patients had the same investigations, including angiography, it may be difficult to draw any more conclusions of the impact of these test results on outcome. The reason for this was primarily that it was felt to be unethical to submit all patients to invasive investigations as part of a study and so invasive investigations and interventions were only performed by a clinician blinded to the notion that long term patient outcomes were being studied. Finally as an end point, death was used in survival analysis rather than major adverse cardiac events such as hospitalisation, myocardial infarction (fatal and non-fatal), cardiac failure and cardiac death. The reason for this was that as patients were frequently followed up at another hospital, the investigator was unable to be certain regarding the cause of hospitalisation or diagnosis of a major adverse cardiac event. Defining myocardial infarction or cardiac failure are difficult in ESRD due to the high prevalence of ECG abnormalities, frequent rises in biomarkers of myocardial necrosis such as troponin in the absence of coronary artery occlusion and development of pulmonary oedema due to hypervolaemia rather that primary cardiac dysfunction.

6.4.6 Conclusions and further studies required in the role of cardiovascular assessment for renal transplantation

There is a high mortality amongst patients screened as potential renal transplant recipients with 20% of patients dying over a median follow up period of 32.5 months. Patients who received a renal transplant had a better survival than those who did not, with the major predictors of death being the presence of, or risk factors for ischaemic heart disease. CMR with contrast indicated patients at greater risk of death, but not independent of other variables. Based on the results of these data, an appropriate strategy would be to attempt to reduce the risk of ischaemic heart disease a long time prior to the development of ESRD and the need for renal transplantation. Further study of reduction of perioperative risk in patients undergoing renal transplantation is required, examining whether coronary revascularisation has any benefits in this cohort of patients as well as investigation of optimal medical therapy for the potential renal transplant recipient.

Chapter 7

A study of the diagnostic utility of brain natriuretic peptide in end stage renal disease

7.1 INTRODUCTION

Brain natriuretic peptide (BNP) is a hormone synthesised and secreted primarily by the ventricles of the heart(270). Natriuretic peptides, specifically brain natriuretic peptide (BNP) are released from the heart in response to chamber distension and thus are increased in the presence of volume expansion and cardiac overload. Their physiological role is to cause vasodilatation and promote natriuresis to maintain volume homeostasis(271). Increasingly serum levels of BNP are used to both diagnose and manage cardiovascular disorders(272). However, the diagnostic role of serum BNP levels in patients with advanced renal dysfunction remains to be defined. Patients with ESRD have a high prevalence of left ventricular disorders, specifically LVH, which may reduce the diagnostic utility of BNP. In addition, ventricular stretch may be determined by intravascular volume status rather than by cardiac dysfunction.

Nonetheless, since the prognosis of patients with end stage renal failure and co-existing heart failure is so poor, the availability of a further marker of cardiac “distress” may in future become a useful diagnostic tool and in due course may become a primary goal for titration and tailoring of therapy. Whilst population studies demonstrate that BNP is a useful test for targeted screening for LVSD in the general population(272), in the ESRD population echocardiographic studies have suggested that BNP correlates with LV dimensions, but appears to be more closely related to LV mass than cardiac dysfunction(273). Similarly, BNP may be a predictor of long term survival in these patients(274), but there are limited ‘real world’ studies of patients with ESRD and superimposed ischaemic heart disease and/or congestive heart failure.

The following studies were performed in order to determine the diagnostic accuracy of serum BNP in patients with ESRD for both the presence of LVH and LVSD. Additionally the relationship between serum BNP and long term survival was examined.

7.2 METHODS

7.2.1 CMR technique and analysis

All patients were studied with the CMR technique described in detail in Chapter 2 (2.3.2).

7.2.2 Measurement of serum brain natriuretic peptide levels

Prior to CMR scanning 30ml of venous blood was drawn. 5ml of blood was added to a potassium-EDTA tube and centrifuged for 10 minutes at 3000 revolutions per minute prior to freezing at -20^0C. Samples were analysed in batches in the neuroendocrine laboratory (Dr Ian Morton, University of Glasgow). Serum BNP was measured using by a one step radioimmunoassay (ShionoRIA, Shinogi, Japan).

7.2.3 Statistical methods

Differences between groups of patients were analysed by Chi-squared or Fisher's exact test (as appropriate) for categorical data and paired t-test and Mann–Whitney–U testing for parametric or nonparametric data. One way analysis of variance (ANOVA) was used for comparisons between more than two groups. As BNP demonstrated a skewed distribution log-transformation was performed. Correlations between cardiac dimensions, volume of myocardial fibrosis and BNP were assessed with Pearson and Spearman correlation co-efficient as appropriate. Receiver operator characteristic (ROC) curves were plotted to assess diagnostic utility of serum BNP. BNP levels between patients who died or had a

vascular event during the follow up period were divided into tertiles and subjected to an unadjusted survival analysis by the Kaplan–Meier method. All analyses were performed using the SPSS 13.0 statistical software package (SPSS Inc., Chicago, IL., USA), except ROC curves which were performed with the MedCalc software package (MedCalc 8.1, MedCalc Software, Belgium).

7.3 RESULTS

7.3.1 Dialysis modality and serum BNP levels

Blood sampling and CMR scanning was performed on 114 patients (60 haemodialysis, 37 peritoneal dialysis and 17 pre-dialysis patients). Serum log_{10}BNP was significantly lower in pre-dialysis patients than in patients on haemodialysis (log_{10}BNP in pre-dialysis patients 1.70 vs. 2.28 in HD patients, p=0.004) but not peritoneal dialysis (1.70 vs. 1.77 in CAPD patients, p=0.717). Although the elevation in BNP levels in HD may reflect differences in LV dimensions (mean LVMI- HD 101.3g m^{-2}, PD 80.4 g m^{-2}, pre-dialysis 80.1 g m^{-2}, p=0.005), to minimise any potential effect of renal excretory function on serum BNP levels, only results for patients established on renal replacement therapy were analysed.

7.3.2 Baseline demographics and serum BNP levels

Baseline demographics, LV dimensions and BNP levels are shown in Table 7.1. Significantly more male patients studied were on haemodialysis compared to peritoneal dialysis. Correspondingly patients on HD were significantly taller. A significantly greater proportion of patients with diabetes were on PD. Patients on PD had been receiving renal replacement therapy for a shorter duration. LV dimensions were significantly larger in patients on HD (LVMI 101.3 vs. 80.4 g m^{-2}, p=0.001; end diastolic volume/BSA 85.0 vs.

66.6 p=0.003). Serum BNP and log_{10}BNP levels were greater in patients on HD (mean log_{10}BNP was 2.28 in HD patients vs. 1.77 in PD patients, p<0.001).

7.3.3 Relationship between left ventricular dimensions and serum BNP levels

Serum log_{10} BNP positively correlated with LVMI, end diastolic volume/BSA and end systolic volume/BSA. Serum BNP negatively correlated with LVEF. Similar degrees of correlation were demonstrated in both patients receiving haemodialysis and peritoneal dialysis (Table 7.2).

7.3.4 Serum BNP levels in patients with left ventricular hypertrophy and/or left ventricular systolic dysfunction

Patients with LVH had significantly higher BNP levels than those without LVH (patients with LVH mean Log_{10}BNP was 2.32 vs. 1.75 in patients without LVH, p<0.001) as did patients with LVSD (mean Log_{10}BNP was 2.67 in patients with LVSD vs. 1.99 in patients without LVSD, p=0.001; Figure 7.1). The utility of BNP levels as a diagnostic test for LVH or LVSD was tested using area under receiver operator characteristic (ROC) curves. The area under the curve (AUC) for the diagnosis of LVH for the whole cohort population using BNP was 0.74 and 0.78 for a diagnosis of LVSD (Figure 7.2). Table 7.3 gives the sensitivity, specificity, and positive and negative predictive values for a BNP concentrations determined by optimum cut-point of the ROC for diagnoses of LVH and LVSD in the whole cohort, as well as patients subdivided by dialysis modality. The sensitivity of BNP as a test for LVH was 50.0% and specificity was 85.4% and for LVSD the sensitivity and specificity were 69.2% and 83.3% respectively.

7.3.5 Relationship between BNP and presence of late gadolinium enhancement

From this cohort, 92 patients underwent contrast enhanced CMR with intravenous Gd-DTPA-BMA underwent as described in Chapter 2. 30 (32.6%) patients had evidence of myocardial fibrosis indicated by LGE, of which 18 (19.6%) had subendocardial LGE representing old myocardial infarction and 12 (13.0%) had diffuse LGE. Patients with evidence of myocardial fibrosis indicated by LGE had higher serum BNP levels than LGE negative patients (LGE positive mean $\log_{10}$BNP 2.38 vs. 1.94 in LGE negative patients, p=0.003). Comparing subtypes of LGE, patients with subendocardial LGE had significantly higher BNP levels than LGE negative patients (subendocardial LGE $\log_{10}$BNP mean 2.54 vs. 1.94 in LGE negative patients, p<0.001). There were no significant differences in BNP between patients with the two subtypes of LGE (subendocardial LGE mean $\log_{10}$BNP 2.54 vs. 2.14 in diffuse LGE patients, p=0.158), nor were there significant differences between patients with diffuse LGE and LGE negative patients (Figure 7.3).

Overall there was a significant correlation between serum BNP levels and the mass of myocardial fibrotic tissue indicated by LGE (R=0.38, p=0.041). Patients with subendocardial LGE demonstrated a significant correlation between serum BNP and mass of tissue indicated by LGE (R=0.50, p=0.034) shown in Figure 7.4, whilst in patients with diffuse LGE there was not a significant correlation between BNP and mass of myocardial fibrotic tissue (R=0.15, p=0.640).

	Haemodialysis		Peritoneal Dialysis		p value
Number (%)	60		37		
Age	51.4	(11.3)	53.7	(11.0)	0.322
Male (%)	46	(76.7)	19	(51.4)	0.010
Height (m)	170.9	(9.4)	166.2	(9.7	0.024
Weight (kg)	75.1	(16.9)	74.3	(13.8	0.817
RRT time (months)	26.4	(84.4)	6.2	(11.1)	<0.001
Past history of IHD (%)	11	(18.3)	7	(18.9)	0.942
Diabetes (%)	9	(15.0)	(16)	(43.2)	0.002
Smoker (%)					
Never	32	(53.3)	17	(45.9)	
Current	18	(30.0)	13	(35.1)	0.226
Ex	10	(16.7)	7	(15.8)	
SBP (mmHg)	136.9	(27.3)	140.6	(22.8)	0.511
DBP (mmHg)	81.8	(14.9)	82.5	(12.0)	0.814
Ejection fraction (%)	66.6	(9.5)	66.5	(13.0)	0.978
LV mass index (g m^{-2})	101.3	(32.5)	80.4	(20.6)	0.001
End diastolic volume/BSA (ml m^{-2})	85.0	(31.0)	66.6	(20.6)	0.003
End systolic volume/BSA (ml m^{-2})	29.6	(18.4)	22.7	(13.1)	0.052
BNP	191.0	(750)	54.0	(91)	<0.001
Log_{10} BNP	2.28	(0.70)	1.77	(0.53)	<0.001

Table 7.1

Background demographics for dialysis patients. Results are show as mean with standard deviation in parenthesis or number with percentage in parenthesis as appropriate except for RRT time and BNP which are displayed as median and inter quartile range. Tests of significance are t-test and Chi-squared between groups, except for RRT time and BNP where Mann-Whitney-U was used

	All patients		Haemodialysis		Peritoneal dialysis	
	Spearman's R	p	Spearman's R	p	Spearman's R	p
Ejection fraction	-0.33	0.001	-0.33	0.011	-0.45	0.005
LV mass	0.51	<0.001	0.47	<0.001	0.40	0.015
End diastolic volume	0.51	<0.001	0.47	<0.001	0.40	0.013
End systolic volume	0.52	<0.001	0.48	<0.001	0.54	0.001
LV mass index	0.61	<0.001	0.58	<0.001	0.48	0.004
End diastolic volume/BSA	0.58	<0.001	0.56	<0.001	0.43	0.011
End systolic volume/BSA	0.56	<0.001	0.52	<0.001	0.58	<0.001

Table 7.2

Correlation co-efficients between ventricular dimensions and serum BNP

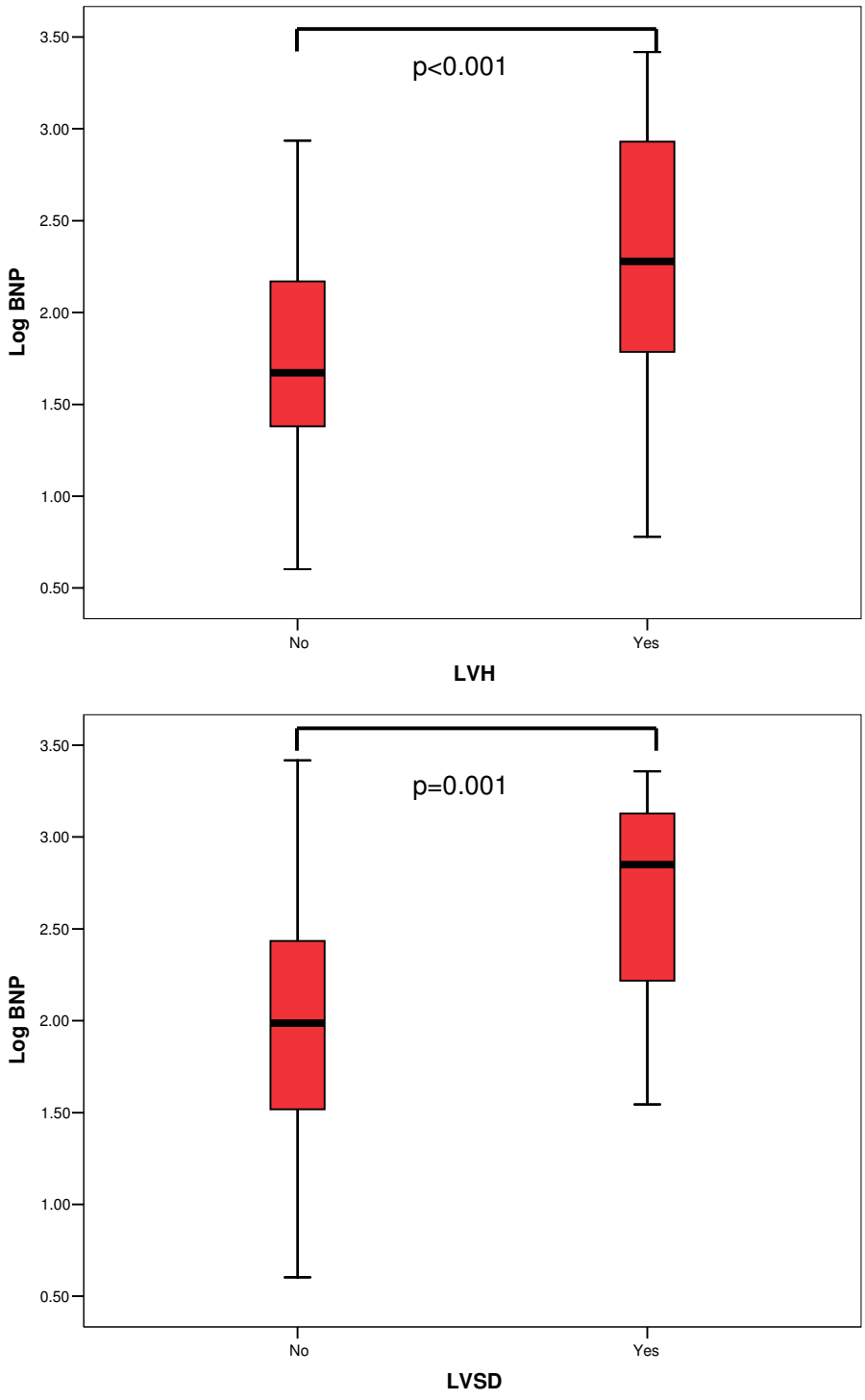


Figure 7.1 Box plots for Log_{10}BNP in patients with and without LVH defined by CMR evidence (above) and LVSD (below)

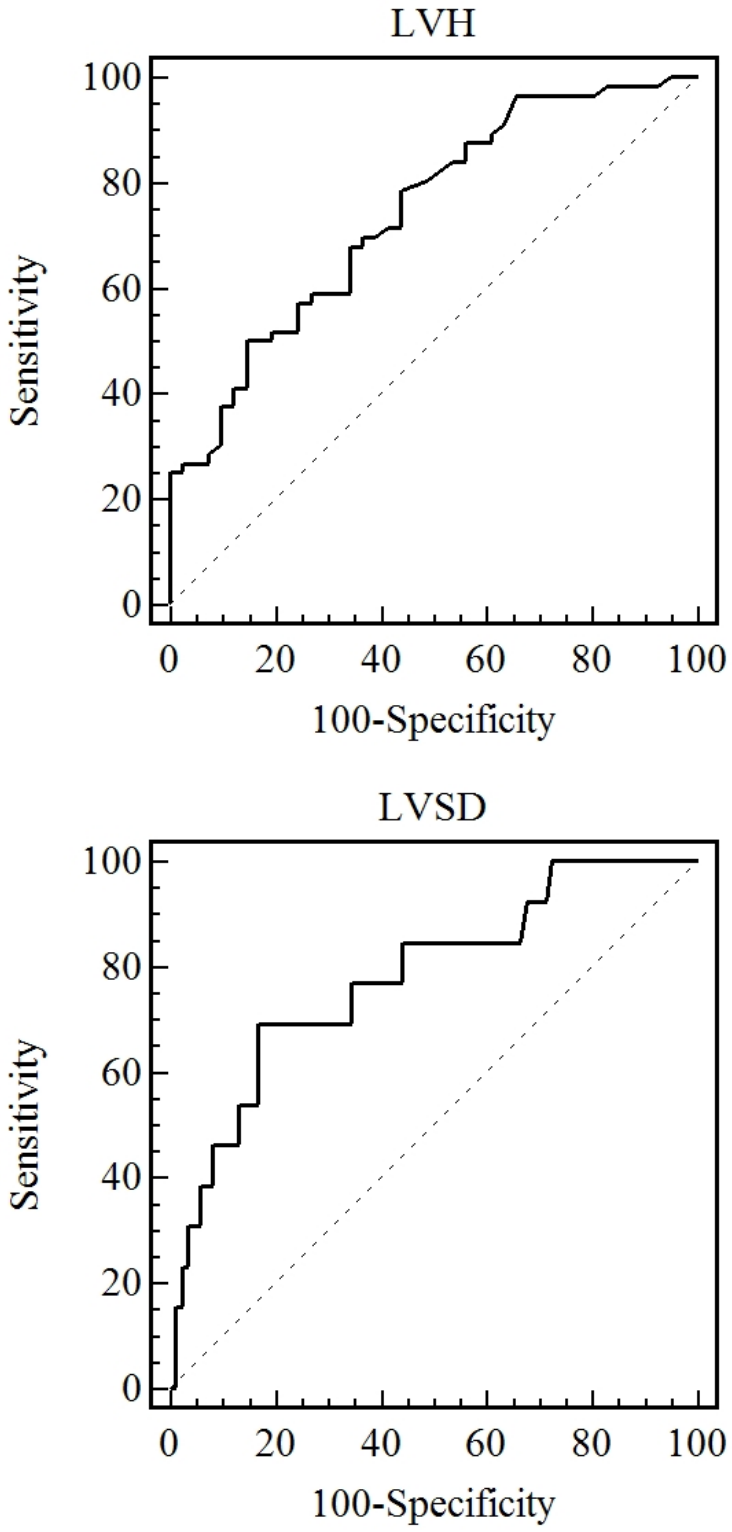


Figure 7.2 Receiver operator characteristic curves BNP for the diagnosis of LVH (above) and LVSD (below)

	Best cut-off	AUC for ROC curve		p	Sensitivity	Specificity	PPV	NPV
	pmol/L	(95% CI)			% (95% CI)			
All patients								
LVH	196	0.74	(0.64-0.83)	<0.001	50.0 (36.3-63.7)	85.4 (70.8-94.4)	82.4	55.6
LVSD	397	0.78	(0.68-0.86)	<0.001	69.2 (38.6-90.7)	83.3 (73.6-90.6)	39.1	94.6
Haemodialysis								
LVH	196	0.73	(0.60-0.84)	<0.001	61.5 (44.6-76.6)	76.2 (52.8-91.7)	82.8	51.6
LVSD	1082	0.82	(0.70-0.91)	0.002	71.4 (29.3-95.5)	90.6 (79.3-96.8)	50.0	96.0
Peritoneal dialysis								
LVH	54	0.71	(0.54-0.85)	0.017	70.6 (44.1-89.6)	75.0 (50.9-91.2)	70.6	75.0
LVSD	114	0.87	(0.72-0.96)	<0.001	83.3 (36.1-97.2)	87.1 (70.1-96.3)	55.6	96.4

