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THE MECHANISMS AND CONSEQUENCES OF HAEMODIALYSIS INDUCED ACUTE CARDIAC INJURY

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Thesis submitted to the

School of Graduate Entry Medicine and Health,

University of Nottingham for the degree of

DOCTOR OF MEDICINE

January 2009

‘The important thing is not to stop questioning.’

Albert Einstein (1879 – 1955)

For

Hen, Felix and Charlotte.

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Abstract

Patients on dialysis are subject to a hugely elevated risk of cardiovascular mortality. Incidence and prevalence of, and mortality and morbidity from heart failure is significantly higher in the haemodialysis population than the general population as a whole. This thesis describes research work focusing on the large scale haemodynamic changes that occur during haemodialysis and how they may negatively impact on the cardiovascular system.

Our results show that the haemodynamic disturbances which occur during haemodialysis are capable of causing a reduction in myocardial blood flow sufficient in magnitude to induce myocardial ischaemia. This is associated with a matched reduction in regional left ventricular (LV) function and is entirely in keeping with other published work describing haemodialysis induced myocardial stunning reflecting subclinical myocardial ischaemia (myocardial stunning).

In addition, we now know that this phenomenon of haemodialysis induced myocardial ischaemia and stunning is common and associated with both short and long term complications including ventricular arrhythmias, left ventricular dysfunction, an increased hazard of death and time to first cardiovascular event. This is pertinent as in non-dialysis patients repeated episodes of myocardial stunning lead to chronic heart failure, and in dialysis patients the presence of LV dysfunction dramatically increases the risk of death.

We also identified a number of factors associated with the presence of myocardial stunning including age, raised biochemical markers of cardiac damage (troponin-T), higher ultrafiltration volumes and lower intradialytic blood pressure. This is of crucial importance as ultrafiltration volumes and intradialytic haemodynamics are potentially modifiable risk factors that could provide targets for dialysis based interventions aimed at improving cardiovascular outcomes in the haemodialysis population.

Declaration

Except where acknowledged, I declare that this thesis is entirely my own work and is based upon research carried out in the School of Graduate Entry Medicine and Health, University of Nottingham and Department of Renal Medicine, Derby Hospitals NHS Foundation Trust between January 2006 and April 2008.

Ethical and local approval for this work (except the myocardial perfusion data) was sought and gained by me. Funding was successfully granted after applications to Kidney Research (UK) and with an unrestricted educational grant from Roche Pharmaceuticals.

All measures of myocardial function (echocardiograms) were undertaken and analysed by me (total of 930 apical scans) after appropriate training and assessment by British Society of Echocardiography accredited instructors and examiners.

Myocardial perfusion scans were kindly analysed by medical staff at the MRC Clinical Sciences Unit, Hammersmith Hospital, London, UK.

Assessment of electrophysiological abnormalities with 24-hour Holter monitoring was done in conjunction with a student from the Department of Clinical Measurement.

Biochemical and haematological data were provided by the laboratories at Derby Hospitals NHS Foundation Trust. Separate ELISAs were performed where necessary by laboratory staff in the School of

Graduate Entry Medicine and Health, University of Nottingham Medical School at Derby.

All statistical analysis was initially performed by me and then verified prior to publication by an independent statistician, especially in the case of more complex statistical models.

James O Burton

January 2009.

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I am hugely grateful to a number of people who helped at various times with a number of aspects of this thesis. Individually, these are:

- First and foremost I would like to thank my supervisor Chris McIntyre. He has been interested, involved and supportive all through my time in Derby, both professionally and personally. He has inspired and encouraged me to see the enjoyable side of research and paved the way to my career in academic medicine.

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- And finally to my wife who kept (and keeps) me going.

Publications and abstracts arising from this thesis

Peer reviewed publications

- Burton JO, Jefferies HJ, Selby NM, McIntyre CW. Haemodialysis induced repetitive myocardial stunning results in global and segmental reduction in systolic cardiac function. *Eur J Heart Fail* 2009 (under review).

- Burton JO, Selby NM, Jefferies HJ, McIntyre CW. Haemodialysis induced cardiac injury: determinants and associated outcomes. *Clin J Am Soc Nephrol* 2009; (in press).

- Burton JO, Korsheed S, Grundy BJ, McIntyre CW. Hemodialysis induced left ventricular dysfunction is associated with an increase in ventricular arrhythmias. *Ren Fail* 2008; 30(7): 1-9.

- McIntyre CW, Burton JO, Selby NM, Leccisotti L, Korsheed S, Baker CS, Camici PG. Haemodialysis induced cardiac dysfunction is associated with an acute reduction in global and segmental myocardial blood flow. *Clin J Am Soc Nephrol* 2008; 3:19-26.

Oral presentations

- Burton JO, Jefferies HJ, Korsheed S, McIntyre CW. Repeated episodes of haemodialysis induced myocardial stunning lead to myocardial hibernation and reduction in overall systolic function. *BRS / RA Annual Conference* 2008; O33.

- Burton JO, Jefferies HJ, Korsheed S, McIntyre CW. Haemodialysis induced myocardial stunning is associated with a reduced 12-month survival. *BRS / RA Annual Conference* 2008; O34.

- Burton JO, Jefferies HJ, Korsheed, McIntyre CW. Serum cardiac troponin-T concentrations are associated with outcome in patients with myocardial stunning [Abstract]. *NDT Plus* 2008; 1(S2): ii420.

- Burton JO, Korsheed S, John SG, McIntyre CW. Haemodialysis induced cardiac injury is associatd with asymptomatic hypotension [Abstract]. *J Am Soc Nephrol* 2007; 18: 67A.

- Burton JO, Korsheed S, John SG, McIntyre CW. The prevalence and variability of haemodialysis induced acute myocardial stunning. *Renal Association Annual Conference* 2007; O22.

Poster presentations

- Burton JO, Korsheed S, Jefferies HJ, McIntyre CW. Haemodialysis induced myocardial stunning is associated with a reduced 12-month survival [Abstract]. *NDT Plus* 2008;1(S2): ii132.

- Burton JO, Korsheed S, Jefferies HJ, McIntyre CW. Ultrafiltration volume is an independent risk factor for myocardial stunning in haemodialysis patients [Abstract]. *NDT Plus* 2008;1(S2): ii130.

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- Burton JO, Selby NM, Korsheed S, Leccisotti L, Camici PG, McIntyre CW. Haemodialysis causes an acute reduction in global and regional myocardial blood flow as measured by $H_2{}^{15}O$ PET scanning [Abstract]. *J Am Soc Nephrol* 2007;18: 508A.

- Burton JO, Korsheed S, Grundy BJ, McIntyre CW. Haemodialysis induced myocardial stunning is associated with an increase in ventricular arrhythmias [Abstract]. *J Am Soc Nephrol* 2007;18: 508A

- Burton JO, Selby NM, Leccisotti L, Korsheed S, McIntyre CW, Camici PG. Haemodialysis Induced Contractile Dysfunction is

Associated with Acute and Reversible Reduction in Global and Segmental Myocardial Blood Flow [Abstract]. *Circulation* 2007;116(16): 757a.

- Burton JO, Korsheed S, McIntyre CW. The association of circulating cardiac biomarkers with the frequency and severity of left ventricular wall motion abnormalities [Abstract]. *Nephrol Dial Transplant* 2007;22(S6): vi311.

- Burton JO, Korsheed S, John SG, McIntyre CW. The prevalence and variability of haemodialysis induced acute myocardial stunning [Abstract]. *Nephrol Dial Transplant* 2007;22(S6): vi327.

Chapter 1

Introduction

1 Introduction

1.1 End stage renal disease

1.1.1 Background

End stage renal disease (ESRD) is the final stage of chronic kidney disease (CKD) and is defined as the failure or near failure of the kidneys to perform their normal functions. These functions include: excretion; maintenance of acid-base, fluid and electrolyte balance and the synthesis of hormones such as erythropoietin and renin.

ESRD usually results from a progressive and irreversible loss of renal function and is defined by a glomerular filtration rate (GFR) of less than 15 ml/min. The Kidney Disease Outcomes Quality Initiative (K/DOQI 2002) guidelines have CKD into five stages of which ESRD is the fifth (table 1.1.1a).

When a patient reaches ESRD, renal replacement therapy (RRT) in the form of dialysis or transplantation must be considered as without treatment, symptoms will likely significantly deteriorate within weeks or months.

Stage	GFR	Description
1	90+	Normal kidney function but urine findings or structural abnormalities or genetic trait point to kidney disease
2	60-89	Mildly reduced kidney function, and other findings (as for stage 1) point to kidney disease
3	30-59	Moderately reduced kidney function
4	15-29	Severely reduced kidney function
5	<15	Very severe, or **established renal disease**.

Table 1.1.1a: The stages of chronic kidney disease (CKD).

1.1.2 Epidemiology

Chronic kidney disease remains a significant health problem and it is now appreciated that it plays a significant role in increasing risk for many other disease processes. It is also apparent that the number of patients with CKD in the population is increasing year on year. The prevalence of CKD in the United States in 1999-2004 was significantly higher than it was in 1988-1994 with an increase in the total prevalence of CKD stages 1-4 from 10% to 13.1% over that time [1] (see figure 1.1.2a). The same study also showed that a higher prevalence of diagnosed diabetes, hypertension and higher body mass index explained the entire increase in prevalence of albuminuria but only part of the increase in the prevalence of decreased GFR.

Estimation of the prevalence of earlier stages of CKD in the population and understanding trends over time is central to disease management and prevention planning, particularly given the increase in the prevalence of obesity, diabetes and hypertension, the leading risk factors for CKD and cardiovascular disease.

The early stages account for most of the individuals with CKD but because individuals with any stage of CKD have a higher risk of cardiovascular disease morbidity and mortality than their risk of progression to ESRD, cardiovascular risk factor management in this group is critical. The high prevalence of CKD overall, and particularly among older individuals and persons with hypertension and diabetes, suggests that CKD needs to be a central part of future public health planning. In addition, the increasing prevalence of diagnosed diabetes and hypertension may in turn lead on to higher rates of complications and ESRD requiring dialysis or transplantation.

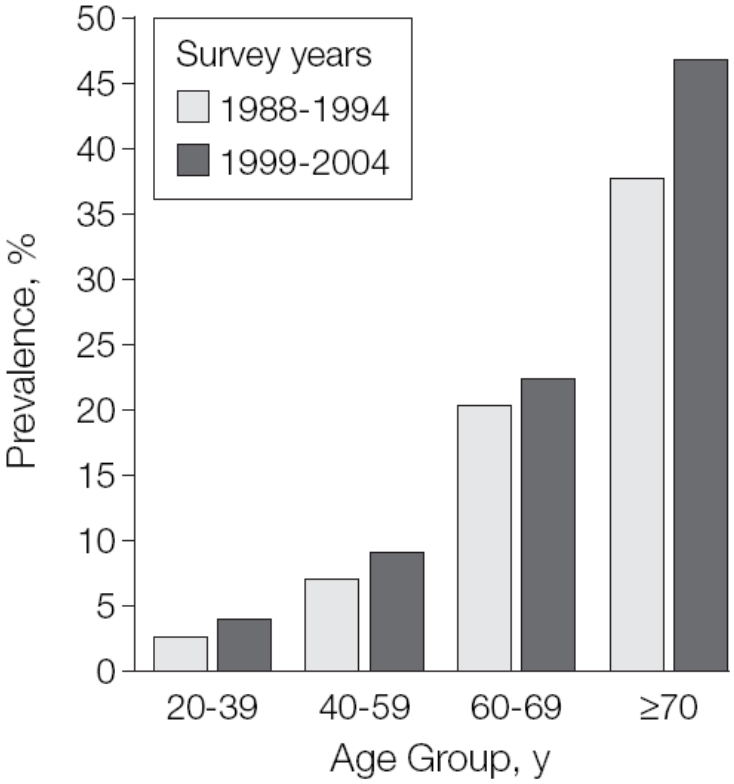


Figure 1.1.2a: Prevalence of chronic kidney disease (stages 1-4) by age group in the National Health and Nutrition Examination Surveys (NHANES) 1988-1994 and 1999-2004 [1].

The projected number of ESRD patients is also expected to rise. In the United Kingdom, the incidence of ESRD, as measured by the number of patients commencing renal replacement treatment, varies between 80 and 110 new patients per million of population (pmp) per year (UK Renal Registry 2002). In the United States, the incidence of ESRD is much higher at around 315 pmp in 1999 (USRDS 2001). In general, the

incidence of ESRD increases with age, reaching around 1300 pmp per year in patients aged over 65 years.

Year	Projected Incidence	Projected Prevalence
2005*	106,896	484,995
2010	120,253	579,105
2015	134,978	679,918
2020	150,772	784,613

*Table 1.1.2a: Expected incidence and prevalence rates for ESRD in the United States up to 2020 (*Actual figures are quoted for 2005)* [2].

In 2001, a projected analysis undertaken in the United States suggests that the incidence of ESRD will continue to increase until 2010 at a rate of 6–7 per cent per year [3]. More recent studies have put the projected number of ESRD patients at approximately 785,000 by 2020 – an increase of 60 percent compared to the number of ESRD patients in 2005 [2] (see table 1.1.2a). Again, this takes into account the rising rates of a number of factors including obesity and diabetes. Given the costs associated with all treatment modalities for ESRD requiring renal replacement therapy, this has significant health service implications.

1.1.3 Health service implications

The increase in the number of prevalent patients requiring renal replacement therapy (RRT) has a significant impact on the provision of services within a health service that has a limited budget.

The cost of a kidney transplant is £20,000 per patient per transplant with immunosuppression costs of £6,500 per patient per year subsequently. The average cost of dialysis is £30,000 per patient per year. Despite the recognised health and social benefits of transplantation (which remains the gold standard for treating ESRD) dialysis remains the treatment for the majority of patients due to a shortage of donor kidneys and an increasingly older ESRD population, many of whom are unfit for transplantation surgery.

In 2003, 67.5 per cent of RRT patients in the UK were receiving haemodialysis and 29.2 per cent peritoneal dialysis (3.3 per cent had a transplant). This large proportion of patients on haemodialysis (HD) has a significant impact on cardiovascular morbidity and mortality, which will form the basis of this thesis.

1.2 Renal replacement therapy

1.2.1 Background

The basic principles of dialysis were described by Thomas Graham over 100 years ago. Even though the first treatments for acute renal failure were performed in the 1920s, chronic dialysis treatment for end

stage renal failure did not become a reality until 1960. In the following few years a series of breakthroughs, in both dialysis technology and vascular access, enabled chronic renal replacement therapy to be established in both the US and Europe by the mid 1960s.

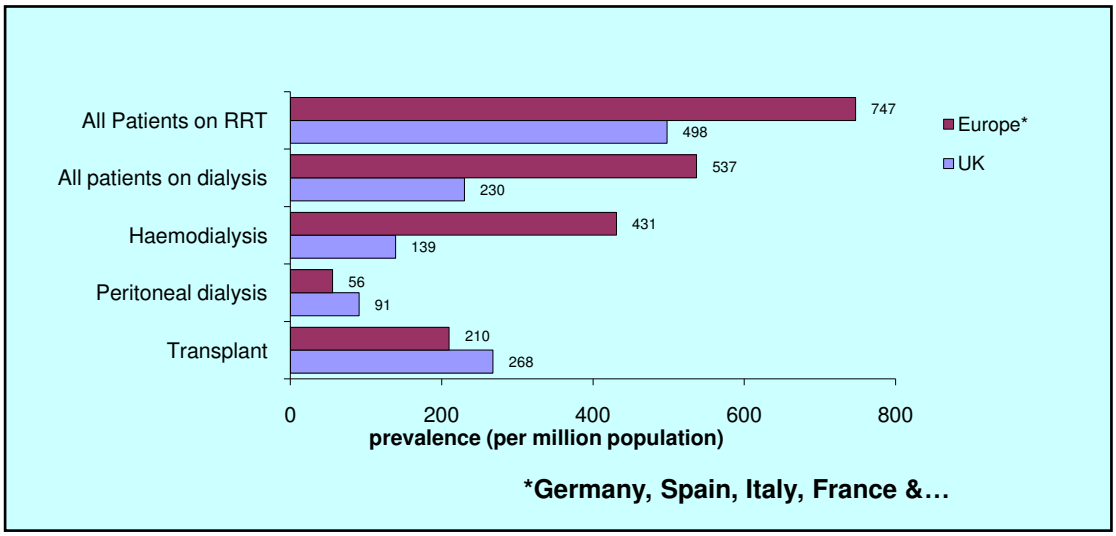


Figure 1.2.1a: Patient numbers and modalities of treatment in ESRD patients requiring renal replacement therapy.

Chronic haemodialysis (HD) became widely available in the UK in the early 1970s (largely as a home based therapy) and continuous ambulatory peritoneal dialysis (CAPD) became increasingly popular during the early 1980s. There are now some 1.5 million patients receiving regular dialysis worldwide and around 25,000 in the UK alone (see figure 1.2.1a).

1.2.2 Haemodialysis

Standard haemodialysis is performed three times a week, for 3–5 hours each session, with low-flux membranes (see figure 1.2.2a). Since the 1960s there has been a general technical improvement in dialysis machines, dialysers, and dialysis solutions. These factors, as well as accurate control of the ultrafiltration rate by new dialysis machines and more precise control of electrolyte dialysate composition, have improved haemodynamic stability during treatment. The substitution of acetate as the dialysate buffer with bicarbonate has also improved vascular stability.

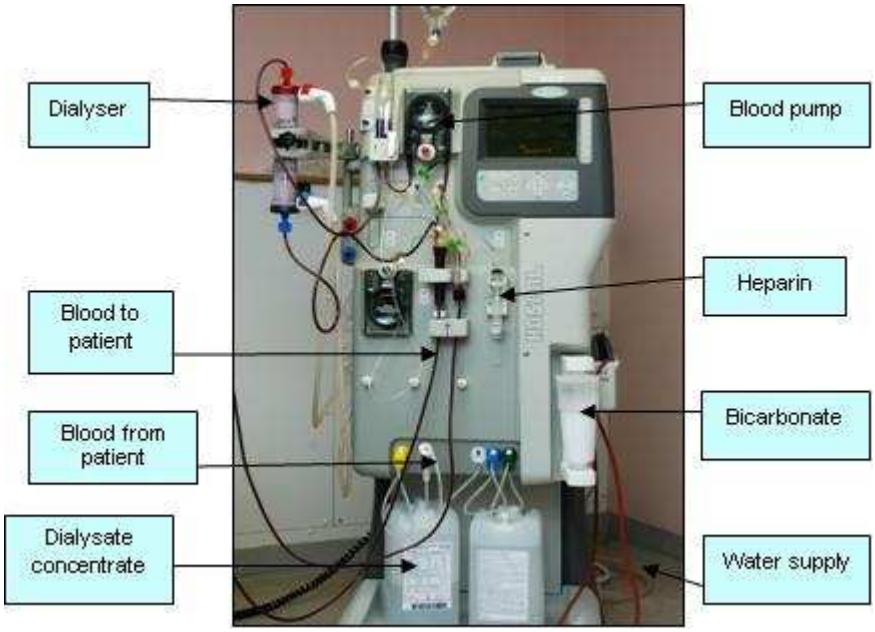


Figure 1.2.2a: A standard haemodialysis machine [4]

The principle of haemodialysis is relatively straightforward. Blood flows on one side of a semi permeable membrane, and dialysis fluid, an osmotically balanced solution of electrolytes, buffer, and glucose in water, flows on the other.

Haemodialysis (HD) was first described in the early 1900s. However, it was not until the 1960s that HD became a viable technique for the long-term treatment of patients with ESRD. This was enabled by the development of a reliable and safe Teflon arteriovenous shunt that allowed adequate rates of blood flow, and also by the availability of heparin to prevent clotting in the extracorporeal circuit.

Although viable, early HD was beset with problems. Poor predictability of the dialysers in terms of solute removal led to long treatment times. Ultrafiltration (UF) was controlled manually by adjustment of the blood and dialysate pumps to create a pressure gradient that led to unpredictable UF rates. Blood leaks in the dialysers and lines were common and there were concerns about bacterial contamination of the dialysate fluid. Also, it was not possible to produce a commercially viable dialysate solution that contained bicarbonate as a buffer because of bacterial contamination of the liquid bicarbonate. Therefore, non-physiological acetate was employed as a buffer that diffused into the patient and was subsequently converted into bicarbonate by the liver. However, acetate transfer into the patient strongly predisposes to intradialytic symptoms, in particular hypotension (IDH), headaches and hypoxia [5].

Significant advances have been made over the subsequent years. The advent of hollow fibre dialysers greatly increased the efficiency of small solute removal and led to shorter treatment times, although shorter dialysis sessions with greater UF rates may themselves contribute to IDH. Dialysis membranes are now synthetic, which improves their biocompatibility. Bicarbonate-based dialysis became possible using dry bicarbonate concentrates that overcame the problems of precipitation and storage of large amounts of bicarbonate solution. In the 1980s, the concept of Kt/V, a measure of the amount of urea cleared by dialysis in relation to body size, became widely accepted. Importantly, it was shown that too little dialysis (as measured by a low Kt/V) was associated with increased mortality [6,7]. Finally, various modifications of the dialysis technique have become common practice. The most widespread of these is haemodiafiltration (HDF), a technique based on additional convective clearance due to high UF volumes and subsequent fluid reinfusion. HDF may confer benefit in terms of stability on dialysis and greater clearance of some of the larger uraemic toxins.

However, despite these technological advances HD remains an imperfect treatment. Dialysis only partially replicates the removal of fluid and uraemic toxins by the kidney, and does not replace any of its hormonal functions. The intermittent nature of dialysis dictates repeated, rapid shifts between fluid overload and euvolaemia, and IDH remains a complication of up to a third of treatments [8]. In addition, the cardiovascular death rate of haemodialysis patients is extraordinarily high [9].

1.3 Cardiovascular disease in dialysis patients

1.3.1 Epidemiology

The frequency of fatal and non-fatal cardiovascular events is increased even in the earliest stages of chronic kidney disease [10-13] (see figure 1.3.1a).

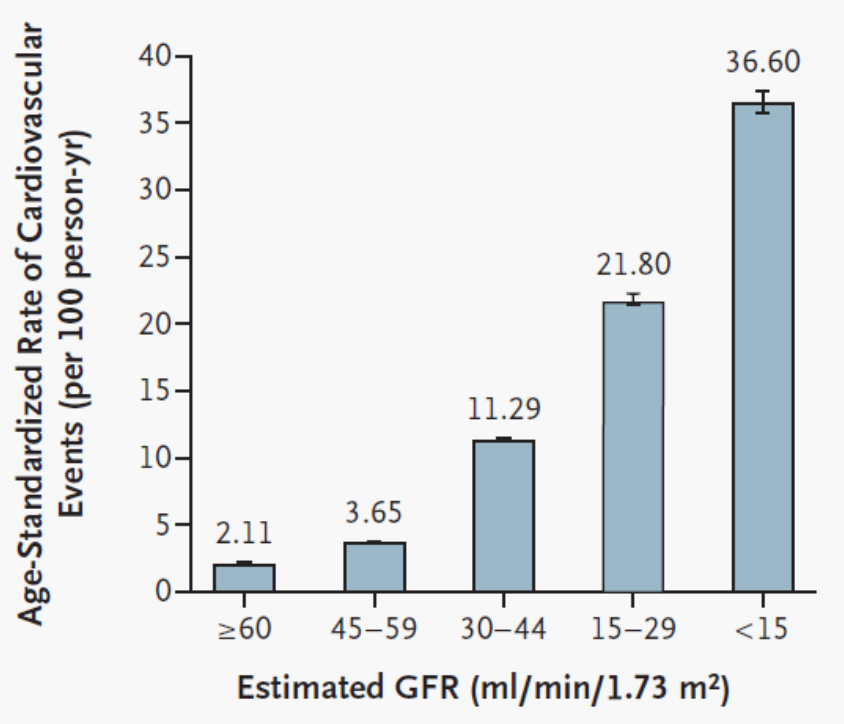


Figure 1.3.1a: The relative risk of cardiovascular events with respect to eGFR. Those patients with CKD stages 4 or 5 have the highest risk [13].

This risk is even higher within dialysis patients who display hugely elevated rates of cardiac mortality, at least thirty-times greater than age-matched controls [9] (figure 1.3.1b).

In the *HEMO* study, the most common cause of death in dialysed patients was ischaemic heart disease (20.4 per cent) followed by cardiac rhythm disorder (10.4 per cent), cerebrovascular disease (8.6 per cent), and infections (7.7 per cent) [14]. According to the Annual Report 2001 of the United States Renal Data System (USRDS), the incidence of new myocardial infarction in the first year of renal replacement therapy was 7.0 per cent, of cerebrovascular accidents 7.1 per cent, and of surgery for peripheral vascular disease 8.4 per cent.

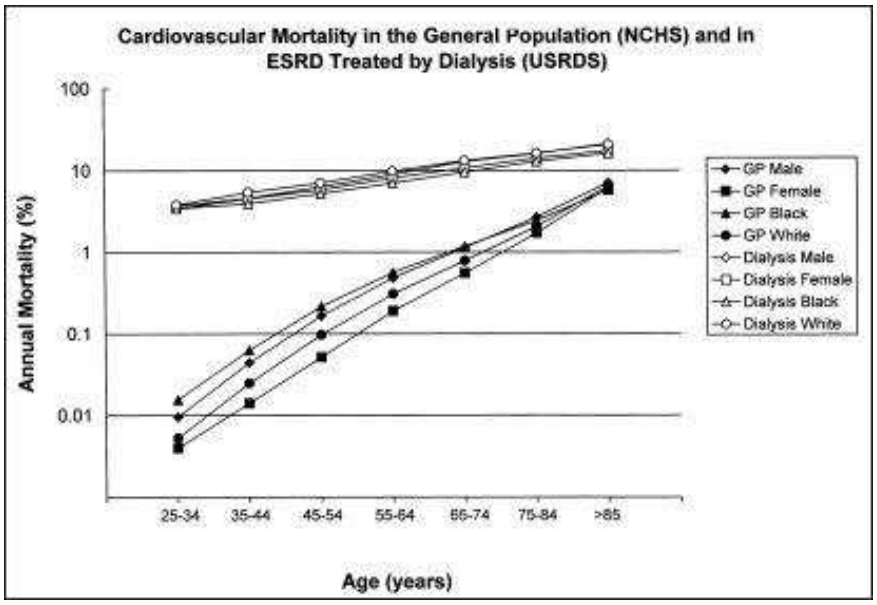


Figure 1.3.1b: Graph showing the cardiovascular mortality rates by age and ethnicity for dialysis patients and healthy controls. The y-axis is a logarithmic scale, emphasising the huge excess of cardiovascular death seen with dialysis patients [9].