Table 7.3

Areas under receiver-operator-characteristic curves for the use of BNP for the diagnosis of LVH and LVSD in the whole cohort, patients receiving haemodialysis and patients receiving peritoneal dialysis. Percent and 95% confidence intervals are shown for the threshold of best cut-point values. The best cut-point values are defined as those which maximise both sensitivity and specificity

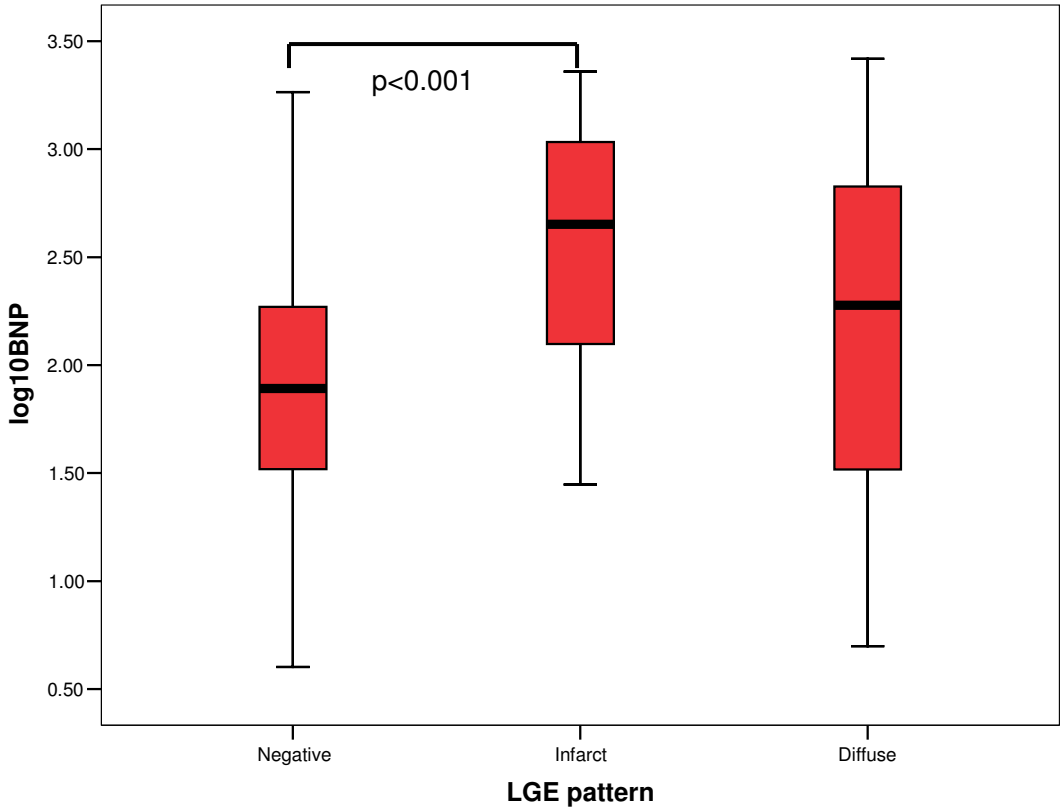


Figure 7.3

Box plots for Log_{10}BNP in patients defined by subtype of LGE demonstrated by CMR

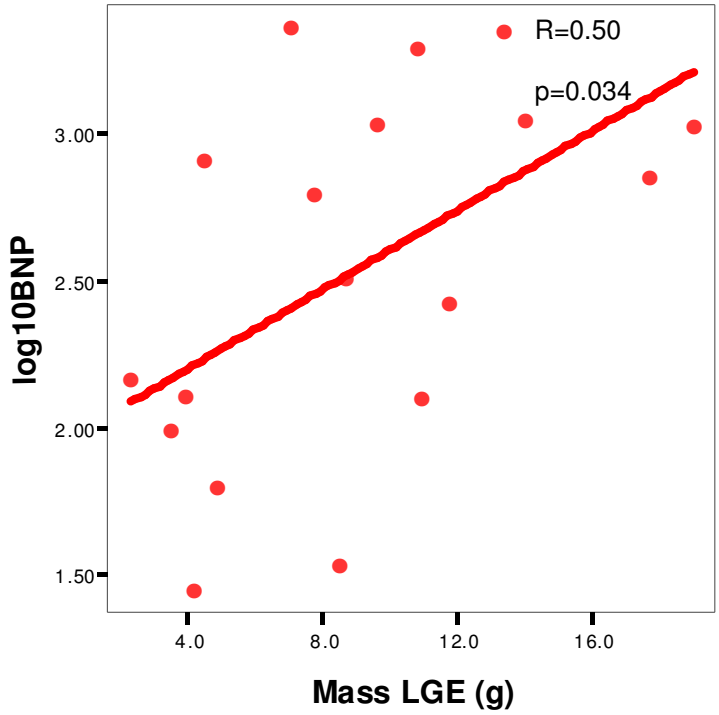


Figure 7.4

Log transformed serum BNP level plotted against mass of subendocardial myocardial fibrosis indicated by LGE

7.3.6 Prognostic value of brain natriuretic peptide

During the follow up period eleven patients died. The median follow up period was 838 days (inter quartile range 385). There were no significant differences in BNP between survivors and patients who died during the follow up period (survivors mean $\log_{10}$BNP 2.09 vs. 1.98 patients who died, p=0.626). The influences of the measures of BNP on survival are shown as Kaplan-Meier survival curves in Figures 7.5.

During the follow up period there were an additional seven non-fatal cardiovascular events (five patients had a myocardial infarction; one patient underwent coronary revascularisation and one patient had a cerebrovascular event). Again there were no significant differences in serum BNP between than survivors who remained event-free and patients who had a non-fatal cardiovascular event (event-free patients mean $\log_{10}$BNP 2.06 vs. 2.46 in patients who had a cardiovascular event, p=0.074).

Combining death with non-fatal cardiovascular events as a combined cardiovascular end point (total of 18 events), there were no significant differences in serum BNP between than survivors who remained event-free and patients who had a non-fatal cardiovascular event (mean $\log_{10}$BNP 2.06 vs. 2.17 in patients who died, p=0.562).

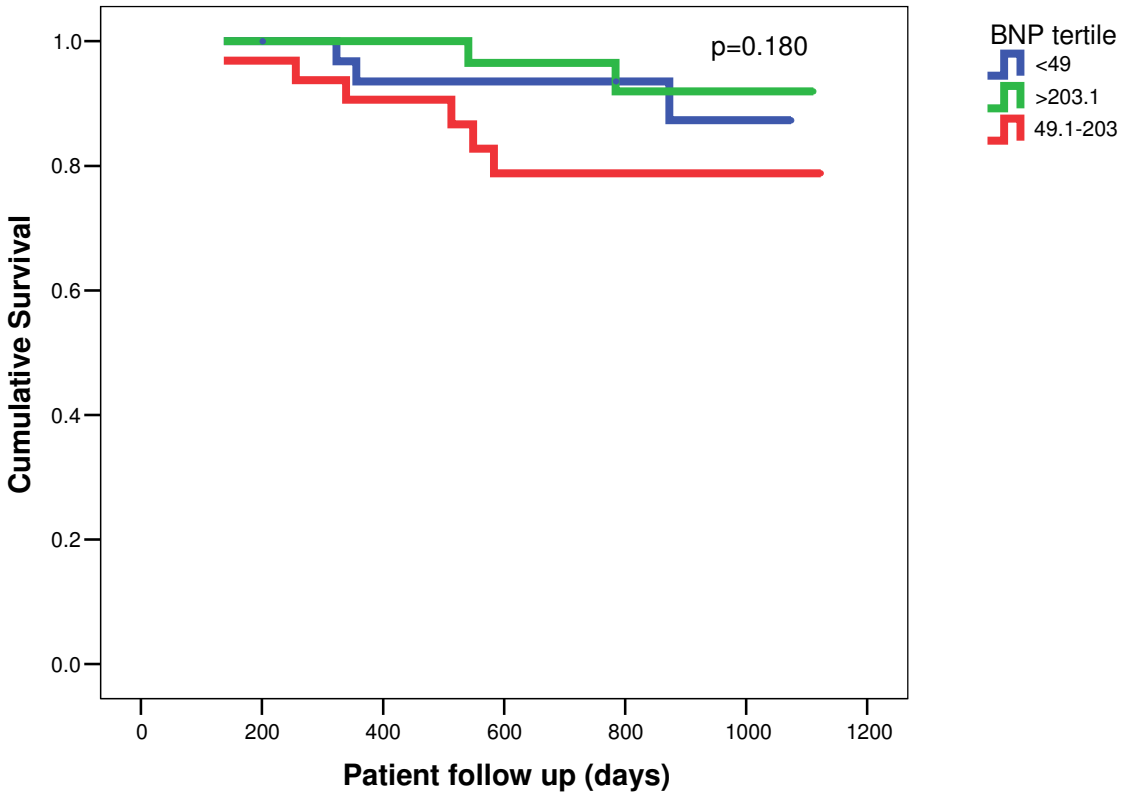


Figure 7.5

Kaplan-Meier survival curves for all cause mortality with patients stratified by serum BNP tertile

7.4 DISCUSSION

7.4.1 Serum BNP in cardiovascular disorders in the general population and ESRD

BNP was originally isolated from porcine brain, and is produced in small amounts by the glomerular epithelial cells(275). The past decade has seen a developing role for the use of natriuretic peptides (both BNP and atrial natriuretic peptide- ANP) to screen for left ventricular dysfunction in the general population(272;276). Additionally, it is likely that using serum BNP levels will become a routine tool to assess response to therapeutic interventions in patients with chronic heart failure (CHF)(277). To date the situation is less clear in patients with ESRD. The prognostic implications of left ventricular disorders in patients with ESRD are well established with LVH, left ventricular dilatation and LVSD being associated with worse survival(76;77). There is a pressing need for new diagnostic and prognostic tools to identify patients with ESRD at increased risk of premature cardiovascular events, specifically those with structural and functional abnormalities of the left ventricle, to facilitate appropriate and timely therapeutic intervention. However, in dialysis patients there is difficulty in defining ventricular chambers volumes which are dependant on hydration status and vary in the intra-dialytic period. Furthermore, the levels of circulating natriuretic peptides depend on renal function, due to renal metabolism and excretion(278). Thus natriuretic peptide levels may be increased by volume overload due to intravascular volume and reduced renal clearance in the absence of any cardiac dysfunction(279). The high incidence of ventricular disorders in the dialysis, compared to the general population, may further alter the diagnostic potential of BNP as a screening test.

7.4.2 Basic pathophysiology of mechanisms of production, action and excretion of brain natriuretic peptide

BNP is a 32 amino acid polypeptide (molecular weight 3.5 kDa), with a plasma half life of approximately 22 minutes in healthy individuals(280). It is secreted mainly by cardiac ventricles and, to a small extent, by renal glomerular cells. Cardiac secretion is in response to ventricular volume expansion, pressure overload and increased wall tension. Thus, plasma BNP levels reflect the level of left ventricular overload and, in patients with CHF, BNP levels correlate with LV filling pressures, NYHA class and are inversely related to left ventricular ejection fraction, or exercise performance(281). BNP is synthesised as high molecular weight preproBNP in the ventricular myocardium, and enzymatically cleaved to proBNP in response to myocyte stretching. It is thereafter released as hormonally active BNP and inactive N-terminal-proBNP (NT-proBNP)(282).

Serum levels of both BNP and NT-proBNP are elevated in patients with CHF and tend to change in parallel. However, due to differences in molecular size and metabolism there is variation between the utility of BNP and NT-proBNP in the diagnosis of ventricular dysfunction or hydration status, specifically in patients with renal failure. BNP is eliminated from plasma primarily by neutral endopeptidase receptors and degraded. Although these enzymes are found in the kidney, glomerular filtration has only a minor role in elimination of BNP. NT-proBNP is a larger molecule (molecular weight 8.5kDa) and is not cleared significantly by the clearance receptor or neutral endopeptidases(283). It has been speculated that clearance of NT-proBNP is principally by renal excretion. These confounding factors may explain the direct correlation between renal function and serum NT-proBNP levels and therefore the limited utility of serum NT-proBNP in patients with CKD(284).

The physiological actions of BNP, and other natriuretic peptides, oppose the effects of over-activity of the renin angiotensin-aldosterone system, the sympathetic nervous system and endothelin-1. In the kidney, BNP inhibits sodium resorption, promoting natriuresis and increases glomerular filtration rate. The natriuretic peptides also relax vascular smooth muscle, causing both arterial and venous dilation, thereby reducing blood pressure and ventricular preload(271).

Even in normal individuals there is considerable inter-individual variability in plasma BNP levels. This may contribute to susceptibility to volume overload and hypertension. From the Framingham dataset it appears that heritable factors may have a substantial role in determining plasma BNP levels, with a region suggestive of genetic linkage on chromosome 12p13(285). This finding provides insight into the mechanisms present in patients with normal ventricular dimensions and no obvious cause for a raised BNP. Moreover, due to its short half-life BNP production is dependent on continuing gene expression and translation.

Several studies have investigated the impact of CKD on serum levels and metabolism of BNP. This issue is particularly vexed by the increasing prevalence of LVH in patients with progressive renal disease(78). Myocyte hypertrophy causes increased serum BNP even in the presence of normal renal function(286). As BNP can be found in relatively normal to high levels in the urine of patients with CKD, it can be assumed that BNP is cleared sufficiently by the endopeptidase system to ensure that it maintains some diagnostic utility in the presence of renal impairment(287).

7.4.3 Results of study of diagnostic properties of serum brain natriuretic peptide in end stage renal disease

In this cohort of patients BNP correlated more closely with LVMI than any parameter indicating LV stretching (LV end diastolic or end systolic volume) or LV function, although there were also significant correlations between these factors and serum BNP levels. However, using ROC analysis serum BNP appeared to be a worthwhile diagnostic tool in the diagnosis of either LVH or LVSD. In particular BNP had a high negative predictive value (94.6%) for excluding the diagnosis of LVSD. Conversely, when used as a diagnostic tool for LVH, the reverse was true, with serum BNP having a high positive predictive value for LVH but a much lower negative predictive value. Either way, when compared to LV dimensions it would appear that BNP may be a useful indicator of patients with LV abnormalities requiring further assessment of LV function (with either echocardiography or CMR). In patients who underwent contrast enhanced CMR, perhaps unsurprisingly; higher BNP levels appeared to indicate patients with previous myocardial infarction, in keeping with the findings of Chapter 4 suggesting that LVSD in uraemia is almost inevitably linked to underlying coronary artery disease. In the small number of patients with myocardial infarction, BNP directly correlated with the mass of infarcted tissue. Whether this indicates that BNP is produced primarily by infarcted tissue or as a secondary response to the presence of infarction is unclear from this study. Laboratory studies of cultured cardiac fibroblasts suggest that BNP is likely to be produced in response to, and to counteract the production of myocardial fibrosis(288).

BNP was higher in patients on haemodialysis than peritoneal dialysis. Whilst this is likely to be related to greater LV mass, some studies have suggested that the presence of

an arteriovenous fistula as vascular access for haemodialysis may additionally lead to raised natriuretic peptide levels by increasing cardiac output(112).

7.4.4 Diagnostic properties of brain natriuretic peptide in the general population

One of the most promising uses of serum BNP is in the assessment of left ventricular dysfunction. Population based studies have shown that it may be an effective tool for screening for asymptomatic left ventricular dysfunction. By identifying patients with asymptomatic left ventricular dysfunction it is likely that evolution to clinical heart failure will be prevented by instigating evidence based therapy with angiotensin converting enzyme (ACE) inhibitors and beta-blockers. Examples of studies using BNP to screen or diagnose echocardiographic LVSD or clinical heart failure are listed:

- In one population based study the presence of a raised BNP concentration (greater than 5.2 pmol/L) gave an overall sensitivity of 77% and specificity of 87% (92% and 72%, respectively, in participants aged 55 years or older) for the presence of echocardiographic LVSD. However, this population based study has either not reported renal dysfunction in the main analysis and subsequently found renal failure to be associated with the presence of a raised serum BNP (or NT-BNP) despite normal echocardiographic findings(272;289).
- In patients with of acute dyspnoea presenting to hospital, the large “Breathing Not Properly” study found a raised BNP to be better than any clinical variable at predicting a final diagnosis of heart failure One of the most useful points to emerge from this study is that in this setting BNP appears to be an extremely useful tool for ruling out heart failure with a negative predictive value of 90% for a BNP threshold of 28.9 pmol/L with a sensitivity of 90% for a diagnosis of heart failure(290). It should be noted that patients with severe renal failure (GFR <15ml/min/1.73m^2 or dialysis dependent) were excluded from this study

- In the BNP for Acute Shortness of Breath Evaluation (BASEL) study, a BNP based strategy for the initial assessment and management of patients with dyspnea can prevent hospitalisation, the need for intensive care and reduce length of hospital stay in patients presenting with acute dyspnoea. Furthermore, these improvements ensure that treatment of these patients is carried out in a cost effective manner(291)

The obvious difference between these population based studies and the cohort of ESRD patients is that the diagnostic cut points for either LVSD or LVH is much greater in ESRD than in the general population (196 pmol/L for LVH and 397pmol/L for LVSD). This is in keeping with findings of a previous study from our group and is likely to reflect both the reduced clearance of BNP in ESRD and the greater prevalence of LVH in patients with ESRD. From these and other studies, it is apparent that serum BNP is a promising diagnostic tool for the diagnosis of left ventricular dysfunction in the general population, either used as a targeted screening measure in asymptomatic patients, as part of the diagnostic workup of patients presenting with acute dyspnoea or to monitor response to therapy. The current study suggests that BNP may be a useful tool in the ESRD population, although our previous findings suggest than the same diagnostic utility does not hold true across a spectrum of CKD(292).

7.4.5 Diagnostic properties of BNP in end stage renal disease

The high prevalence of LVH and LVSD in ESRD (compared to the general population) poses an additional problem that may limit the diagnostic screening potential of BNP, notwithstanding the additional problems of defining appropriate serum BNP levels in ESRD. Nevertheless, there have been some other promising studies that assess the diagnostic potential of BNP in dialysis patients:

- In an echocardiographic study combining both haemodialysis and peritoneal dialysis patients, a close correlation was found between serum BNP and LV mass. BNP had a high sensitivity (88%) for the diagnosis of LVH, with a positive predictive value of 87%. In the same study BNP was a less impressive marker for LVSD with a sensitivity of 94% but a specificity of only 22%(273)
- It has been reported that BNP levels are lower in PD patients than in HD patients(293). Whether this is due to reduced cardiac stress in PD patients, as suggested by the authors, or due to a reduction in BNP related to improved residual renal function in PD patients is not addressed by this small study
- Although BNP levels are generally higher in ESRD patients with LVSD, the relationship between BNP and LV ejection fraction does not appear to be as strong as its correlation with LV mass(273). In studies assessing the relationship between BNP and the presence of atherosclerotic coronary disease, it appears that HD patients with CAD have higher levels of serum BNP that those with normal coronary arteries(294). This supports the notion of a close association between LVSD and coronary artery disease in ESRD as demonstrated in Chapter 3

Many of these studies specifically excluded patients with a history of CHF, exposing limitations on these investigations into the relationship between serum BNP and severity of LVSD as the patients with most severe cardiac dysfunction have been excluded. This current study suggests that, while BNP shows potential as a marker of LVH and has been proposed to be a further therapeutic target to assess regression of LVH, it is unlikely that serum BNP will be able to replace echocardiography (or CMR) in the diagnosis of LVSD. Further study is required of a larger number of dialysis

patients to define BNP levels consistent with normal LV dimensions, LVH and LVSD taking into account hydration status, to improve the diagnostic utility of BNP.

7.4.6 Prognostic role of serum brain natriuretic peptide in end stage renal disease

Serum BNP was highest in the patients who were at highest cardiac risk, based on CMR findings and previous reports on the prognostic implications of uraemic cardiomyopathy. In this study however, BNP did not predict patients likely to die or have a cardiac event during the follow up period. Possible limitations of this study include the small cohort of patients, relatively short follow up period and limited number of events. On the basis of this study alone BNP does not provide useful prognostic information for the management of patients with ESRD. This finding conflicts with other similar studies in this ESRD population. As well as providing diagnostic value, a number of studies have shown the prognostic value of serum BNP in dialysis patients:

- The CREED investigators have demonstrated that serum BNP appears to be an important predictor of mortality in a group of haemodialysis and peritoneal dialysis patients, independent of LV mass and LV function(274). This is an important finding given the interdependence of these factors already outlined
- Other studies of shown that in patients on maintenance HD with no prior history of cardiac events, an elevated BNP is significantly associated with an increased risk of cardiovascular mortality. These studies however all had a relatively low number of events (13-16 events) and it of interest that despite fairly similar cohort size and follow up time, their results appear to conflict with the current study(295;296)

Therefore based on these and the current study it is clear that the role of serum BNP as a prognostic marker for patients on renal replacement therapy, needs to be defined in larger studies, specifically controlling for the presence of coronary artery disease, itself a major determinant of both LVSD and poor cardiovascular outcomes.

7.4.7 Other potential roles for brain natriuretic levels in end stage renal disease

BNP has been touted as a potential marker to assess hydration status in the dialysis patient. As natriuretic peptides are secreted in response to myocardial stretch, and are therefore increased in patients with hypervolaemia, and BNP measurement may guide assessment of 'dry weight' in the haemodialysis patient. From a physiological standpoint, atrial natriuretic peptide has been shown to increase in response to volume overload, and theoretically would appear to the most promising for assessment of volume status. A number of studies have tried to compare the use serum BNP to assess hydration status with measurement of extracellular water content by bioimpedance or inferior vena caval diameter(297;298). Results of these studies have also been disappointing, with a lack of a correlation between BNP levels and pre- and post-dialysis hydration status, although pre-dialysis BNP appeared to a significant predictor of pre dialysis over hydration indicated by extra-cellular fluid/total body water ratio assessed by bioimpedance(298). Again difficulty exists in interpreting these studies if the confounding factor of increased LVM is taken into account.

As BNP has been shown to predict survival in some studies in ESRD a role may develop for the use of BNP as a goal of treatment in interventional studies in ESRD. This approach has been used in patients with heart failure. One short study, using BNP levels to guide titration of ACE inhibitor dosage, showed a more favorable response in heart rate and other neurohumoral factors in patients whose therapy was titrated

specifically to reduce BNP levels(299). However, this possibility has yet to be tested in this population in large trials in either heart failure or the ESRD population. One small study has shown a significant reduction in BNP levels in 14 haemodialysis patients with LV dilatation in response to metoprolol therapy, with a corresponding improvement in ventricular dimensions(300).

7.4.8 Effect of dialysis on serum BNP

In considering the effect of haemodialysis therapy on serum BNP levels, there are two factors to take into account. Firstly, BNP has a half life in non-uraemic patients of approximately 22 minutes, and is cleared by endopeptidases. It is likely that ultrafiltration (during dialysis treatment) will lead to reduced ventricular stretch and reduced serum BNP levels, due to reduced production. Secondly, any clearance by the dialysis process itself must be taken into account. However BNP is a relatively large molecule and there is conflicting evidence for this effect. In one study assessing the relationship between BNP and hydration status there was no significant difference observed in pre- and post-dialysis serum BNP(298). Other studies have shown BNP to be reduced by haemodialysis and it appears that dialytic clearance is of importance as in both these studies greater clearance of BNP is achieved with a high flux dialyzer. Moreover, whilst BNP is reduced by dialysis with both high and low flux dialysis membranes NT-BNP appears to only be significantly reduced by a high flux membrane. Crucially, in one of these studies dialysate BNP was measured and both BNP and NT-BNP were found to be partially cleared by dialysis(301). These results are intriguing and it is clear that larger studies of this nature are required to assess thoroughly the impact of the dialysis process on serum BNP levels.

7.4.9 Conclusions

Serum BNP has emerged as a rapid, convenient diagnostic tool for identifying and managing patients with heart failure. In the ESRD population BNP appears to have a role in assessing LVH and complementing echocardiography or CMR in evaluating patients with symptoms or clinical signs of LV dysfunction. A raised BNP carries at least important diagnostic value and may provide further prognostic information (although not in this study). It may yet have a potential role as a target for intervention in the attempt to reduce cardiovascular disease in the ESRD population. It is likely that the role of BNP-targeted therapy requires better definition in the general population patients with heart failure (in which there is evolving evidence) prior to translating this to ESRD. A cut-off point in ESRD needs to be defined for which further assessment of ventricular function is required in patients with ESRD. Such cut-points have been demonstrated in this study and these now require prospective validation. This is particularly true in asymptomatic patients who may benefit from additional therapeutic interventions to prevent the evolution of either progressive LVH or LVSD which are associated with reduced survival.

Chapter 8

A study of vascular function with cardiovascular magnetic resonance imaging in end stage renal disease

8.1 INTRODUCTION

Arterial function is deranged in patients with ESRD. This is due to a combination of both accelerated arteriosclerosis and atherosclerosis. Epidemiological studies have shown that increased arterial stiffness, most commonly assessed by measurement of pulse wave velocity (PWV) or augmentation index, is independently associated with cardiovascular morbidity and mortality in these patients(302;303). However, although these studies are generally felt to be robust, using applanation tonometry as an indirect measure of central arterial function may be prone to limitations. Cardiac MRI permits visualisation of large arteries and blood flow. It thereby facilitates direct measurement of aortic compliance and aortic pulse wave velocity. Although blood flow and pulse wave velocity have been extensively studied with MRI in healthy volunteers, few studies have been performed using MRI to assess vascular function in any pathological disease state.

CMR potentially offers an integrated method of assessing vascular as well as ventricular function during the same examination. Given the strong relationship between vascular stiffness and outcome in ESRD, CMR measures of vascular function may identify patients at increased cardiovascular risk. As patients with ESRD are at extremely high cardiovascular risk with an established link between altered arterial compliance and outcome, they represent an ideal patient population to validate this technique for translation for use in other patient groups at risk of cardiovascular disease.

The following pilot studies were performed in order to:

- develop methods for measuring vascular function using CMR
- validate the relationship between CMR measures of vascular function, namely aortic distensibilty, aortic volumetric arterial strain(VAS) and PWV

- assess the relationship between CMR measures of vascular function and conventional cardiovascular risk factors
- establish a cohort of patients to determine the long term prognostic implications of CMR measures of vascular function

8.2 METHODS

8.2.1 CMR measures of aortic distensibilty and pulse wave velocity

All patients and volunteers underwent CMR imaging of both the left ventricle and thoracic aorta as described in detail in section 2.3.2. Gd-DTPA-BMA was administered to all patients bar exceptions as described as exceptions in section 3.4. Contrast was not administered to controls. Scan time to perform only the measures of vascular function was 12 minutes.

8.2.2 Measurement of aortic distensibilty

This was performed as described in detail in section 2.4.3. Briefly aortic distensibilty was calculated from change in aortic volume and simultaneous brachial blood pressure acquired during the scan using the formula:

$$\text{Aortic distensibilty} = \frac{[(\text{Aortic volume})_{max} - (\text{Aortic volume})_{min}]}{[(\text{Aortic volume})_{min} * \text{pulse pressure}]}$$

where $(\text{Aortic volume})_{max}$ and $(\text{Aortic volume})_{min}$ are the maximal and minimal calculated aortic volumes obtained during the cardiac cycle.

Aortic volumetric arterial strain (VAS), which is non-pressure dependent, was calculated from the formula:

$$VAS = \frac{[(\text{Aortic Volume}_{max} - \text{Aortic Volume}_{min})]}{(\text{Aortic Volume}_{min})}$$

8.2.3 CMR measurement of pulse wave velocity

PWV was measured as described in detail in section 2.4.3. Briefly, velocity was calculated from blood flow along the aorta at three points- the ascending aorta at the level of the bifurcation of the pulmonary trunk, the transversely contiguous position of the descending aorta and the junction of the thoracic and abdominal aorta at the level of the diaphragm.

PWV was calculated from the distance between these points and the time delay between propagation of the flow wave from each point using the equation:

Pulse wave velocity = Distance/Time

Pulse wave velocity was calculated over two distances- ascending aorta-diaphragmatic level aorta (PWV 1-3), and ascending aorta-contiguous descending thoracic aorta (PWV 1-2). This was to account for cases where PWV from ascending aorta-diaphragmatic level aorta measurement was not possible due to the tortuous vessel preventing accurate aortic length and flow patterns being recorded.

8.2.4 Applanation tonometry measurement of pulse wave velocity

Healthy volunteers underwent measurement of PWV was using the SphygmoCor system (AtCor Medical, West Ryde, Australia) on the same day as CMR scanning by Dr Lukas Zimmerli (University of Glasgow). After 15 minutes of rest in the supine

position, pulse wave velocity measurements were performed by applanation tonometry. Aortic PWV was assessed by the time difference between pulse wave upstrokes, which were consecutively measured at the right carotid artery and right femoral artery and aligned by ECG-based trigger.

8.2.5 Follow up

Follow up data was collected from date of the CMR scan to 9th November 2006 using electronic patient records from the Western Infirmary, Glasgow and Glasgow Royal Infirmary Renal Units. With this cohort, death and cardiovascular events (myocardial infarction, cerebrovascular event, coronary revascularisation and amputation for peripheral vascular disease) were collected as end points.

8.2.5 Statistical methods

Correlations between cardiac dimensions, measures of arterial function and continuous clinical variables by the various methods were assessed with Pearson and Spearman correlation co-efficient as appropriate. Differences between groups were tested by t-test and Mann-Whitney-U test. Measures of vascular function were compared between patients who died or had a vascular event during the follow up period with these measures divided into tertiles or quartiles and subjected to an unadjusted survival analysis by the Kaplan–Meier method. Statistical significance was determined by the log-rank test. The data were then analysed by Cox survival analysis to assess the influence of multiple variables on outcome. Variables identified as possibly influential on outcome by univariate analysis were then entered into a forward stepwise regression model. All analyses were performed using the SPSS 13.0 statistical software package (SPSS Inc., Chicago, IL., USA) except comparison between SphygmoCor and CMR

measurement of PWV which was performed using Bland-Altman plots on the MedCalc 8.1 software package (MedCalc Software, Belgium).

8.3 RESULTS

8.3.1 Patient and control demographics

Patient demographics at the time of scan are shown in Table 5.1. 147 patients with advanced renal failure and 20 controls were studied. The groups of controls and uraemic patients had a similar age and sex distribution. A total of 144 patients had aortic VAS measurement and 122 patients had measurement of aortic distensibilty available for analysis. 22 patients did not have blood pressure readings to calculate distensibilty (either due to bilateral arteriovenous fistulae for dialysis making recording impossible, sphygmomanometer failure or due to blood pressure being recorded non-synchronously with the aortic distensibilty trace). The automated sphygmomanometer was broken for a portion of the study reducing the number of successful blood pressure recordings. Combined aortic compliance and PWV were available in 19 control subjects and 116 patients. 28 PWV traces performed with CMR were not of sufficient technical quality for analysis either due to tortuous vessels, patient movement or failure of computer analysis of the trace.

8.3.2 Measures of vascular function

Demographic and vascular function data are shown in Table 8.1. No significant differences in patient demographics or vascular function were exhibited between patients with advanced renal failure not yet on dialysis therapy (24 patients, 16.3% of the uraemic group) and those established on renal replacement therapy (123 patients, 83.7% of the uraemic group) and therefore these patients were combined as a single

'uraemic' group. Systolic and diastolic blood pressures were higher in uraemic patients than controls as expected.

Cross sectional aortic volume (i.e. cross sectional aortic area multiplied by the slice thickness) was significant greater in uraemic patients. Aortic distensibilty and VAS were significantly lower in uraemic patients than controls (median distensibilty 2.4 x 10^{-3} vs. 4.9 x 10^{-3} $mmHg^{-1}$, p<0.001; VAS 0.13 vs. 0.19, p=0.001), whilst PWV 1-3 was non-significantly higher compared to controls (median 7.5 vs. 6.7 m s^{-1}, p=0.069). PWV 1-2 was numerically higher in uraemic patients compared to controls (median 7.1 vs. 6.4 m s^{-1}, p=0.620).

8.3.3 Comparison between CMR and applanation tonometry for measurement of pulse wave velocity

Combined measurement of PWV by applanation tonometry and CMR was performed in 14 healthy volunteers. Mean difference between the two measures was 0.2 m s^{-1}. The comparison between the two methods of measurement of PWV is shown as a Bland Altman plot (Figure 8.1).

8.3.4 Haemodynamic relationship between pulse wave velocity, aortic distensibilty and volumetric arterial strain

As both PWV and aortic distensibilty exhibited skewed distributions log transformation was performed. Pulse wave velocity displayed a significant negative correlation between aortic distensibilty (PWV 1-3 R= -0.48, p<0.001; PWV 1-2 R= -0.37, p<0.001) and VAS (PWV 1-3 R= -0.47, p<0.001; PWV 1-2 0.38, p<0.001) in uraemic patients but not controls (aortic distensibilty-PWV 1-3, R= -0.35, p=0.139; VAS-PWV 1-3, R= -0.38, p= 0.111). Overall Spearman correlation co-efficient for the whole group

(combined uraemic and control) for PWV1-3 was R= -0.49, p<0.001 for aortic distensibilty and R= -0.48, p<0.001 for aortic VAS (Figure 8.2).

Assessing the relationship between central haemodynamics and aortic vessel characteristics, there was a significant correlation between cross sectional aortic volume and diastolic blood pressure in both uraemic patients (R = 0.242, p=0.007) and controls (R = 0.54, p=0.015), but not between aortic cross sectional area and either systolic blood pressure or pulse pressure. PWV correlated directly with both systolic (PWV 1-3 R= 0.32, p=0.004, PWV 1-2 R= 0.28, p=0.006) and diastolic blood pressure (PWV 1-3 R= 0.26, p=0.020, PWV 1-2 R= 0.33, p=0.001).

	Uraemic		Control		p value
Number	147		20		
Age	51.5	(11.2)	49.6	(9.4)	0.469
Male (%)	93	(63.3)	12	(60)	0.777
Height (m)	169.6	(9.7)	170.7	(8.8)	0.640
Weight (kg)	76.0	(16.0)	74.9	(10.3)	0.770
On dialysis (%)	123	(83.7)	-		-
RRT time (months)	8.0	(55.6)	-		-
Past history of IHD (%)	24	(16.3)	-		-
Diabetes (%)	47	(32.0)	-		-
Smoker (%)					
Never	85	(57.8)	13	(65.0)	
Current	39	(26.5)	2	(10.0)	0.220
Ex	23	(15.6)	5	(25.0)	
SBP (mmHg)	139.7	(24.6)	116.4	(9.9)	<0.001
DBP (mmHg)	82.6	(13.0)	74.8	(7.4)	0.010
Cross sectional aortic volume (mL)	5.0	(2.0)	3.5	(0.9)	<0.001
Aortic distensibilty (x 10^{-3} $mmHg^{-1}$)	2.4	(2.0)	4.9	(2.9)	<0.001
Aortic volumetric arterial strain	0.13	(0.09)	0.19	(0.11)	0.001
PWV 1-2 (m s^{-1})	7.1	(4.5)	6.4	(2.5)	0.620
PWV 1-3 (m s^{-1})	7.5	(3.1)	6.7	(2.9)	0.069

Table 8.1

Background demographics for uraemic patients and controls. Results are show as mean with standard deviation in parenthesis or number with percentage in parenthesis as appropriate, except for RRT time and measures of vascular function which are displayed as median and inter quartile range. Tests of significance are t-test and Chi-squared between groups, except for measures of vascular function where Mann-Whitney-U was used

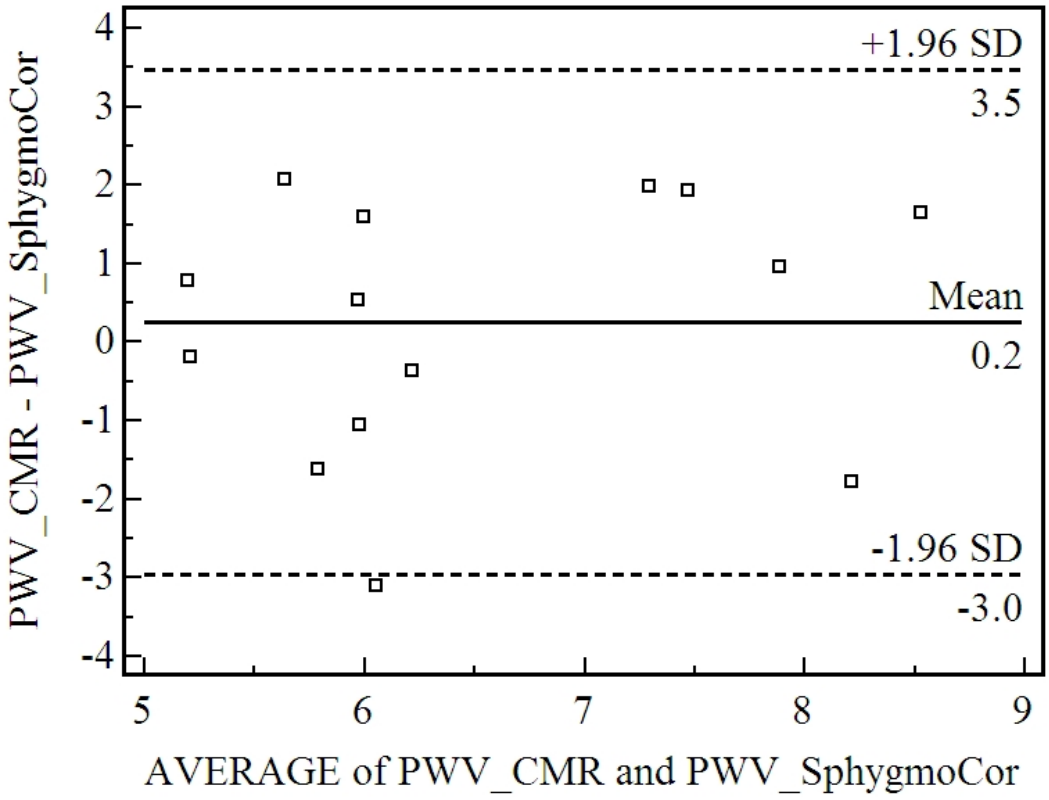


Figure 8.1

Bland Altman plot comparing CMR and applanation tonometry (SphygmoCor) methods for measuring pulse wave velocity in healthy volunteers

8.3.5 Clinical correlates of aortic distensibilty and volumetric arterial strain

Aortic distensibilty demonstrated a significant negative correlation with age in both uraemic patients (aortic distensibilty R= -0.44, p<0.001, VAS R= -0.44, p<0.001; Figure 8.3) and controls (aortic distensibilty R= -0.55, p=0.013, VAS R= -0.45, p=0.047).

In uraemic patients there were no significant correlations between haemoglobin, dialysis adequacy (either urea reduction rate in haemodialysis or creatinine clearance in peritoneal dialysis), time on renal replacement therapy, any lipid parameter, C - reactive protein, calcium, phosphate or calcium phosphate product and aortic distensibilty or VAS. There were no significant differences in aortic distensibilty or compliance between genders. Aortic distensibilty and VAS were significantly reduced in patients with diabetes mellitus (diabetics – median aortic distensibilty 1.8 x 10^{-3} vs. non-diabetics 2.8 x 10^{-3} $mmHg^{-1}$, p=0.001; VAS 0.11 vs. 0.15, p=0.001), patients with a past history of ischaemic heart disease (median aortic distensibilty 1.8 x 10^{-3} vs. 2.6 x 10^{-3} $mmHg^{-1}$, p=0.028; VAS 0.11 vs. 0.15, p=0.009; Figure 8.4) or peripheral vascular disease (median aortic distensibilty 1.2 x 10^{-3} vs. 1.8 x 10^{-3} $mmHg^{-1}$, p=0.005; VAS 0.14 vs. 0.07, p=0.015).

No significant differences in aortic distensibilty or VAS between patients treated with statin therapy, or those requiring antihypertensive therapy. There were no significant differences between aortic distensibilty between dialysis modalities nor was there any correlation between time on renal replacement therapy and aortic distensibilty or VAS.