As a crude comparison, the Framingham Heart Study reported that the annual risk of re-infarction in subjects surviving a first recognised myocardial infarction was 4 per cent per year, almost half the risk of new myocardial infarction in HD patients [15] for whom the additional risk factor of uraemia is compounded by also being on dialysis.

1.3.2 Risk factors

Such high levels of cardiovascular disease in dialysis patients are in part due to the high prevalence of 'traditional' risk factors (diabetes, hypertension, hyperlipidaemia, smoking, and physical inactivity). Unfortunately, aggressive management of these traditional risk factors in dialysis patients has failed to adequately control the progression of cardiovascular disease. For example, in uraemic patients, LV hypertrophy progresses with time on dialysis even when patients are kept normotensive [16,17]. In the study of Parfrey, 71 per cent of non-diabetic dialysis patients without dilated cardiomyopathy had LV hypertrophy and in the majority of patients this progressed over a period of 3–4 years. Progression was not predicted by blood pressure, hyperparathyroidism or anaemia (although these factors are definitely involved), suggesting that additional factors may play a part. Therefore this excess of cardiovascular disease in dialysis patients must be explained by the presence of other unique metabolic and haemodynamic derangements specific to the uraemic patient – the so-called 'uraemic' specific risk factors. These uraemic risk factors are less

well defined but are multiple, including some of those mentioned above already.

It is not only systolic and diastolic blood pressures which determine LV work and LV mass. Vascular calcification with increased vessel stiffness is common, associated with the development of left ventricular hypertrophy (LVH) [18] and also independently predicts mortality [19]. Stiffening of the aorta and the associated increased impedance play a major role in the development of LVH [20]. High pulse pressure is a surrogate marker for aortic stiffness and pulse wave velocity is a potent predictor of LV mass and cardiovascular events [7,21,22].

Diabetic patients with ESRD have particularly high cardiovascular morbidity and mortality [23]. Even diabetic patients without nephropathy have major abnormalities of cardiac structure. In observational studies, when compared to non-diabetic individuals, diabetic patients have more severe LV hypertrophy and also develop ischaemic heart disease more frequently [24].

Anaemia is a common consequence of ESRD. Sustained anaemia leads to vasodilatation, increased venous return, cardiac enlargement and increased cardiac output [25,26]. Numerous observational studies documented that anaemia is associated with increased LV mass in patients with CKD [27] and this is true even for apparently trivial degrees of anaemia [28]. Observational studies have also suggested that anaemia is an independent predictor of mortality [29,30]. Partial correction of anaemia partly corrects LV hypertrophy [31-33] but there is little or no

controlled evidence that reversal of anaemia reduces cardiovascular mortality. In the Canadian Normalisation of Hemoglobin Trial, haemodialysis patients with asymptomatic echocardiographic enlargement were randomly treated to haemoglobin of 10 or 13.5 g/dl. The higher haemoglobin failed to show a regression in left ventricular dilatation but subsequent studies have shown that a normal haemoglobin will prevent the development of new LV dilatation [34].

Recurrent volume overload with rapid fluid shifts (that occur on dialysis) increases cardiac filling pressures and venous return, imposing an increased work load on the left ventricle. Eventually this increase in preload results in LV dilatation and LVH. A correlation is found between LV volume and blood volume. In patients with fluid overload the heart diameters usually return to normal a few hours after ultrafiltration, but chronic overload may lead to eccentric LV hypertrophy and irreversible dilatation. The importance of hypervolaemia is illustrated by the observation of Ozkahaya *et al*. that volume control by low salt diet and aggressive ultrafiltration reversed LV dilatation and hypertrophy despite no administration of antihypertensive agents [35,36].

Cardiac mortality in dialysis patients has also been linked to chronic inflammation, often manifest as hypoalbuminaemia and elevated C-reactive protein (CRP) levels. Inflammation leads to accelerated atherosclerosis, vascular calcification and increased muscle catabolism [37]. Certainly, the elevation of several cytokines (in particular CRP and

interleukin-6) have also been shown to be associated with an increase in mortality [38,39].

1.3.3 Left ventricular hypertrophy

The presence of LVH itself predicts a worse long term outcome, and is associated with an increased propensity to cardiac arrhythmias [40,41]. LVH is more frequent in early stages of chronic kidney disease and increases progressively, so that it is found in approximately 70 per cent of patients starting renal replacement therapy [28]. Initially concentric LVH is seen, while in later stages more frequently eccentric LVH prevails. LVH tends to be associated with hypertension, anaemia, high arteriovenous fistula flow and poor control of volume overload [31] as mentioned above. At an early stage, systolic function is usually normal or even increased, but evidence of diastolic malfunction – including reduced compliance and abnormal passive filling of the LV – can be found even in asymptomatic patients. LVH is not an innocent academic finding: Silberberg *et al*. had clearly documented that it was an independent predictor of death on dialysis [42] (figure 1.3.3a).

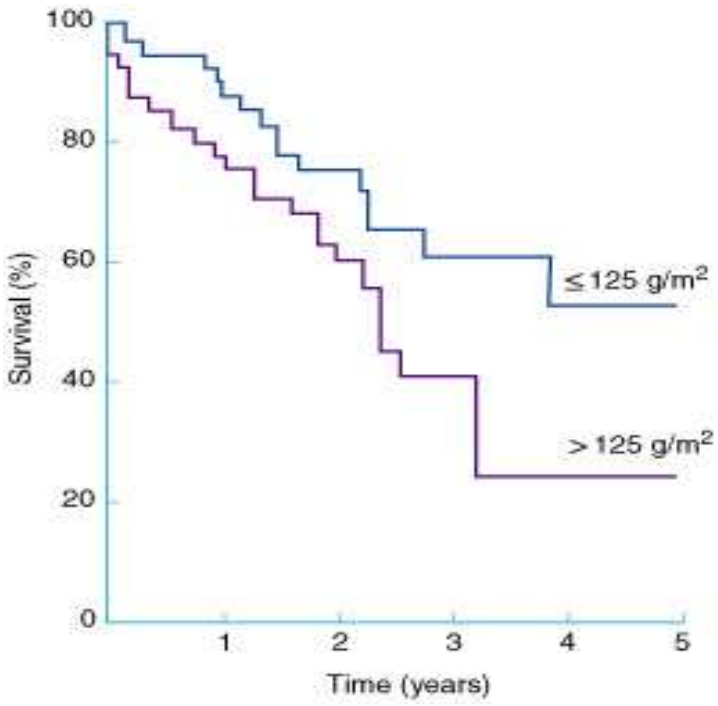


Figure 1.3.3a: LVH reduces survival in dialysis patients. Cumulative survival according to echocardiographic LV hypertrophy defined as LV mitral inflow greater than 125 g/m2. This cut-off point corresponds to the 95th centile of the normal population [42].

1.3.4 Myocardial ischaemia and haemodialysis

It has long been suspected that myocardial ischaemia may be precipitated by haemodialysis, with the first evidence of silent ST segment depression during dialysis reporting back to 1989 [43]. However, this concept of dialysis induced subclinical ischaemia (occurring without

acute atherosclerotic plaque rupture) has received remarkably little attention, despite its theoretical plausibility.

Short intermittent haemodialysis treatments exert significant haemodynamic effects, and 20-30 per cent of treatments are complicated by intra-dialytic hypotension (IDH) [44-46]. In conjunction with this, haemodialysis patients are particularly susceptible to myocardial ischaemia. In addition to the high prevalence of coronary artery atheroma [47,48], diabetic dialysis patients have been shown to have a reduced coronary flow reserve (CFR) – the ability of coronary arteries to dilate when myocardial demand is increased – even in the absence of coronary vessel stenoses [49].

There is preliminary evidence that the same phenomenon is also seen in non-diabetic dialysis patients [50] which may be due to LVH, that leads to both structural and functional reductions in the myocardial microcirculation. The presence of LVH on its own reduces CFR even in the absence of large vessel coronary disease [51]. Because of the increased extravasal resistance, coronary reserve is reduced as illustrated in the patient with aortic valve stenosis. They suffer from angina pectoris resulting from ischaemia despite patent arteries. This is particularly important in HD patients in whom ischaemia is often asymptomatic [52].

Similarly the uraemic patient with LV hypertrophy may have ischaemia intolerance when oxygen demand is increased. In addition, the presence of concentric LVH renders the ventricle more sensitive to

acute changes in filling pressure, exactly as occurs during haemodialysis [53]. Increased peripheral artery stiffness is also recognised to have an adverse effect on myocardial perfusion and reduces the ischaemic threshold [54]; therefore, LVH in conjunction with increased vascular stiffness leads to a propensity to reduced subendocardial blood flow [55].

Since the initial report by Zuber et al [43], there have been further studies that have demonstrated silent ST segment depression occurring during dialysis [56-64]. These studies report the occurrence of dialysis induced ST depression at rates that vary between 15 and 40 per cent. However, there has been ongoing debate as to whether these electrocardiographic abnormalities reflect silent ischaemia or changes in electrolyte concentrations. Other than this, there has been only one subsequent study that has demonstrated ischaemia using an alternate technique. Singh et al assessed dialysis induced ischaemia using sestamibi single photon emission computed tomography (SPECT) [65]. In an unselected group of ten dialysis patients who were not known to have coronary artery disease, seven developed perfusion defects during dialysis. Importantly, concurrent ST depression occurred with the perfusion defects in only 3 patients, suggesting that electrocardiographic assessment alone may underestimate the incidence of dialysis induced ischaemia.

Unfortunately, none of these studies included an intervention to attempt to reduce the frequency of dialysis induced ischaemia, nor did any

search for potential long term sequelae on LV function. Also, no studies have, as yet, looked at the direct effect of haemodialysis on both myocardial perfusion and function.

1.3.5 Dialysis induced acute myocardial stunning

In patients with coronary artery disease but without chronic kidney disease, transient myocardial ischaemia may lead to LV dysfunction that can persist after the return of normal perfusion.

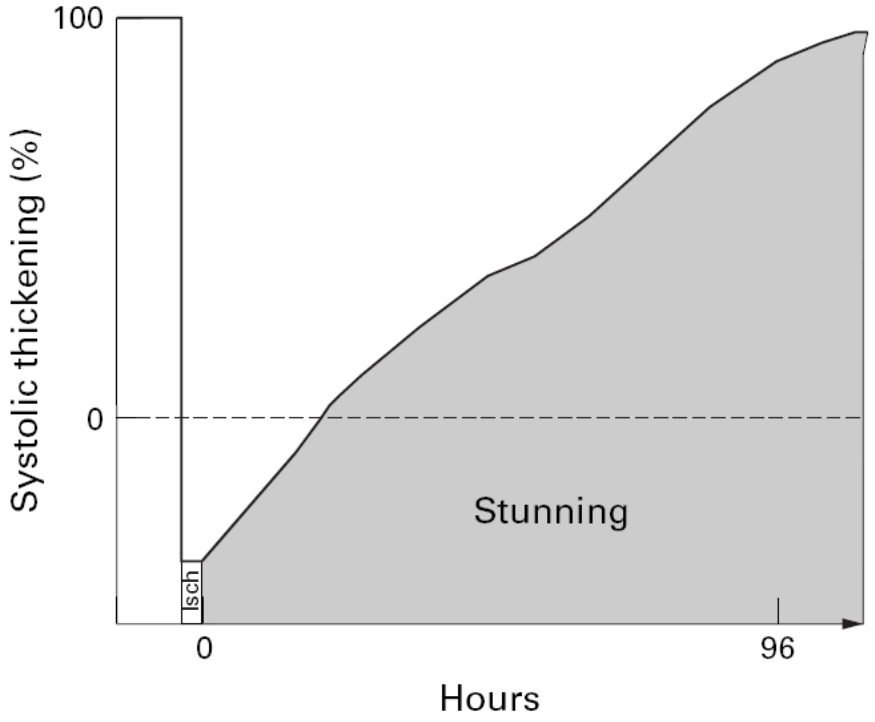


Figure 1.3.5a: The effect of brief coronary occlusion on systolic function. The box 'isch' represents a short episode of ischaemia. The myocardium rapidly becomes dyskinetic but there is a gradual return to normal function. This period between restoration of normal flow and recovery of function is known as myocardial stunning [66].

Animal models have shown that after a brief reduction in coronary blood flow caused by occlusion to the coronary vessels, myocardial contractility falls and becomes dyskinetic. After restoration of normal flow, there is a mismatch between flow and function and this prolonged dysfunction (which can take days to recover) is known as myocardial stunning [66] (figure 1.3.5a).

Myocardial stunning has been demonstrated in humans after exercise and dobutamine stress in patients with coronary artery disease [67,68]. It is thought that transient wall motion abnormalities occur in areas of the myocardium that are subtended by a coronary stenosis of <40% that is not severe enough to diminish resting myocardial blood flow but will impair coronary flow reserve. As a consequence, the myocardium may become ischaemic following exercise (or other haemodynamic stressor) and before it has had time to completely recover, another episode of ischaemia may occur. Such repeated episodes of ischaemia can be cumulative and result in an apparent chronic reduction in left ventricular function [69] (figure 1.3.5b).

It has previously been demonstrated that myocardial stunning occurs as a direct consequence of haemodialysis (and can be ameliorated by improving systemic haemodynamics whilst on treatment) [70,71]. Therefore, if myocardial ischaemia and stunning are induced by haemodialysis then the process of haemodialysis itself, repeated three times a week, may potentially contribute to chronic cardiac damage in this patient group. Myocardial stunning is therefore increasingly thought

to be an underappreciated causative mechanism for heart failure in the haemodialysis population.

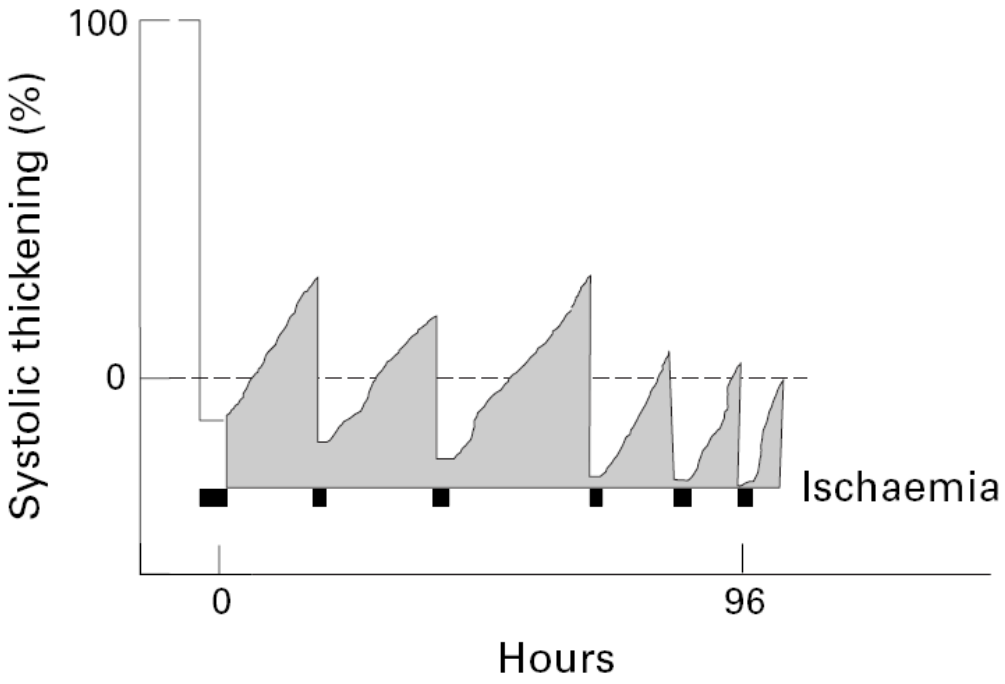


Figure 1.3.5b: The effect of repetitive myocardial stunning on contractile function. The black boxes indicate short episodes of ischaemia. After restoration of flow, function begins to improve but before returning to normal a further episode of ischaemia results in a further reduction in contractility. Repeated short ischaemic episodes may therefore result in a chronic impairment of myocardial function [72].

Recent studies in the non-dialysis population have provided evidence that repetitive episodes of ischaemia can be cumulative and lead to prolonged left ventricular dysfunction through the process of

myocardial hibernation [66,68,73-77]. However, none of these mechanisms have been studied in the haemodialysis population.

1.3.6 Myocardial hibernation and fibrosis

So, standard conventional thrice weekly haemodialysis may cause repeated episodes of sub-clinical myocardial ischaemia and therefore be a potential cause of repetitive myocardial stunning leading to chronic myocardial hibernation and left ventricular dysfunction. In the non-dialysis population, repeated episodes of demand ischaemia and stunning (before full recovery has taken place) can result in an apparent chronic reduction in left ventricular function [72,78].

This led to the hypothesis that in areas with reduced CFR but normal resting myocardial blood flow, repeated episodes of demand associated myocardial ischaemia led first to stunning and then to chronic myocardial hibernation [79]. Whilst in myocardial stunning there is a flow-function mismatch (return to normal flow but delayed return to normal function), myocardial hibernation is characterised by a matched deterioration in flow and function. Unlike stunned myocardium which can respond to inotropes like dobutamine, hibernating myocardium has little or no response and continues to display fixed areas of systolic dysfunction. Fortunately, both of these adaptive processes are reversible and in patients with obstructive coronary artery disease, revascularisation can lead to restoration of varying degrees of ventricular function [80,81].

Although myocardial hibernation may represent a functional adaptation to chronic hypoperfusion that can be reversed with restoration of regional MBF (the 'smart heart' hypothesis) [82], there is evidence to suggest that hibernating myocardium is still highly vulnerable to increases in demand or reductions in oxygen supply [83]. Therefore, ongoing recurrent episodes of ischaemia precipitated by HD may have negative consequences on this adaptive balance. Hibernation then, in a clinical context is most likely caused by repetitive stunning and these two processes form a continuum on their way to eventual myocardial fibrosis and ventricular dysfunction. Although it is often difficult to distinguish practically between them, both respond to revascularisation and so it is crucial to identify patients at this stage of the pathological process so that they can be targeted for appropriate intervention.

Interestingly, the withdrawal of haemodialysis as the method of renal replacement therapy after kidney transplantation was associated with an increase in left ventricular ejection fraction, an improvement in functional status of congestive heart failure and increased survival [84]. This is similar to the improvement seen in patients with congestive heart failure after coronary revascularisation and lends credence to the argument that it is the process of haemodialysis itself that causes repetitive myocardial ischaemia that in turn may contribute to the increased incidence, prevalence and mortality from heart failure in HD patients.

1.3.7 Ischaemic preconditioning

Interconnected with the processes of stunning and hibernation is the concept of ischaemic preconditioning. This was originally defined as a short period of ischaemia that protected the myocardium from a subsequent, more prolonged and potentially catastrophic period of ischaemic damage [85]. Initially it was believed that there had to be a period of reperfusion between the preconditioning ischaemia and the subsequent, more prolonged episode. This would fit with the idea of repetitive stunning leading to the adaptive process of hibernation through the mechanism of ischaemic preconditioning.

Subsequent studies have shown however that a period of reperfusion is not always necessary and that the protective / adaptive phenomenon of preconditioning can also occur if the onset of lethal ischaemia is more gradual (sub-lethal) rather than sudden, so called 'intra-ischaemic preconditioning' [86]. This model is less like the mechanism of repetitive stunning described above but could still be induced if the ischaemic insults were close enough together that full restoration of flow and recovery of function were never attained.

What is clear is that both of these models could potentially result from haemodialysis induced repetitive cardiac injury and, whilst initially being protective, may eventually lead to maladaptive processes resulting in fibrosis and ventricular dysfunction due to the inexorable nature of current dialysis treatment regimes.

There is some preliminary data into the therapeutic effects of pre-ischaemic conditioning in donor kidneys prior to renal transplantation [87] however, as yet there is no research into the effects (positive or negative) of ischaemic myocardial preconditioning in dialysis patients.

1.3.8 Cardiac troponins in haemodialysis patients

It is well reported that cardiac troponins are elevated in haemodialysis patients, and that these elevated levels predict mortality [88,89]. Interestingly, HD patients have an increased mortality not just from cardiovascular death but also all cause mortality including infections [90] (see figure 1.3.8a). This may suggest that those patients with more severe cardiovascular disease (as evidenced by significant troponin leak) have insufficient cardiovascular reserve to cope with additional insults such as sepsis.

Although both troponin T (cTnT) and troponin I (cTnI) levels are raised in dialysis patients, there is more data with respect to troponin T due to the uniformity of assays and greater homogeneity of cut off points employed. In addition, cTnT is elevated more frequently than cTnI, suggesting this molecule may be a more sensitive marker [88,91] and although the exact origin of elevated troponins in renal disease was initially unclear, it is now well established that the troponins are cardiac in origin [92].

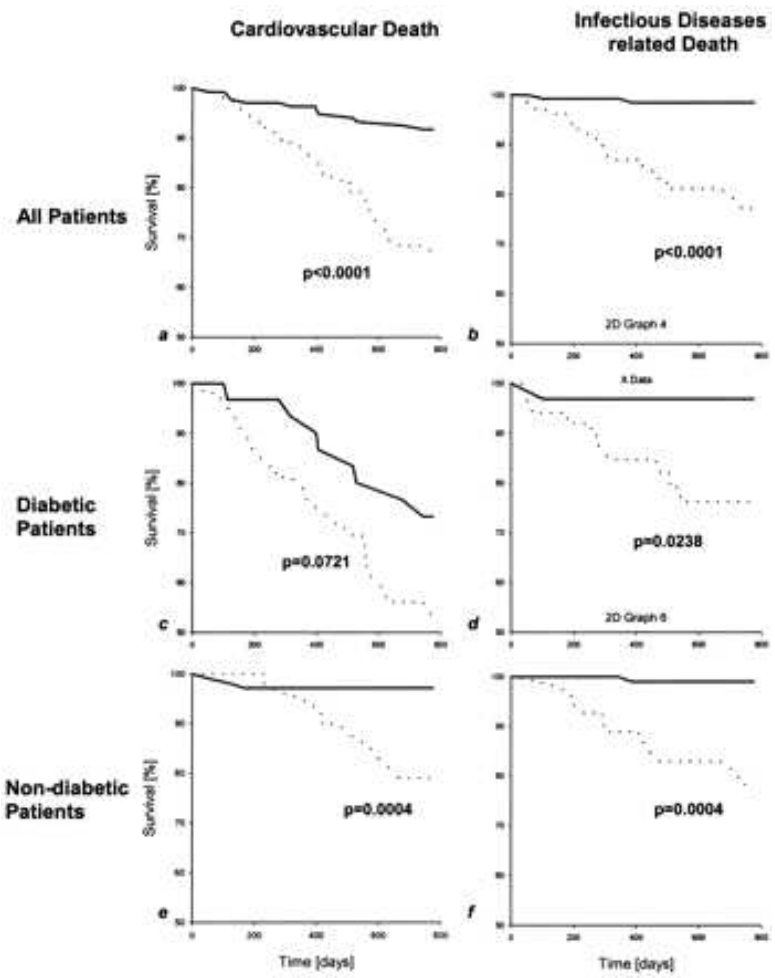


Figure 1.3.8a: Kaplan-Meier survival plots for haemodialysis patients with normal (solid line) and clinically raised (dotted line) troponin-T levels. Cardiovascular and non cardiovascular mortality was raised in both diabetic and non-diabetic patients [90].

There is however still some continuing uncertainty as to whether troponin rises acutely following dialysis. Several authors have reported significant rises in cTnT post dialysis [91,93-95], whereas others have found no difference in pre- and post- dialysis cTnT or cTnI levels, or found that any difference disappears after correction for haemoconcentration [96-98].

However, measuring post dialysis troponin levels to look for dialysis induced ischaemia may be flawed; it is well recognised that plasma troponin levels may only become elevated after 6 to 12 hours following an episode of ischaemia. Therefore, the studies that found no difference in pre- and post-dialysis troponin levels do not refute the development of dialysis induced myocardial cell damage.

Higher cardiac troponin T levels have been reported in patients prone to intradialytic hypotension as compared to stable patients [99]. However, this does not necessarily reflect dialysis induced cTnT release and alternatively could be explained by a greater cardiac disease burden in those unstable patients [94]. However, it has been demonstrated that cTnI rises significantly when measured 44 hours after dialysis sessions complicated by intradialytic hypotension (i.e. measured at the subsequent dialysis session) as compared to sessions in which patients were stable [100]. This provides additional evidence that subclinical myocardial injury does occur during dialysis complicated by hypotension and haemodynamic instability.

1.3.9 Haemodialysis and cardiac arrhythmias

Sudden cardiac death appears to correlate with the peri-dialytic period [101]. Consequently, there have been a number of studies looking at the potential pro-arrhythmogenic effects of HD, which reported the frequency of dialysis induced arrhythmias as anywhere in the range of 5 – 75% of treatments [102,103]. The presence of both potentially life-

threatening complex ventricular arrhythmias (CVAs) and premature ventricular complexes (PVCs) has been associated with increased morbidity and mortality. CVAs (defined as Lown score of 3 and above) are reported in up to 35% of HD patients during treatment [56] and associated with negative prognostic factors including new coronary events [104] and silent myocardial ischaemia [60]. In the general population, people with PVCs are more than twice as likely to die from coronary artery disease (CAD) [105] and their presence may play a vital role in assessment of cardiovascular risk.

The frequency and prevalence of intra- and post-dialytic ventricular arrhythmias are higher in patients with significant coronary stenoses [106], suggesting that myocardial ischaemia plays a direct role in the induction and persistence of ventricular arrhythmias during and after HD. Myocardial ischaemia is also known to lead to the development of regional wall motion abnormalities (RWMAs) and myocardial stunning as a direct consequence of HD [70,71]. This arises secondary to ischaemia caused by a reversible reduction in myocardial blood flow during the HD treatment session even in the absence of significant CAD [107]. In addition HD patients have been shown to have reduced coronary flow reserves [49], increased prevalence of left ventricular hypertrophy and impaired microcirculation [108], all of which predispose to demand myocardial ischaemia.

A number of therapeutic strategies directly targeting the increased incidence of CVAs and PVCs in HD patients have been utilised. Unfortunately the majority of these involve additional pharmacological

agents that may be poorly tolerated and have undesirable side-effects [109].

No evidence currently exists that myocardial ischaemia may contribute to the development of both RWMAs and ventricular arrhythmias. As ischaemia induced RWMAs are potentially preventable [70,71], the identification of a common pathophysiological process connecting both sudden and ischaemic cardiac death in HD patients may offer single therapeutic targets to reduce both causes of mortality.