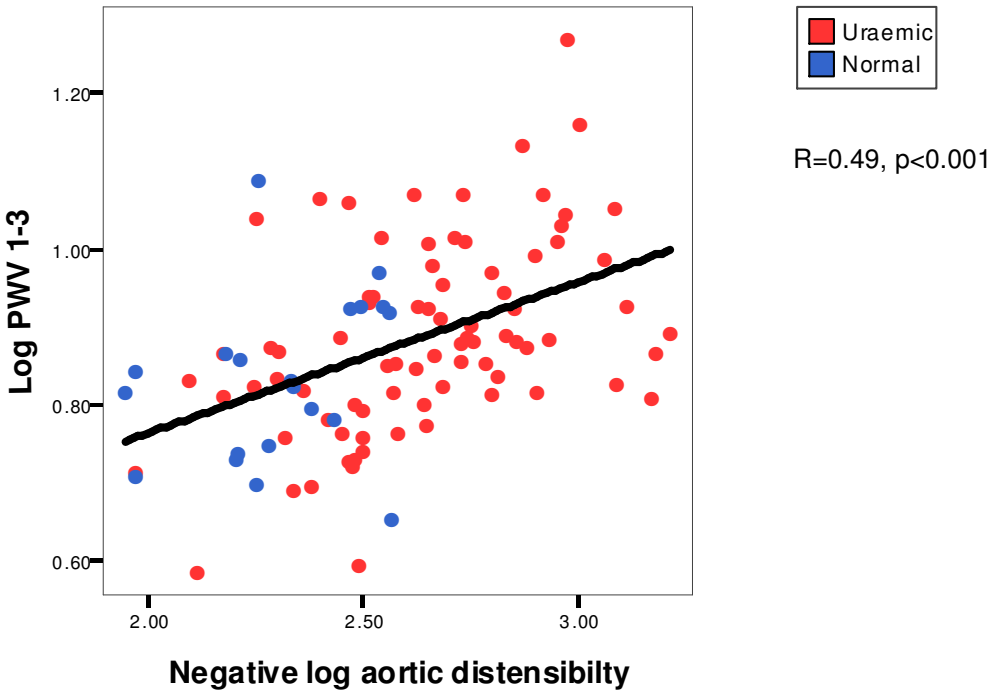


Figure 8.2 Scatter plot of log transformed pulse wave velocity plotted against negative log transformed aortic distensibilty

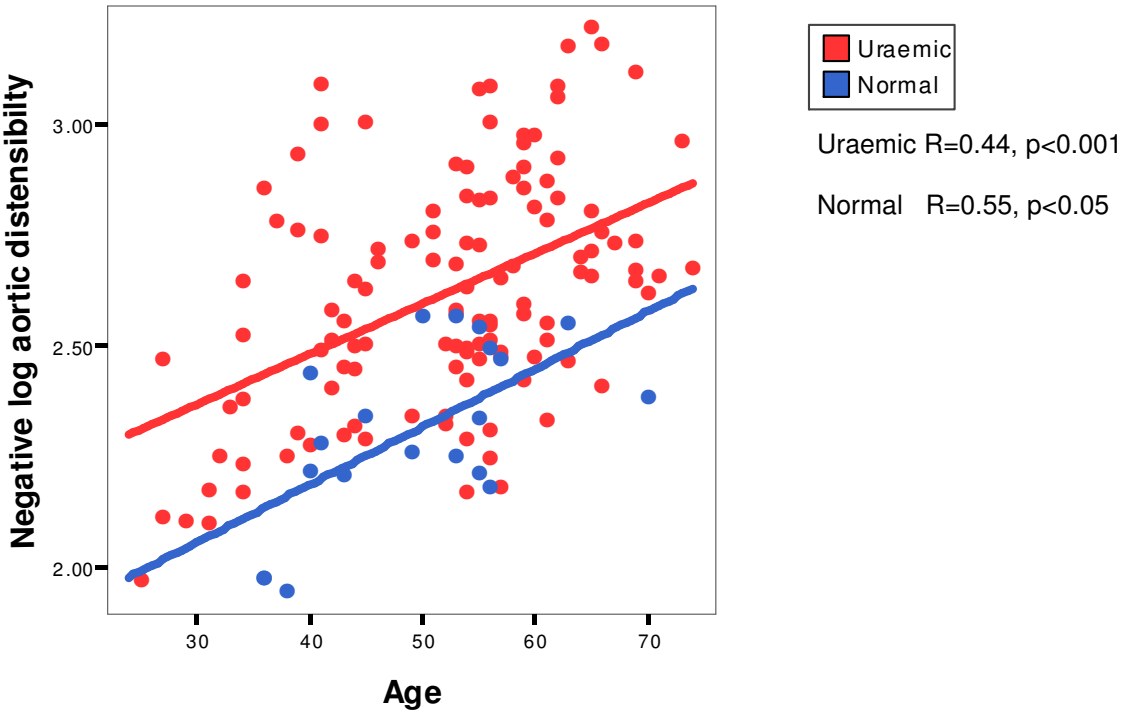
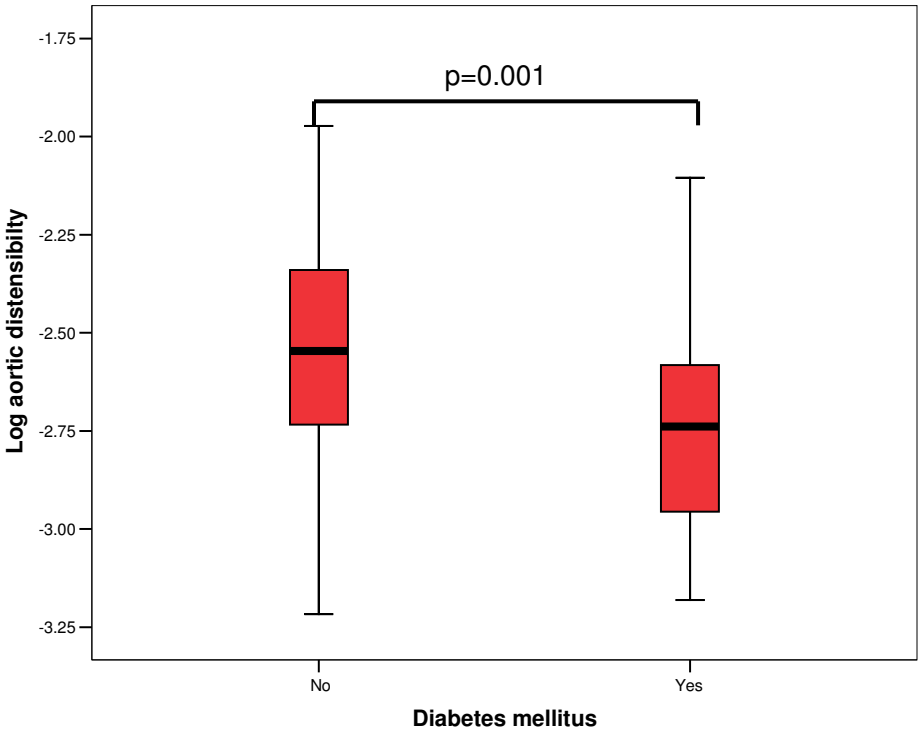


Figure 8.3 Scatter plot of negative log transformed aortic distensibilty plotted against age for normal controls and uraemic patients



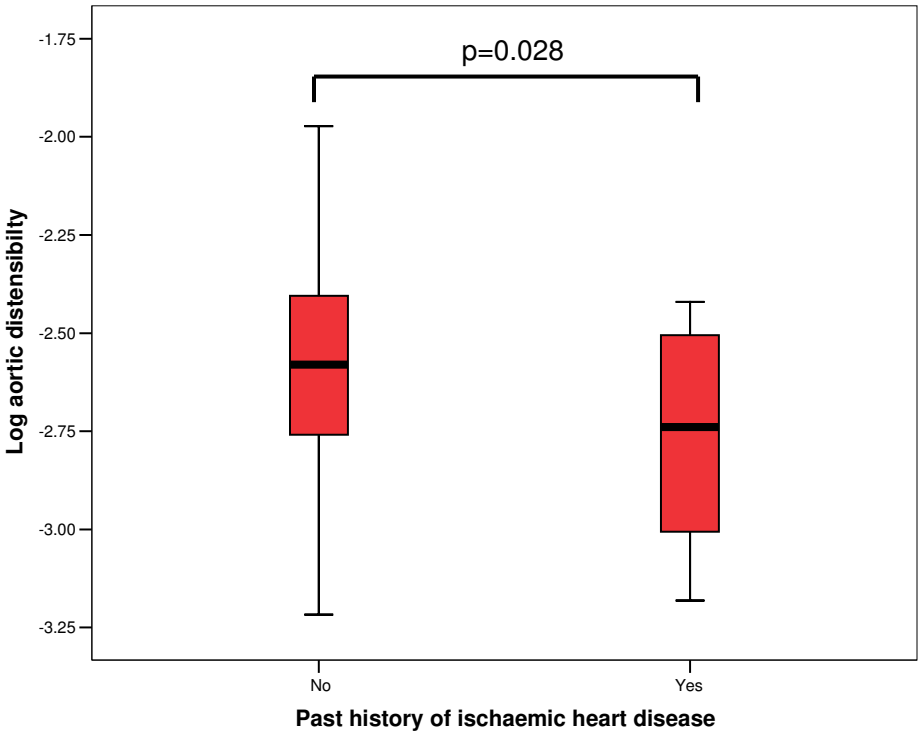


Figure 8.4

Box and whisper plots of log transformed aortic distensibilty in patients with and without a past medical history of diabetes mellitus and ischaemic heart disease

8.3.6 Relationships between aortic distensibilty and left ventricular dimensions and late gadolinium enhancement

Significant negative correlations were demonstrated between aortic distensibilty and LVMI (R= -0.21, p=0.021) and end systolic volume (R= -0.18, p=0.048) but no other LV dimension. Aortic VAS correlated with markers of cardiac function - ejection fraction (R= 0.23, p=0.006) and stroke volume (0.19, p=0.024). Of the 119 patients who had combined vascular function measures with Gd-DTPA-BMA contrast enhanced studies, 37 (31.1%) patients had evidence of myocardial fibrosis indicated by LGE as described in Chapter 3 of which 20 (14.6%) patients had subendocardial LGE representing previous myocardial infarction and 17 (12.4%) had diffuse LGE. Patients who were positive for LGE had lower aortic distensibilty and VAS than LGE negative patients (median aortic distensibilty 2.0 x 10^{-3} for LGE positive vs. 2.8 x 10^{-3} $mmHg^{-1}$ for LGE negative patients, p=0.027; VAS 0.11 vs. 0.14, p=0.056). Patients with subendocardial LGE representing previous myocardial infarction had significantly lower aortic distensibilty and VAS than patients who were LGE negative (median aortic distensibilty 1.6 vs. 2.2 x 10^{-3} $mmHg^{-1}$ p=0.008; VAS 0.09 vs. 0.14, p=0.006). There were no significant differences in aortic distensibilty or VAS between patients with diffuse LGE and patients with subendocardial LGE. Additionally there were no significant differences between patients with diffuse LGE and LGE negative patients (Figure 8.5).

In patients with subendocardial LGE, there was a significant negative correlation between aortic distensibilty and the absolute mass of fibrotic myocardial tissue represented by subendocardial LGE (R= -0.57, p=0.032). There was additionally a negative correlation between percentage of the LV mass occupied by fibrotic myocardium and aortic distensibilty (R= -0.54, p=0.047; Figure 8.6). A significant

correlation between aortic distensibilty or VAS and mass of myocardial fibrosis was not present in patients with diffuse LGE.

8.3.7 Clinical correlates of pulse wave velocity

In uraemic patients, but not controls, increased PWV correlated significantly with age (PWV 1-3 R= 0.41, p<0.001, PWV 1-2 R= 0.34, p<0.001; Figure 8.7). In uraemic patients there were no significant correlations between haemoglobin, dialysis adequacy (either urea reduction rate in haemodialysis or creatinine clearance in peritoneal dialysis), time on renal replacement therapy any lipid parameter, C - reactive protein, phosphate or calcium phosphate product and PWV. There was however a significant negative correlation between serum corrected calcium and PWV (PWV 1-3 R= -0.22, p=0.034) and with serum albumin (PWV 1-2 R= -0.25, p=0.007) There were no significant differences in PWV between genders.

Although PWV was numerically higher in patients with diabetes, a past history of ischaemic heart disease of peripheral vascular disease compared to those patients without this conditions none of these differences were statistically significant. There were no significant relationships demonstrated between PWV and cardiac mass and function or between the presence or extent of myocardial fibrosis demonstrated by LGE.

There were no differences in systolic or diastolic blood pressure at the time of scanning between patients not requiring antihypertensive therapy (n=68) and those treated with either calcium channel antagonist (n=29) or angiotensin converting enzyme inhibitor/angiotensin receptor blocker monotherapy (n=27) or a combination of these agents (n=19). However PWV was non-significantly higher in patients treated with calcium channel antagonist monotherapy compared to those treated with angiotensin

converting enzyme inhibitor/angiotensin receptor blocker monotherapy (calcium channel antagonist group - median PWV 1-2 7.1 m s^{-1} inter quartile range (IQR) 7.3 vs. angiotensin converting enzyme inhibitor/angiotensin receptor blocker group - median PWV 1-2 7.2 m s^{-1} IQR 2.9, p=0.067; Figure 8.8).

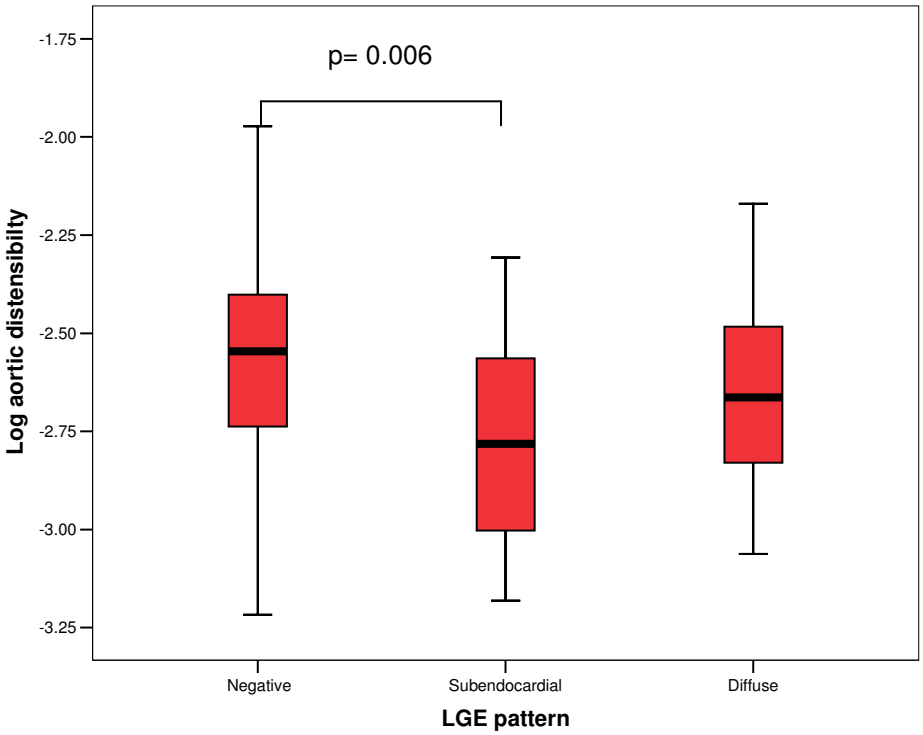


Figure 8.5

Box and whisper plots of log transformed aortic distensibilty in patients divided by pattern of late gadolinium enhancement

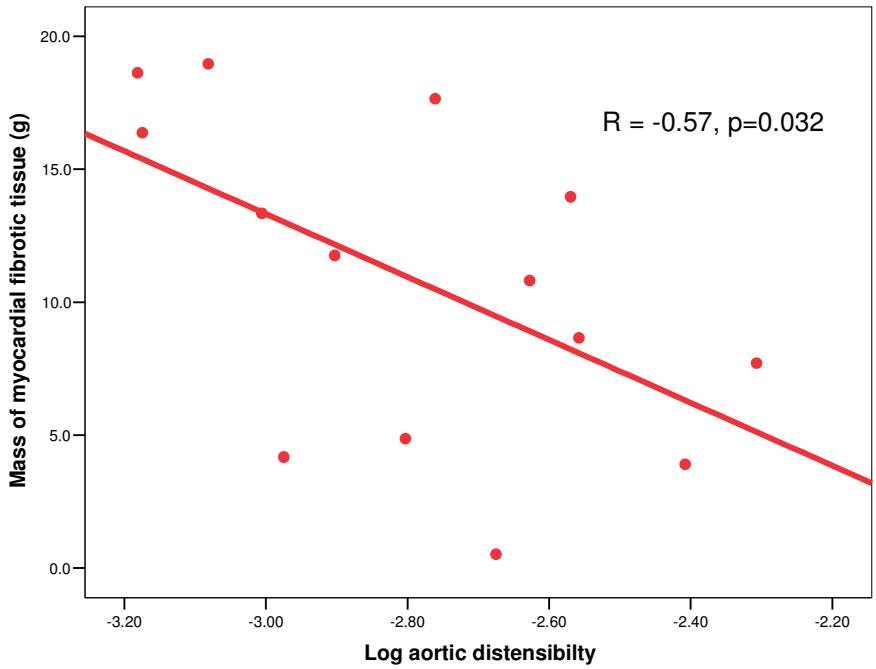


Figure 8.6 Mass of subendocardial myocardial fibrosis indicated by LGE plotted against log transformed aortic distensibilty

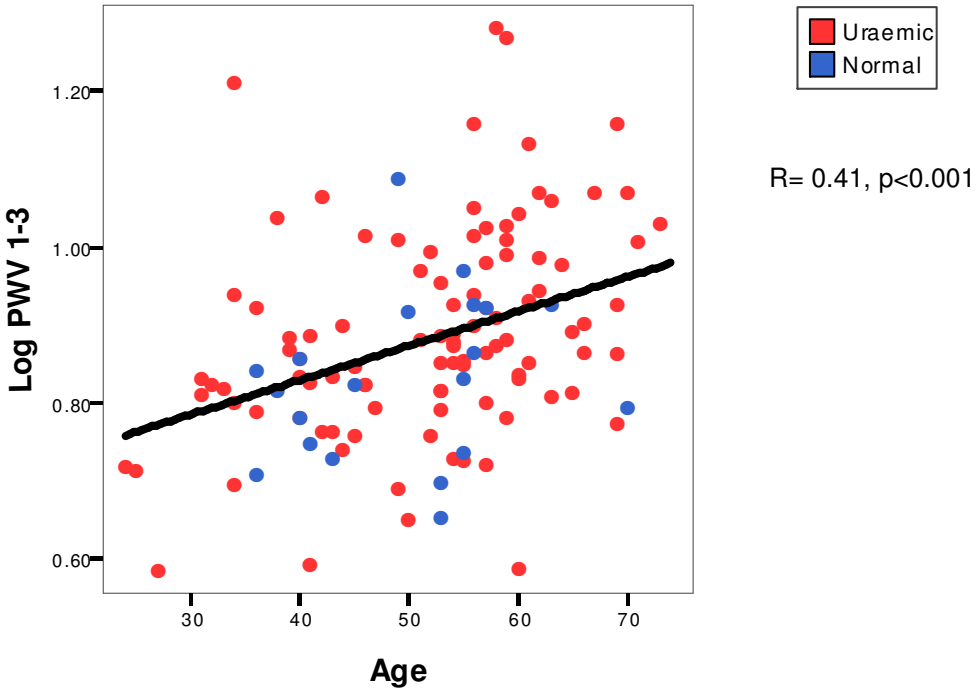


Figure 8.7 Scatter plot of log transformed pulse wave velocity plotted against age for normal controls and uraemic patients

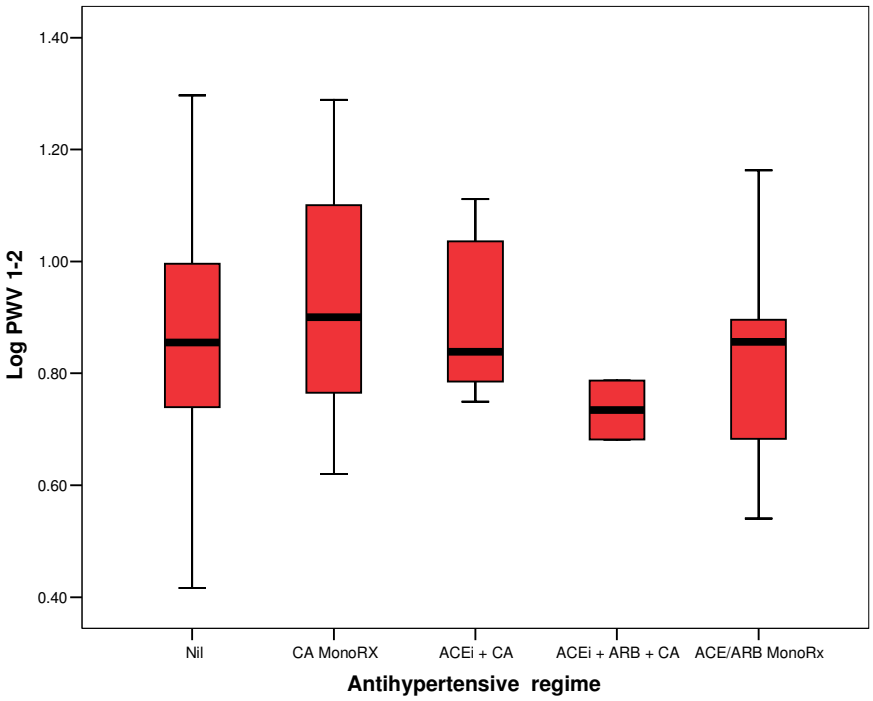


Figure 8.8 Box and whisper plots of log transformed pulse wave velocity in patients divided by antihypertensive therapy. Abbreviations CA – calcium channel antagonist, MonoRx – monotherapy, ACEi - angiotensin converting enzyme inhibitor, ARB - angiotensin receptor blocker

8.3.8 Relationship between CMR measures of vascular function on survival and combined mortality and cardiovascular events

Follow up data combined with vascular function measurements were available for 144 patients. The median follow up period was 719 days (IQR 375 days). Over the follow up period there were 20 deaths, giving an overall mean death rate in this cohort of 76.2 per 1000 patient years. Patients who died had significantly lower aortic distensibilty than those who where alive at the end of the follow up period. There were no significant differences in VAS or PWV between survivors and patients who died. Analysing the effect of blood pressure at the time of the scan on survival demonstrated that patients who died had significantly higher systolic and pulse pressure than survivors, but there were no significant differences in diastolic blood pressure (Table 8.2). The influences of the measures of vascular function on survival are shown as Kaplan-Meier survival curves in Figures 8.9 and 8.10.

During the follow up period there were an additional 12 non-fatal cardiovascular events (five patients had a myocardial infarction; four patients underwent coronary revascularisation, two patients undergoing amputation for peripheral vascular disease and one patient had a cerebrovascular event). These patients had significantly lower aortic distensibilty and VAS than survivors who remained event free. Pulse pressure in these patients was higher. There were no significant difference in blood pressure between than survivors who remained event free and patients who had a non-fatal cardiovascular event.

Combining death with non fatal cardiovascular events as a combined cardiovascular end point, overall patients who reached this combined end point had significantly lower aortic distensibilty (median 1.7×10^{-3} vs. 2.7×10^{-3} $mmHg^{-1}$, $p<0.001$) and VAS (0.12

vs. 0.14, p=0.006) and higher systolic blood pressure (mean 152.9 vs. 136.5 mmHg, p=0.001) and pulse pressure (mean 67.0 vs. 54.5 mmHg, p=0.001) than those who did not. The influences of the measures of vascular function on cardiovascular vents are shown as Kaplan-Meier survival curves in Figures 8.11 and 8.12. There were no significant differences in either diastolic blood pressure (mean 85.9 vs. 82.0 mmHg, p= 0.154) or pulse wave velocity (median PWV 1-2 6.5 vs. 7.1 m s^{-1} p=0.387; median PWV 1-3 7.4 vs. 7.5 m s^{-1}, p= 0.711) between those who reached the combined end point and those who did.

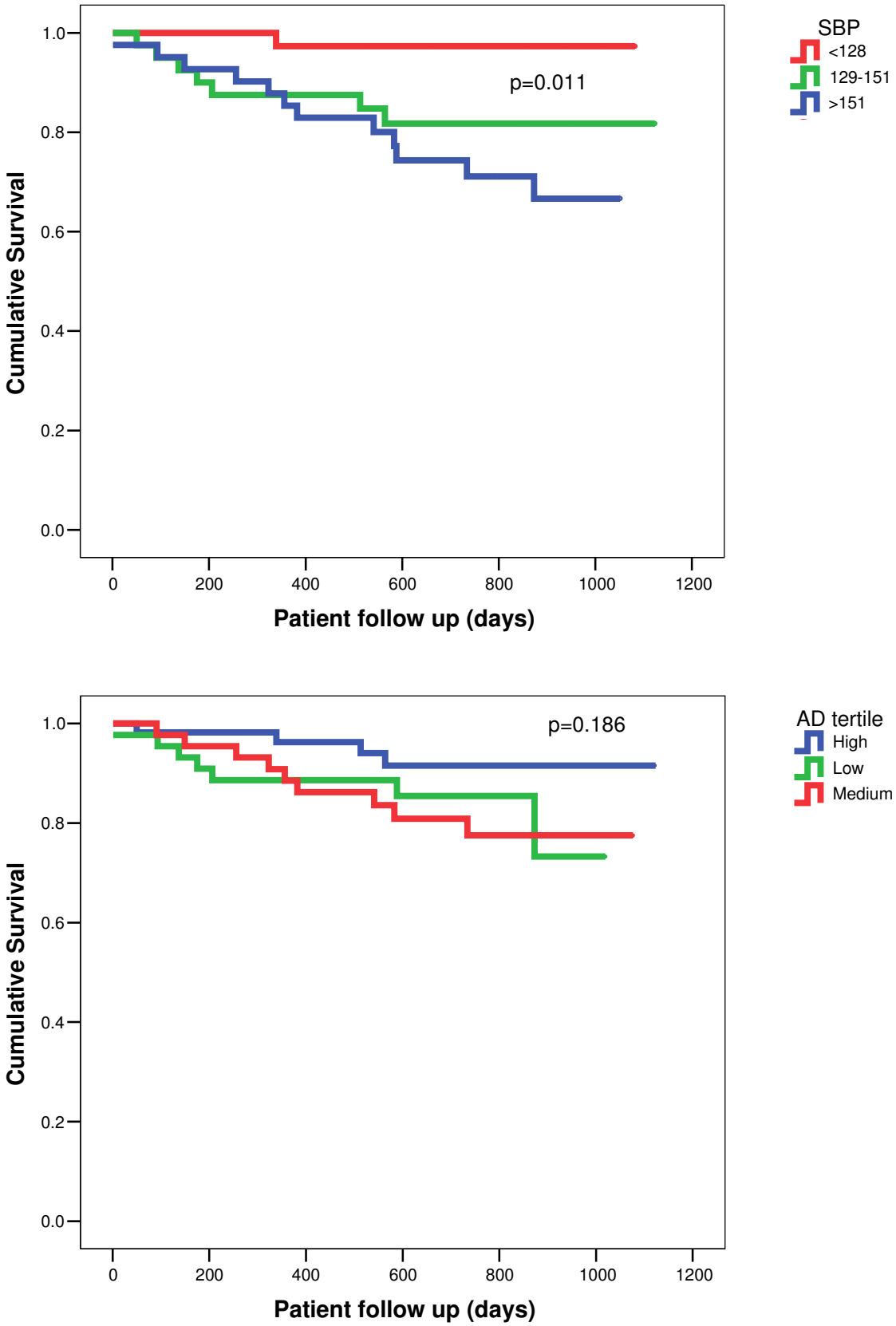
8.3.9 Multivariate analysis of relationship between CMR measures of vascular function and survival

In a Cox forward stepwise regression model assessing patient survival; diabetes, systolic blood pressure and aortic distensibilty were identified as significant independent predictors of patient survival. In a similar analysis assessing the combined cardiovascular end point of death or a vascular event; diabetes, aortic distensibilty and aortic VAS were significant predictors of events. Due to their close interdependence, blood pressure variables and aortic distensibilty were entered individually into the each model to determine their influence. Similarly either aortic distensibilty or VAS were entered individually to the model but not together.

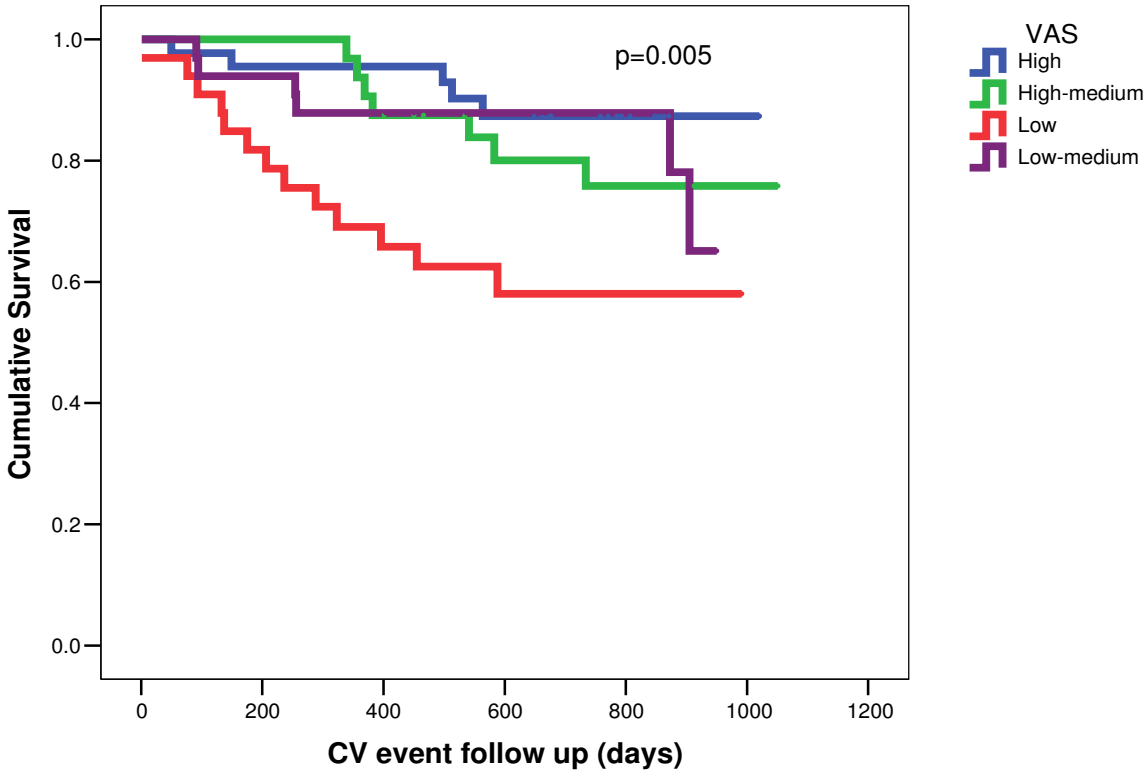
	Alive		Dead		p value
Number	124	(86.1)	20	(13.9)	
Age	51.5	(11.3)	51.9	(11.1)	0.876
Male (%)	80	(64.5)	10	(50.0)	0.213
On dialysis (%)	102	(82.3)	18	(18.0)	0.363
RRT time (months)	3.9	(45.6)	18.4	(83.4)	0.018
Past history of IHD (%)	19	(15.3)	5	(25.0)	0.281
Diabetes (%)	34	(27.4)	12	(60.0)	0.004
Smoker (%)					
Never	73	(58.9)	10	(50.0)	
Current	30	(24.2)	8	(40.0)	0.304
Ex	21	(16.9)	2	(10.0)	
SBP (mmHg)	137.3	(23.6)	156.3	(21.4)	0.001
DBP (mmHg)	82.1	(12.9)	87.4	(11.7)	0.092
PP (mmHg)	55.20	(17.1)	69.0	(17.1)	0.001
Cross sectional aortic volume (mL)	5.0	(1.9)	5.1	(2.3)	0.634
Aortic distensibilty (x 10^{-3} $mmHg^{-1}$)	2.4	(2.0)	2.1	(2.1)	0.009
Aortic volumetric arterial strain	0.12	(0.09)	0.15	(0.10)	0.176
PWV 1-2 (m s^{-1})	7.1	(4.1)	6.5	(5.8)	0.807
PWV 1-3 (m s^{-1})	7.6	(2.9)	7.3	(2.3)	0.330

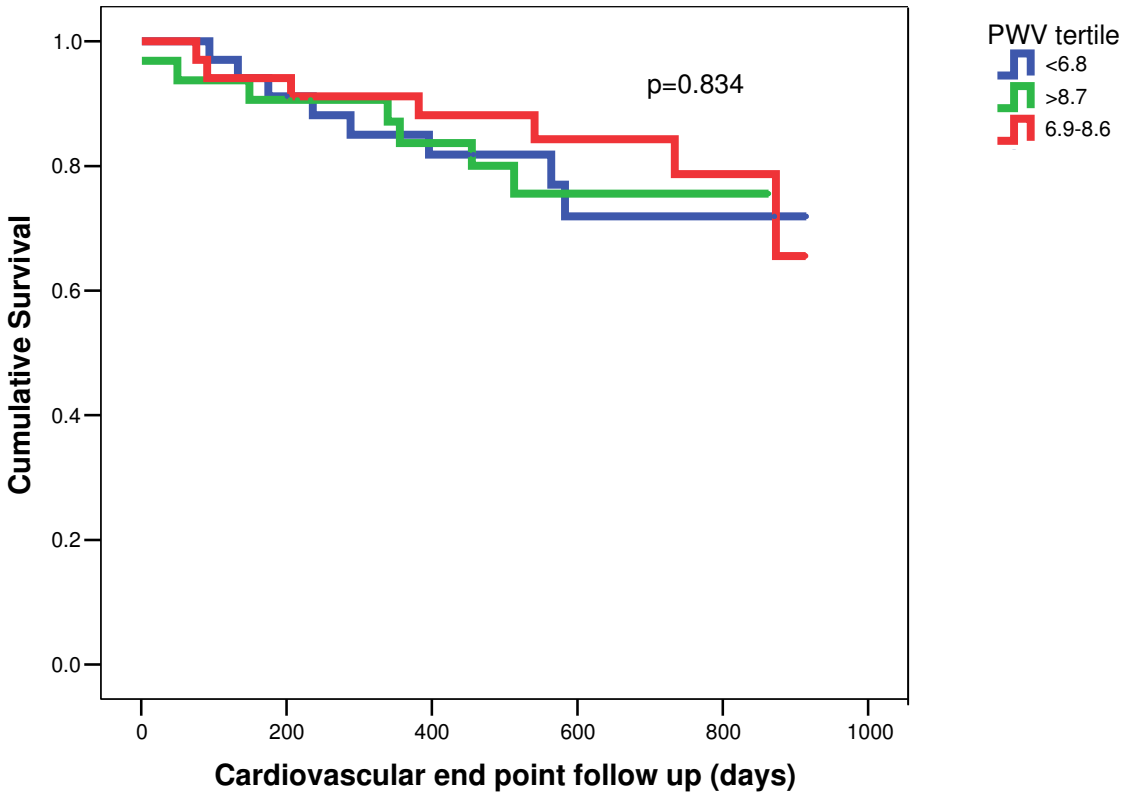
Table 8.2

Demographic data for patients were alive and dead at the end of the follow up period for uraemic patients and controls. Results are show as mean with standard deviation in parenthesis or number with percentage in parenthesis as appropriate except for RRT time and measures of vascular function which are displayed as median and inter quartile range. Tests of significance are t-test and Chi-squared between groups, except for measures of vascular function where Mann-Whitney-U was used



Figures 8.9 and 8.10 Kaplan-Meier survival curves for all cause mortality with patients stratified by systolic blood pressure tertile and aortic distensibilty tertile





Figures 8.11 and 8.12

Kaplan-Meier survival curves for survival to either CV end point or death with patients stratified by aortic VAS quartile and pulse wave velocity tertile

	Patient survival							
	Univariate analysis				Multivariate analysis			
Variable	**HR**	**(95.0% CI)**		**p**	**HR**	**(95.0% CI)**		**p**
Diabetes	3.031	(1.036,	8.866)	0.043	4.214	(1.631,	10.886)	0.003
SBP (mmHg)	1.015	(0.992,	1.038)	0.197	1.022	(1.000,	1.044)	0.049
Log aortic distensibilty	0.072	(0.008,	0.694)	0.023	0.135	(0.019,	0.948)	0.044
Log aortic VAS	0.107	(0.007,	1.732)	0.116				
Duration RRT (days)	1.000	(1.000,	1.000)	0.421				
Age (years)	1.015	(0.969,	1.063)	0.526				
Gender (ref male)	0.833	(0.312,	2.226)	0.716				
Haemoglobin (g/dL)	0.910	(0.670,	1.236)	0.547				
Albumin (g/L)	0.942	(0.839,	1.058)	0.311				

Table 8.3

Cox survival analysis for patient survival. Because of their close correlation SBP aortic distensibilty and aortic VAS were entered separately into the model. Results for other variables are shown with systolic blood pressure in the model

	Combined CV event and death survival							
	Univariate analysis				Multivariate analysis			
Variable	**HR**	**(95.0% CI)**		**p**	**HR**	**(95.0% CI)**		**p**
Diabetes	2.989	(1.276	7.004)	0.012	2.409	(1.087	5.337)	0.030
Log aortic distensibilty	0.052	(0.009	0.319)	0.001	0.066	(0.013	0.347)	0.001
Log aortic VAS	0.021	(0.002	0.206)	0.001	0.026	(0.004	0.175)	<0.001
Duration RRT (days)	1.000	(1.000	1.000)	0.560				
SBP (mmHg)	1.014	(0.995	1.032)	0.142				
Age (years)	1.023	(0.984	1.062)	0.248				
Gender (ref male)	0.827	(0.368	1.860)	0.646				
Haemoglobin (g/dL)	0.958	(0.748	1.225)	0.731				
Albumin (g/L)	0.984	(0.896	1.080)	0.728				

Table 8.4

Cox survival analysis for patient survival to either death or cardiovascular event. Because of their close correlation SBP aortic distensibilty and aortic VAS were entered separately into the model. Results for other variables are shown with systolic blood pressure in the model

8.4 DISCUSSION

8.4.1 Basic physiology of aortic function

The aorta as a large artery has two discrete functions. Firstly, as a conduit, it delivers blood and hence oxygen to the tissues. This role is expressed by mean blood pressure and blood flow. Disruption to this role, primarily by atherosclerosis, potentially results in distal ischaemia. Additionally, large arteries have a buffering function, to dampen pressure oscillations from intermittent cardiac pumping (the 'Windkessel' effect), therefore transforming the pulsatile blood flow into a steady stream as required by peripheral organs and tissue. Alterations in shear stress or tensile stress within the arterial wall lead to arterial remodelling, with blood pressure being the principal determinant of these stresses. The characteristics of arterial remodelling are dependant on the nature of the haemodynamic stimuli applied to the vessel. Chronic increase in arterial blood flow will result in subsequent increase in the vessel lumen whilst increase in tensile stress will lead to thickening of the arterial walls, in keeping with the law of Laplace, which states that transmural stress directly proportional to the transmural pressure and radius and inversely proportional to the arterial wall thickness(304). Atherosclerosis may further affect arterial remodelling due to alterations in blood flow patterns and shear stresses in the vessel wall as well as by direct endothelial injury. However this is likely to be of lesser importance in the thoracic aorta, as atherosclerosis occurs preferentially in the medium sized conduit vessels.

8.4.2 Alterations of aortic function in ESRD

The prime action of atherosclerosis is to disrupt the conduit function of the artery. Although patients with ESRD have many risk factors to promote the development of atherosclerosis (diabetes, hypertension, age, dyslipidaemia and smoking) and

endothelial function is clearly deranged in uraemia(305), it still remains unclear whether the nature of the interactions between the vascular endothelium and atherogenic plaque are different to the general population. In any case, these studies focus on the thoracic aorta, and whilst aortic plaque may be found in this region, as basal blood flow is unchanged until the lumen diameter is reduced by 50%, altered conduit function unlikely to be of prime importance as a pathological mechanism in the aorta(304).