1.3.10 Intra-dialytic hypotension and ultrafiltration rate

Intra-dialytic hypotension (IDH) is a very serious clinical problem and remains a significant cause of morbidity in the haemodialysis population, occurring in 20-30 per cent of treatments [46]. In addition, a fall in blood pressure during dialysis predicts mortality [109]. Furthermore, IDH could potentially contribute to myocardial hypoperfusion during dialysis. The main mechanism of IDH is rapid reduction of blood volume owing to ultrafiltration and decrease in extracellular osmolarity during the dialysis session. If the ultrafiltration rate exceeds the plasma refill rate, this will lead to a reduction in circulating volume. Hypotension occurs when this reduction in blood volume surpasses the compensatory mechanisms of the cardiovascular system. Although no data currently exists as to the direct effect of cumulative ultrafiltration volumes over time on mortality there is evidence to suggest that patients greater fluid retention between two subsequent HD treatment sessions is associated with worse survival (figure 1.3.10a) [110].

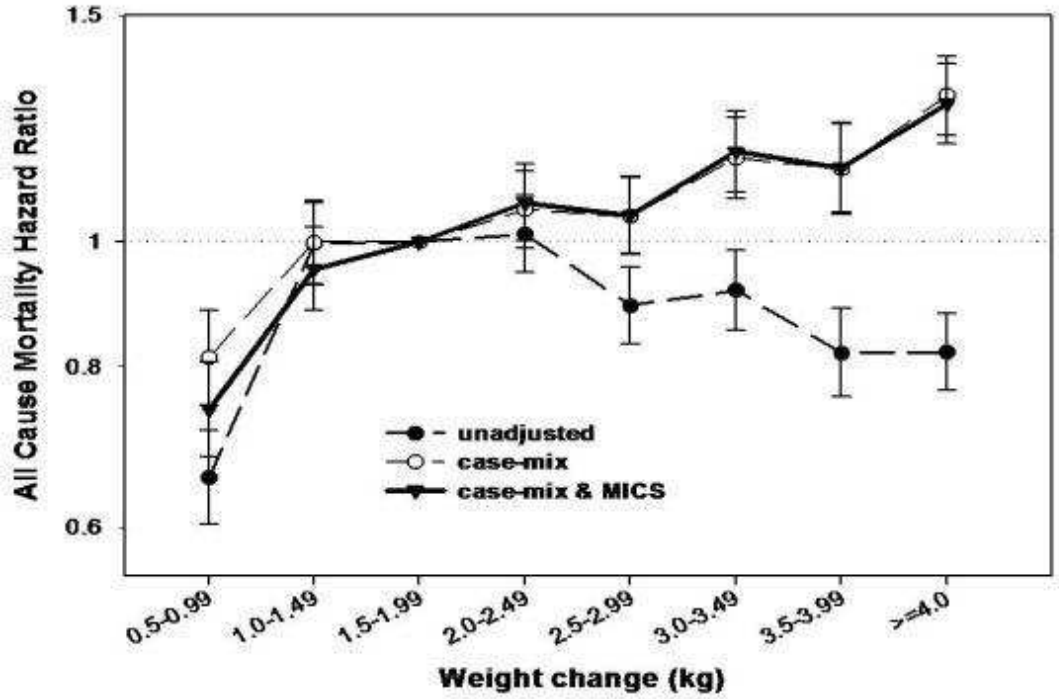


Figure 1.3.10a: After adjustment for demographics (case-mix) and surrogates of malnutrition-inflammation complex syndrome (MICS), higher weight gain between HD sessions was associated with increased all-cause mortality [110].

Coexisting illnesses, especially cardiovascular diseases, particularly common in older and diabetic patients play a significant role in the development of IDH. Efficient treatment of IDH is difficult as there are no universally accepted approaches to its management [111]. Multilevel strategy of IDH management includes emergency replacement of intravascular volume in acute episodes of hypotension, accurate assessment of 'dry weight', education of the patient, adequate hypertension treatment, and assessment.

Methods related directly to the haemodialysis procedure may also play a role in reducing the incidence of IDH. UF rate is important in determining blood volume [112]. Other than reducing the interdialytic weight gain, which to a large extent is patient-dependent, other strategies are being tested to reduce hypotension. Several studies have shown biofeedback dialysis to be effective in reducing IDH frequency in both IDH prone and resistant patients [113-115].

Dialysis using a low dialysate temperature have been shown to be beneficial [116]. Standard dialysis (with a dialysate temperature of 37°C) leads to an increase in patient body temperature [117]. The reasons for this are not entirely clear, but may include heat transfer to the patient from warm dialysate (especially as many dialysis patients have low baseline core temperatures), reduced heat loss from the skin due to vasoconstriction or possibly increased thermogenesis from an inflammatory response to a blood-membrane reaction. Cooling the temperature of the dialysate has been shown to reduce the incidence of both IDH [117] and myocardial stunning [70]. However, cooling the dialysate remains a relatively under utilised technique. In part this may be due to fears of causing unacceptable symptoms of cold and shivering, as empirical reduction of dialysate temperature may in some patients lead to excessive cooling. In addition, there has also been concern that cooling the dialysate will lead to a reduction in dialysis adequacy due to peripheral solute sequestration as a result of greater peripheral vasoconstriction.

Dialysis techniques that use proportionally greater degrees of convection to remove uraemic toxins (haemofiltration, HF or haemodiafiltration, HDF) have also been linked to improvements in stability during dialysis (in addition to their other potential advantages on solute clearance) [118]. However, there is accumulating evidence that most, if not all of this benefit on stability is due to thermal effects [119].

Short daily dialysis has a protective effect against IDH compared to conventional HD three times per week [120]. This was thought to be due to a number of factors including lower ultrafiltration volumes from smaller interdialytic volume gain. The benefits of short daily dialysis can also be seen at a biochemical level. Keeping the total number of hours of dialysis per week constant, patients on short daily dialysis have a reduction in pre-dialysis brain natriuretic peptide (BNP) dialysis [121]. Given the body of evidence that BNP levels correlate with LV dysfunction in dialysis patients (see below), short daily dialysis appears to result in less myocardial injury than conventional dialysis.

There is also a possibility for pharmacological treatment with the use of such agents as the well described midodrine, or other drugs such as caffeine, effedrin, and vasopressin analogs. Besides the discussed strategies, efficient treatment of congestive heart failure, a common reason of hypotension in uraemic patients, should not be overlooked.

1.3.11 Cardiac failure

The development of cardiac failure, which can occur in as many as 25-50 per cent of haemodialysis patients, reflects a particularly poor prognosis [122]. In a cross-sectional study congestive heart failure (defined by persistent or recurrent heart failure when patients were considered to be at dry weight), was found in 10 per cent of non-diabetic dialysis patients [16,51,123]. A third of the patients had developed their congestive heart failure before reaching end-stage renal disease; 53 per cent of these had dilated cardiomyopathy and 47 per cent had hypertrophic hyperkinetic disease. The most common cause of congestive heart failure in these patients is ischaemic heart disease, but other factors for example; diabetic cardiomyopathy, myocardial calcification, iron overload, and thiamine deficiency may also be involved.

Congestive heart failure carries a poor prognosis; records from the US Renal Data System have shown that HD is an independent risk factor for the development of both *de novo* and recurrent heart failure with a two-year mortality after a diagnosis of congestive heart failure as high as 51% [124] (as compared with 80 per cent in patients without congestive heart failure), making it the one of the most common causes of cardiovascular mortality in this patient group. In non-uraemic patients with congestive heart failure, cardiomyocyte drop (by apoptosis or necrosis) is an important element. It is therefore of interest that

cardiomyocyte loss has been documented in experimental uraemia as well [125].

However, in addition to the direct effects of uraemia, as discussed above, if myocardial ischaemia is induced by haemodialysis then the process of repeated haemodialysis itself may well potentially contribute to the development of congestive cardiac failure.

1.3.12 Summary

Despite the well documented increase in cardiovascular morbidity and mortality in haemodialysis patients, very little is known about the exact effect that haemodialysis has on myocardial blood flow and myocardial stunning or their relative contribution to the development of complications including hibernation, fibrosis and heart failure. This is despite the fact that HD patients are subject to a grossly elevated rate of cardiovascular decline and that raised levels of the biochemical markers of cardiac damage support the hypothesis that haemodialysis induces some form of cardiac injury.

It is becoming apparent that the current prevailing paradigm of considering this as a result of a particularly severe form of the cardiovascular risk factors seen in the general population is not true. This results in the well appreciated failure of Framingham risk models to predict events in this group, and the almost universal failure of attempts to successfully apply interventions that are well proven to be effective in the general population. There is an urgent need to enhance our

understanding of the actual pathophysiology at work in haemodialysis patients, and to utilise that knowledge to generate and apply novel therapeutic interventions, configured and conformed to those mechanisms.

Chapter 2

Thesis Aims

2 Thesis aims

2.1 Hypothesis

This thesis has been planned to test the following hypothesis:

Haemodialysis directly induces subclinical myocardial ischaemia and myocardial stunning, which may, over time, progress to myocardial hibernation and fibrosis. This could be a previously underappreciated (and potentially modifiable) pathophysiological process in the development of heart failure in haemodialysis patients.

2.2 Research questions

To test this hypothesis, the following interrelated research questions will be addressed:

- Is it possible to confirm that haemodialysis induces myocardial ischaemia through a reduction in myocardial blood flow and if so, does this cause myocardial stunning as defined using current literature?
- What is the prevalence of myocardial stunning in the general haemodialysis population and what, if any, are the factors associated with its development and severity?

- Is haemodialysis induced myocardial ischaemia and stunning associated with any acute electrophysiological complications?
- What are the long term functional cardiovascular consequences of repetitive haemodialysis induced cardiac injury in the form of myocardial stunning?
- Is there an association between myocardial stunning and an increase in morbidity and mortality?

Chapter 3

Methodology

3 Methodology

All methods are dealt with in detail in this chapter and then referred back to for reference in the relevant results sections. Certain methods and techniques were used in more than one study outlined below.

3.1 Applications for ethical approval

Ethical approval was sought and granted for all aspects of the research detailed in this thesis. In the first instance, application was made to the Central Office of Research Ethics Committees (COREC) now the National Research Ethics Service. Multi-centre research ethics committee (MREC) applications were made for every study either because data collection or patient recruitment took place on (or was planned for) more than one site. Site specific assessments were undertaken and submitted for each of these areas. The author attended the meetings of the Committee to answer questions and address concerns.

Approval from the Derby Hospitals NHS Foundation Trust Research and Development Department was also sought using the centralised research and development application form that was integrated into the COREC application system. Joint sponsorship was granted between the Hospital Trust and the University of Nottingham to allow for the collection, analysis and storage of data and pathological samples (plasma and serum) within both institutions.

3.2 Patient recruitment

All patients were recruited from the haemodialysis unit at Derby City General Hospital where the average number of prevalent HD patients is around 200. Because of the limited number of patients within the cohort to be studied, some patients undertook several different measurements that have been reported in more than one chapter. This is outlined in Figure 3.2a.

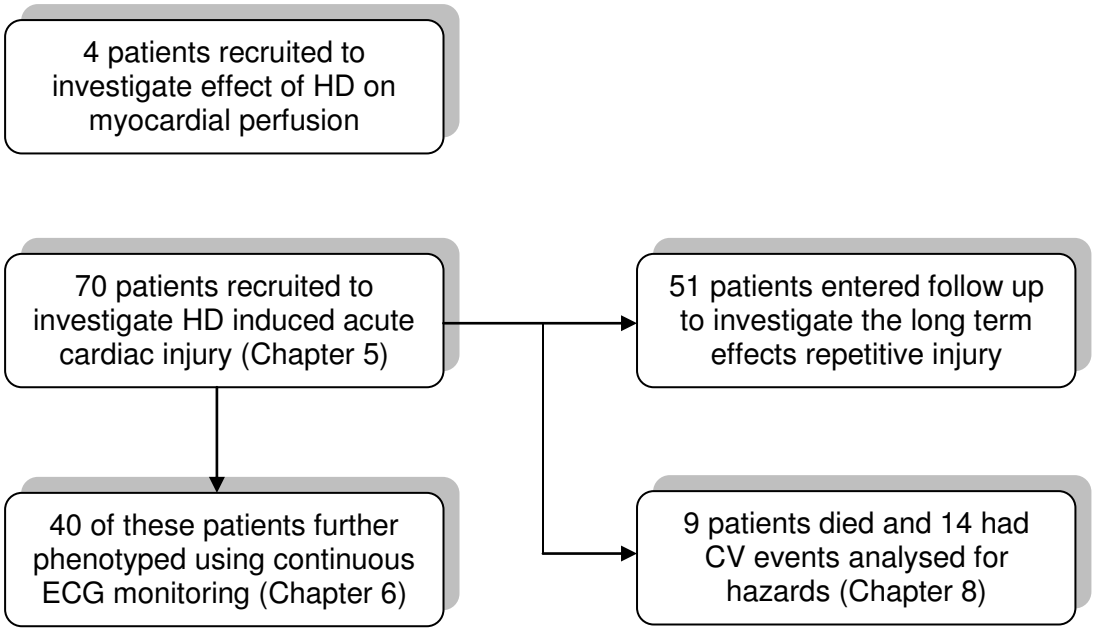


Figure 3.2a: Patients recruited and their contribution to the collection of data and analysis of results in each chapter.

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

Four patients agreed to take part in the study to investigate the associations between HD induced contractile dysfunction and myocardial blood flow (chapter 4). The data collection from these individuals was separate and distinct from any other aspects of the research in this thesis.

Seventy patients were recruited to investigate the prevalence, associated factors and long-term outcomes of HD induced cardiac injury (chapter 5). The demographical information for this cohort is detailed in table 5.4.3a below. A sub-set of these seventy were further analysed with continuous ECG Holter monitoring for any associations between functional and electrophysiological abnormalities during (and after) HD. These data are displayed in chapter 6. Fifty-one patients entered follow up after 12 months (details of those censored are given in section 7.2.1 below) and were further investigated for the long-term consequences of HD induced myocardial stunning (chapter 7). A further

subset of the original 70 patients were analysed: those who died (n=9) and those who suffered a cardiovascular event (n=14) in the proceeding 12 months to quantify hazards associated with HD and myocardial injury (chapter 8).

3.3 Non invasive haemodynamic measurements

Two methods were employed to measure haemodynamic variables that were either continuous or intermittent at regular time intervals. Continuous measurements were undertaken using the Finometer. This was used in studies involving smaller numbers of patients to gain records for variables including blood pressure, cardiac output, stroke volume and total peripheral resistance. Intermittent readings for blood pressure alone were taken in studies with larger numbers of patients using an automated digital oscillometric device. Both of these individual methods are outlined in more details below.

3.3.1 Continuous measurement of blood pressure (the Finometer®)

The Finometer (Finapres Medical Systems, Arnhem, The Netherlands) is a tool for blood pressure and haemodynamic monitoring (figure 3.3.1a).

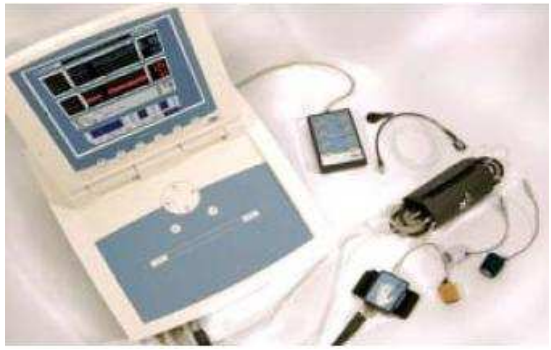


Figure 3.3.1a: The Finometer in isolation and connected to a subject.

The advantage of the Finometer is that it is accurate and robust and provides continuous measurement of multiple cardiovascular variables in one device. As a result, it is a versatile, non-invasive monitoring system that measures multiple haemodynamic variables on a beat-to-beat basis. The accompanying BeatScope® software allows online monitoring, control, storage and offline review of all the data on a PC.

The Finometer is particularly useful due to its non-invasive nature and ability to provide continuous readings over a period of several hours. The Finometer works by continuous pulse-wave analysis at the digital artery and utilises the finger-clamp method, in which changes in digital

arterial diameter are detected by means of an infrared photoplethysmograph [126] and opposed by an ultra-fast pressure servo controller that changes pressure in an inflatable air bladder, both mounted in a finger cuff. This generates an arterial waveform that is measured on a beat-to-beat basis and is used to reconstruct a central aortic waveform [127]. This allows calculation of a full range of haemodynamic variables on a continuous basis; these include heart rate (HR), blood pressure (BP), stroke volume (SV), cardiac output (CO) and total peripheral resistance (TPR). All data are subsequently downloaded to the PC based analysis program, allowing averaging of results over defined time periods. This technology provides unprecedented resolution of changes in the critical cardiovascular variables. Previous work has validated the Finometer against invasive haemodynamic measurements in normal individuals, unstable intensive care patients and in cardiac surgery patients, a proportion of whom had vascular calcification [127-129]. This has shown the Finometer to be accurate in tracking relative change. Data are therefore presented as percentage change from baseline except for BP, which is calibrated against brachial readings using a return to flow method and absolute values can therefore be used [130].

More recently, the Finometer has been increasingly used to assess chronic dialysis patients [44,45,131] and has now been validated within this patient group.

3.3.2 Serial measurements of blood pressure

For larger studies, blood pressure was measured pre-dialysis and then serially every 15 minutes during haemodialysis using an automated digital oscillometric device (Model UA-767, A&D Instruments, Japan) calibrated to research standards. For each time point, 3 measurements were obtained and the average of those readings was recorded. Haemodynamic instability was defined by the magnitude of reduction in systolic blood pressure (SBP) at each time point compared to the pre-dialysis reading. Patients were defined as having intradialytic hypotension if they experienced as systolic blood pressure of <100 mmHg even in the absence of symptoms, or a fall in systolic blood pressure of >10 per cent of the pre-dialysis reading in association with any of the classical symptoms of hypotension including: abdominal discomfort; yawning; sighing; nausea; vomiting; muscle cramps; restlessness; dizziness or fainting; and anxiety.

3.4 Echocardiography

We used two-dimensional echocardiography to assess regional LV function as a marker of ischaemia. The development of new LV regional wall motion abnormalities (RWMAs) during physiological or pharmacological stress occurs in response to ischaemia and its onset precedes that of symptoms and electrocardiographical changes. This principle underlies dobutamine stress echocardiography [132].

Left ventricular regional wall motion was assessed by recording standard apical views (with the patient in the left lateral position) before, during and after dialysis procedures and then performing specialised semi-automated analysis on the images. Images were acquired using commercially available equipment (Sonos 5500; Hewlett Packard, Andover, Massachusetts, USA or Vivid 3® with 1.5-3.6 MHz 3S probe, GE medical systems, Sonigen, Germany).

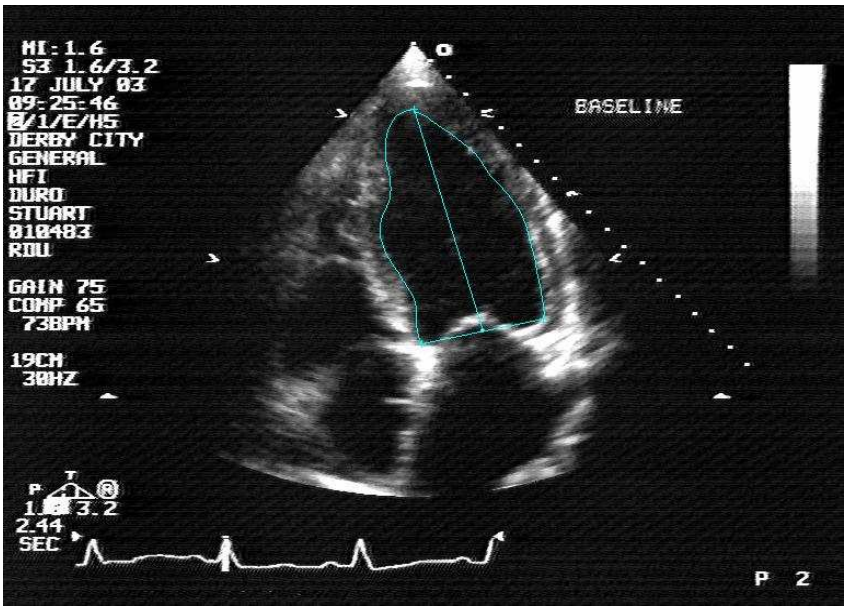


Figure 3.4a: Echo CMS software allows semi-automatic tracing of the endocardial border that can be manually corrected for anomalies. The movement of the endocardial border is then measured over 100 chords around the left ventricle.

A single experienced technician (the author) carried out all examinations and if alternate dialysis modalities were used, they were blinded to dialysis treatment. Timing of the echocardiography varied between studies but is stated in each relevant chapter. Standard apical 2-chamber and 4-chamber views (to visualise the LV endocardial border in 2 planes at 90° to each other) were recorded. For the Sonos 5500 (Hewlett Packard) ultrasound machine, images were recorded directly onto super-VHS videotape for off-line analysis. For the Vivid 3®, the images were saved onto the internal hard drive and subsequently recorded onto compact disc using the standardised digital imaging and communications in medicine (DICOM) format. This enabled more effective backup of patient data and maintained digital quality images for offline analysis using the PC based software program (see below).

Videotaped and digitised image sequences were subsequently analysed using a personal computer based software programme (Echo-CMS, MEDIS, Leiden, The Netherlands) as previously described [133]. Three consecutive heartbeats were analysed for each time point (extrasystolic beats were excluded). Endocardial borders (excluding papillary muscles) were traced semi-automatically for each video frame of the 3-beat sequence, and any anomalies corrected manually (see figure 3.4a). Maximal displacement of the endocardial border from a centrepoint was then measured over each of 100 chords around the LV wall, corrected for end-diastolic LV circumference and expressed as percentage shortening fraction (SF), as shown in figure 3.4b. Each apical view was divided into 5 segments and SF for the chords in each

segment was averaged so 10 regions of the left ventricle were assessed at each time point. New RWMAs were defined as those segments that demonstrated a decline in SF of >20 per cent from baseline. This technique uses endocardial borders as the sole marker of abnormal contraction, and therefore could be criticised as it does not take account of wall thickening or transmyocardial heterogeneity. However, this method does have the advantage that it is repeatable and quantitative.

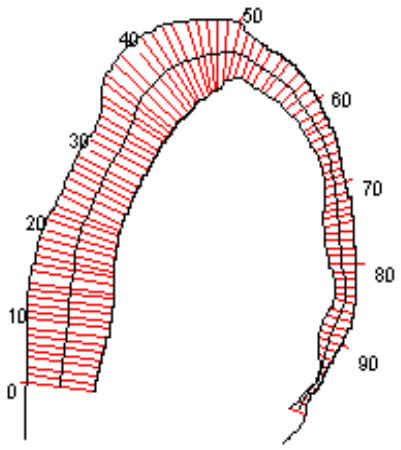


Figure 3.4b: The movement of the endocardial border is measured over 100 chords around the left ventricle with each red line representing an individual measurement.

We also used echocardiography to assess measure cardiac dimensions and global LV function. Ejection fraction (EF) was calculated using LV volumes at end systole and end diastole, measured by the biplane disk

method. M-mode echocardiography using the para-sternal long axis view was utilised to measure LV dimensions and LVMI was calculated from each patient's original baseline images using the Devereux formula corrected for height$^{2.7}$.

3.4.1 Measurement of intra-observer reliability

Echocardiographical training was delivered by British Cardiac Society instructors and accuracy and quality of appropriate windows (apical two chamber, apical four chamber, para-sternal long axis and M-mode) was assessed by accredited examiners. These results were verified by a Consultant Cardiologist. Competency was reviewed annually thereafter.

Ten patients' images were selected at random for reassessment. This was done to test reliability of both the detection of regional wall motion abnormalities (numbers and position) and the severity in terms of reduction in %SF. There was a strong correlation with respect to the numbers of regions affected (r=0.94) with no patients being re-classified as >2 RWMAs or ≤2 RWMAs. The correlation with %SF was less but still high (r=0.85). Despite the often subjective nature of echocardiographical assessment, the intra-observer reliability was good, meaning the results are reproducible and robust. As all echocardiographical images were analysed by a single operator, inter-observer reliability is not discussed.

3.5 Dialysis methodology

3.5.1 Blood volume measurement

Blood volume measurement was performed by dialysis monitors in several of our experiments. Measurement of the change in the concentration of blood components (blood cells, proteins, haemoglobin) during dialysis reflects the balance between fluid removal from the circulation (ultrafiltration) and the plasma refill rate. There are two commonly available methods to measure blood volume - optical and ultrasonic. The optical method measures haemoglobin concentration by measuring the light absorbance of blood at two different frequencies. Measurement at two frequencies allows correction for the degree of oxygenation, which can alter the light absorbance characteristics of blood.

The ultrasonic method calculates the total protein concentration in blood (plasma proteins and haemoglobin) by measuring the velocity of sound in blood (which is determined by protein concentration) and compares this to the velocity of sound in isotonic saline. For this method, the temperature of the blood and saline must be accurately fixed, and the haematocrit is deduced from the total protein concentration using simple formulae.

In our experiments, we used the optical method that has been commercially developed and incorporated into dialysis monitors [134].

3.5.2 Conductivity measurement and ionic dialysance

The conductivity of a substance is defined as the ability to carry electrical current. In dialysate fluid, the conductivity is determined by the concentration of ions capable of carrying electrical current, providing the temperature remains constant. This is almost entirely dependent on sodium concentration, although the other ions (such as potassium and calcium) that are present in much smaller quantities do also exert a small effect. Therefore, by measuring conductivity of the dialysate it is possible to derive a value for dialysate sodium concentration [135]. This technique has a variety of applications. A conductivity monitor can be placed at the dialysate inlet and outlet ports of the dialyser and by changing the inlet conductivity by a set amount and measuring the resultant change in dialysate outlet conductivity, it is possible to calculate ionic dialysance (value representing the amount of electrolytes that have passed from plasma to dialysate). From the degree of ionic dialysance that occurs at a set dialysate conductivity, it is possible to calculate plasma conductivity and this can be used a surrogate for plasma sodium concentration. As the transfer characteristics of sodium and urea are similar, the ionic dialysance also reflects the clearance of urea. Therefore, using this technology it is possible to estimate from each dialysis session the ionic mass balance (amount of sodium removed), the dialysis adequacy for small solute removal (Kt/V, providing the patient's volume of distribution is known) and the plasma conductivity at the end of the treatment [135].

3.5.3 Dialysis technique

All dialysis described in this thesis was performed using Hospal Integra® monitors (Gambro-Hospal, Mirandola, Italy) unless otherwise stated. In all cases, we used low-flux polysulphone dialysers either 1.8m^2 or 2.0m^2 as per individual patients' usual prescription (LOPS® 18/20, Braun Medical Ltd, Sheffield, UK) unless otherwise stated. Dialysate contained sodium 138 mmol/l, potassium 1 mmol/l, calcium 1.5 mmol/l, magnesium 5 mmol/l, bicarbonate 32 mmol/l, glucose 1 g/l and acetate 3 mmol/l. All treatments were of 4 hours duration and anti-coagulation was achieved with unfractionated heparin. Dialysate flow was 500 ml/min, dialysate temperature was set at 37°C and blood flow was 250-460 ml/min. For paired sessions, care was taken to ensure blood flows were similar for each patient. Net fluid removal was set on an individual basis according to ideal dry weight. No patients underwent sodium or ultrafiltration profiling.

3.6 Positron emission tomography

3.6.1 Measurement of regional myocardial perfusion

Positron emission tomography (PET) with $H_2{}^{15}O$ or $^{13}NH_3$ is the only technique that allows the non-invasive measurement of regional absolute myocardial blood flow.

PET is a nuclear medicine imaging technique which produces a three-dimensional image or picture of functional processes in the body. It is

both a medical and research tool and is used heavily in clinical oncology for the imaging of metabolically active tissues such as tumours and metastases, and for clinical diagnosis of certain diffuse brain diseases such as those causing various types of dementias. PET is also an important research tool to map normal human brain and heart function.

The system detects pairs of gamma rays emitted indirectly by a positron-emitting tracer, which is introduced into the body on a biologically active molecule. Images of tracer concentration in 3-dimensional space within the body are then reconstructed by computer analysis. In this case, reconstruction was accomplished with the aid of a transmission scan performed on the patient during the same session, in the same machine.

To conduct the scan, a short-lived radioactive tracer isotope (in this case $H_2{}^{15}O$, see below) is injected into the patient's circulation and becomes concentrated in tissues of interest. As the radioisotope undergoes positron emission decay (also known as positive beta decay), it emits a positron, a particle with the opposite charge of an electron. After travelling up to a few millimetres the positron encounters and annihilates with an electron, producing a pair of annihilation (gamma) photons moving in opposite directions. These pairs of gamma photons are detected in the PET scanner and converted into images of bioactive tissue, photons which do not arrive in pairs (i.e. within a timing window of few nanoseconds) are ignored.

Radionuclides used in PET scanning are typically isotopes with short half lives such as carbon-11 (~20 min), nitrogen-13 (~10 min), oxygen-15 (~2 min), and fluorine-18 (~110 min). These radionuclides are incorporated either into compounds normally used by the body such as glucose (or glucose analogues), water or ammonia, or into molecules that bind to receptors or other sites of drug action. Such labelled compounds are known as radiotracers. For this study, we utilised oxygen-15 ($H_2{}^{15}O$) for two main reasons: firstly the short half life enabled us to perform repeated scans within the time frame of the study session without confounding effect of residual radiotracer and secondly because it is more readily utilised in the myocardium compared to other radiotracers and therefore less likely to be affected by dialysis. Due to the short half lives of most radioisotopes, the radiotracers must be produced using a cyclotron and radiochemistry laboratory that are in close proximity to the PET imaging facility, hence the need to transport the patients from the local HD unit in Derby to the PET centre with an onsite cyclotron at the Hammersmith Hospital in London.

The PET scans were performed in a three-dimensional imaging mode with a 962 (HR+) scanner (Siemens, Knoxville, TN, USA) (see figure 3.6.1a). The scanner enables the acquisition of 15 planes of data over a 10.5-cm axial field of view, allowing the whole heart to be imaged.

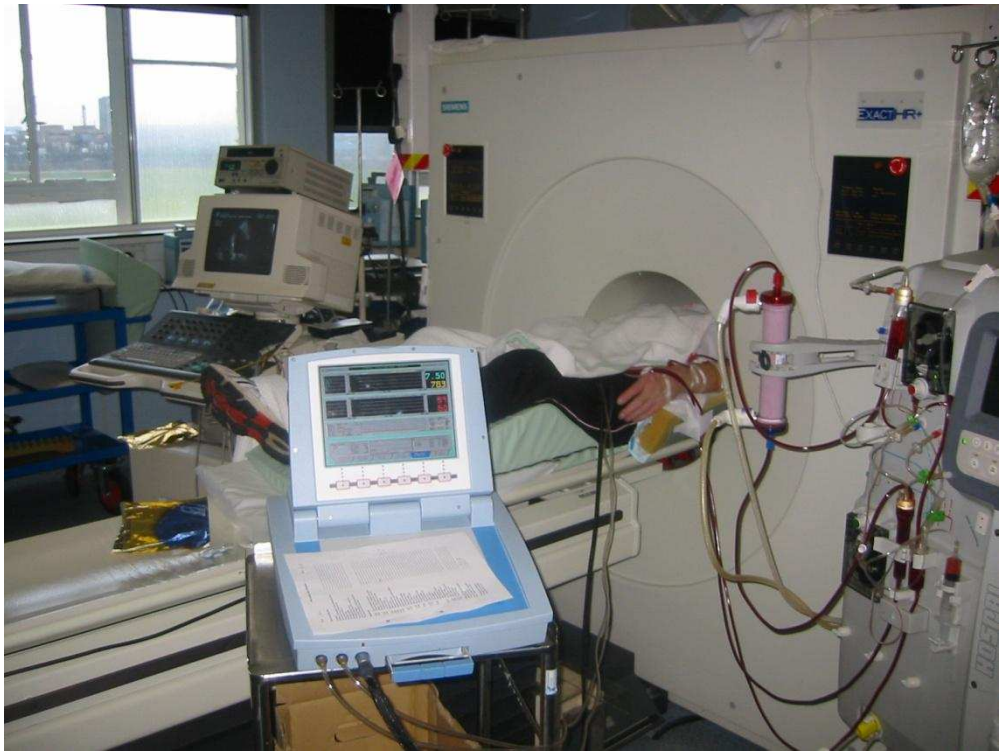


Figure 3.6.1a: Patient connected to the haemodialysis machine being studied in the PET scanner. The Finometer and cardiac ultrasound scanner are also visible in this picture.

All emission and transmission data were reconstructed with a Hanning filter with a cut-off frequency of 0.5 units of reciprocal of the sampling interval of the projection data, achieving an image resolution of 8.4x8.3x6.6 mm^3 full-width half maximum at the centre of the field of view. All subjects were asked to abstain from caffeine containing beverages for 24 hours before the scan.

Subjects were positioned in the scanner, and after exposure of a retractable ^{68}Ge ring source, a 5 minute rectilinear transmission scan was acquired to determine the optimal imaging position of the left ventricle within the field of view. A 20 minute transmission scan was subsequently performed for the purpose of attenuation correction of all emission scans. To maintain the optimal imaging position of the subject within the scanner, a low power laser beam was superimposed on a cross-shaped ink mark on the subject's chest and this position was kept constant throughout all the emission scans. Starting after a 30 second background frame, a bolus of oxygen-15 labelled water $H_2{}^{15}O$ (185 MBq) was injected intravenously over 20 seconds at an infusion rate of 10 ml/min. The venous line was then flushed for another 2 minutes. The following scanning protocol was used: 1x30 seconds (background); 1x20 seconds; 14x5 seconds; 3x10 seconds; 4x20 seconds and 4x30s for a total scanning time of 350 seconds.

3.6.2 Analysis of myocardial perfusion data

The acquired sinograms were corrected for attenuation and reconstructed on a Microvax II computer (Digital Equipment, Marlboro, MA, USA) using dedicated array processors and standard reconstruction algorithms. Images were then transferred to a Sun Sparc 2 workstation (Sun Microsystems, Mountainview, CA, USA). Images were analysed with customised MATLAB software (The Math-works Natick, MA, USA. Myocardial images, for the definition of regions of interest, were generated directly from the dynamic $H_2{}^{15}O$, as previously

reported [136]. The creation of factor sinograms requires estimates of vascular (right and left heart) and myocardial tissue time-activity curves [136,137]. Factor images describing tissue and blood distributions were generated by iterative reconstruction as previously described [138]. Factor images were re-sliced into short-axis images in an orientation perpendicular to the long axis of the left ventricle.

Sixteen regions of interest, corresponding to the territories of distribution of the 3 major coronary arteries, were drawn within the left ventricular myocardium on 10 consecutive image planes, according to the recommendations of the American Society of Echocardiography [139]. The regions were drawn semi-automatically by use of a centre line within the myocardium. For our analysis, however, the original 16 left ventricular regions of interest were regrouped into 12 segments. A separate set of regions of interest was defined for the right ventricular cavity and the left atrium.

Myocardial and blood time-activity curves were then generated from the dynamic image and fitted to a single–tissue compartment tracer kinetic model to give values of MBF (in millilitres per minute per gram) [137]. Because resting MBF is determined by cardiac work-load [140], we also corrected resting MBF for the rate-pressure product (RPP), an index of myocardial oxygen consumption: MBF = (MBF/RPP) 10^4 [141].

3.7 Continuous ECG recording

3.7.1 Holter monitoring

Continuous ECG (Holter) monitoring was performed using four Northeast Monitoring Inc, DR180+ Digital Recorders. After correct skin preparation, the Holter monitor was connected to the patient with ten leads for the conventional 12-lead configuration using lead *RL* as the reference and nine signal leads. This utilised nine channels for the highest possible quality data collection. Signal quality was visually verified for proper amplitude, electrode placement and lack of artifact in all channels by the operator using the Holter display. Lead quality was then further verified using built-in software that displayed signal condition for each lead based on impedance detected between the two electrodes for each channel. The best possible reading was five; any channel with a reading of less than four was reapplied. The sampling rate was set to the maximum of 720 samples per second. Interval between recordings and length of ECG recordings were both set to zero in order to obtain continuous data readings.

Monitoring commenced immediately before the start of HD and continued for 24 hours subsequently. Patients were encouraged not to change their activities of daily living for the duration of the study time. Recording stopped automatically after the 24 hour period. Patients disconnected themselves and returned the units for data retrieval at their next dialysis session.

3.7.2 Analysis of Holter data

Data was stored and subsequently downloaded from compact flash digital storage cards for offline analysis (NorthEast Monitoring Inc Holter LX Enhanced Software) by a single, experienced technician who was blinded to echocardiographical and biochemical results.

Frequency of ectopy was classified as a percentage of the total beats during the time period studied [142]: rare (≤0.1%), occasional (>0.1 to 1.0%), frequent (>1.0 to 10%) and very frequent (>10%). Ventricular arrhythmias were stratified according to the Lown classification [143] (see table 3.7.2a); classes 3 and above were taken as complex ventricular arrhythmias (CVA). All patients who were classified as having a class 3 arrhythmia or above had all their abnormal complexes visually reviewed to exclude false positive results caused by artifact.

Class	Arrhythmia
0	None
1	Unifocal; <30/hour
2	Unifocal; ≥30/hour
3	Multiform
4A	2 consecutive
4B	≥3 consecutive
5	R-on-T phenomenon

Table 3.7.2a: Lown Classification of ventricular arrhythmias

Beat-by-beat analysis of ST segment changes using the auto-detection software was impracticable due to the large volume of incorrectly identified significant beats. Consequently, 12-lead ECGs were recorded and subsequently analysed for significant changes from baseline every 15 minutes during HD.

3.8 Haematological and biochemical assays

All pre-dialysis blood tests were drawn immediately after insertion of access needles, and post-dialysis levels were taken from the arterial line 10 seconds after reduction of blood pump speed to 50 ml/min. Biochemical analysis was performed on a multichannel autoanalyser. Full blood count measurements were performed using a compact analyser. Other assays and measurements are detailed below.

3.8.1 Cardiac troponin-T

Cardiac troponin-T (cTnT) analysis was performed using a third-generation electrochemiluminescence assay (Roche Diagnostics, Lewes, UK). Postdialysis cTnT values were corrected individually for haemoconcentration with reference to percentage change in haematocrit and blood volume using the following formula:

$$\text{Adjusted cTnT} = \text{cTnT}_{post} \times \frac{\text{BV}_{post} \times (1 - \text{Hct}_{post})}{\text{BV}_{pre} \times (1 - \text{Hct}_{pre})}$$

where $cTnT_{post}$ is postdialysis cTnT, Hct_{post} is postdialysis haematocrit, Hct_{pre} is predialysis haematocrit, BV_{post} is end dialysis blood volume, and BV_{pre} is start dialysis blood volume.

3.8.2 High sensitivity C-reactive protein

High sensitivity C-reactive protein levels were measured using an enzyme linked immunosorbent assay (ELISA).

Briefly, a monoclonal antibody against the molecule to be detected is adsorbed onto the surface of microwells. Samples or standards are then added to the wells and the molecule binds to these solid phase antibodies. A labeled antibody against the molecule is then also added and this also binds to the molecule to form a sandwich (solid phase antibody - test molecule - labeled antibody). After incubation, the wells are washed to remove unbound labeled antibody and a chromogenic reagent is added that reacts with the labeled antibody to produce a colour change. A stop solution is added to prevent excess colour development and the absorbance of each well is measured spectophotometrically. The absorbance is proportional to the concentration of test molecule present. A standard curve is drawn from the standards and values for the concentration of samples in each well can then be calculated. We used standard ELISA kits that provided pre-coated 96 well plates (DRG diagnostics, Marburg, Germany).

Serum was isolated from blood collected in plain tubes and immediately centrifuged at 3500rpm for 10 minutes. The plasma was then removed

and frozen at -80℃ until time of assay. Samples were centrifuged again upon thawing at 17,000rpm for 3 minutes to remove particulates. Before assaying, the serum was diluted 100-fold. The C-reactive protein (CRP) high sensitivity ELISA uses monoclonal mouse antibody for the solid phase and a goat anti-CRP antibody labeled with horseradish peroxidase (HRP).

3.8.3 Dialysis adequacy

Dialysis adequacy was measured using single-pool Kt/Vurea values which were calculated from pre- and postdialysis urea levels, post dialysis weight and ultrafiltration volume based on the Daugirdas II formula [144].

3.9 Statistical methods

The exact statistical methodology varied depending on the individual analyses used and is dealt with in detail in the relevant results sections below.

The author undertook all statistical analyses using the software packages Prism 5 for Windows (GraphPad Software Inc, San Diego, USA) and SPSS v10 (SPSS Inc, Chicago, USA). More complex multivariate analyses were verified by an independent statistician employed by Derby Hospitals NHS Foundation Trust Research and Development Department.

Chapter 4

Results:

Haemodialysis induced contractile dysfunction is associated with acute and reversible reduction in global and segmental myocardial blood flow.

4 Results: Haemodialysis induced contractile dysfunction is associated with acute and reversible reduction in global and segmental myocardial blood flow.

4.1 Introduction

Previous small studies have shown that HD induces acute and reversible regional wall motion abnormalities (as measured using echocardiography). Such regional wall motion abnormalities are usually indicative of ischaemia however; for conclusive evidence of dialysis induced myocardial stunning, myocardial blood flow needs to be measured in conjunction with functional assessment of the left ventricle. This would allow demonstration of the initial ischaemic insult followed by persistent dysfunction upon resolution of flow [145].

This study was designed to investigate whether haemodialysis directly induces myocardial ischaemia and myocardial stunning through a reduction in myocardial blood flow using PET scanning (to measure myocardial blood flow), echocardiography (to measure left ventricular regional wall function) and non-invasive haemodynamic monitoring. We also aim to compare the effects of standard dialysis and biofeedback dialysis. This is an entirely novel concept, and one with great clinical relevance. The work may potentially reveal an additional aetiological

factor in the development of cardiovascular disease in dialysis patients, which could be a target for future therapy or prevention.

We hypothesise that observed reductions in segmental contractile function consistent with myocardial stunning are as a result of ischaemia. Furthermore, the repeated nature of this insult (thrice weekly) may be important in the development of dialysis associated heart failure.

Additional aims included: the evaluation of the acute effects of both standard and modified (biofeedback controlled) dialysis on global and segmental myocardial blood flow utilising intradialytic $H_2{}^{15}O$ PET scanning (the most accurate dynamic measure of myocardial perfusion); and the effect of reduced myocardial blood flow on segmental function using echocardiography.

4.2 Methods

4.2.1 Patients

Four patients on chronic haemodialysis who were recruited for a randomised cross-over study. All patients were male and all had been on dialysis for longer than six months. Angiograms had been performed as part of routine clinical care (e.g. for investigation of ischaemic heart disease or for work up for transplantation). Angiographic results and remaining characteristics are shown in table 4.2.1a. All patients dialysed via native arteriovenous fistulae and all were anuric.

Patient	Age (yr)	Months on Dialysis	Cause of ESRF	Smoker	Angiogram Indication	Angiogram Result	Antianginal or BP-Lowering Drugs
1	54	17	Diabetes	Yes	Chest pain	Normal	None
2	63	68	APKD	No	Assessment for transplantation (equivocal ETT)	Moderate Cx disease (50%); normal RCA, LMS, and LAD.	None
3	56	46	Diabetes	No	Assessment for transplantation (equivocal ETT)	Mild LAD disease	Lisinopril 5 mg OD, nifedipine 30 mg OD, doxazosin 2 mg OD
4	64	44	Diabetes	Yes	Assessment for transplantation (unable to perform ETT)	Trivial coronary disease	Atenolol 25 mg OD, ISMN 60 mg OD
Mean ± SD or *n* (%)	59 ± 5	44 ± 21		2 (50)		4 (100)	

[a]APKD, adult polycystic kidney disease; Cx, circumflex artery; ESRF, end-stage renal failure; ETT, exercise tolerance test; IHD, ischemic heart disease; LAD, left anterior descending artery; LMS, left main stem; OD, once daily; RCA, right coronary artery; ISMN, isosorbide mononitrate.

Table 4.2.1a: Patient demographics including angiographic indications and findings.

Patients were excluded if they had significant symptomatic cardiac failure (NYHA ≥ 3) or experienced an acute coronary syndrome in the preceding four months, had previously received a cardiac transplant or if it was not possible to obtain echocardiographic images of sufficient quality to allow meaningful analysis.

4.2.2 Study protocol

Upon entry to the study, patients' dry weight and anti-hypertensive medications remained unchanged for the duration. Patients were then randomised to two groups. Group A patients were commenced on standard thrice weekly haemodialysis (HD); group B patients started

thrice weekly dialysis with the Hemocontrol™ biofeedback system (BFD). Patients, but not dialysis unit staff, were blinded to the intervention. Both groups underwent two weeks of the dialysis therapy at the HD unit in Derby after which patients attended their initial monitored dialysis session at the Hammersmith Hospital in London. Beta-blocking agents and calcium antagonists were withdrawn 72 hrs prior to the study (as they are known to protect against myocardial stunning), and other anti-anginal medication omitted on the day. Patients were asked to avoid activities that might precipitate their angina for 12 hours before the study and were excluded if they have suffered angina or used their GTN spray within 4 hours of commencement. After the conclusion of the first study, patients then crossed over to the other dialysis modality thereby acting as their own controls. After two weeks on the alternate modality, patients underwent a second monitored session on the same day of the week as the first study session.

For each monitored dialysis treatment, myocardial blood flow was measured pre-dialysis, during treatment at two and four hours and again 30 minutes into the recovery using $H_2{}^{15}O$ PET scanning. Serial echocardiography was performed at the same time points and immediately prior to the PET scans. Non-invasive haemodynamic monitoring of blood pressure (BP) and heart rate was undertaken using a Finometer. To obtain baseline values, monitoring was started 30min prior to commencement of dialysis. Pre-dialysis blood tests were drawn immediately after insertion of access needles, and post levels were

taken from the arterial line 10sec after reducing blood pump speed to 50ml/min. Single pool Kt/V_{urea} values were calculated from pre and post urea levels [144].

The primary endpoint was the frequency of new LV regional wall motion abnormalities during HD in relation to global and regional MBF.

All patients gave informed consent prior to commencement, and ethical approval for the project was granted by Derbyshire Local Research Ethics Committee.

4.2.3 Haemodialysis details

Dialysis was performed as described in chapter 3. For standard HD, dialysate sodium conductivity was set at 13.6 mS/cm. For biofeedback dialysis (BFD), conductivity limits were set at 13.0 mS/cm and 14.0 mS/cm. Automatic adjustment of dialysate conductivity by the dialysis monitor during Hemocontrol has been shown to achieve equivalent overall dialysate conductivity and therefore equal change in plasma water sodium concentration [146]. Limits for relative blood volume were set on an individual basis depending on measurements taken during the week prior to echocardiographic assessment.

4.2.4 Positron emission tomography (PET)

Measurement of myocardial blood flow (MBF) using $H_2{}^{15}O$ PET is described in detail in chapter 3. Initial transmission scans and baseline measurements of MBF were taken before the commencement of

haemodialysis. Subsequent scan were taken at 120 minutes and 240 minutes into dialysis and still on treatment, each being done immediately after echocardiographical imaging for assessment of left ventricular regional wall motion abnormalities.

4.2.5 Echocardiography

Echocardiography and subsequent analysis were performed as described in chapter 3. Images were recorded prior to commencing dialysis (baseline); at 120 minutes and 240 minutes during dialysis and immediately prior to the measurement of myocardial blood flow using PET; and 30 minutes after dialysis was finished (recovery). Ten regions of the left ventricle were assessed for the development of new regional wall motion abnormalities (RWMAs) at each time point. We calculated mean SF for all ten segments ($SF_{(mean)}$) and for those segments that developed new RWMAs ($SF_{(WMA)}$). Peak stress was defined for each patient as the point during the first monitored dialysis session when most RWMAs were present (either 120 minutes or 240 minutes). When comparing dialysis modalities, the same time point was used in the second dialysis session.

4.2.6 Finometer

The Finometer was used as described in chapter 3 and connected up before the first measurement of MBF was taken. This enabled: correct calibration of the device before commencement of haemodialysis; for accurate measures of blood pressure and heart rate during the PET

scan to allow for exact correction of the rate pressure product; and allowed for measurements of baseline haemodynamic variables over 20 minutes for comparison to be made at subsequent timepoints on HD.

4.3 Statistical analysis

This power calculation is based on detecting a significant change in myocardial blood flow from baseline during dialysis (primary objective) and does not calculate the numbers required to detect a difference in blood flow between the two dialysis types. Data on myocardial blood flow are currently only available from patients with known coronary artery disease without renal failure. These data indicate that myocardial blood flow changes by about 60-70% in response to a dobutamine stress. Using the standard deviation (SD) from these studies, a sample size of 6 would appear to be sufficient to detect a difference of 62% change from baseline. A sample size of 6 is also similar to other studies using similar techniques [67,147]. However, although this power calculation is not aimed at detecting a difference between the 2 types of dialysis, previous work with 8 patients has shown that we can detect a difference in LV regional wall motion with these 2 types of dialysis.

Results are expressed as mean ± standard deviation if parametric or median (interquartile range, IQR) if non-parametric unless otherwise stated. Echocardiographic, BP and haemodynamic data were analysed using one-way analysis of variance (ANOVA) with a design for repeated measures and Bonferroni's test to correct for multiple comparisons. For

other data, either the paired *t*-test or Wilcoxon rank sum test was used depending on normality of the distribution. Significant deviations from a normal distribution were excluded with the Kolmogorov-Smirnov test. An alpha error at $P<0.05$ was judged to be significant.

4.4 Results

4.4.1 Echocardiographic Data

Throughout the study all patients were in sinus rhythm and none had significant valvular disease or pulmonary hypertension.

At two and four hours a total of 39/130 (33%) RWMAs developed during both types of dialysis. There was no significant difference in the number of RWMAs that developed between two and four hours. At 30 minutes post dialysis (recovery period) compared to four hours, 81% of RWMAs had improved and 23% had regained normal function, confirming the presence of myocardial stunning in this group. There was no significant difference in either the number of RWMAs or their severity in terms of shortening fraction (SF) between HD and BFD during dialysis or after the recovery period ($P>0.05$ by ANOVA).

4.4.2 PET Data

All results were corrected for rate pressure product (RPP). However, no significant difference was observed between corrected and non-corrected values at given time points. Previous studies have shown

reduced baro-reflex sensitivity and heart rate variability in this group so results shown are therefore non-corrected unless otherwise stated.

Global, mean pre-dialysis MBF for both modalities was within the normal range. Global MBF was acutely reduced during dialysis and became progressively worse over time. There was some restoration of flow (but not complete) after the 30 minute recovery period (figure 4.4.2a).

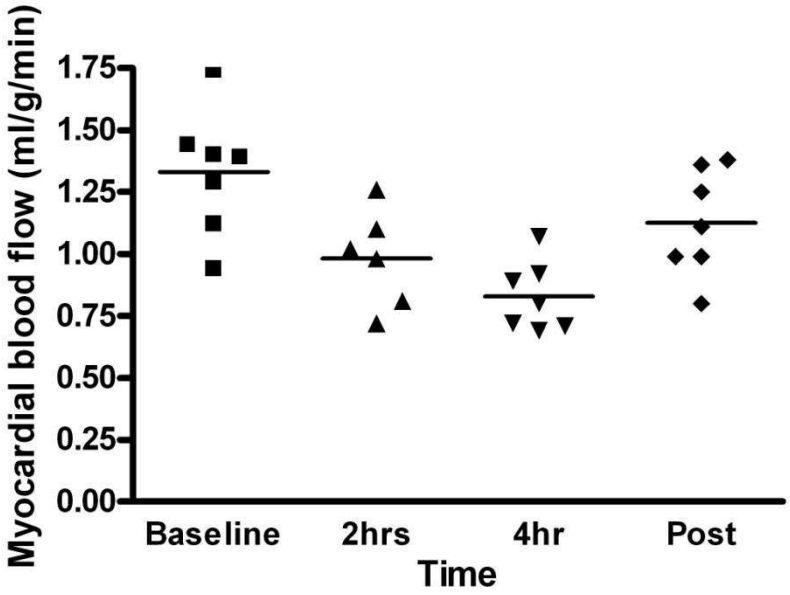
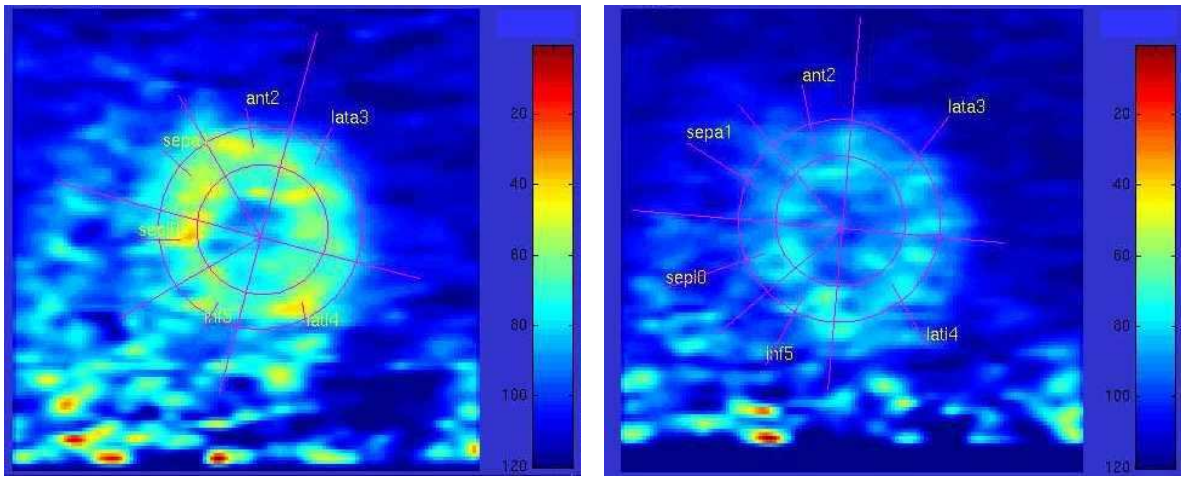


Figure 4.4.2a: Mean global myocardial blood flow reduced significantly over time during dialysis compared to baseline with partial restoration in the recovery period.