In ESRD the large arteries classically undergo remodelling characterised by dilatation and hypertrophy of the arterial intima-media. This remodelling is associated with stiffening and the dampening role of the vessel is affected(306). The consequence of this is primarily an increase in systolic and reduction in diastolic blood pressure resulting in higher pulse pressure. This is due to two mechanisms. Firstly a higher pressure wave is generated by the direct effect of the LV pumping blood into the stiff arterial tree. Additionally as vessel stiffness increases this is associated with a rise in the PWV over any given arterial segment, in keeping with the Moens-Korteweg equation. Thereafter this pressure wave (forward travelling) is reflected at any point of discontinuity in the arterial tree, generated an echo wave travelling backward toward the ascending aorta. The resultant sum of these incident and pressure waves is the measured pulse pressure wave and is dependant on their amplitude and timing. In young healthy individuals this reflected wave occurs during diastole and is the associated increase in early diastolic pressure augments coronary perfusion. However in conditions where the dampening function of the arterial tree is reduced such as aging or uraemia, this beneficial mechanism is disturbed. As PWV increases the reflected waves occur earlier, and are more likely to occur during systole than diastole, ‘closer’ in phase with the incident wave(307). This therefore leads to an increase in aortic and LV pressure during

systole. This increases cardiac work and the LV after load whilst additionally reducing coronary perfusion(308).

Haemodynamic factors which promote arterial remodelling present in ESRD patients, although not specific to uraemia, include age and blood pressure. Factors specific to uraemia include hypervolaemia due to water and sodium retention, arteriovenous shunts, chronic volume and flow overload secondary to anaemia(306). Arterial medial calcification is pronounced in patients in ESRD and is a key mediator of increased arterial stiffness(309). This occurs due to changes in the phenotype of vascular smooth muscle cells. Factors which may affect this in ESRD include altered calcium and phosphate metabolism, chronic inflammation and associated low levels of fetuin-A or osteprotegerin (inhibitors of arterial calcification), genetic factors or the presence unknown uraemic toxins up regulating expression of genes known to promote calcification in vascular smooth muscle cells(142;147;310;311).

8.4.3 Relationship between aortic distensibilty, volumetric arterial strain and pulse wave velocity

As different methods of measuring arterial stiffness aortic distensibilty, VAS and PWV were demonstrated to be significantly correlated with each other. Using CMR to measure aortic distensibilty or VAS visualises changes in the aortic cross sectional volume throughout the cardiac cycle. Therefore these measures are true representations of vascular function. This study therefore confirms that PWV (an indirect measure of arterial stiffness) and either aortic distensibilty or VAS (direct measures) are directly related. To an extent this represents validation of the technique for measurement of PWV.

8.4.4 Results of studies of aortic distensibilty

These data represent the first report of using CMR to assess vascular function in uraemic patients. The uraemic patients had significantly increased aortic stiffness compared to age matched normal subjects, represented by reduced aortic distensibilty and VAS with associated vessel dilatation. In keeping with the relationship between increased arterial stiffening and advancing age, there was a significant relationship between reduced aortic distensibilty and VAS and increasing age, both in uraemic patients and in healthy controls.

In the uraemic patients, aortic distensibilty and VAS were demonstrated to be reduced in patients at highest cardiovascular risk, i.e. those with diabetes and/or a past medical history of ischaemic heart disease or peripheral vascular disease. No other factors such as drug therapy, adequacy of dialysis, duration of renal replacement therapy, dialysis modality or any laboratory parameter appeared to be significantly associated with aortic distensibilty or VAS. Therefore overall increased aortic stiffness, as indicated by reduced aortic distensibilty or VAS, was demonstrated to be present in uraemic patients with the presence of classical cardiovascular risk factors.

The relationship between these markers of aortic function and LV dimensions show a weak but significant relationship between increased aortic stiffness and increasing LVMI and end systolic volume, suggesting that reduced aortic distensibilty may promote LV afterload and hence cardiac hypertrophy. Aortic VAS, which is independent of blood pressure, correlated with markers of cardiac systolic function, namely ejection function and stroke volume, suggesting that in the failing heart where systolic blood pressure is lower, increased arterial stiffness remains deleterious to ventricular performance.

Finally in the small group of patients with subendocardial LGE indicating previous myocardial infarction, the relationship between volume of infarcted tissue and aortic distensibilty offers a direct demonstration of the interaction between increased arterial stiffness and reduced diastolic coronary artery perfusion(308) leading to increased area of infarction. This may be one explanation long term outcome post myocardial infarction is poor in patients with ESRD.

8.4.5 Limitations of studies with aortic distensibilty and aortic volumetric arterial strain

Calculation of aortic distensibilty is based on cross-sectional volume of the vessel wall and pulse pressure. For practical reasons, direct aortic blood pressure measurement has been substituted by non-invasive indirect brachial blood pressure measurement. Therefore it is difficult to dissociate any clinical effect associated with changes in aortic distensibilty from that due directly to pulse pressure, which is well described as an independent predictor of outcome in ESRD. Nonetheless, as it is otherwise impossible to truly assess aortic stiffness, without documentation of pressure within the vessel lumen, the approach used in this study has been used widely in other patient groups(312-314). For this reason the aortic VAS, the fractional increase in aortic volume during the cardiac cycle was also used. This does not depend on blood pressure variables and in this study appeared to display fairly similar relationships with clinical variables and outcome. There is however limited literature to support its use. Additionally, whilst this is the largest study to date using aortic distensibilty in any patient group, the number of patients in any subgroup, such as diabetes or with previous ischaemic heart disease was small, therefore limiting conclusions that can be drawn from this study.

Although it is highly likely that arterial calcification is the intricately involved as a pathomechanism for reduced aortic compliance, CMR is unable to display calcific lesions due to their absence of water (and hence proton) content. This compares relatively unfavourably with multi-slice computed tomography (CT), which has emerged as a tool for assessing cardiac calcification(144). However, multi-slice CT is not able to accurately distinguish between intimal and medial calcification and requires ionising radiation. Therefore, serial CT scans to test a therapeutic intervention is an unattractive option compared to using cardiac MRI as a surrogate measure of calcification.

8.4.6 Results of studies of pulse wave velocity

This represents one of the largest studies of pulse wave velocity with CMR in any pathological disease state. Previous, smaller studies have demonstrated the feasibility of measuring PWV in healthy volunteers using CMR(312;315;316). In this study, PWV was numerically higher in uraemic patients compared to age matched controls in keeping with findings of previous studies using alternative methods to measure pulse wave velocity(315). Additionally, PWV rose significantly with advancing age in both uraemic and control patients. In keeping with the findings for aortic distensibilty and VAS, PWV tended to be higher in patients at highest cardiovascular risk (patients with diabetes, ischaemic heart disease or peripheral vascular disease), although with PWV none of these differences reached statistical significance. Like aortic distensibilty, generally there were no associations between PWV and dialysis modality, adequacy, time on renal replacement therapy or biochemical or haematological data. It is difficult to interpret a negative correlation between serum corrected calcium and PWV as other studies have shown the converse to be true. However a negative relationship between PWV and albumin may be due to low serum albumin levels reflecting over hydration

leading to vessel dilatation, or may indicate patients with the malnutrition-inflammation-atherosclerosis complex.

The differences in PWV between patients on various classes of antihypertensive therapy, alludes to potential differences between actions of antihypertensive agents on central haemodynamics but due to the limited numbers in each group, firm conclusions cannot be drawn. One study however has observed beneficial influences on long term survival in similar patient groups with ACE inhibitors compared to other agents(317), which may reflect beneficial haemodynamic effects. No prospective randomised controlled trials exist in this population to confirm this observation.

8.4.7 Limitations of study of PWV with CMR

Certain limitations of using CMR to measure PWV were highlighted by this study. Developing the technique required extensive collaboration to develop the Cardiowarp analysis software to measure aortic lengths and and interpret flow curves generated with CMR imaging of blood flow in the aorta. Although the results for PWV are broadly similar to the findings of other studies of healthy volunteers using either CMR or applanation tonometry to measure PWV, PWV in the uraemic patients appears lower than found in other studies(303). This was a cohort of uraemic patients being assessed for renal transplantation and may be healthier than other cohorts studied in the literature. Unfortunately direct comparison of CMR derived PWV with applanation tonometry was not possible in the uraemic patients.

Potential sources of error in measurement of PWV include the assessment of aortic length. A number of studies were discarded due to unfolding of the aorta taking it out of the plane of imaging and therefore not suitable for analysis. Of the scans analysed,

although the aorta was entirely visualised in the sagittal plane of imaging, tortuousity of the vessel in and out of the sagittal plane used for image acquisition may not have been detected. This potentially may have lead to underestimation of aortic length and hence PWV.

By assessing flow at three points along the aorta, the technique assumes that all scans are acquired at the same time points relative to the cardiac cycle. Although in the majority of scans this appears to be the case, this represents a further source of error. Additionally the aorta itself is regionally heterogeneous and in terms of its composition and hence its viscoelastic properties. Finally, the flow sensitive phase contrast CMR images were acquired at a temporal resolution of approximately 15ms. This compares poorly to the temporal resolution of Doppler probes typically used for applanation tonometry of 10 MHz.

8.4.8 Prognostic implications of CMR studies of vascular function

Independent predictors of mortality during the follow up period were diabetes, aortic distensibilty and systolic blood pressure. Due to their close interdependence, systolic blood pressure and aortic distensibilty could not be independently assessed. However when a combined end point of death, non-fatal myocardial infarction, cardiac revascularisation, amputation for peripheral vascular disease and cerebrovascular event was used, only diabetes and aortic distensibilty or VAS were independent predictors of events. Age was not a predictor of outcome in this group of patients. Therefore aortic distensibilty or VAS predict outcome whatever age the patient, rather than merely being reflective of older uraemic patients having stiffer vessels as demonstrated earlier. In keeping with other studies, time on renal replacement therapy was significantly longer in those patients who died during the follow up period(303), but this factor was not

independently associated with outcome. Therefore in this patient group, aortic distensibilty was a predictor of mortality and/or vascular events whatever the age of the patient and even if the CMR was performed long after the patient had started renal replacement therapy.

8.4.9 CMR measures of vascular function as surrogate markers of cardiovascular risk

As a potentially novel marker of increased cardiovascular risk, no data exist on the natural history of progression of aortic distensibilty in uraemic patients, or whether it can be improved by therapeutic intervention. The assumption is that reduced aortic distensibilty is reflective of arterial calcification. As arterial calcification has been demonstrated by electron beam CT to progress in patients on dialysis therapy and be related to oral intake of calcium (primarily as calcium containing phosphate binders)(143), one strategy to improve aortic distensibilty may be to use non-calcium containing phosphate binders such as sevelamer hydrochloride or lanthanum chloride to reduce to calcium load whilst controlling serum phosphate. Analogues of inhibitors of arterial calcification are in the early stages of development and may be of clinical benefit. CMR measures of vascular function, in particular aortic distensibilty, represent potentially useful surrogate markers of cardiovascular risk for future trials of such interventions.

8.4.10 Assessment of vascular function using CMR

A variety of studies have attempted to address the role of aortic distensibilty as a marker of either cardiovascular risk or relating aortic distensibilty to cardiac performance. Like the current study, it appears that CMR measures of arterial function represent attractive surrogate markers for intervention for other groups at high risk of cardiovascular

disease. Currently data relating CMR measures of arterial function on long term outcome are scant and similarly there a few studies to suggest that arterial function assessed by this method can be modulated:

- In otherwise healthy young individuals, obese subjects have been shown by CMR to have increased aortic cross sectional area and decreased aortic elasticity(318)
- In patients with either systolic or diastolic heart failure, aortic distensibilty was reduced compared to controls and correlated with exercise capacity as assessed by peak volume of oxygen consumption at cardiopulmonary exercise testing(313)
- A small study of 33 hypertensive patients demonstrated that aortic distensibilty increased in patients following treatment with either nicardipine or alacepril but not trichlormethiazide over a 12 week treatment period. This effect appeared to be independent of changes in pulse pressure, which did not significantly differ between the three groups(319)
- In Marfan syndrome, where the leading cause of death is aortic dissection, both aortic distensibilty and aortic diameter have been shown to be independent predictors of progressive aortic dilatation(320). A further CMR study has shown that beta-blocker therapy significantly improves aortic distensibilty in these patients(321)
- An interesting pilot study has demonstrated that arterial endothelial function can be studied using CMR to by assessing cross sectional flow mediated dilation of the brachial artery(316)

8.4.11 Other studies using CMR to assess pulse wave velocity

A small number of studies have used CMR to assess PWV, either validating the technique against established methods or comparing the results to clinical characteristics of the subjects studied. Generally, studies assessing validation of CMR measures of vascular function have focused on measurements in normal volunteers:

- Rogers *et al* compared applanation tonometry and CMR measured PWV in healthy volunteers across a wide age range. Similar to the current study they found that PWV measured with both tonometry and CMR increased with age(315)
- One study in healthy volunteers validated aortic PWV using complex mathematical derivation of PWV by alternative methods. This study assumes that in young adults, by the time of maximum blood flow, flow direction is unidirectional (no reflected waves) and uses fluid dynamic theory to estimate PWV from maximum blood flow rates and changes in aortic cross sectional area. Although an attractive concept it is unclear how this translates to older patients (all subjects were 21-30 years old) as aortic stiffening occurs. The scanning protocol used was similar to the current study(312)
- One CMR study of healthy volunteers demonstrated a close correlation between aortic compliance and PWV in the thoracic aorta, and again show significant correlations between aortic stiffness and age by both methods (322)
- PWV assessed by CMR has been show to correlate with age but no other clinical parameters in patients with juvenile idiopathic arthritis(314)

8.4.12 Arterial stiffness in uraemia - clinical implications and long term survival

A large number of studies have been performed assessing both the determinants of increased arterial stiffness, measured with alternative methods, and its relation to long

term survival in patients with ESRD, using either elastic incremental modulus, PWV or augmentation index as markers of arterial stiffness:

- Factors consistently associated with increased arterial stiffness include age, diabetes, and systolic blood pressure (or pulse pressure)(323-325)
- A number of studies have identified other factors associated with increased arterial stiffness including serum calcium or the presence of inflammation indicated by C-reactive protein(325)
- Using electron beam CT, Covic *et al* have shown that PWV measured with the SphygmoCor apparatus correlates with the degree of coronary artery calcification, even when corrected for confounding variables such as age and duration of renal replacement therapy(193)
- A number of survival studies from the group of London *et al* demonstrate that PWV is an independent predictor of both all cause and cardiovascular mortality in patients on haemodialysis(302;303;326). Interestingly in one of these studies in a cohort of 180 dialysis patients followed up for 52 months brachial blood pressure was a less impressive predictor of outcome compared to central measures of aortic PWV(326)

8.4.13 Conclusions

This study has demonstrated that vascular function can be measured using CMR in uraemic patients. Increase in aortic stiffness, indicated by reduced aortic distensibilty, reduced VAS and increased PWV are all associated with risk factors for cardiovascular disease including diabetes, previous ischaemic heart disease, peripheral vascular disease and advancing age. Although PWV measured by CMR was not associated with long term outcome, both aortic distensibilty and VAS were independent predictors of combined vascular events and mortality. Aortic distensibilty predicted all cause

mortality. It is likely that these derangements in aortic function are due to arterial calcification although this requires correlation with conventional methods of assessing calcification such as CT scanning. CMR offers a novel non-invasive tool to assess vascular function in these patients. Aortic distensibilty and VAS are potential targets for therapeutic intervention to reduce cardiovascular risk in uraemia.

Chapter 9

A study of dermatological complications of contrast enhanced magnetic resonance imaging

9.1 INTRODUCTION

The main goal of the studies contained in Chapter 3 was to assess the relationship between myocardial tissue abnormalities, indicated by contrast enhanced CMR and uraemic cardiomyopathy defined by CMR. Furthermore, these abnormalities were shown to predict long term outcome. Therefore, the next intention was to use this information to power a prospective study with the goal of either preventing the development of myocardial fibrosis or reducing the progression of myocardial fibrosis using findings at contrast enhanced CMR as a study end point.

However in April 2006 a case series emerged from Austria postulating a link between gadolinium based MRI contrast agents and the development of nephrogenic systemic fibrosis (NSF)(327), previously labelled nephrogenic fibrosing dermopathy, in patients with advanced chronic kidney disease. This was subsequently followed by a caution issued by the United States Food and Drug Administration, regarding the use of gadolinium based MRI contrast agents and the development of NSF.

NSF was first identified in 1997 and presents as a scleromyxoedema-like condition affecting patients with advanced kidney disease(328). Approximately 200 cases have been reported to the NSF registry(329). The clinical manifestations include thickened, oedematous, indurated or discoloured skin affecting the limbs and trunk. The face is typically spared. Progression may occur over weeks resulting in joint contractures and severe restriction of movement. Multi-organ involvement has been observed and mortality is high with death occurring often from sepsis as a complication of immobility or hypoventilation if lung and diaphragm involvement occurs(330).

Although clinical cases of NSF had been documented prior to the commencement of these studies at the hospitals from which the patients were recruited, no causative link between gadolinium and NSF had been proven. Whilst this uncertainty existed, future contrast enhanced CMR studies were suspended and a retrospective case-control study was performed to assess any link between exposure to gadolinium based MRI contrast agents and development of NSF.

9.2 METHODS

9.2.1 Identification of patients and MRI data

A retrospective analysis was undertaken of all prevalent patients on renal replacement therapy within the renal units of Glasgow Royal Infirmary and the Western Infirmary Glasgow between 1st January 2000 and 1st July 2006 to identify all cases of NSF. The computerised electronic patient record (EPR) was interrogated to using the keywords "nephrogenic", "dermopathy", "fibrosing", "scleromyxoedema" to identify all cases ("fibrosis" and "systemic" were not used as many more cases were generated of unrelated conditions such as pulmonary fibrosis or systemic lupus erythematosus without identifying further cases of NSF). Additionally all consultant nephrologists working at these two units were asked to forward details of any patients they had cared for with NSF. Patients with NSF therefore formed the group of cases. Each case was reviewed thoroughly with respect to the clinical history and histopathological records.

As controls all patients undergoing dialysis therapy for established renal failure (defined as dialysis dependent for >90 days) at the Western Infirmary, Glasgow over the same time period were identified via the EPR. A search was performed of the radiology computer system for all records of magnetic resonance imaging with the number of

gadolinium-containing scans and cumulative gadolinium contrast dosage and type recorded for each patient. Doses were recorded in the radiology database. Where this was unavailable the MRI logbook was checked. Gadolinium exposure was calculated over the six and a half-year period for all non-NSF patients and for the period 1st January 2000 until the first presentation of disease for all patients in the NSF group. Patients who had functioning renal grafts throughout the study period and thus did not receive dialysis were excluded during the time they were not on dialysis therapy.

9.2.2 Statistical Methods

Non-parametric testing (Mann-Whitney-U test) was used to assess differences between median doses of gadolinium contrast and total number of gadolinium-enhanced MRI between NSF and non-NSF groups. Statistical analysis was performed by SPSS version 13.0 (SPSS Inc, IL, USA).

9.3 RESULTS

9.3.1 Demographics of patients undergoing renal replacement therapy at the Western Infirmary, Glasgow 2000-2006

A total of 814 patients were identified who had received renal replacement therapy for ESRD at the Western Infirmary during the period 01/01/2000 to 01/07/2006. The mean age at starting RRT was 56.5 years and 471 (7.9%) patients were male. The cause of ESRD was diabetic nephropathy in 124 (15.2%), adult polycystic kidney disease in 51 (6.3%), glomerulonephritis in 168 (20.6%), chronic pyelonephritis in 95 (11.7%), renovascular disease in 98 (12.0%), miscellaneous other known causes of ESRD in 65 (8.0%) and 213 (26.2%) with an unknown or unclassifiable cause of their renal failure.

9.3.2 Cases of nephrogenic systemic fibrosis identified in North Glasgow

A total of 15 patients were identified with a diagnosis of NSF as evidenced by clinical and pathological findings noted within the clinical records. Four cases were identified at the Western Infirmary Glasgow and 11 cases at Glasgow Royal Infirmary. 13 of the 15 cases were biopsy proven. Compared to the control cohort of patients undergoing renal replacement therapy for ESRD at the Western Infirmary, patients with NSF were significantly younger at the time of starting renal replacement therapy (median age NSF group 51.7 vs. non-NSF group 60.9, p= 0.031). A greater proportion of patients who developed NSF were female compared to those who did not (NSF group 66.7% female vs. non-NSF group 41.9% female, p=0.054). The demographics of patients with and without NSF are shown in Table 9.1. At the end of the study period 6/15 (40.0%) of patients with NSF had died.