Segmental MBF was reduced in 67% and 90% of regions at two hours and at 4 hours respectively (figures 4.4.2b and 4.4.2c). Three of the four

patients had universal (100%) involvement with reduction of MBF in all segments during both dialysis modalities.



B C

Figures 4.4.2b and 4.4.2c: Regional myocardial blood flow is reduced over time on haemodialysis. Higher amounts of $H_2{}^{15}O$ tracer are present in the ventricular myocardium at baseline (b) compared to 4 hours into dialysis treatment (c) indicating reduced blood flow to that area.

Between dialysis modalities (HD and BFD) baseline MBF was compared on an individual basis for each. There were no significant differences in three of the four patients ($P>0.05$) however one patient showed a significant improvement in resting MBF after the two weeks of BFD. There were also no significant differences in MBF between dialysis modalities at two or four hours although when observing

individual patients, one had a significant improvement in MBF at four hours during BFD (P<0.001) without any significant difference in MBF at baseline.

After the recovery period, there was an improvement in MBF in 85% of segments demonstrating partial but not complete restoration of blood flow after 30 minutes. There was a greater overall restoration of MBF after BFD compared to HD (P<0.001) (see figure 4.4.2d).

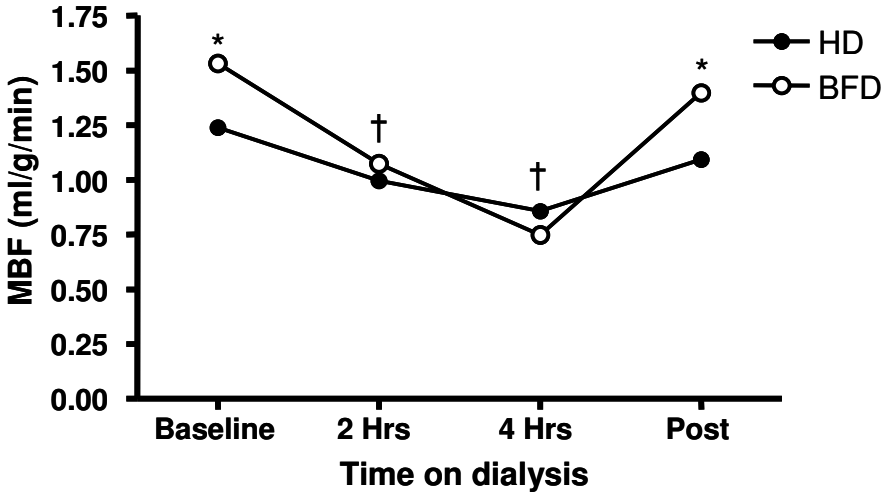


*Figure 4.4.2d: Mean MBF during HD and BFD. There was no significant benefit between dialysis modalities during treatment (†P>0.05) however, there was an improvement in MBF in the recovery period after BFD (*P<0.001).*

The development of RWMAs demonstrated by echocardiography was significantly associated with both: a greater overall percentage reduction in MBF (-30 ± 27.7% vs. -12.3 ± 12.3%) (P=0.001, see figure 4.4.2e); and a greater reduction in MBF at each time point studied (P<0.001 by ANOVA, see figure 4.4.2f).

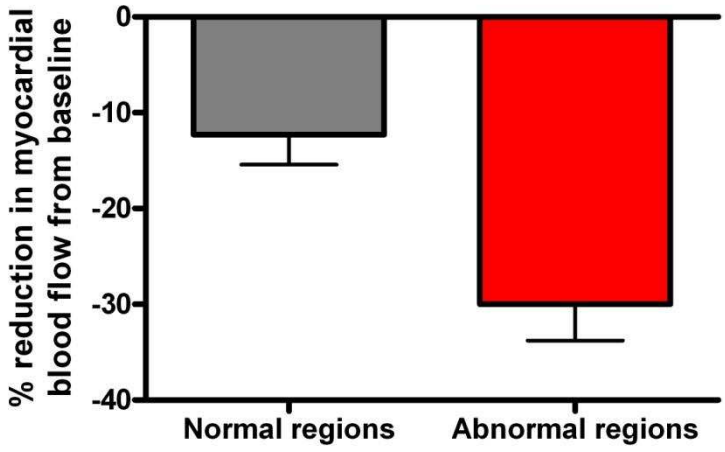


Figure 4.4.2e: The development of RWMAs (abnormal regions) was associated with a greater reduction in MBF from baseline than those areas that maintained normal movement (normal regions) (P=0.001)

Not all regions with reduced MBF were functionally affected; however a reduction in MBF ≥30% from baseline (mean reduction of MBF in all regions = 29%) was significantly associated with the development of RWMAs (P<0.01). Also, in areas where reduction in MBF was <30%,

there was actually a mean overall increase in percentage wall motion (see figure 4.4.2g).

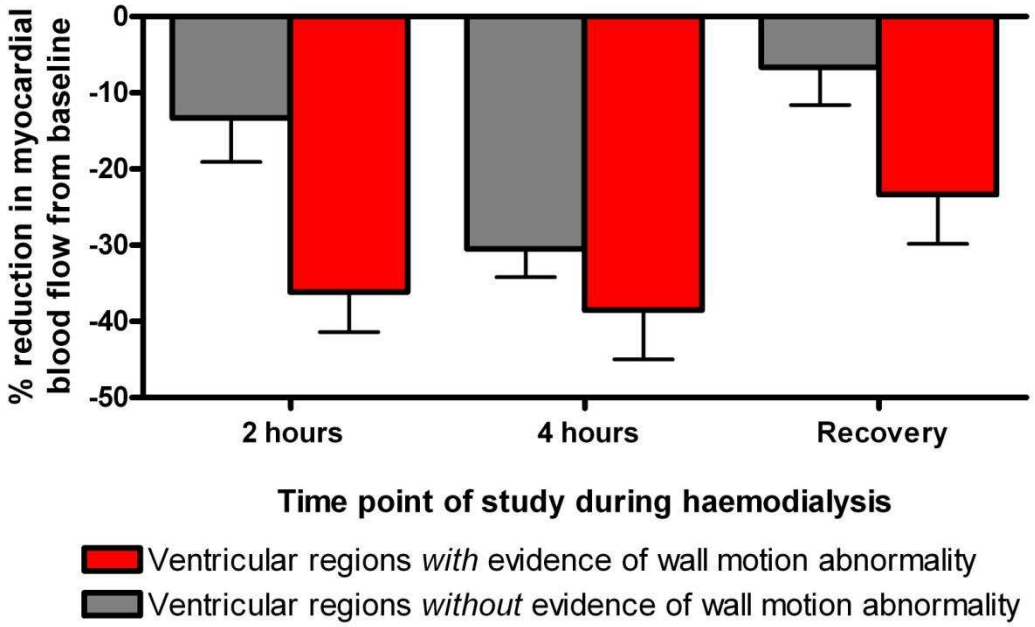


Figure 4.4.2f: The development of RWMAs (red bars) was associated with a greater reduction in MBF at each timepoint than those areas that maintained normal movement (grey bars) (P<0.001)

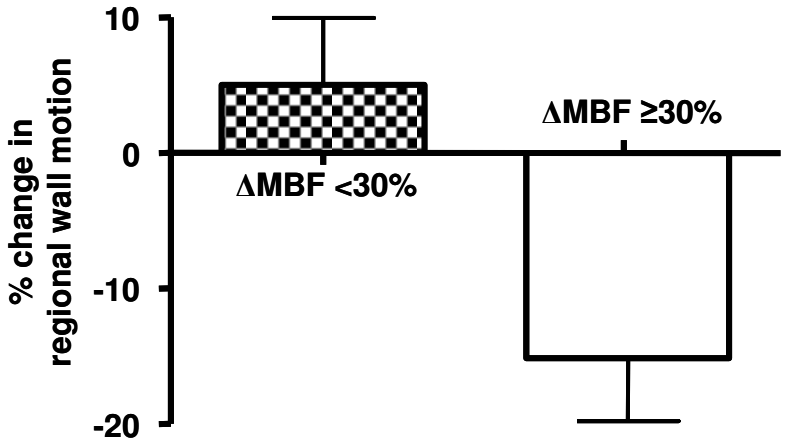
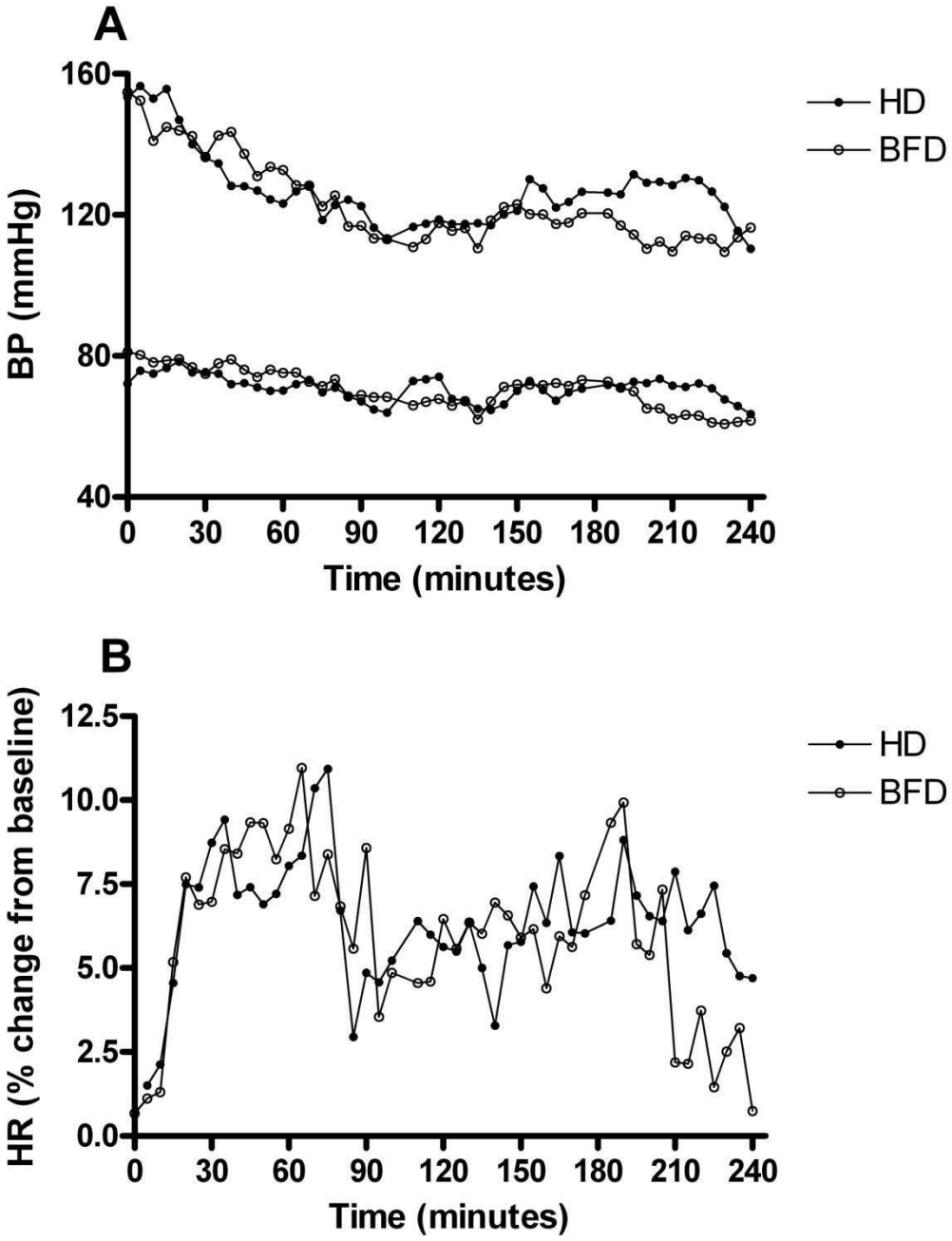

10
0
-10
-20
% change in
regional wall motion
ΔMBF <30%
ΔMBF ≥30%

Figure 4.4.2g: A MBF reduction of ≥30% was associated with a mean reduction in wall motion of -15.2% however a MBF reduction of <30% was associated with a mean increase in wall motion of 5% (P<0.01).

4.4.3 Haemodynamic data

Haemodynamic data are summarised in figures 4.4.3a and 4.4.3b. Mean systolic BP was maintained slightly higher during BFD (127 ± 10 mmHg) than HD (124 ± 12 mmHg) (P<0.05). There were no significant differences between diastolic BP or mean arterial pressure (MAP) between HD and BFD. None of the patients had episodes of symptomatic or asymptomatic intra-dialytic hypotension.

Heart rate increased throughout both modalities to a similar degree, with means of 6 ± 2% during HD and 6 ± 3% during BFD (NS).



Figures 4.4.3a and 4.4.3b: A) Overall mean blood pressures between HD and BFD. BP was maintained higher on BFD (P<0.05). B) Heart rate increased similarly during both HD and BFD (P=ns).

4.4.4 Laboratory data

There were no differences in any of the biochemical parameters when the two types of dialysis were compared. Cardiac troponin-I (cTnI) levels were similar between the 2 modalities and did not change significantly after dialysis. Three of the four patients had normal cTnI levels of <0.032 (table 4.4.4a).

Parameter	Standard dialysis (HD)		Biofeedback dialysis (BFD)	
	Pre dialysis	Post dialysis	Pre dialysis	Post dialysis
Haemoglobin (g/dl)	12.10 (10.7 to 13.4)	12.5 (11 to 14.6)	11.9 (11.3 to 13.6)	12.9 (11.3 to 13.1)
Haematocrit	0.36 (0.33 to 0.44)	0.37 (0.34 – 0.43)	0.37 (0.35 to 0.4)	0.38 (0.35 to 0.4)
Na^+ (mmol/l)	137 (135 to 149)	137.5 (136 to 138)	137 (136 to 138)	137 (135 to 138)
Corr. Ca^{2+} (mmol/l)	2.32 (2.22 to 2.39)	2.28 (2.15 to 2.35)	2.46 (2.36 to 2.46)	2.33 (2.33 to 2.33)
Phosphate (mmol/l)	1.75 (1.49 to 2.05)	0.89 (0.77 to 0.99)	1.93 (1.49 to 3.1)	1.05 (0.77 to 1.13)
Albumin (g/l)	32 (31 to 33.5)	36.5 (35 to 39)	32 (32 to 33)	34 (33 to 36)
CRP (mg/l)	7.5 (3 to 12)	7.5 (2 to 13)	4 (3 to 9)	5 (4 to 10)
cTnI (μg/l)*	<0.032 (<0.032 to 0.05)	<0.032 (<0.032 to 0.46)	<0.032 (<0.032 to 0.41)	<0.032 (<0.032 to 0.4)
Kt/V_{urea}	1.32 (1.3 to 1.42)		1.44 (1.42 to 1.52)	

*Table 4.4.4a: Laboratory data. Data are median (interquartile range). There were no significant differences between haemodialysis (HD) and biofeedback dialysis (BFD) for any parameters, including Kt/V_{urea}. CRP, C-reactive protein; cTnI, cardiac troponin-I; Corr. Ca^{2+}, corrected calcium. *Lowest value quoted 0.032 (normal).*

4.5 Discussion

This study demonstrates for the first time that HD is associated with significant reductions in MBF and that HD stress–induced myocardial ischemia results in the development of RWMA. Furthermore, dialysis-induced myocardial stunning occurs in the absence of large-vessel epicardial coronary disease.

All of the patients in all of the HD treatments studied developed RWMA during HD. Both the pattern of abnormalities and the subsequent partial resolution after dialysis are in keeping with previous reports of small studies done in this area [70,71]. In an additional cross-sectional study of 70 unselected HD patients, we also found significant dialysis-induced RWMA in 63% of patients who were undergoing conventional thrice-weekly HD [148].

Cardiac PET scanning using $H_2{}^{15}O$ revealed significant dialysis-induced changes in global and segmental MBF. Resting values in these patients were broadly in keeping with published values in patients without CAD or uremia [149]. This is expected because ischemic potential of myocardium is determined by reduced CFR rather than resting perfusion. The values were also consistent within an individual between two study sessions. All patients experienced global reduction of MBF, which increased during the HD session and was partially restored to normality 30 minutes after HD. This observed pattern of change is mirrored in the development of RWMA during HD, both within this study and in others [70,71] where the numbers of segments affected and the

severity (in terms of the magnitude reduction in percentage shortening fraction) are increased over time. The magnitude of the reduction in MBF at peak stress during HD is also broadly comparable with that observed in studies of pharmacologically stressed myocardial ischemia in patients with known significant CAD [67].

The reduction of MBF was significantly more marked in segments of myocardium that also displayed significant dialysis-induced reduction in contractile function. This was true both globally and at each given time point measured. Regional subendocardial and transmural MBF has been well correlated to reduction in segmental ventricular function in studies in conscious dogs that were subjected to graded coronary restriction [150]. We observed a similar scale of response in our patients who were subjected to HD.

Uraemic patients have a high prevalence of coronary atherosclerotic lesions [47,48] but although these patients did not have angiographic evidence of significant CAD (indeed, two of the patients had entirely normal coronary angiograms), HD was still capable of inducing myocardial ischemia. Previous descriptions of dialysis-induced myocardial ischemia and, indeed, implied ischemia from dialysis-associated RWMA, indicate that this is a common feature, with a prevalence well in excess of even the more pessimistic estimates of significant CAD in this patient group. As already discussed, patients with chronic kidney disease (especially those who receive HD) are uniquely well primed to experience demand ischemia as a result of a

variety of factors relating to structural and functional alterations in the cardiac microcirculation [151], as well as abnormalities in myocardial metabolism [152]. Specifically, a reduction in capillary density ('myocyte-capillary mismatch') has been described [153,154] which is, in part, explained by left ventricular hypertrophy. Myocardial perfusion reserve has been found to be decreased in patients with chronic renal failure [155], in diabetic HD patients [49] and in young adults after renal transplantation [156] in the absence of coronary artery disease. Even patients with mild renal insufficiency but without significant CAD have significantly reduced CFR measured at coronary artery catheterisation with adenosine challenge [155]. Other factors also play a role. LVH renders the ventricle more sensitive to acute changes in filling pressure during UF-induced hypovolaemia [53]. Increased peripheral artery stiffness also has an adverse effect on MBF and reduces the threshold for myocardial ischemia in patients with coronary artery disease [54]. LVH together with increased vascular stiffness predispose to reduced subendocardial blood flow [55]. Left ventricular hypertrophy, and peripheral arterial stiffness [157].

The haemodynamic response to HD was typical of that reported previously. The relative lack of change in heart rate with ultrafiltration-induced changes in BP is also typical. Baroreflex sensitivity is characteristically significantly impaired in this patient group [158] and may account at least in part for the cardiovascular response to HD [159]. Such heart rate stability also brings into question the validity of correcting MBF for RPP. Such correction, however, does not alter the pattern or

significance of the results reported in this study. Patients who underwent dialysis using biofeedback control did demonstrate significantly higher recovery of MBF in the postdialysis period. The reduction in BP was more marked when patients underwent dialysis using conventional rather than biofeedback HD. Although these patients did not experience any episodes of intradialytic hypotension (IDH), such biofeedback treatment has been associated with improvement of clinical indices of treatment tolerability even in patients who were characterised as being IDH resistant [113]. The difference in BP was significant only in the latter part of the HD treatment. This was in contrast to a previous study of biofeedback dialysis on the development of RWMA [71]. Comparison of this study with subsequent reduction in dialysis-induced RWMA using cooling of dialysate [70] suggests that the degree of protection from dialysis-induced cardiac injury is at least in part proportional to the maintenance of BP achieved.

4.6 Conclusions

The results in this chapter demonstrate that the cardiovascular stress of HD is capable of inducing significant global and segmental reductions in MBF. These changes in myocardial perfusion are repetitive and sufficient to result in reduced ventricular contraction. Such repetitive ischemic insults in patients with CAD are widely recognised to be important in the pathogenesis of cardiac failure. Stunning has been shown to be a powerful predictor of a dismal prognosis in patients with coronary artery disease [160]. HD-induced RWMA are common in dialysis

patients. Dialysis-induced myocardial stunning may be an important and previously unappreciated factor in the development of cardiac failure in HD patients. Additional attention to the way in which we dialyse patients may not only be important in the subjective patient tolerability of the treatment but also a key therapeutic target to reduce the vast cardiovascular morbidity and mortality to which these patents are subjected.

The following chapters aim to further knowledge into the extent of the problem of acute haemodialysis induced cardiac injury and functional decline in the haemodialysis population as a whole.

Chapter 5

Results:

Haemodialysis induced acute cardiac injury: prevalence and associated factors.

5 Results: Haemodialysis induced cardiac injury: prevalence and associated factors.

5.1 Introduction

The data contained in the previous chapter demonstrates that acute, dialysis-induced regional wall motion abnormalities are associated with a significant reduction in segmental myocardial blood flow, consistent with ischaemia. This was true even in the absence of significant macrovascular coronary artery disease. As previously mentioned, a number of small studies have described the phenomenon of HD induced myocardial stunning, as well as the potential (at least in the short term) to abrogate dialysis induced cardiac injury with dialysis techniques which improve haemodynamic tolerability [70,71].

The details of the mechanisms involved in HD induced myocardial stunning are currently largely unresolved and unfortunately, little is known about the extent to which the haemodialysis population actually suffers from this phenomenon. What is known however is that during HD, patients are particularly susceptible to myocardial ischaemia and in the four patients studied in chapter 4, all had a reduction in myocardial blood flow during dialysis and all had some degree of functional impairment as measured by the development of RWMAs. That study was however not powered adequately to look for either the prevalence or associated factors of myocardial stunning. A deeper understanding of

the prevalence of acute haemodialysis induced myocardial stunning may help to identify patients at risk of intra-dialytic myocardial ischaemia, RWMAs and haemodynamic instability. This may in turn help to develop dialysis based interventions to combat these complications and improve mortality.

This study was designed to ascertain the prevalence of myocardial stunning in the HD population to better understand the longer term consequences of this phenomenon on LV function, intradialytic haemodynamics and survival. In addition this study provided an opportunity to define the risk factors and their interactions in relation to the development of acute cardiac injury.

5.2 Methods

5.2.1 Patients

Seventy prevalent HD patients were recruited for a 12-month observational cohort study from a single hospital based haemodialysis unit. Patients were excluded if they had pre-existing severe LV systolic dysfunction (NYHA IV) or inadequate echocardiographical windows to obtain images of sufficient quality. Only one patient was excluded on this basis.

As patients dialysed as part of a conventional thrice weekly treatment regime (four hour dialysis sessions), all studies were conducted after the first two-day interdialytic period, as arrhythmias and cardiac events

are known to be increased after the 3-day interdialytic break. All patients were undergoing standard haemodialysis treatments with no sodium or biofeedback profiling.

5.2.2 Study protocol

After entry to the study, patients' were interviewed both to confirm consent and for the purpose of taking a medical history and performing a physical examination. All medical details were then confirmed using patients' notes.

After that initial meeting, all patients (n=70) subsequently underwent an initial (baseline) monitored haemodialysis session. During this monitored dialysis treatment, global and regional left ventricular function was measured using serial echocardiography at four separate time points: pre-dialysis; during treatment at both two and four hours; and again 30 minutes into the post-dialysis recovery phase. Non-invasive haemodynamic monitoring of blood pressure (BP) was undertaken every 15 minutes. Pre-dialysis blood tests were drawn immediately after insertion of access needles, and post levels were taken from the arterial line 10sec after reducing blood pump speed to 50ml/min. Single pool Kt/V_{urea} values were calculated from pre and post urea levels [144].

The primary endpoint was to assess the prevalence of HD induced RWMAs in a standard HD population. Secondary endpoints included the identification of variables associated with the development of LV RWMAs.

Ethical approval for the project was granted by Nottingham Local Research Ethics Committee.

5.2.3 Haemodialysis details

Dialysis was performed as described in chapter 3 using Hospal Integra monitors (Hospal, Mirandola, Italy) using low-flux polysulfone dialysers, either 1.8 or 2.0 m^2, per individual patients' usual prescriptions (LOPS 18/20; Braun Medical Ltd, Sheffield, UK).

Dialysate fluid contained sodium, 138 mmol/L; potassium, 1 mmol/L; calcium 1.25 mmol/L; magnesium, 0.5 mmol/L; bicarbonate, 32 mmol/L; glucose, 5.6 mmol/L; and acetate, 3 mmol/L. Dialysate sodium conductivity was set at 13.6 ms/cm.

All treatments were of four hours' duration, and anticoagulation was with unfractionated heparin. Dialysate flow was 500 mL/min, and dialysate temperature was set at 37°C. For each session, net fluid removal was set on an individual basis according to ideal dry weight. Blood pump speed varied between 250 and 450 mL/min, depending on the patient's vascular access.

5.2.4 Echocardiography

Echocardiography and subsequent analysis were performed as described in chapter 3. Images were recorded prior to commencing dialysis (baseline); at 120 minutes and 240 minutes during dialysis; and 30 minutes after dialysis was finished (recovery).

Ten regions of the left ventricle were assessed for the development of new regional wall motion abnormalities (RWMAs) at each time point. A wall motion abnormality was defined as a reduction in wall motion of >20% from baseline readings. Regions that showed a functional decline of >20% during HD compared to rest with evidence of functional recovery in the post-dialysis period were classed as stunned segments. The presence of three or more such RWMAs (out of a maximum of ten regions) in any individual patient was counted as evidence of clinically significant myocardial stunning.

5.2.5 Measurement of blood pressure

Non-invasive, serial blood pressure readings were taken as described in chapter 3. Blood pressure was measured pre-dialysis and then serially every 15 minutes during haemodialysis using an automated digital oscillometric device. Each value recorded was the mean of three individual readings.

5.2.6 Measurement of haematological and biochemical variables

Blood samples were analysed as detailed in chapter 3. Pre-dialysis blood tests were drawn immediately after insertion of access needles, and post-dialysis levels were taken from the arterial line 10 seconds after reduction of blood pump speed to 50 ml/min.

5.3 Statistical analysis

This power calculation is based on detecting significant changes in LVEF using echocardiography between baseline and 12 months of 3.6% [161] with a standard deviation of 8.987 [162] for which the required sample size was 68 patients. This achieved a power of 90% at a significance level of 5%.

Results are presented as the mean value ± SD or the median and interquartile range (IQR) unless otherwise stated. BP data were analysed using two-way analysis of variance (ANOVA) with Bonferroni's post tests for multiple comparisons. Categorical variables between the two groups were analysed using Fisher's exact test. Depending on Gaussian distribution (significant deviations from a normal distribution were excluded with the Kolmogorov-Smirnov test), all other data were analysed using either the paired or unpaired *t* tests or the Mann-Whitney or Wilcoxon matched pairs tests. An alpha error at $P<0.05$ was judged to be significant. An alpha error at $P<0.05$ was judged to be significant.

5.4 Results

5.4.1 Prevalence of regional wall motion abnormalities

64% of patients (45/70) developed a significant number of regional wall motion abnormalities during haemodialysis (figure 5.4.1a). There was

no significant difference between the number of regions affected at two hours compared to four hours (4.48±1.7 vs. 4.52±1.9, P=0.8).

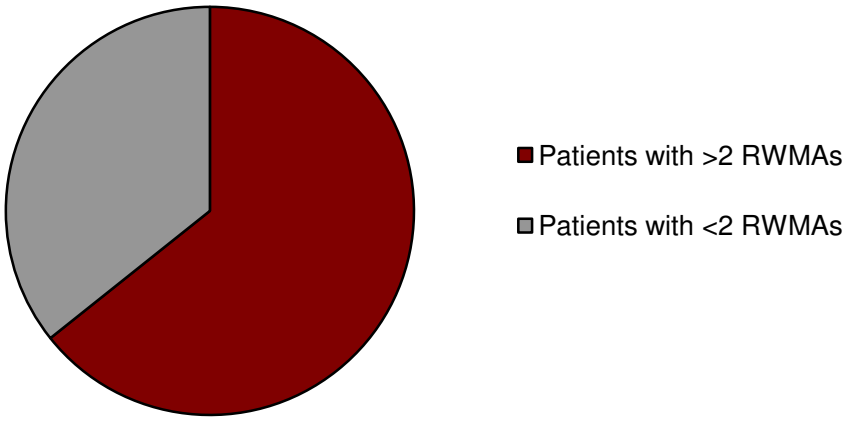


Figure 5.4.1a: Prevalence of haemodialysis induced left ventricular regional wall motion abnormalities (RWMAs).

5.4.2 Severity of regional wall motion abnormalities

In those regions affected, there was a significant reduction in percentage shortening fraction (%SF) at each timepoint compared to baseline (baseline 3.17±1.28%; two hours 1.75±0.67%, P<0.001; four hours 1.59±0.69%, P<0.001; recovery 2.3±0.94%, P<0.01). There was also a significant return towards pre-dialysis values for %SF between both timepoints during HD and the recovery period (P<0.001), confirming the presence of myocardial stunning. Although the mean

%SF was lower in affected regions at four hours compared to two, this was only a trend and did not reach statistical significance (P=0.14). All of these data are represented in figure 5.4.2a.

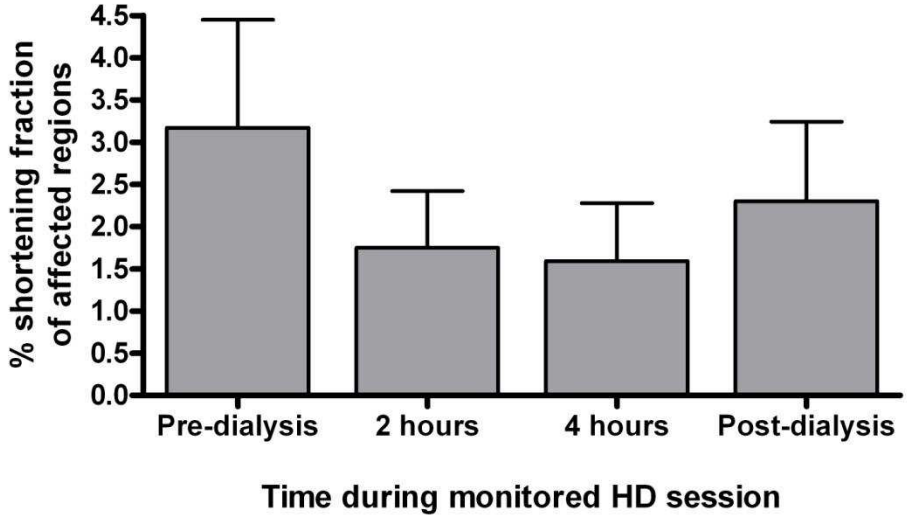


Figure 5.4.2a: Severity of regional wall motion abnormalities over time during haemodialysis. Percentage shortening fraction (%SF) was significantly lower compared to baseline at each timepoint. Post dialysis %SF was significantly higher than at both 2 and 4 hours during treatment, confirming at least partial recovery of function in those areas and consistent with myocardial stunning.

5.4.3 Patient characteristics and dialysis related factors associated with the presence of myocardial stunning

Patient demographics and other dialysis related factors are shown in table 5.4.3a. Univariate analysis of these variables showed a number of factors associated with the development of haemodialysis induced myocardial stunning. These were advancing age (P=0.03); higher intradialytic ultrafiltration volumes (P=0.01) and the presence of diabetes mellitus as a co-morbid condition (RR=1.7; CI 1.2 to 2.4; P=0.002), see figures 5.4.3a and 5.4.3b.

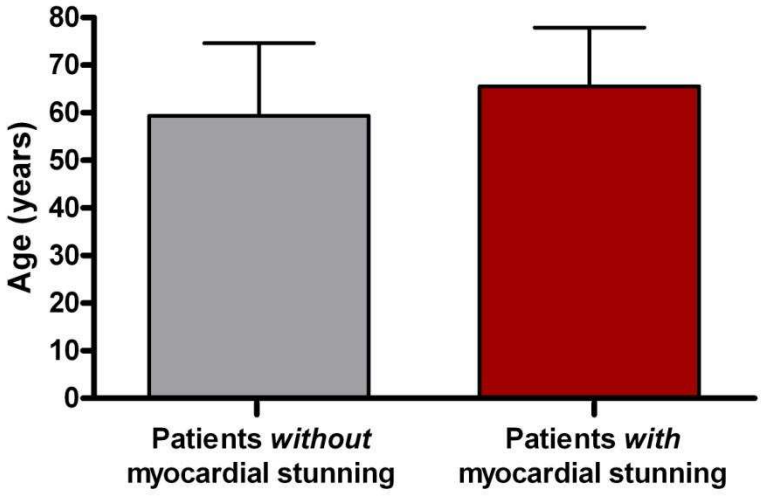


Figure 5.4.3a: Myocardial stunning and patient age. The development of HD-induced myocardial stunning was associated with a significantly higher age (P=0.03).

Characteristics	*All* Patients (n=70)	Patients *with* RWMAs (n=45)	Patients *without* RWMAs (n=25)	*P value*
Age (mean, years)	63.1 ± 13.7	65.6 ± 1.9	59.4 ± 3	0.03
Male:Female	47 : 23	28 : 17	19 : 6	0.29
Dialysis vintage (mean, months)	45.2 ± 32.3	43.5 ± 31	48.1 ± 34.8	0.57
Intradialytic UF volume (mean, L)	1.95 ± 0.83	2.13 ± 0.91	1.59 ± 0.75	0.01
Kt/V urea	1.3 ± 0.2	1.3 ± 0.2	1.3 ± 0.3	0.88
Smoker	11 (16%)	7 (16%)	4 (16%)	1
***Ethnicity* (n)**				
Caucasian	65 (93%)	43 (96%)	22 (88%)	0.34
Afro-caribbean	1 (1%)	1 (2%)	0 (0%)	1
Asian	4 (5%)	1 (2%)	3 (12%)	0.13
***Etiologies* (n)**				
Diabetic nephropathy	22 (31%)	18 (40%)	4 (16%)	0.06
Glomerular disease	12 (17%)	8 (18%)	4 (16%)	1
APKD	7 (10%)	5 (11%)	2 (8%)	1
Urological	6 (9%)	3 (7%)	3 (12%)	0.66
Unknown	9 (13%)	5 (11%)	4 (16%)	0.71
Other	14 (29%)	6 (13%)	8 (32%)	0.12
***Co-morbidities* (n)**				
Ischaemic heart disease	24 (34%)	19 (42%)	5 (20%)	0.071
Diabetes mellitus	28 (40%)	24 (53%)	4 (16%)	0.002
Hypertension	42 (60%)	27 (60%)	15 (60%)	1
Hyperlipidaemia	31 (44%)	21 (47%)	10 (40%)	0.62
Left ventricular hypertrophy	42 (60%)	27 (60%)	15 (60%)	1

Table 5.4.3a: Patient characteristics and dialysis related factors in all patients and in groups based on the presence (with) or absence (without) of dialysis induced regional wall motion abnormalities (RWMAs). APKD = adult polycystic kidney disease; UF = ultrafiltration.

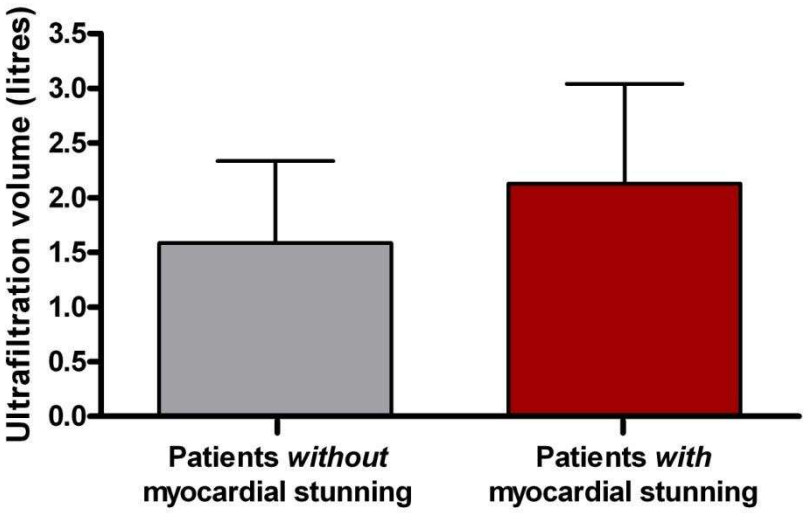


Figure 5.4.3b: Myocardial stunning and ultrafiltration volume. The development of HD-induced myocardial stunning was associated with a significantly higher ultrafiltration volume (P=0.01).

Patients with ischaemic heart disease (IHD) were more likely to develop HD-induced RWMAs but this was only a trend and did not reach statistical significance (P=0.07).

There were no other statistically significant differences between those patients with or without HD-induced myocardial stunning with respect to underlying aetiology, dialysis vintage, drug therapies or other co-morbid conditions.

5.4.4 Haematological and biochemical variables associated with the presence of myocardial stunning

Haematological and biochemical values are summarised in table 5.4.4a.

Parameter	Pre-dialysis blood testing			Post-dialysis blood testing		
	Patients *with* RWMAs	Patients *without* RWMAs	P value	Patients *with* RWMAs	Patients *without* RWMAs	P value
Haemoglobin (g/dL)	11 ± 1.3	11.5 ± 1	0.15	11.4 ± 1.4	12 ± 1.5	0.14
Haematocrit (L/L)	0.36 ± 0.04	0.36 ± 0.02	0.35	0.36 ± 0.04	0.37 ± 0.04	0.44
Na^+ (mmol/L)	138 ± 3	139 ± 4	0.18	138 ± 2	139 ± 3	0.1
K^+ (mmol/L)	4.8 ± 0.8	4.7 ± 0.9	0.89	3.0 ± 0.4	3.0 ± 0.5	0.69
Urea (mmol/L)	21.1 ± 5.4	19.8 ± 5.0	0.33	6.2 ± 2.2	6.3 ± 2.5	0.81
Creatinine (µmol/L)	668 ± 192	746 ± 220	0.13	259 ± 88	301 ± 132	0.12
Phosphate (mmol/L)	1.7 ± 0.4	1.6 ± 0.6	0.42	0.8 ± 0.2	0.8 ± 0.2	0.61
Bicarbonate (mmol/L)	22.7 ± 3.0	22.8 ± 3.2	0.87	27.8 ± 2.8	28.4 ± 2.5	0.44
Corrected Ca^{2+} (mmol/L)	2.42 ± 0.16	2.4 ± 0.15	0.58	2.36 ± 0.09	2.4 ± 0.11	0.84
Albumin (g/L)	35 ± 4.1	37 ± 3.4	0.02	37 ± 5.2	39 ± 5.7	0.04
hsCRP	11.02 ± 9.5	7.9 ± 6.3	0.13	-	-	-
cTnT (µg/L)	0.098 ± 0.08	0.036 ± 0.04	0.001	-	-	-

Table 5.4.4a: Baseline haematological and biochemical data stratified based on the presence (with) or absence (without) of haemodialysis induced myocardial stunning taken before and after HD. Post dialysis cardiac troponin-T (cTnT) levels were not taken due to an insufficient time lapse needed to detect a significant change. hsCRP = high sensitivity C-reactive protein.

Univariate analysis of these variables also showed a number of factors associated with the development of HD-induced myocardial stunning. These were lower albumin levels (p=0.02) and elevated cTnT concentration (P=0.001), see figures 5.4.4a and 5.4.4b.

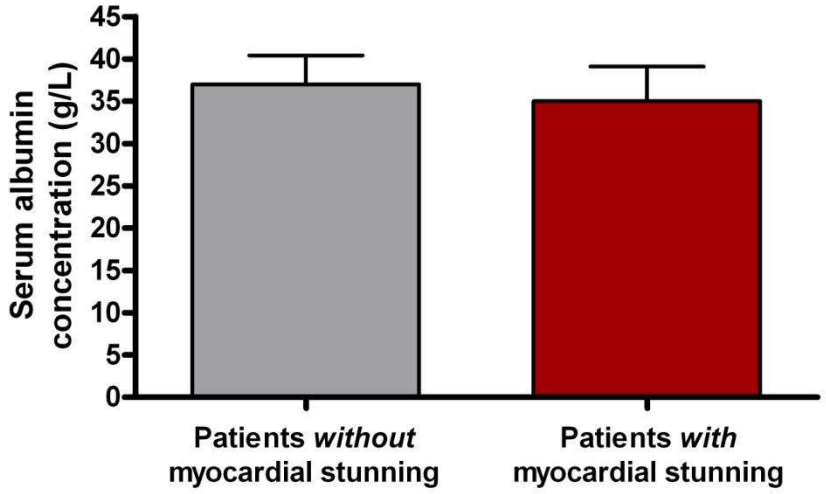


Figure 5.4.4a: Myocardial stunning and serum albumin. The development of HD-induced myocardial stunning was associated with a significantly lower serum albumin concentration (P=0.02).

There were no other statistically significant differences between those patients with or without HD-induced myocardial stunning with respect to haematological or biochemical markers that were measured.

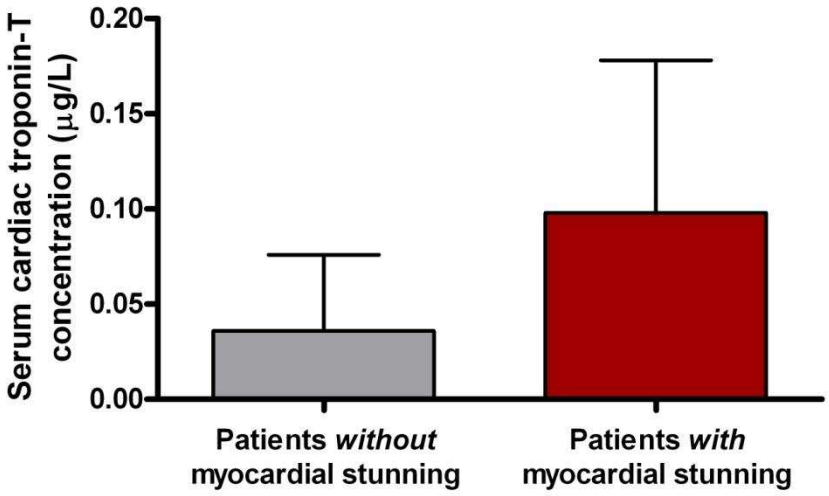


Figure 5.4.4b: Myocardial stunning and cardiac troponin-T. The development of HD-induced myocardial stunning was associated with a significantly higher serum cardiac troponin-T concentration (P=0.001).

5.4.5 Intradialytic blood pressure and myocardial stunning

There was a significant reduction in systolic blood pressure (SBP) during treatment in patients with HD-induced myocardial stunning (P<0.0001) compared to those without who had no such reduction in SBP (P=0.16), see figure 5.4.5a.

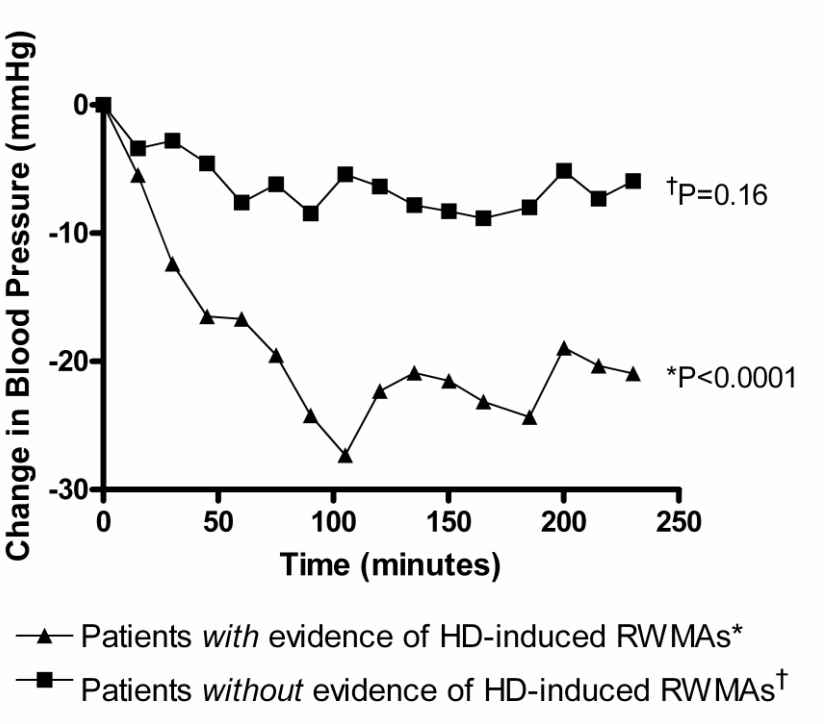


Figure 5.4.5a: Reduction in systolic blood pressure (SBP) during haemodialysis (HD) in patients with and without HD-induced regional wall motion abnormalities (RWMAs) consistent with myocardial stunning (P<0.0001).

The total number of episodes of intradialytic hypotension (as defined in chapter 3) was significantly higher in patients with myocardial stunning than those without (2.1 ± 3.5 episodes vs. 0.2 ± 0.5 episodes, P<0.01). Sub analysis revealed however that it was only asymptomatic intradialytic hypotension that was significantly different (1.9 ± 3.3 episodes vs. 0.3 ± 0.9 episodes, P=0.02) between patient groups – episodes of symptomatic IDH were not statistically different (0.2 ± 0.5 episodes vs. 0.04 ± 0.2 episodes, P=0.1).

5.4.6 Factors associated with the severity of myocardial stunning

Further analysis using Spearman univariate correlation revealed significant correlations between a number of these same variables mentioned above and the severity of myocardial stunning as measured by magnitude of reduction in %SF including: reduction in SBP (r^2 0.4, P=0.001); higher UF volumes (r^2 0.28, P=0.02); higher plasma cTnT concentration (r^2 0.29, P=0.02) and increasing age (r^2 0.29, P=0.014), see table 5.4.6a.

Variable	r^2	P value
Maximum SBP reduction	0.4	0.001
Ultrafiltration Volume	0.28	0.02
Asymptomatic IDH	0.28	0.02
Cardiac troponin-T	0.29	0.02
Age	0.29	0.014

Table 5.4.6a: Independent factors associated with the severity of myocardial stunning. SBP, systolic blood pressure; IDH, intradialytic hypotension.

5.4.7 Multivariate analysis of factors associated with myocardial stunning

Factor associated with development of myocardial stunning	Odds Ratio	P value
UF volume during HD of 1L	5.1	
UF volume during HD of 1.5L	11.6	0.007
UF volume during HD of 2L	26.2	
Maximum SBP reduction during HD of 10 mmHg	1.8	
Maximum SBP reduction during HD of 20 mmHg	3.3	0.002
Maximum SBP reduction during HD of 30 mmHg	6.0	

Table 5.4.7a: The effect of increasing ultrafiltration (UF) volume and worsening intradialytic haemodynamics on the development of HD-induced myocardial stunning.

Stepwise multivariate analysis of those factors contributing to the presence of myocardial stunning revealed that the following were all independent variables associated with the development of HD-induced RWMAs: maximum reduction in SBP, per mmHg (OR 1.1, CI 1.02-1.1, P=0.002); UF volume per litre (OR 5.1, CI 1.33-19.7, P=0.007); age per year (OR 1.26, CI 1.04-1.54, P=0.004) and cTnT concentrations per µg/L (OR 1.07, 1.01-1.13, P=0.018) (Nagelkerke R^2=0.6 of the model overall). The non linear increasing effects of UF volume and BP on risk

of developing HD induced cardiac injury are illustrated in table 5.4.7a. All other factors, including diabetes mellitus, albumin levels and IHD did not enter the final analysis.

5.5 Discussion

This chapter demonstrates for the first time that dialysis induced recurrent cardiac injury is common, and associated with a number of factors including reduction in myocardial contractile function. Furthermore we have identified specific elements of the dialysis process (and the patients' response to it) as previously unappreciated adverse factors and potential drivers of heart failure in HD patients.

Almost two-thirds of patients had evidence of acute, dialysis induced LV dysfunction. This was present at both timepoints studied, but was more severe towards the end of the HD session as treatment time progressed. After HD had completed there was evidence of partial (but not complete) recovery. However recovery was assessed at 30 minutes post HD, largely determined by patient willingness to remain in the unit after termination of therapy. We have previously demonstrated that HD can directly induce RWMAs [70,71] and that these are associated with a corresponding decrease in segmental myocardial blood flow with recovery post-HD [107] consistent with myocardial stunning

There was a significant association between the development of HD-induced myocardial stunning and diabetes mellitus, but not for patients with a history of IHD. In the HD population, decline in LVEF is usually

progressive over time and often accelerated [163], and there is increasing evidence that this is not due to traditional cardiac risk factors or conventional atherosclerotic coronary artery disease [164,165]. However, evidence suggests that HD patients with diabetes, but normal epicardial coronary anatomy, have significantly reduced coronary flow reserve [49], that predisposes to the development of demand ischaemia. It is therefore not surprising that the effects of diabetes and coronary artery disease *per se* on the development of HD-induced myocardial stunning might be dissociated from each other.

Lower serum albumin levels were also associated with the development of dialysis induced myocardial stunning (on a univariate basis only). There is a large body of evidence linking malnutrition and inflammation to the development of atherosclerosis in patients with end-stage renal disease (ESRD) [37,166-169]. Unfortunately, serum albumin in isolation (especially with no significant difference between high sensitivity C-reactive protein levels) is a poor diagnostic marker. The role of other biochemical markers such as interleukin-6 and fetuin-A may provide additional information on the association between HD-induced myocardial stunning, malnutrition, calcification and inflammation [170,171] but was outside the scope of this study.

Higher ultrafiltration volumes, intradialytic haemodynamic instability and raised biochemical markers of myocardial damage (cTnT) were all associated with the development and severity of HD-induced myocardial stunning. Logistic regression analysis confirmed that each of

these factors were independent determinants of myocardial stunning and that the risk associated with higher UF volumes and greater drops in SBP increased disproportionately with each additional unit of measure. It is certainly not unusual in clinical practice for patients to have ultrafiltration volumes set at two litres or more and to experience a drop in SBP of more than 20 mmHg.

It is well recognised that cardiac troponin-T (cTnT) levels are often elevated in dialysis patients and that elevated levels predict mortality [89-91,95,172-175]. This study has shown a direct association between troponin-T concentrations and the development and severity of myocardial stunning. A recent study of cardiac troponins and outcome in acute heart failure revealed associations with lower systolic blood pressure, lower left ventricular ejection fraction and higher mortality [88]. However the identification of 'at risk' patients who may benefit from specific dialysis based interventions remains a clinical challenge. The usefulness of cTnT and other biochemical markers of cardiac damage (in conjunction with other clinical factors like left ventricular ejection fraction and systolic blood pressure) remains unknown but may in the future provide a useful tool to identify vulnerable patients who would benefit from modified therapies and additional treatments.