9.3.3 Contrast enhanced magnetic resonance imaging performed during the period 2000-2006

Over the six and a half-year period, 204 (24.7%) of the total 825 patient cohort (NSF cases from both hospitals plus controls from the Western Infirmary dialysis population) patients underwent a total of 248 gadolinium-enhanced MRI scans. Six (2.4%) of these MRIs used gadobenate-dimeglumine (Multihance, Bracco S.p.A., Milan, Italy) as the contrast agent with median dose volume of 15ml. 228 (91.9%) of these scans were performed with gadodiamide (Omniscan, GE Healthcare, Chalfont St Giles, UK) with a median dose volume of 32ml. 11 (4.4%) scans used gadopentetate dimeglumine (Magnevist, Berlex, Canada) as the contrast agent with median dose volume of 15ml, and three (1.2%) scans used gadobutrol (Gadovist, Schering, West Sussex, UK) as the contrast agent with median dose volume of 15ml.

9.3.4 Relationship between exposure to MRI contrast agents and development of NSF

Of the patients who developed NSF only one patient had not been exposed to MR contrast (Table 9.2). All other patients who developed NSF had undergone MR scanning with gadodiamide used as the contrast agent. None of these patients had been exposed to other MR contrast agents. When compared to the 190 patients who had undergone contrast-enhanced MR and not developed NSF, the 14 NSF patients were found to have been exposed to a significantly higher median dose of gadodiamide-based contrast than their non-NSF counterparts (median 51.5ml v 32ml, $p<0.001$). Additionally the median dose of gadodiamide-based contrast used for their first MRI scan was significantly higher in patients who developed NSF (45.0 vs. 32ml, $p<0.001$). There was additionally a significantly greater number of MRI scans performed on patients who developed NSF (median of two scans per patient in the NSF group and one per patient in the non-NSF patients, $p<0.001$). The frequency of performance of contrast-enhanced MR examinations steadily increased over the study period as shown in Figure 9.1 with the cumulative numbers of diagnoses of NSF following a similar trend.

9.3.5 Characteristics of patients who developed nephrogenic systemic fibrosis

The baseline demographics and outcome for the patients who developed NSF is displayed in Table 8.2. Other than exposure to MR contrast in all but one patient, there were no consistent recurrent clinical features present in all these patients, either in terms of underlying renal diagnosis, time on dialysis, concurrent medication or co-morbidity (data not shown). The median time period from MRI scan to diagnosis of NSF was 70 days with an interquartile range of 313.5 days. Although one patient (Patient 5) had a time interval of 2395 days between first MRI scan and onset of symptoms of NSF, a

second MRI was performed after development of skin changes. As a retrospective study it is not clear what, if any, exacerbation was related to the first or second scan. Skin biopsy was performed after the second scan in this patient. Six patients died during the follow up period, one patient spontaneously regained renal function sufficiently to discontinue dialysis (Patient 6) and one patient has undergone cadaveric renal transplantation (Patient 12). The patient who has regained renal function has also been treated with photophoresis therapy and has experienced improvement but not resolution in her skin condition. One patient (Patient 15) has been treated with immunosuppression (prednisolone and sirolimus) and at the time of data collection this has been poorly tolerated and has not led to any dermatological improvement.

Characteristic		Non-NSF		NSF		p
Number of patients		810		15		-
Age at start of RRT		60.9	(28.8)	51.7	(20.9)	0.031
Male (%)		471	(58.1)	5	(33.3)	0.054
Diagnosis	(%)					
	Diabetes	124	(15.4)	4	(26.7)	-
	APKD	51	(6.3)	1	(6.7)	-
	GN	161	(20.0)	5	(33.3)	-
	CPN	94	(11.7)	3	(20.0)	-
	Renovascular	98	(12.2)	0	(0.0)	-
	Other	63	(7.8)	0	(0.0)	-
	Unknown	213	(26.5)	2	(13.3)	-
Number undergoing MRI		190	(23.5)	14	(93.3)	<0.001
Mortality at end of study period		405	(50.0)	6	(40.0)	0.604

Table 9.1. Clinical characteristics of the complete of cohort of patients comparing those with and without a diagnosis of NSF. All variables expressed as value (%) expect age expressed as median and interquartile range.

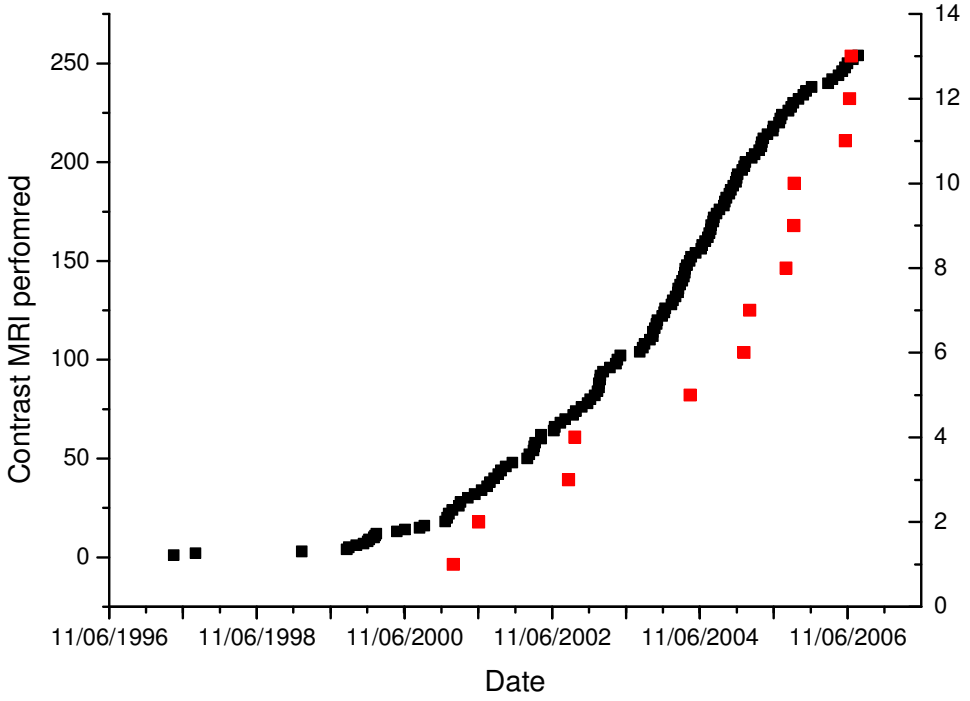


Figure 9.1

Graph displaying the cumulative number of contrast enhanced MRI scans performed in this cohort of patients – left axis (black) with the cumulative frequency of diagnosis of nephrogenic systemic fibrosis displayed - right axis (red)

CASE	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Sex	M	F	M	F	M	F	F	M	F	F	M	F	F	F	F
Primary diagnosis	U	CPN	DM	GN	DM	GN	U	U	AP	CPN	DM	CPN	GN	GN	GN
Skin biopsy	Y	Y	Y	Y	Y	Y	Y	N	Y	Y	Y	Y	N	Y	Y
Age at diagnosis (years)	63.2	61.0	43.5	54.3	67.1	49.0	63.7	43.7	37.7	20.5	60.4	58.5	59.8	73.2	50.50
Age starting RRT (years)	61.6	56.6	42.4	22.2	67.0	49.0	56.7	38.9	35.1	17.0	59.9	51.7	59.1	66.2	48.97
Time on RRT (years)	1.6	4.4	1.1	32.1	0.1	0.0	7.0	4.8	2.6	3.5	0.5	6.8	0.7	7.0	1.53
RRT modality	PD	HD	HD	HD	HD	HD	HD	HD	HD	HD	HD	HD	PD	HD	PD
MRI	Y	Y	Y	N	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
MRI-NSF time (days)	76	83	477	-	2395	35	2	4	16	52	64	289	99	441	6
Previous MRI	2	2	1	-	1	1	3	1	1	2	1	1	2	5	0
MRI post NSF	1	3	0	-	1	1	1	1	0	3	0	0	1	1	0
HCO_3 (mmol/l)	22	24	26	25	18	28	18	36	17	25	23	26	29	21	28
Treatment	-	Ph	-	-	Ph	-	-	-	-	-	-	-	-	-	Im
Renal function	-	-	-	-	-	Sp	-	-	-	-	-	Tx	-	-	-
Outcome	S	I	M	M	S	I	M	M	N/A	M	M	S	S	N/A	D

Table 9.2 Baseline characteristics of NSF cases. Abbreviations: M - male; F - female; U - unknown; CPN - pyelonephritis; DM - diabetes mellitus; GN - glomerulonephritis; AP - adult polycystic kidney disease; Y - yes; N - no; Time on RRT - at diagnosis with NSF; RRT modality - at the time of NSF diagnosis; PD - peritoneal dialysis; HD - haemodialysis; MRI-NSF time - time interval between gadolinium-enhanced MRI and NSF onset; Previous MRI - total number of MRI scans prior to NSF diagnosis; MRI post NSF - number of MRI scans performed after a diagnosis of NSF; HCO3 - serum bicarbonate level within 30 days of MRI; Ph – photophoresis; Im – immunosuppression; - no change; Sp - spontaneous improvement; Tx - renal transplant; Outcome - of NSF; S - stable; I - improved; M - dead; D – deteriorating; N/A - not applicable/follow up time too short for comment.

9.4 DISCUSSION

9.4.1 Results of this study

These data support an association between the use of the gadodiamide-based contrast agent (Gd-DTPA-BMA, Omniscan) and the development of nephrogenic systemic fibrosis. Patients who developed NSF were exposed to higher initial and subsequent doses of gadodiamide than those who did not and additionally underwent more contrast enhanced MRI scans. However one patient developed NSF without ever undergoing contrast enhanced MRI scanning suggesting that gadodiamide is not the sole cause of NSF. Conversely although 6.6% in this cohort of ESRD patients undergoing MRI scanning with gadodiamide developed NSF, cases of NSF were over-represented in this group by including cases from two hospitals (Western Infirmary, Glasgow and Glasgow Royal Infirmary) but only using a control group of a cohort of patients on renal replacement therapy from one hospital (Western Infirmary, Glasgow). This approach was used to ensure accuracy of the data collected regarding contrast agents used for MRI scanning. If the entire dialysis population from each centre was selected then there would be a prevalence of 15 cases for a total of 1826 patients on maintenance dialysis. Between both centres 425 contrast enhanced MRI scans were performed, giving an incidence of NSF of 3.5% in patients with ESRD who have undergone previous MRI scanning.

None of the alternative gadolinium based contrast agents were associated with the development of NSF suggesting that it may be properties of the chelating molecule or an interaction between the gadolinium chelate and the uraemic milieu that leads to development of NSF. Based on these findings, caution should be used in future before using gadolinium based contrast agents in the ESRD population, both by reducing the

dose of contrast used and by focusing on strategies to minimize exposure to the gadolinium chelate compound.

9.4.2 Pathogenesis of nephrogenic systemic fibrosis

Currently the pathogenesis of NSF remains unclear. Approximately 90% of patients with the condition are established on dialysis therapy. The condition has been described in both patients receiving haemodialysis and peritoneal dialysis and no one underlying renal disease, dialysis technique; manufacturer, dialysate or dialysis unit has been consistently associated with NSF(331). Hypercoagulable states have been reported in NSF including patients experiencing deep vein thrombosis and pulmonary embolus as well as thrombosed dialysis access (both femoral grafts and arteriovenous fistula). These have not been a universal finding(332). Other associated findings reported in patients with NSF have included underlying liver disease, most commonly, hepatitis B- or C-induced cirrhosis(333). The significance of any of these associations is unclear but suggests that vascular injury or thrombosis may set in motion a process of aberrant processes akin to early wound healing, leading to abnormal collagen deposition.

More recently the spindle cells present in the dermis of patients with NSF has been demonstrated to be a CD34-postive dendritic fibroblast, giving rise to the concept of a ‘circulating fibrocyte’ of bone marrow origin migrating from the circulation to the dermis and differentiating into cells which histologically resemble dermal fibroblasts(334). These circulating cells suggest that NSF is a systemic disorder. The trigger for the migration and differentiation of these CD34-postive fibrocytes is not clear but thrombosis and/or endothelial injury are possibly responsible.

9.4.3 Clinical features of nephrogenic systemic fibrosis

The cutaneous features of NSF include skin-coloured to erythematosus papules which classically coalesce to the characteristic brawny plaque with peau d'orange surface changes. The skin becomes marked thickened with a woody texture. The condition usually follows a symmetrical distribution involving the limbs and trunk, most typically between the ankles and mid-thighs and wrists and upper arms. The face is usually spared(332). Patients may complain of pain in the affected areas. Arthritis is not seen but joint contractures may develop over a time course of weeks. Loss of function is extremely common and patients may become wheelchair bound.

Although the condition was initially thought to be confined to the skin, systemic involvement has been described including diaphragmatic and pleural involvement indicated by hypoventilation and reduced gas transfer as well as pericardial and myocardial disease manifest as cardiomyopathy and arrhythmia(330). The natural history of the disease is not yet well understood. Some patients report a gradual improvement in mobility and slight softening of the skin over time, either spontaneously or in response to therapy. Complete spontaneous healing in a patient with ongoing kidney disease has not yet been reported. Some patients with NSF (approximately 5% or less) have an exceedingly rapid and fulminant disease course that may result in death.

9.4.4 Histological findings in nephrogenic systemic fibrosis

As the disease extends into the subcutaneous fascia a deep biopsy specimen is required. Characteristic biopsy findings include increased cellularity in the dermis and in the subcutaneous septa. This increased cellularity consists of dermal spindle-cell proliferation (CD34-postive) with variable mucin deposition and little inflammation; occasional multinucleated giant cells, elongated elastic fibres, and dystrophic

calcification. An Alcian blue stain is often used to highlight the increased deposition of acid mucin in the dermis(335). Histological features of NSF are demonstrated from a skin biopsy of one of the patients in this study in Figure 9.2. In keeping with the concept of NSF as a deranged process of aberrant wound healing increased expression of transforming growth factor β1 has been demonstrated in the affected skin and muscle(336).

Differential diagnoses of NSF include scleromyxoedema, eosinophilia–myalgia syndrome, eosinophilic fasciitis, systemic sclerosis/morphea, porphyria cutanea tarda, fibroblastic rheumatism, Spanish toxic oil syndrome, vinyl chloride exposure and β_2-microglobulin amyloidosis.

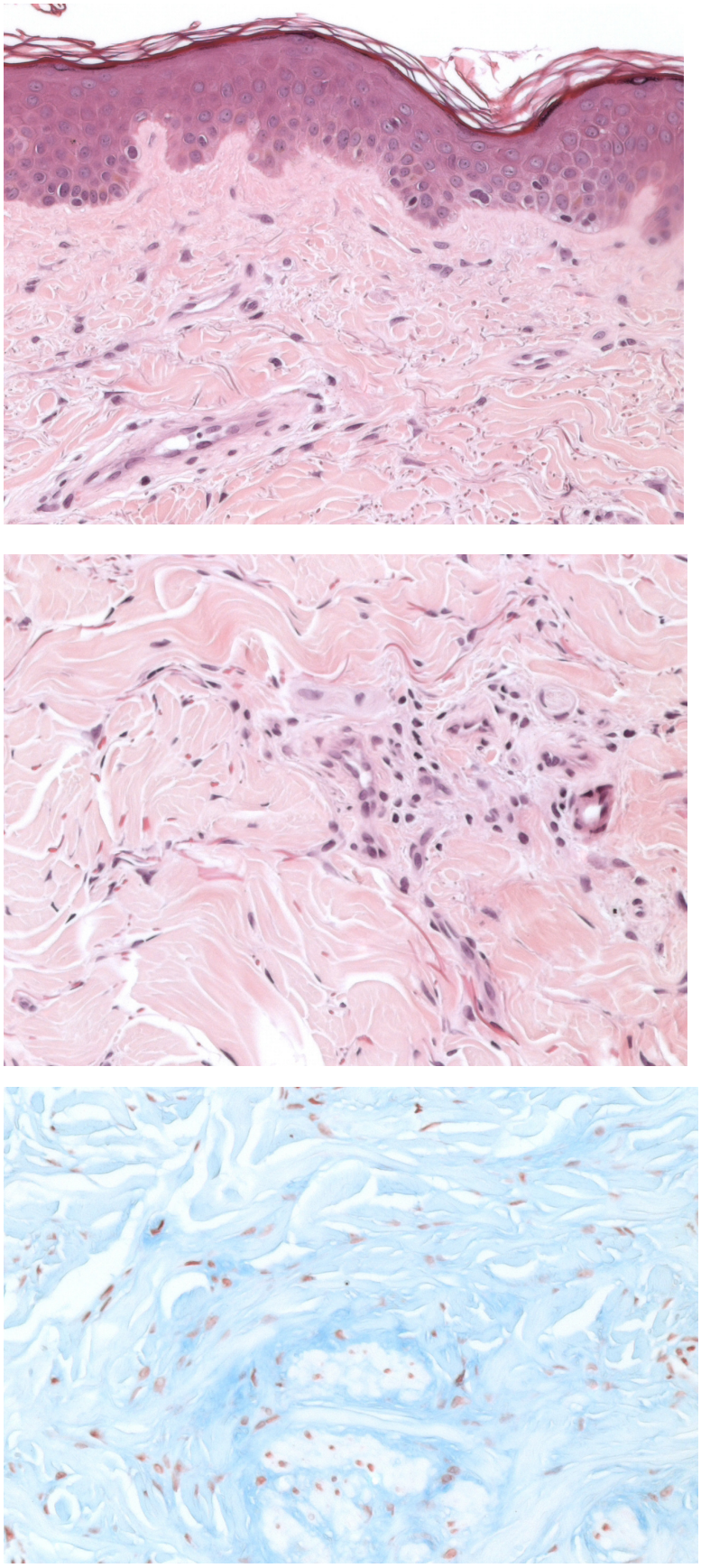


Figure 9.2

NSF lesions showing normal epidermis, thick collagen bundles with numerous clefts and plump fibroblast-like cells, and Alcian blue staining. Photograph courtesy of Dr Michael Edward, University of Glasgow

9.4.5 Postulated triggers for the development of nephrogenic systemic fibrosis

As endothelial or vascular injury has previously been implicated in the pathogenesis of NSF a number of case reports have suggested a temporal relationship between vascular insults, either operations or thrombotic events, and development of NSF. Approximately 15% of patients report a surgical procedure (other than renal or hepatic transplantation) immediately antecedent to the onset of NSF. If transplantation is included, the percentage climbs to 48%. Inclusion of formation of dialysis fistula and central dialysis catheter placement increases the total of patients experiencing a vascular intervention prior to development of NSF approximately 90%. However this finding must be interpreted in the context that vascular interventions are extremely common in patients with ESRD, irrespective of their NSF status(332).

An alternative proposal has suggested that erythropoietin therapy may be implicated in the pathogenesis of NSF. Patients with NSF have been observed to have been treated with higher doses of erythropoietin(337). Whether this suggests a common aetiological link between erythropoietin resistance (due for example to chronic inflammation) and NSF is not clear. Erythropoietin resistance may indicate bone marrow fibrosis as part of the NSF syndrome, although this has not been demonstrated in clinical practise. Moreover high-level exposure to erythropoietin may contribute to the disease by increasing numbers of circulating haematopoietic stem cells and endothelial progenitors (and hence CD34-postive circulating fibrocytes). Erythropoietin has also been shown in vivo to trigger an exaggerated fibrin induced wound-healing response that is histologically similar to NSF(338). However it will be almost impossible to determine the role, if any, of erythropoietin in the development of NSF.

9.4.6 Pharmacokinetics of gadolinium based contrast agents in end stage renal disease

Gadodiamide (Gd-DTPA-BMA; Omniscan) is distributed in the extracellular fluid and entirely excreted from the body through the kidneys by glomerular filtration, with an elimination half-life in normal renal function of 80–100 min. Therefore this compound gadolinium is excreted from the body as a complex, i.e. unchanged from the administered form. 98% of administered gadodiamide is excreted at 24 hours in normal renal function)(339). A number of small studies have been performed to assess the pharmacokinetics of gadolinium based contrast agents in end stage renal disease:

- In patients with severely reduced renal function (glomerular filtration rate 2-10ml/min, not on dialysis therapy), the elimination half-life of gadodiamide injection is prolonged (34.3 hours +/- 22.9). In the same group of studies 65% of the gadodiamide injected was eliminated during a haemodialysis session(340)
- In another study using gadopentetate dimeglumine (Gd-DTPA; Magnevist) the average excretory rates of the total administered agent was 78.2%, 95.6%, 98.7% and 99.5% in the first to fourth hemodialysis sessions, respectively(341)
- Peritoneal clearance appears to much less efficient for removal of gadodiamide. Of the studies available it appears that approximately 69% of the gadodiamide is excreted over a 22 day period(340)
- One concern with gadodiamide compared to the other contrast agents relates to the risk of transmetallation. Gadodiamide has excess chelate compared to the other agents and is theoretically less stable with a higher risk of transmetallation resulting in release of toxic free Gd^{3+}. Transmetallation has not been observed in these pharmacokinetic studies in ESRD patients and its role in development of NSF is unproven(342)

9.4.7 Adverse effects of gadolinium based contrast agents in end stage renal disease

Common side effects following administration gadolinium based contrast agents include headache, nausea, taste disturbance and urticaria. Typically with gadodiamide the incidence of these side effects is approximately 2% for headache and <1% for all other effects. As the excretion is delayed these side effects are likely to be exacerbated in renal impairment. Anaphylaxis is a rare occurrence.

- Reports of nephrotoxicity in patients with advanced renal impairment exist with gadolinium based agents. Nonetheless the risk of precipitating dialysis is rare and serum creatinine usually returns to baseline. Risk factors for developing gadolinium induced nephrotoxicity include a lower glomerular filtration rate at baseline and diabetes(343;344)
- Massive life threatening free iron mobilisation has been reported in an ESRD patient with biochemical iron overload following administration of Gd-DTPA(345)
- Pseudohypocalcaemia is commonly observed following administration of gadolinium based contrast agents due to gadolinium's interference with the colorimetric assays used to measure serum calcium. Serum calcium is normal when measured by mass spectrometry. This effect is prolonged in renal impairment(346)

9.4.8 Relationship between gadolinium based contrast agents and nephrogenic fibrosing dermopathy

A link between gadolinium based contrast agents, in particular gadodiamide, and NSF was first postulated in April 2006 by Grobner(327), with further subsequent reports emerging from Denmark(347). To date only gadodiamide has been implicated with

NSF. The findings of the current study are entirely in keeping with these observations and extend this association by demonstrating a dose dependent relationship and indicating a theoretically risk of developing NSF following administration of gadodiamide of approximately 3.5%. The first report postulating this link suggested that acidosis was a prominent feature at the time of MRI scanning in patients who developed NSF. This has not been observed in subsequent studies and was not the case in the current study.

Two recent studies using spectroscopy have been able to demonstrate the presence of gadolinium particles in the skin of patients with NSF(348;349). However gadolinium particles were not present in the skin of all affected patients. Therefore these studies, along with this work, strengthen the epidemiological association between gadolinium based contrast agents and development of NSF but do not provide causation or a mechanism for its pathogenesis. The authors speculate that gadolinium retained in tissue is phagocytosed by macrophages and this intracellular gadolinium results in the up regulation of profibrotic cytokines and/or growth factors that ultimately eventuate in dermal or systemic fibrosis.

9.4.9 Treatment of nephrogenic systemic fibrosis

No consistently successful treatment for NSF has emerged. Various therapies have been tried including oral and topical corticosteroids, thalidomide, ultraviolet therapy, plasmapheresis and intravenous immunoglobulins. Isolated reports suggest that some of these interventions are beneficial in individual cases(350). Extracorporeal photophoresis has been advocated most widely with a number of reports published to suggest benefit(333;351). Whether this is due to a direct effect on the circulating fibrocyte is speculative. Restoration of renal function, whether by treatment of the underlying renal

disease or by renal transplantation is likely to be of benefit as impaired renal function is one consistent finding in all patients with NSF.

9.4.9 Conclusions

This study has confirmed an association between used of gadodiamide as a MRI contrast agent and development of NSF. This relationship appears to be dose related and development of NSF occurs following the demonstration of gadodiamide and is exacerbated by multiple further exposures. Based on these findings, in combination other studies, gadodiamide should no longer be considered safe for use in patients with advanced chronic kidney disease or patients with established ESRD. If there is no alternative to its use, the lowest diagnostic dose should be used and prompt dialysis after the scan to remove the gadodiamide. It is not clear is alternative gadolinium based agents are safe or it the preponderance of cases of NSF related to gadodiamide merely reflect that this agent accounts for >90% of the scans performed. Use of contrast enhanced CMR as a tool for future research in this population has been jeopardised by the finding of the association between NSF and gadolinium based contrast agents.

Chapter 10

General discussion and conclusions

10.1 Definition of uraemic cardiomyopathy with cardiac magnetic resonance imaging

The principal aim of this project was to determine whether a cardiomyopathy, specific to uraemia could be described *in vivo* using CMR. The chief method used to investigate this was using the contrast agent gadolinium-DTPA-BMA. A further question was to unravel the relationship between ischaemic heart disease (or coronary artery disease) and uraemic cardiomyopathy. Contrast enhanced CMR allowed the identification of myocardial infarction, thereby allowing a clear distinction between the presence of cardiomyopathy potentially related to uraemia and that where the major determinant was premature coronary artery disease.

The results contained in Chapter 3 demonstrate two distinct pathological processes or subtypes of uraemic cardiomyopathy. Firstly, the presence of left ventricular systolic dysfunction is closely associated with subendocardial late gadolinium enhancement, in keeping with previous myocardial infarction. This is in keeping with the aetiology of the majority of cases of left ventricular systolic dysfunction in the general population. The one feature specific to uraemia in this setting is that the progression of coronary artery atherosclerosis is accelerated in uraemia, leaving patients with ESRD vulnerable to future myocardial ischaemia. Second, and specifically to uraemia, diffuse LGE representing fibrosis through the left ventricular myocardium was observed in some, but not all, patients with left ventricular hypertrophy. There were no precedent reports of this finding, but this observation is in keeping with histopathological studies of cardiac tissue in uraemia. Since these findings have been published, a number of authors have commented on the novelty of this observation using CMR, which appears to demonstrate a further strength of this technique(352;353).

10.2 Long term implications of uraemic cardiomyopathy and other cardiovascular risk factors in uraemia

Both subtypes of uraemic cardiomyopathy described with contrast enhanced CMR appeared to be associated with poorer survival. However using the conventional definitions of cardiomyopathy with left ventricular function or dimensions, neither LVH nor left ventricular systolic dysfunction were demonstrated to be independently associated with poor outcome, although there was a trend towards poorer survival with left ventricular systolic dysfunction. This may relate purely to small sample size of the cohort of patients. By contrast with other similar studies, all these patients were being considered for renal transplantation, and may have by definition been fitter, with less severe cardiac abnormalities than studied in other cohorts(75-77). A centre or era effect is possible, where patients with left ventricular hypertrophy are less likely to experience a premature cardiovascular event in the first decade of the twenty-first century compared to the last decade of the twentieth. In the West of Scotland, with such a high rate of coronary artery disease, patients with only left ventricular hypertrophy, without coronary artery atherosclerosis, may be relatively protected from cardiovascular events compared to the rest of the population, although this seems unlikely. Survivor bias may be present in patients who survive to commence dialysis therapy as the majority of patients with cardiovascular disease and renal impairment will die of cardiovascular disease before requiring dialysis. Ultimately it is less clear, why in this cohort of patients, the presence of uraemic cardiomyopathy, detected by cardiac morphology appeared to have less of an impact on survival than expected.

The outcome studies contained in Chapter 6, observed that the greater determinants of long term outcome were conventional risk factors such as age, diabetes and presence of ischaemic heart disease. Clearly the ability to receive a renal transplant, the only

definitive treatment to reverse uraemia, improves survival, but there is an inevitable selection bias, in choosing relatively fitter patients for transplantation. One practical application emerged by studying this cohort of patients. An objective method of documenting functional capacity using an exercise tolerance test conveys useful prognostic information for long term prognosis. This may help indicate which patients at much higher risk, who still require invasive investigations with angiography, and in whom further cardioprotective strategies may be contemplated, e.g. beta blockade, anti-platelet therapy or statins. Unfortunately, an evidence base for many of these interventions is still lacking in this patient group.

10.3 Investigation of vascular function in uraemia using cardiac magnetic resonance imaging

At the point of inception of the series of all the studies contained within this project, CMR was still a relatively new technique. Although clearly an attractive method for studying vascular function, there were very limited data published on the use of CMR for assessing vascular function as part of integrated cardiovascular risk assessment in any patient population(315;316). As there is a well established link between cardiovascular outcome and arterial stiffness, studied with alternative methods(302;303), in patients with ESRD; this appeared to be an ideal cohort to develop this technique with.

The studies contained within Chapter 8 clearly demonstrate arterial stiffness is distorted in ESRD compared to normal individuals, and is further reduced by the presence of additional cardiovascular risk factors, superimposed upon uraemia. Interestingly, both parameters for measuring aortic stiffness, aortic distensibilty or aortic volumetric arterial strain, were independent predictors of cardiovascular events in these patients.

This finding requires further exploration. One hypothesis would suggest that long term dialysis patients have acquired extremely stiff vessels, which may not be amenable to intervention with corrective therapy. Study is required to identify ESRD at highest risk of developing reduced arterial stiffness, perhaps using circulating markers of either calcification or atherosclerosis, such as fetuin-A, osteprotegerin, homocysteine or matrix Gla protein(130;310;311).

10.4 Nephrogenic systemic fibrosis- a devastating new disorder in uraemic patients

The identification of a link between CMR contrast agents, in particular gadolinium-DTPA-BMA, and nephrogenic systemic fibrosis was an unexpected and catastrophic event. Of the patients in this study, as far as can be identified, no patient who developed NSF received an isolated MRI scan for research purposes, with patients with NSF undergoing multiple scans, particularly for planning vascular access for dialysis or to image large vessels to assess peripheral vascular disease. Further reports of the association of this condition with gadolinium containing contrast agents have emerged. Currently, there are no plans to restart research based MRI scanning in ESRD patients using gadolinium based contrast agents until this issue is resolved.

10.5 Limitations of these current studies

One major goal of this study was to identify the relationship between CMR findings, particular LGE and arrhythmia and sudden cardiac death. As part of the initial investigation, a 24-hour ambulatory ECG monitor recording was proposed in all patients, with the intention of studying minor rhythm abnormalities(78). Unfortunately for technical reasons this was not possible, leaving an unexplored gap between CMR findings and arrhythmia. Similarly, although details on cardiovascular events, hospital

admissions and mortality were collected from the renal electronic patient records, causes of death and cardiovascular event data were not collected. Where this data is included it is verified information. Potentially this information is available for all subjects, but was deemed too costly to collect, following discussions with the administrative group of the Scottish Renal Registry, who in turn can access records held by the Information Services of the National Health Service for Scotland.

Vascular function studies would have been improved by detailed correlation with measures of vascular function using established methods such as the SphygmoCor system, particularly in the ESRD cohort. This was not technically feasible due to the geographical location of the examination room used for SphygmoCor examination. When piloted on a number of ESRD patients, it was reported to require too long a session to make to the study day an acceptable duration to retain patient comfort. One solution would have been to perform SphygmoCor examination on a separate occasion from CMR, but as vascular dimensions are dependent on hydration status, this may have lead to inaccuracies in the correlation between the various methods.

10.5 Cardiac magnetic resonance imaging- a novel surrogate end point for clinical trials in end stage renal disease?

As discussed previously, CMR is ideally placed as an end-point for trials assessing left ventricular remodelling both in the ESRD and in other populations(354). Methods to reduce left ventricular hypertrophy include drug therapy, alterations to the dialysis schedule or minimising the haemodynamic instability of dialysis with techniques such as haemodiafiltration. Methods such as haemodiafiltration are attractive as they may increase clearance of ‘uraemic factors’ responsible for promotion of left ventricular growth. Additionally measures of vascular function could be studied in using CMR,

using interventional strategies to reduce arterial stiffness, such as calcium free phosphate binders.

10.6 Future work with cardiac magnetic resonance in end stage renal disease

The initial findings of these studies demonstrating the presence of diffuse LGE as a clear marker for uraemic cardiomyopathy along with the suggestion that it may be a marker for patients at risk of sudden cardiac death, suggested that a clinical trial aimed at either preventing the development or progression of cardiac fibrosis in ESRD would be desirable, using the presence and extent of diffuse LGE as an end point. Therapeutic agents which have negative effects on myocardial fibrosis, which were proposed, include angiotensin receptor blockers(355), or an aldosterone antagonist such as spironolactone or eplerenone, which may oppose the direct effect of aldosterone in promoting myocardial fibrosis(356).

Similarly from a clinical perspective, the ability to non-invasively detect functionally significant coronary artery stenosis using stress perfusion CMR is extremely attractive. This technique would be ideal for indicating which patients require coronary angiography as part of work up for renal transplantation, as well as helping to plan coronary revascularisation in patients with critical coronary stenosis. One recent study had demonstrated that CMR was able to distinguish haemodynamically relevant from non-relevant coronary lesions, delineated by the use of fractional flow reserve at angiography, with a high sensitivity and specificity(357). A prospective trial to assess stress perfusion CMR in patients being assessed for renal transplantation was planned, comparing findings at CMR with angiography. Unfortunately, with the emergence of the link between gadolinium and nephrogenic systemic fibrosis, all plans to perform prospective trials using gadolinium contrast were halted.

One CMR application which is likely to provide further insight into myocardial metabolism in uraemia is phosphorous-31 magnetic resonance spectroscopy (^{31}P-MRS). This does not require a contrast agent. By analysing left ventricular high-energy phosphates the derived phosphocreatine/adenosine triphosphate ratio can be calculated which indicates the energy metabolism and the phosphate potential (energy charge) of the myocardium. In a cross sectional pilot study, uraemic patients had higher LV mass, lower phosphocreatine/adenosine triphosphate ratio in compared to patients with a kidney transplant and healthy controls(204). The prognostic implications of these findings are unclear, but this may be an attractive target for intervention, with the goal of improving myocardial metabolism.

Identifying vulnerable coronary artery plaque has in patients with coronary artery disease has been an area of intense research with CMR. As the coronary arteries are difficult to image due to motion artefact, the carotid arteries offer the most easily accessible site for non-invasive assessment of atherosclerotic disease. Assessment of atherosclerotic plaque anatomy, size and characterisation by MR is based on the signal intensities and morphological appearance of the plaque on T1-weighted, proton dense-weighted and T2-weighted sequence. Current level of resolution on MRI allows the assessment of the composition of the carotid atherosclerotic plaque, between fibrous tissue, lipid and necrotic core, calcium, haemorrhage, thrombus, and the state of the fibrous cap, based on their different signal intensities(358). This technique would be applicable to study in patients with ESRD, where it is not clear if rates of cardiovascular events are related to the stability of the atherosclerotic plaque, or the subsequent haemodynamic effects which follow plaque rupture and thrombosis, occluding a less compliant vessel, where compensatory reperfusion may be harder to achieve. CMR can

additionally assess plaque progression or regression with time and in response to therapy(359).

Finally, there has been a great deal of interest in the use of ultrasmall superparamagnetic particles of iron oxide to label macrophages within the vulnerable atherosclerotic plaque. Accumulation of these particles in macrophages in predominantly ruptured and rupture-prone human atherosclerotic lesions has been demonstrated in the vessels of patients undergoing carotid endarterectomy using CMR(360). Whether this technique is safe in ESRD patients is unknown.

10.7 Interventions to reduce cardiovascular risk in end stage renal disease

There remains a pressing need to reduce the risk of future cardiovascular events in patients with ESRD. One clear goal which, although is not currently evidence based is likely to benefit all patients is the aggressive treatment of cardiovascular risk factors in patients with less severe chronic kidney disease, who do not yet (and may never) require dialysis therapy. At this point, these risk factors may still be reversible, and therapies directed at treating hypertension, hypercholesterolaemia, smoking and lifestyle are likely to reduce both cardiovascular risk and progression of renal disease. This strategy has been adopted in the United Kingdom with the advent of initiatives aimed at improving management of patients with less severe degrees of chronic kidney disease in primary care(361).

Once patients have been established on dialysis therapy randomised placebo-controlled clinical trial data on the efficacy of cardioprotective medications patients are sparse. Cice *et al* in a prospective trial, demonstrated the benefits of beta blockers in dialysis patients with dilated cardiomyopathy showing improved 2-yr survival with

carvedilol(362). Using angiotensin converting enzyme inhibitors, the Fosinopril in Dialysis (FOSIDIAL) trial however was unable to show a reduction in cardiovascular events with fosinopril in 397 dialysis patients(363), although a smaller Japanese trial of 80 patients found a reduction in mortality with the angiotensin receptor blocker candesartan(364). Larger randomised clinical trials to assess the efficacy and safety of cardioprotective agents for primary prevention of cardiovascular morbidity and mortality (including cardiac arrest) in ESRD patients would be welcome, but their design and implementation may be fraught with difficulty both in terms of scale and logistics. Many physicians find it hard to withhold beta blockers in patients already considered at such high vascular risk, whilst other nephrologists point to better survival in centres where patients are maintained with long dialysis schedules and minimal use of anti-hypertensives, including beta blocking medication(19). The evidence, where it exists, for lipid lowering therapy in ESRD remains unclear. Although the 4D study showed no benefit in treating diabetic dialysis patients with atorvastatin compared to placebo(36), further large prospective randomised controlled trials of lipid lowering therapy in advanced- or end stage renal disease, such as AURORA (A study to evaluate the Use of Rosuvastatin in subjects On Regular haemodialysis: an Assessment of survival and cardiovascular events) and SHARP (Study of Heart and Renal Protection) will report in the next two to five years(365;366).

Perhaps, rather than targeting conventional cardiovascular risk factors, trialling therapeutic interventions at calcium-phosphate imbalance or hyperparathyroidism, may be a more appropriate approach in ESRD. Already, it has been demonstrated that using sevelamer carbonate compared to calcium containing phosphate binders results in less coronary calcification with a possible mortality benefit(143;148). The treatment of secondary hyperparathyroidism with the calcimimetic cinacalcet leads to significant

improvements in biochemical parameters but patient-based benefits have not yet been demonstrated in trials(367). Potentially this agent may yet have benefits by counteracting the adverse cardiovascular effects of parathyroid hormone in uraemia.

Finally, in patients with a history of cardiac arrest or left ventricular systolic dysfunction, there is growing evidence that implantable cardiac defibrillators improve survival(96). A primary prevention trial of implantable cardiac defibrillators for the prevention of sudden cardiac death in ESRD may be theoretically feasible, particularly among dialysis patients with left ventricular ejection fraction <35% who comprise a high-risk population for sudden death. Potentially the microwave T-wave alternans test may used as a method to identify high-risk patients who would require an implantable cardiac defibrillator and also classify a low-risk group unlikely to benefit from ICD therapy(368).

10.8 CONCLUSIONS

- Cardiovascular disease is the leading caused of death in patients with chronic renal failure, both prior to commencing dialysis and on renal replacement therapy. The incidence is several times greater than that of age matched individuals from the general population
- Increased risk of cardiovascular disease in this population is related to both changes in cardiac structure and function, as well as to alterations in the major blood vessels
- CMR is the current optimal method for assessing cardiac morphology and function as well as interrogating myocardial tissue abnormalities
- Although previous reports suggest uraemic cardiomyopathy is a combination of left ventricular hypertrophy, dilatation and systolic dysfunction, studies in this thesis suggest that there are two main forms of uraemic cardiomyopathy; left ventricular hypertrophy is the cardiomyopathy specific to uraemia whilst left ventricular systolic dysfunction reflects underlying coronary artery disease. Studies in this thesis have demonstrated the presence of diffuse myocardial fibrosis in the presence of left ventricular hypertrophy
- Echocardiography appears to overestimate left ventricular mass compared to CMR in this population
- Although uraemic cardiomyopathy does have an impact on long term survival in this patient cohort, conventional cardiovascular risk factors such as advanced age, diabetes and a history of ischaemic heart disease confer poorer survival, whilst receiving a renal transplant is likely to be protective for future cardiovascular events
- Serum brain natriuretic peptide, whilst elevated in patients with evidence of uraemic cardiomyopathy does not appear to a sensitive or specific enough

biomarker to be used as a diagnostic aid in this patient group and conveys little prognostic value

- Arterial function measured as aortic distensibilty using CMR is a powerful independent predictor of future cardiovascular events
- There appears to be a link between the use of some gadolinium containing CMR contrast agents and development of nephrogenic systemic fibrosis, a potentially fatal condition, characterised by skin thickening, pain and immobility
- Both left ventricular hypertrophy and arterial stiffness measured by CMR are potential targets for intervention to reduce cardiovascular risk in patients with ESRD. Future work to is required to assess whether it is possible to modify arterial stiffness in these patients in which case this parameter may be used as a surrogate marker for treatments to reduce cardiovascular events in this high risk group

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