Similarly, intradialytic hypotension (IDH) has also been shown to be an independent risk factor for mortality in HD patients [109,176,177]. It is interesting to note that the number of episodes of IDH with symptoms was comparable between those patients with myocardial stunning and

those without but that asymptomatic IDH was significantly higher in the myocardial stunning group. This would suggest that the most vulnerable patients, most susceptible to dialysis induced cardiac injury could go undetected on the majority of haemodialysis units. There is currently no research showing that higher ultrafiltration rates during HD are associated with poor outcome. However, there is evidence that both larger inter dialytic weight gain between two subsequent HD treatment sessions and higher ultrafiltration rate are associated with worse survival [178]. There are currently no available data to allow the potential differential effects of ultrafiltration volume and ultrafiltration rate on poor outcome to be appreciated. This finding of the effect of ultrafiltration requirements is crucially important as it represents a potential therapeutic target that might be pursued by a variety of means. More frequent daily or nocturnal HD therapies can improve cardiovascular outcome measures and quality of life [179,180]. Such treatments have also been shown to improve intradialytic haemodynamics by reducing IDH [120] and caused an improvement in plasma concentrations of biochemical markers of myocardial damage and dysfunction [121]. We have already demonstrated that dialysis based strategies such as cooled dialysate and the use of bio-feedback control can improve intradialytic haemodynamics and reduce HD-induced myocardial stunning [70]. Targeting treatments that minimise ultrafiltration rates and improve IDH will prevent the acute development of myocardial stunning, and may improve long term cardiovascular outcomes.

5.6 Conclusions

Conventional HD exerts significant acute stress upon the cardiovascular system. The results in this chapter support the contention that subclinical myocardial ischaemia causing myocardial stunning is commonly precipitated by dialysis. Such episodes of ischaemia are associated with higher ultrafiltration volumes, intradialytic haemodynamic instability and raised biochemical markers of ischaemic myocardial damage. These same variables are also associated with the severity of stunning as measured by the magnitude of reduction in regional shortening fraction. Unfortunately however, the most at risk and vulnerable patients remain asymptomatic and therefore difficult to identify, making it more challenging to target interventions to those patients who would most benefit.

The results in the following chapters will highlight the potential acute and chronic consequences of repetitive haemodialysis induced myocardial injury. A deeper knowledge of these short and long-term sequelae will further help to appreciate the true nature of this phenomenon and its contribution to the appalling cardiovascular morbidity and mortality in chronic HD patients.

Chapter 6

Results:

Haemodialysis induced left ventricular dysfunction is associated with an increase in ventricular arrhythmias.

6 Results: Haemodialysis induced left ventricular dysfunction is associated with an increase in ventricular arrhythmias.

6.1 Introduction

As already discussed, the majority of haemodialysis patients die from cardiovascular causes and a significant proportion of that mortality is attributable to sudden cardiac death. As sudden cardiac death appears to correlate with the peri-dialytic period, a number of studies looking at the potential pro-arrhythmogenic effects of HD and the presence of both potentially life-threatening complex ventricular arrhythmias (CVAs) and premature ventricular complexes (PVCs) has been associated with increased morbidity and mortality.

Separate to that, we have now shown that haemodialysis causes a reduction in regional ventricular function as seen in the form of regional wall motion abnormalities and that this is associated with a matched reduction in myocardial blood flow consistent with ischaemia. Other studies have linked the increase in ventricular ectopy and arrhythmias with alternative surrogate markers of ischaemia (e.g. the presence of coronary vessel stenoses) but no evidence exists to date of a link between dialysis induced acute left ventricular dysfunction (secondary to myocardial ischaemia) and cardiac arrhythmias.

We hypothesise that myocardial ischaemia may contribute to the development of both RWMAs and ventricular arrhythmias. As ischaemia induced RWMAs are potentially preventable [70,71], the identification of a common pathophysiological process connecting both sudden and ischaemic cardiac death in HD patients may offer single therapeutic targets to reduce both causes of mortality. The aim of this study was to investigate any association between the development of left ventricular RWMAs and arrhythmias.

Additional aims included: the evaluation of the roles of co-morbid conditions such as diabetes, left ventricular hypertrophy and ischaemic heart disease (as well as other demographic variables) that may contribute to increased demand ischaemia; and any association between serum electrolyte concentrations and the presence of arrhythmias.

6.2 Methods

6.2.1 Patients

Forty prevalent HD patients were recruited for an observational cohort study from a single hospital based haemodialysis unit. Patients were excluded if they had: a change in target weight in the preceding six weeks, pre-existing severe LV systolic dysfunction or inadequate echocardiographical windows to obtain images of sufficient quality. All patients haemodialysed thrice weekly for four hours. The study session was conducted after the first short inter-dialytic period as arrhythmias

and cardiac events are known to be increased after the long inter-dialytic break. Medications remained unchanged.

6.2.2 Study protocol

After recruitment and consent, patients' dry weight and anti-hypertensive as well as all other medications remained unchanged for the duration of the study. Patients were continued on standard thrice weekly haemodialysis. Patients were encouraged to continue daily activities as usual before the study session. In the event that the patient had suffered symptoms of angina or used their GTN spray within 24 hours of commencement, the session was postponed as this may have affected the continuous ECG monitoring and the frequency of ventricular arrhythmias and ectopics. The session was then rescheduled for four weeks time at which point the same restrictions applied.

For the monitored dialysis treatment, serial echocardiography was performed pre-dialysis, during treatment at two and four hours and again 30 minutes into the recovery. Non-invasive continuous ECG recordings were undertaken using a Holter monitor. Pre-dialysis blood tests were drawn immediately after insertion of access needles, and post levels were taken from the arterial line 10 seconds after reducing blood pump speed to 50 ml/min. Single pool Kt/V_{urea} values were calculated from pre and post urea levels [144].

The primary endpoint was the frequency of ventricular ectopics and complex ventricular arrhythmias during HD in relation to the development of ischaemia induced LV regional wall motion abnormalities.

All patients gave informed consent prior to commencement, and ethical approval for the project was granted by Derbyshire Local Research Ethics Committee.

6.2.3 Haemodialysis details

Dialysis was performed as described in chapter 3 and is not repeated here.

6.2.4 Continuous ECG monitoring (Holter)

Continuous measurement of patients' ECG using the Holter monitors is described in detail in chapter 3. Patients were connected to the monitoring equipment and 5 minutes of baseline data was collected before the commencement of dialysis. This enabled the Holter analysis software (NorthEast Monitoring Inc Holter LX Enhanced Software) to more accurately distinguish intradialytic changes in ECG morphology. The initiation of dialysis was marked as an event using the built in event recognition software.

After 24 hours, the Holter device automatically stopped recording and patients were instructed on how to remove the monitors themselves. All

equipment was returned to the Haemodialysis Unit for data retrieval at their next treatment session 48 hours later.

Frequency of ectopy was classified as a percentage of the total beats during the time period studied [142]: rare (≤0.1%), occasional (>0.1 to 1.0%), frequent (>1.0 to 10%) and very frequent (>10%). Ventricular arrhythmias were stratified according to the Lown classification [143] (class 0, no VEs; class 1, unifocal VEs <30/hour; class 2, unifocal VEs >30/hour; class 3, multiform VEs; class 4a, 2 consecutive VEs; class 4b, ≥3 consecutive VEs; class 5, R-on-T phenomenon). Classes 3 and above were accepted as complex ventricular arrhythmias. All patients who were classified as having a class 3 arrhythmia or above had all their abnormal complexes visually reviewed to exclude false positive results caused by artifact.

6.2.5 Echocardiography

Echocardiography and subsequent analysis were performed as described in chapter 3. Images were recorded prior to commencing dialysis (baseline); at 120 minutes and 240 minutes during dialysis; and 30 minutes after dialysis was finished (recovery). Ten regions of the left ventricle were assessed for the development of new regional wall motion abnormalities (RWMAs) at each time point as before.

6.3 Statistical analysis

Results are expressed as mean ± SD if normally distributed or median (interquartile range, IQR) if non-normally distributed unless otherwise stated. Categorical data was analysed using Fisher's exact test. Both echocardiographic and Holter data were analysed using the unpaired *t*-test or the Mann-Whitney test for unpaired data depending on normality of the distribution. Similarly, for paired data the paired *t*-test or Wilcoxon signed rank test was used. An alpha error at $P<0.05$ was judged to be significant.

6.4 Results

6.4.1 Haemodialysis induced echocardiographic dysfunction

67% (27) of the 40 patients developed significant RWMAs at peak stress on HD defined as a reduction in SF of >20% in >2 LV regions. Of these patients, 58% had shown recovery 30 minutes after HD, confirming the presence of myocardial stunning in this group.

6.4.2 Prevalence and associations of ventricular ectopy

Premature ventricular complexes (PVCs) were detected in 63% (25) of patients with 25% (10) being classed as frequent or very frequent (figure 4.4.2a. The total frequency of PVCs was higher during HD than in the post dialysis phase (0.26% (IQR 0.04 to 1.3) vs. 0.12% (IQR 0.01 to 0.7), $P<0.01$).

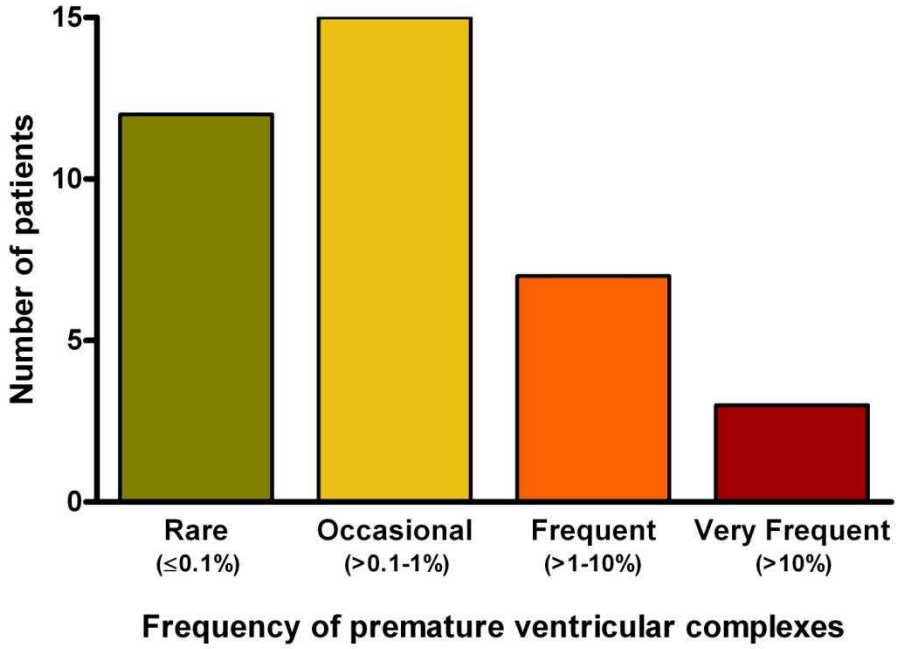


Figure 6.4.2a: Frequency of premature ventricular complexes. 25% (10) were classed as frequent or very frequent.

Demographic data are shown in table 6.4.2a.

Characteristics	All Patients (n=40)	Sub Groups			
		Occasional / Rare PVCs	Frequent / very frequent PVCs	Lown Score <3	Lown Score ≥3
Age (mean, years)	63.7 ± 13.2	62 ± 13.4	69 ± 12	59.6 ± 13.8	66 ± 12.7
Male:Female (n)	28 : 12	16 : 10	12 : 2	12 : 4	14 : 10
Dialysis vintage (mean, months)	50.6 ± 34.3	50 ± 37.9	55.3 ± 28.9	57.2 ± 38.9	46.7 ± 32.5
Ethnicity (n)					
Caucasian	38	26	0	16	22
Asian	2	0	2	0	2
Etiologies (n)					
Diabetes mellitus	13	8	5	5	8
Glomerular disease	8	6	2	4	4
APKD	4	3	1	1	3
HT / Renovascular disease	2	1	1	0	2
Other	9	5	4	5	4
Unknown	4	3	1	1	3
Relevant medication (n)					
ACEi / ARB	8	4	4	3	5
B-blocker	4	2	2	3	1
Calcium channel blocker	7	3	4	3	4
Digoxin	1	1	0	1	0
Amiodarone	2	2	0	2	0

Table 6.4.2a: Demographic characteristics of the patients. PVCs, premature ventricular complexes; APKD, adult polycystic kidney disease; HT, hypertension; ACEi, angiotensin converting enzyme inhibitor; ARB, angiotensin-II receptor blocker.

There were no statistically significant differences with respect to frequency of PVCs with any of the variables described (P>0.05). Of note, the frequency with which the two patients on amiodarone experienced PVCs was classified as rare and occasional.

Parameter	Patients with rare or occasional PVCs		Patients with frequent or very frequent PVCs	
	Pre-dialysis	Post dialysis	Pre dialysis	Post dialysis
Haemoglobin (g/dl)	11.4 ± 1.5	11.9 ± 1.8	10.6 ± 1.0	10.9 ± 1.0
Na^+ (mmol/L)	138.1 ± 3.4	137.9 ± 2.7	138.0 ± 3.6	138.1 ± 2.5
K^+ (mmol/L)	4.9 ± 0.8	3.1 ± 0.4*	4.4 ± 0.8	2.7 ± 0.3*
Urea (mmol/L)	20.4 ± 5.4	6.1 ± 2.2	22.3 ± 4.7	6.2 ± 2.0
Creatinine (µmol/L)	711 ± 169	275 ± 80	723 ± 241	257 ± 97
Phosphate (mmol/L)	1.7 ± 0.4	0.8 ± 0.2	1.7 ± 0.5	0.7 ± 0.2
Bicarbonate (mmol/L)	22.7 ± 2.7	27.0 ± 3.4	22.2 ± 3.3	29.3 ± 2.0
Corrected Ca^{2+} (mmol/L)	2.4 ± 0.2	2.4 ± 0.1	2.4 ± 0.1	2.3 ± 0.1
Albumin (g/L)	36.4 ± 3.8	38.9 ± 6.2	35.1 ± 5.3	36.2 ± 5.9
cTnT (µg/L)	0.09 ± 0.1	-	0.08 ± 0.06	-

*Table 6.4.2b: Biochemical data stratified according to frequency of PVCs (expressed as mean ± SD). Except for post dialysis K^+ levels (*P<0.02), there were no significant differences between those patients with rare or occasional PVCs and those with frequent or very frequent PVCs.*

Laboratory data stratified according to frequency of PVCs are shown in table 6.4.2b. Lower post-dialysis potassium (K^+) levels were associated with the development of PVCs (2.7 ± 0.3 mmol/L vs. 3.1 ± 0.4 mmol/L, P<0.02). There were no other significant differences between patients with rare or occasional PVCs and those with frequent or very frequent PVCs.

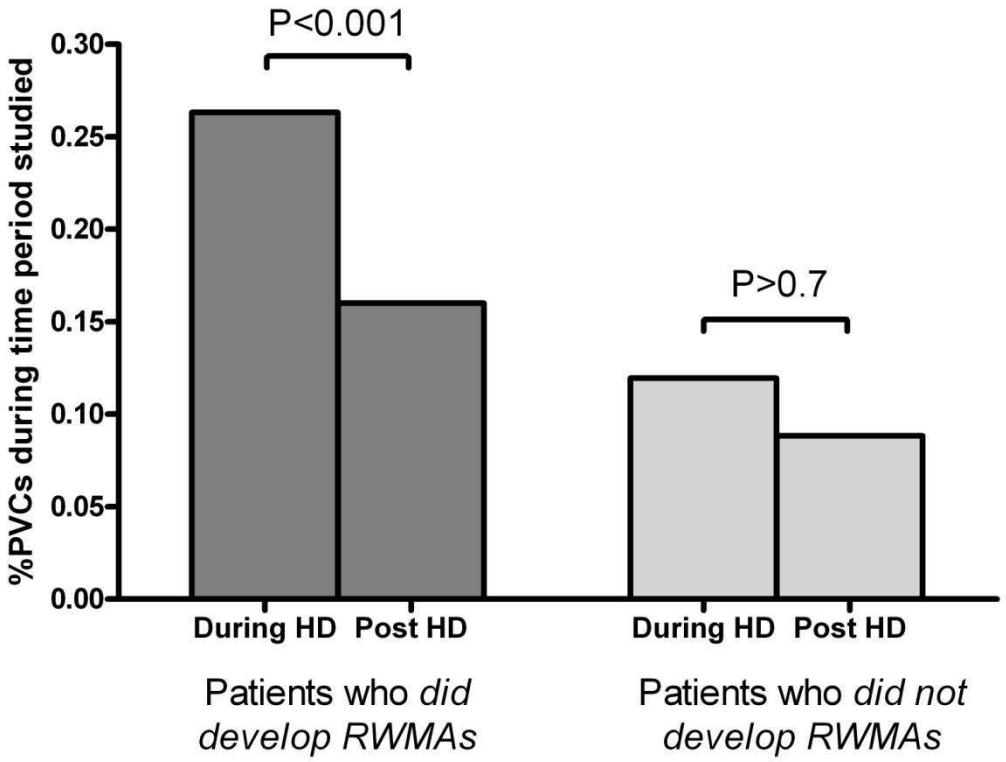


Figure 6.4.2b: The effect of RWMA and HD on %PVCs – The frequency of PVCs was significantly higher during HD than in the post dialysis phase in those patients who developed RWMAs (n=27). There was no such difference in those patients who did not develop RWMAs (n=13).

The frequency of PVCs was higher during HD in patients who developed RWMAs compared to the post dialysis period (0.26% (IQR 0.07 to 1.82) vs. 0.16% (IQR 0.01 to 0.7), P<0.001). However, in those patients who did not develop RWMAs, there was no significant difference in the incidence of PVCs during and after HD (0.12% (IQR 0 to 0.67) vs. 0.09% (IQR 0 to 1.2), P>0.7), see figure 6.4.2b. Although the median incidence of PVCs both during and after dialysis was higher in those patients who developed RWMAs compared to those who did not, this did not reach statistical significance (P>0.05).

Patients with pre-existing ischaemic heart disease (IHD) (n=13) had an increased frequency of PVCs during HD compared to those without (1.19% (IQR 0.15 to 3.46) vs. 0.17% (IQR 0.02 to 0.48), P<0.03) as did patients with LVH (n=18) (0.87% (IQR 0.21 to 3.46) vs. 0.12% (IQR 0.01 to 0.76), P<0.02). Post dialysis, there was no difference in the frequency of PVCs between either group (P>0.2), see figure 6.4.2c.

There was also a difference in median values between the frequency of PVCs during and after HD within the cohorts of patients with IHD and LVH but this did not reach statistical significance (P>0.1). Only four patients had both IHD and LVH and no increased risk for both was observed.

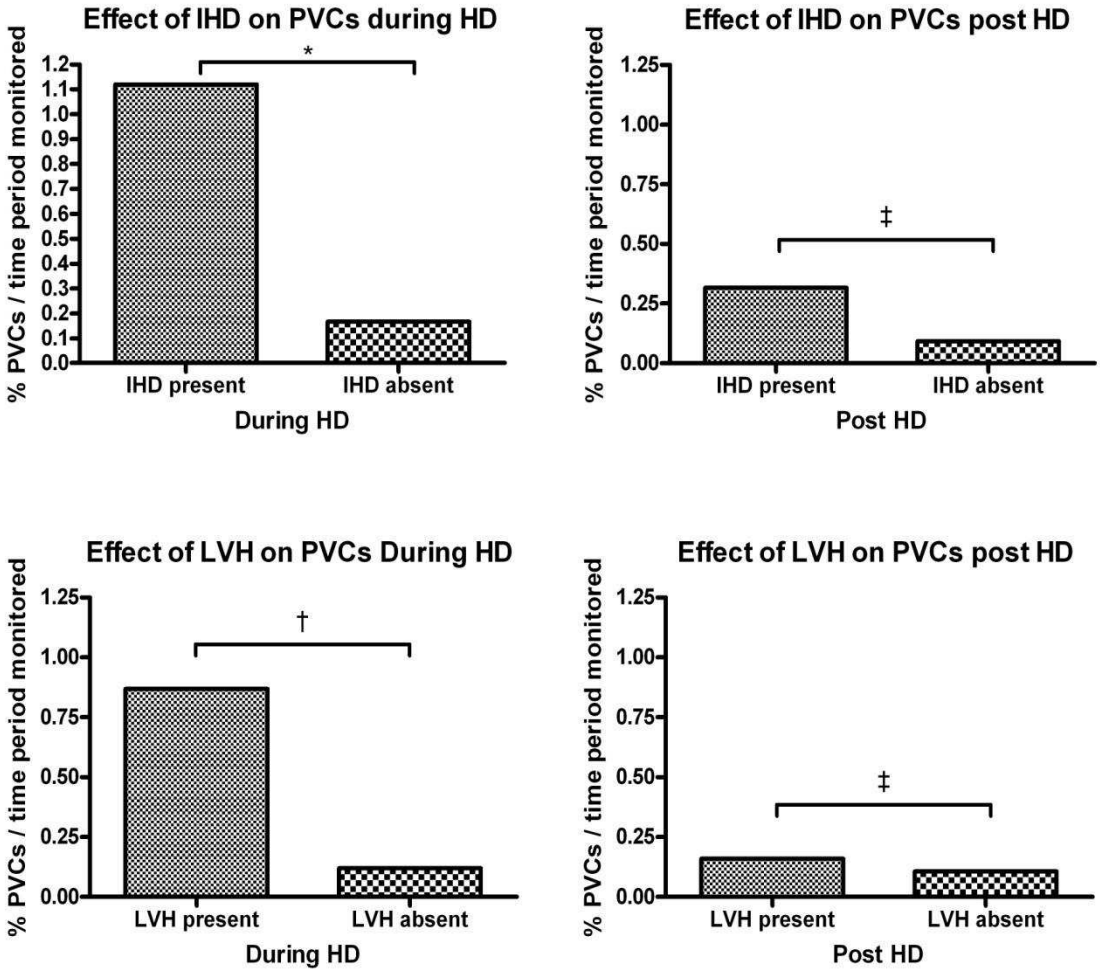


*Figure 6.4.2c: The impact of IHD and LV morphology on PVCs – the frequency of PVCs during HD in patients with IHD was significantly higher than those without (*P<0.02). Similarly, the frequency of PVCs was significantly higher in patients with LVH compared to those without (†P<0.03). There was no difference between the groups post dialysis (‡P>0.2).*

6.4.3 Prevalence and associations of ventricular complexity (Lown Score)

61% (24) of patients were classified as having CVAs with a Lown score of 3 or above (figure 4.4.3a). Of those patients with a Lown score of 4 (a or b), the total number of episodes of ≥2 consecutive PVCs was considerably higher during HD than in the follow up period (57 ± 5.3 vs. 17 ± 7.6, P<0.004).

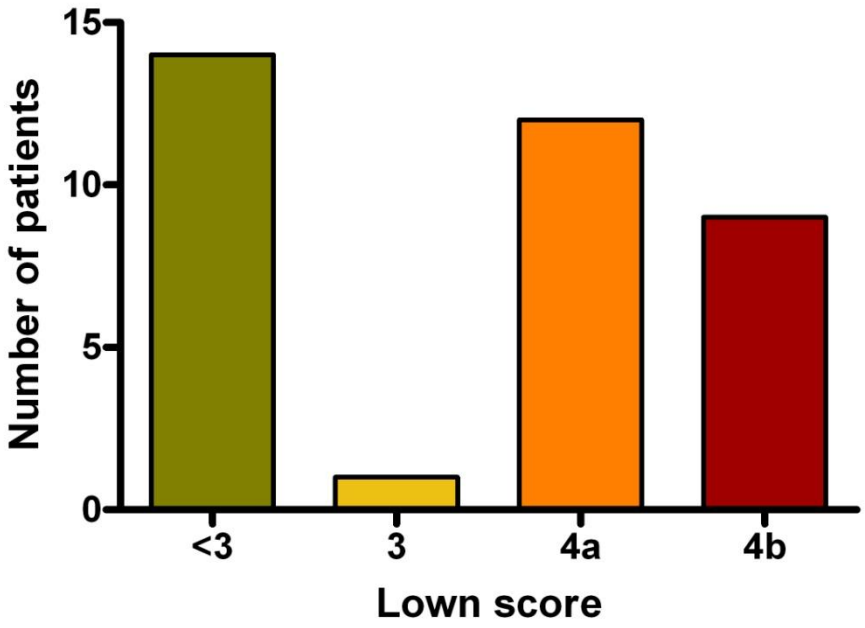


Figure 6.4.2a: Patients stratified according to Lown score. 61% (24) had a score of 3 or above. No patients recorded a score of 5.

Demographic data are shown above in table 6.4.2a. There were no statistically significant differences with respect to Lown score and any of

the variables described (P>0.05). Of note, the two patients on amiodarone had a Lown score of one and zero (i.e. no development of CVAs).

Parameter	Patients with Lown score <3		Patients with Lown score ≥3 (CVAs)	
	Pre-dialysis	Post dialysis	Pre dialysis	Post dialysis
Haemoglobin (g/dl)	10.9 ± 1.3	11.5 ± 1.5	11.2 ± 1.4	11.7 ± 1.7
Na^+ (mmol/L)	138.7 ± 3.1	137.9 ± 2.9	137.8 ± 3.5	138.0 ± 2.5
K^+ (mmol/L)	4.7 ± 0.8	3.1 ± 0.5*	4.8 ± 0.8	2.9 ± 0.3*
Urea (mmol/L)	19.5 ± 5.4	6.1 ± 2.5	21.9 ± 5.0	6.2 ± 1.9
Creatinine (µmol/L)	721 ± 164	282 ± 85	711 ± 209	261 ± 86
Phosphate (mmol/L)	1.6 ± 0.4	0.9 ± 0.2	1.7 ± 0.5	0.7 ± 0.2
Bicarbonate (mmol/L)	22.4 ± 3.2	26.5 ± 4.3	22.6 ± 2.7	28.5 ± 2.1
Corrected Ca^{2+} (mmol/L)	2.4 ± 0.1	2.4 ± 0.05	2.4 ± 0.2	2.4 ± 0.1
Albumin (g/L)	36.1 ± 4.0	38.4 ± 5.8	36.0 ± 4.5	37.7 ± 6.5
cTnT (µg/L)	0.1 ± 0.13		0.08 ± 0.06	

*Table 4.3.3a: Biochemical data stratified according to Lown score (expressed as mean ± SD). Except for post dialysis K^+ levels (*P<0.04), there were no significant differences between patients with a Lown score of <3 and those with a Lown score of ≥3 representing complex ventricular arrhythmias (CVAs).*

Laboratory data stratified according to Lown score are shown in table 4.4.3a. Lower post-dialysis potassium (K^+) levels were associated with a Lown score ≥3 (2.9 ± 0.3 mmol/L vs. 3.1 ± 0.5 mmol/L, P<0.04). There were no other significant differences between patients with a Lown score <3 and those with a Lown score ≥3.

There were no correlations between Lown score and the development of RWMAs (P>0.7) or change in %SF between any of the time points. Patients with IHD were more likely to have a Lown score ≥3 although this was only a trend and did not reach statistical significance (P<0.08). There was no significant association between LVH and Lown score (P>0.4). Again, no increased risk in patients with both IHD and LVH was observed.

There was no evidence of significant ST segment change associated with either the development of CVAs, PVCs or RWMAs (P>0.05).

6.5 Discussion

This study demonstrates a significant number of new, reversible left ventricular RWMAs occurred during HD, consistent with our previous findings [70,71]. We have also confirmed the arrhythmogenicity of HD. Ventricular ectopy in the form of PVCs and CVAs is increased during HD, which is consistent with previously published evidence [181,182]. More significantly, we have demonstrated a significantly higher frequency of PVCs during HD in those patients who develop dialysis induced acute cardiac injury (in the form of RWMAs).

We found no significant association between PVCs or arrhythmias and demographic variables including concomitant medication. Previous evidence has suggested that the high incidence of ventricular arrhythmias detected in previous studies could be due to differences in patient recruitment and variables including age, disease aetiology, dialysis vintage and duration, and concomitant medication [103]. We found no such association between these variables and the frequency of PVCs or arrhythmias. Furthermore, the use of digoxin in patients on maintenance HD has been shown to increase the incidence of occult and potentially serious ventricular ectopy [183,184] and also potentially contribute to the high incidence of PVCs and CVAs in HD patients. Only one (2.5%) out of the 40 patients recruited to this study was on digoxin and they had no evidence of ectopic ventricular activity, with a Lown score of zero.

Lower post dialysis potassium concentrations were associated with a higher incidence of PVCs and an increased Lown score. The evidence relating to the association of end-dialysis potassium concentration with ventricular ectopy is somewhat contradictory. The largest study looking at the use of potassium profiling, designed to avoid rapid shifts in plasma potassium during HD did show a reduction in the arrhythmogenic effect of HD with this technique [185]. However, another study showed no increase in arrhythmias with empirical reductions in fixed dialysate potassium concentration, even when using a dialysate solution containing no potassium at all [186]. There was no association between post-dialysis potassium concentration and the development of

RWMAs. Whilst the association between the development of ischaemia related functional decline and arrhythmias is clear, it is not so easy to clearly define the role of potassium within that relationship.

The incidence of new RWMAs and the prevalence of dialysis induced myocardial stunning were both in keeping with previous work [187]. RWMAs have been shown to develop in response to a decrease in myocardial blood flow [107]. This work has shown a direct correlation between HD induced functional decline of the left ventricular myocardium at a segmental level and falling myocardial blood flow within the corresponding regions.

The incidence of cardiac arrhythmias manifesting as PVCs and CVAs stratified according to Lown score was also consistent with previous published work [24]. We confirmed that these arrhythmias were higher during HD than in the subsequent monitored period post-dialysis [60]. Our results did not show an association between the development of RWMAs and Lown score however there is evidence that increasing Lown scores do not predict mortality in HD patients [188].

Patients who developed RWMAs on HD had a significantly higher incidence of PVCs during HD than in the following 20 hour period. Patients who did not develop left ventricular RWMAs displayed no difference in the incidence of PVCs during and after HD. As mentioned above, the development of RWMAs and myocardial stunning are linked with both global and regional myocardial ischaemia. In this study, IHD and LVH were also associated with an increased frequency of PVCs

during HD. Both of these pathological processes pre-dispose our patients to demand myocardial ischaemia.

There is to date, no published work on the potential link between HD induced myocardial ischaemia and the development of ventricular arrhythmias. In this study, the numbers of intra-dialytic PVCs were significantly increased in patients with evidence of HD induced myocardial ischaemia. This strengthens the hypothesis that HD induces ischaemic damage and that sudden death in HD patients may also be linked to an underlying ischaemic process. Unfortunately, patient numbers did not allow for multivariate analysis to understand in more detail the exact role of each of these variables so no further conclusions are derived from these results. However, this still has important clinical implications.

Thus far, only pharmacological strategies directly targeting the increased incidence of CVAs and PVCs in HD patients have been utilised. Beta blockade, with carvedilol, has been shown to be beneficial in reducing CVAs and heart failure in patients with dilated cardiomyopathy [189]. Furthermore carvedilol is associated with a significant reduction in total PVCs and episodes of ventricular tachycardia in HD patients [190]. Unfortunately, the association of intra-dialytic hypotension (IDH) and poor outcome [109] militate against widespread usage of such vasoactive medication. The use of implantable defibrillators in HD patients surviving cardiac arrest has

also been evaluated [191] and shown to be beneficial. However their use as a preventative treatment is yet to be confirmed.

Conversely, modifying HD using such methods as cooled dialysate can improve haemodynamics and has been shown to reduce the incidence of HD induced RWMAs [71]. If the development of PVCs and CVAs is also driven by an ischaemic pathophysiological process, then these therapeutic strategies may help to reduce morbidity and mortality not just from ischaemia but also from sudden cardiac death related to malignant ventricular arrhythmias.

6.6 Conclusions

The data within this chapter demonstrate that haemodialysis induced myocardial ischaemia may lead to the development of both functional LV abnormalities and ventricular arrhythmias, potentially playing a role in the development of the two commonest causes of cardiovascular death in chronic HD patients. Underlying risk factors for demand myocardial ischaemia could contribute to this phenomenon. Therapeutic strategies targeting improved haemodynamics during dialysis may reduce HD induced ischaemia and the life-threatening arrhythmias that go alongside it. This in turn may reduce the incidence of sudden death in our HD population. Further work is needed to look at the long term consequences of HD induced myocardial ischaemia with respect to both function and arrhythmogenicity and to see whether or not potential modifications (e.g. cooled dialysate) that reduce the number of RWMAs

during the HD procedure have any impact on left ventricular function and the incidence of PVCs or CVAs.

Chapter 7

Results:

Haemodialysis induced repetitive myocardial stunning results in global and regional reductions in left ventricular function.

7 Results: Haemodialysis induced repetitive myocardial stunning results in global and regional reductions in left ventricular function

7.1 Introduction

Data above have shown that haemodialysis can induce reductions in myocardial blood flow that precipitate ischaemia and cause regional left ventricular dysfunction consistent with myocardial stunning. This phenomenon is common and associated with a number of potentially modifiable factors including ultrafiltration volume and intradialytic haemodynamic instability. So far, it has been demonstrated that myocardial stunning is associated with an increase in ventricular ectopy but nothing is currently known about the long term consequences of such repetitive cardiac injury.

In the non-dialysis population, repeated episodes of demand ischaemia and stunning can result in chronic reduction in left ventricular function [72,78]. This led to the hypothesis that repeated episodes of myocardial ischaemia lead to a spectrum of disease encompassing myocardial stunning through to myocardial hibernation [79] and ending in myocardial remodeling and scarring [192]. Standard conventional thrice weekly haemodialysis as a cause of repetitive myocardial stunning may lead to chronic myocardial hibernation and eventually to myocardial scarring resulting in left ventricular dysfunction. Although myocardial hibernation

may represent a functional adaptation to chronic hypoperfusion that can be reversed with restoration of regional MBF (the 'smart heart' hypothesis) [82], there is evidence to suggest that hibernating myocardium is still highly vulnerable to increases in demand or reductions in oxygen supply [83] such as further haemodynamic stress during HD. Therefore, ongoing recurrent episodes of ischaemia precipitated by HD may have negative consequences on this adaptive balance leading to further myocardial injury and eventual non-viable myocardium with irreversible reduction in left ventricular function.

The aim of this analysis was to look for evidence of chronic myocardial dysfunction in haemodialysis patients precipitated by repeated episodes of HD induced myocardial stunning over a 12 month period.

7.2 Methods

7.2.1 Patients

Recruitment of patients is covered in chapter 5 section 5.2.1. Of the 70 prevalent HD patients who were consented for this 12-month observational cohort, nineteen patients were censored from the follow up analysis: patients who had died (n=9), patients who had received kidney transplant (n=6), those who changed dialysis modality (n=3) and withdrawal of consent (n=1).

7.2.2 Study protocol

Shortly before the 12-month anniversary of their baseline study, patients were approached on the haemodialysis unit to verbally confirm their consent to remain in the study. As mentioned above, one patient withdrew consent at that time.

As near to 12 months as practicable, patients underwent an identical study session to that at baseline. Patients were re-interviewed for the purpose of reviewing their medical history and performing a physical examination. All medical details were then again confirmed using patients' notes.

During the follow-up monitored haemodialysis session, the following variables were collected: details of dialysis treatment; global and regional left ventricular function was using serial echocardiography at four separate time points: pre-dialysis; during treatment at both two and four hours; and again 30 minutes into the post-dialysis recovery phase; non-invasive haemodynamic monitoring of blood pressure (BP) was undertaken every 15 minutes; pre-dialysis blood tests were drawn immediately after insertion of access needles, and post levels were taken from the arterial line 10 seconds after reducing blood pump speed to 50ml/min and single pool Kt/V_{urea} values were calculated from pre and post urea levels [144].

The primary endpoints at this stage were to understand the longer term consequences of this phenomenon on LV function and to assess

whether HD induced myocardial stunning would progress over time to the development of fixed systolic dysfunction, consistent with the continuum of myocardial hibernation. In addition, we sought to identify the haemodynamic consequences of this with respect to intradialytic haemodynamics. Secondary endpoints included: the potential role of haematological and biochemical variables (as well as dialysis adequacy, Kt/V) and the use of troponin-T as a risk marker for the progression of regional myocardial dysfunction.

7.2.3 Haemodialysis details

Dialysis was performed as described in chapter 3 and is not repeated here.

7.2.4 Follow-up collection of study data

Measurement of regional and global LV function (using echocardiography), blood pressure, haematological and biochemical variables is outlined in full in sections 5.2.4 – 5.2.6 and not repeated here.

7.2.5 Definitions of HD-induced cardiac dysfunction and rationale

Based on segmental performance, the following definitions of HD-induced cardiac dysfunction were applied.

Myocardial stunning: regions that showed a functional decline of >20% during HD compared to rest with evidence of functional recovery in the post-dialysis period were classed as stunned segments. Previous work using PET has demonstrated a corresponding reduction in MBF with post stress recovery in function and perfusion in those areas.

Myocardial hibernation / fibrosis: a reduction in resting systolic function of >60% within previously stunned myocardial segments that remained fixed during HD was taken to represent chronically dysfunctional myocardium within the disease spectrum of myocardial hibernation / fibrosis. A value of 60% was used based on animal studies showing a similar reduction in wall motion score between chronically stunned and hibernating myocardial segments [193].

7.3 Statistical analysis

Descriptive analyses are presented as mean ± standard deviation. The paired Student *t* test was used for comparison of continuous variables within each cohort. The Student *t* test was used for comparison of continuous variables between the two cohorts. Repeated analysis of variance was used for multiple comparisons of a continuous variable within a group. Linear correlation was used to investigate potential associations between variables of interest. For other data, either the paired *t*-test or Wilcoxon rank sum test was used depending on normality of the distribution. Significant deviations from a normal distribution were excluded with the Kolmogorov-Smirnov test. All

statistical tests were two-tailed with a *P* value less than 0.05 taken to indicate significance.

7.4 Results

7.4.1 Prevalence of regional wall motion abnormalities after 12-months

Of the 51 patients who entered follow-up, 74% (38/51) had significant RWMAs during HD. Of the 31 follow-up patients with evidence of myocardial stunning at baseline, 94% (29/31) continued to have evidence of significant RWMAs during HD and 45% (14/31) had an increase in the number of affected regions. Two patients had a reduction in the number of RWMAs below significance.

7.4.2 Incidence of new regional wall motion abnormalities after 12-months

Of the 20 follow-up patients who did not have evidence of myocardial stunning at baseline, 45% (9/20) showed development of significant RWMAs; however 55% (11/20) continued to be unaffected.

7.4.3 Effect of myocardial stunning on global left ventricular function after 12-months

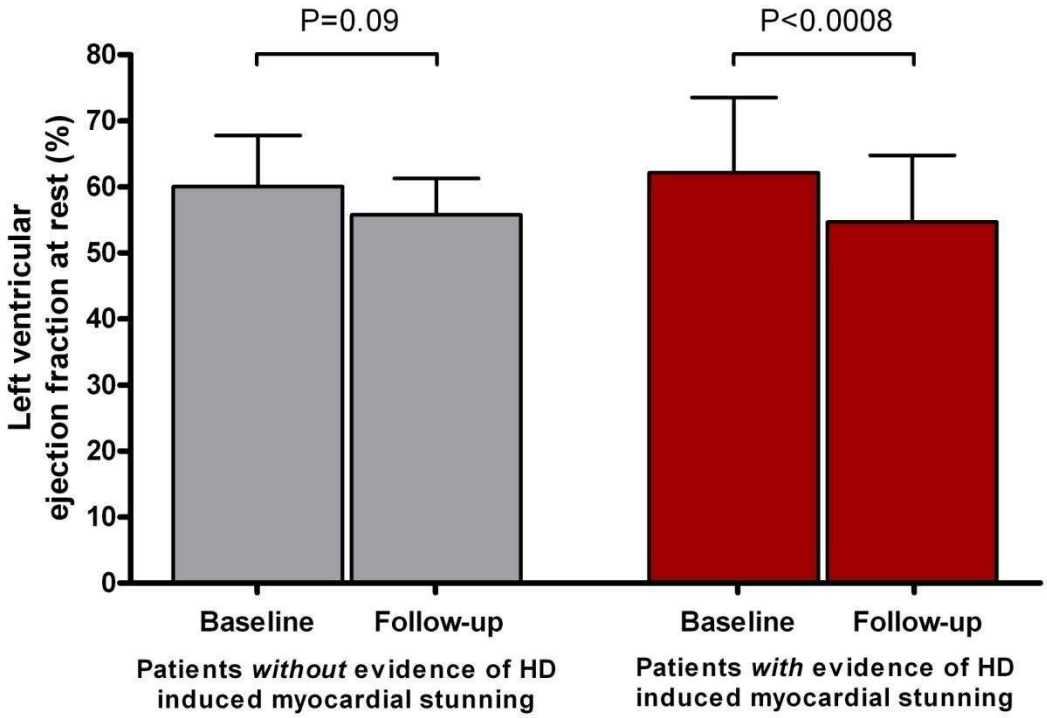


Figure 7.4.3a: Change in left ventricular ejection fraction at rest over 12 months in patients with and without evidence of myocardial stunning at baseline.

At baseline, LVEF at rest ($LVEF_{rest}$) was not significantly different between patients who did, and did not, develop HD-induced RWMAs (62.1±11.4% vs. 60.1±7.7%, P=0.4). After 12 months, $LVEF_{rest}$ had significantly deteriorated in patients with RWMAs (62.1±11.4% vs. 54.7±10.1%, P<0.0008) but remained unchanged in those patients without (60.1±7.7% vs. 55.8±5.5%, P=0.09), see figure 7.4.3a.

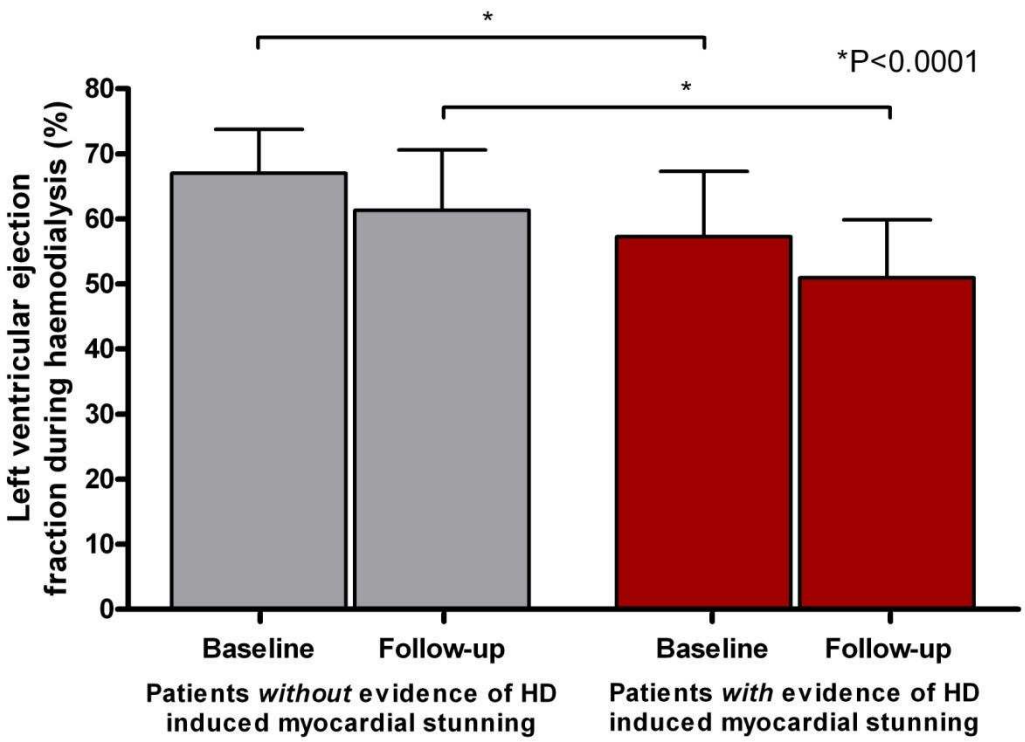


Figure 7.4.3b: Change in left ventricular ejection fraction during haemodynamic stress of HD over 12 months in patients with and without evidence of myocardial stunning at baseline.

Baseline LVEF at peak dialytic stress ($LVEF_{HD}$) was significantly lower in patients who had RWMAs compared to those who did not (67±6.8% vs. 57.3±10.1%, P<0.0001). Although there was a reduction in $LVEF_{HD}$ after 12 months in both those patients with HD-induced RWMAs and those without (57.3±10.1% vs. 50.9±8.9, P<0.001 and 67±6.8% vs. 61.3±9.3, P=0.007 respectively), the difference in $LVEF_{HD}$ between the two groups remained significant (61.3±9.3 vs. 50.9±8.9, P<0.0001), see figure 7.4.3b.

7.4.4 Effect of myocardial stunning on systolic blood pressure over 12 months

After 12-months both groups of patients (with and without evidence of HD-induced RWMAs) exhibited a significant deterioration in SBP during HD. Mean reduction was greater in patients with RWMAs at both timepoints (-18.6±6.9 mmHg to -23.3±7.2 mmHg, P<0.01 and -7±3.6 mmHg to -14±4.8 mmHg, P<0.001 respectively). However, the separation remained between patients developing RWMAs versus those who did not (-23.3±7.2 mmHg vs. -14±4.8 mmHg, P<0.001).

7.4.5 Progression of myocardial stunning to fixed systolic dysfunction (myocardial hibernation)

In order to assess for the progression of myocardial stunning to fixed reductions in segmental functional (myocardial hibernation), a separate analysis of those patients who exhibited signs of HD-induced myocardial stunning at baseline was performed. As stated above, a

reduction in resting systolic function of >60% within previously stunned myocardial segments that remained fixed during HD was classified as a hibernating segment.

Parameters	**Patients *without***	**Patients *with***	***P***
Age (mean, years)	65 ± 14.5	64.8 ± 11.8	>0.9
Male : Female (n)	6 : 5	13 : 6	>0.6
Dialysis vintage (mean, months)	31.9 ± 21.6	41.6 ± 28.5	>0.9
Etiologies (n)			
Diabetes mellitus	4 (36%)	10 (53%)	>0.4
Glomerular disease	2 (18%)	2 (11%)	>0.5
APKD	1 (9%)	3 (16%)	>0.9
Other	3 (27%)	1 (5%)	>0.2
Unknown	1 (9%)	3 (16%)	>0.1
Ischaemic heart disease (n)	6 (45%)	5 (25%)	>0.2
Smoker (n)	3 (27%)	5 (25%)	>0.9
Intradialytic weight gain (mean, kg)	1.6 ± 0.7	1.84 ± 0.7	>0.9
Ultrafiltration volume (mean, L)	2.03 ± 0.6	2.3 ± 0.9	>0.6

Table 7.4.5a: Demographic characteristics and dialysis related factors - based on the presence (with) or absence (without) of new fixed segmental systolic reduction of >60% after 12 months. APKD, adult polycystic kidney disease; HT, hypertension.

Of the 45 patients with evidence of myocardial stunning at baseline, 30 entered follow up. The clinical characteristics of this sub-group are shown in table 7.4.5a. Patients were divided into two groups; patients

with evidence of fixed segmental reduction after 12 months in previously stunned myocardial regions and those without. There were no statistically significant differences between the 2 groups with respect to underlying aetiology, co-morbid conditions (including diabetes mellitus and ischaemic heart disease) or dialysis vintage.

At baseline a total of 146 regions (48.7%) developed myocardial stunning out of a maximum of 300 in 30 patients. The mean resting SF of those regions that developed myocardial stunning on HD was significantly higher than those that did not (3.19 ± 1.18% vs. 2.07 ± 0.75%, P<0.01). At follow-up, 47 of the 146 stunned regions (32.2%) had developed a fixed reduction in systolic function of >60% that did not show an increase on HD. There was a significant decline in resting SF over 12 months in all regions that developed HD-induced myocardial stunning at baseline (3.19 ± 1.18% vs. 1.87 ± 0.7%, P<0.0001). However there was no significant change over 12 months in those regions that were unaffected by HD at baseline (2.07 ± 0.75% vs. 2.06 ± 0.78%, P=0.99), see figure 7.4.5a.

In terms of the effect on EF at rest and on HD, patients who developed myocardial segments with fixed systolic reduction of >60% (n=19) showed a significant decline in EF over 12 months both at rest and at peak stress during HD (61.5 ± 10.1% vs. 52.9 ± 8.6%, P<0.007 and 59.5 ± 10% vs. 49.9 ± 6.5%, P<0.003 respectively).

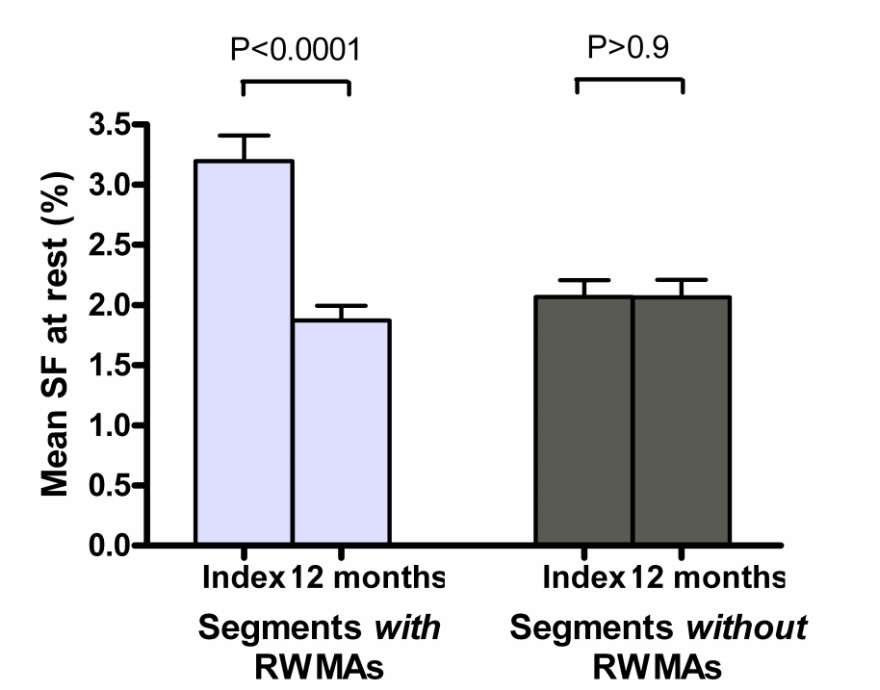


Figure 7.4.5a: Change in regional SF over time - there was a significant reduction in SF over 12 months in those segments that developed RWMAs at baseline (index) compared to those that did not.

In comparison, patients who did not develop such abnormalities showed no significant reduction in these values either at rest or during HD (63.3 ± 14.8% vs. 56.2 ± 13.1%, P>0.1 and 53.9 ± 10.4% vs. 52.4 ± 12.4% P>0.7 respectively). These results are summarised in figure 7.4.5b.

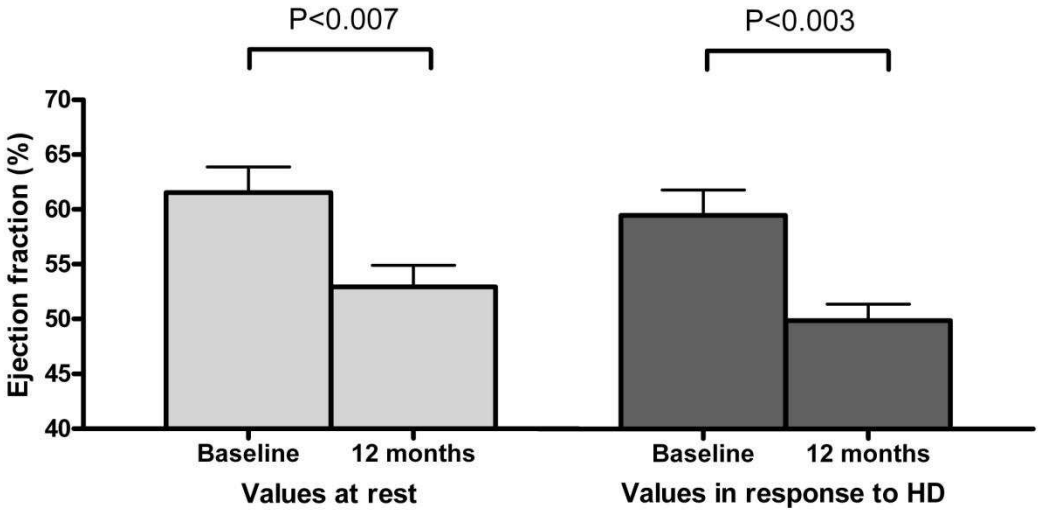


Figure 7.4.5b: Change in EF at rest and during HD over 12 months in patients with fixed reductions in segmental function of >60% - the development of fixed segmental reduction in previously stunned myocardial segments was associated with a significant reduction in LVEF both at rest and during HD. There was no significant reduction in LVEF in patients who did not develop fixed reductions in segmental function.

Looking at Intradialytic changes in blood pressure, using a two-way ANOVA, there was a significant reduction in SBP during HD and over 12 months in the patient group that developed fixed segmental systolic dysfunction of >60% ($F(16,336) = 4.71$; $MSE = 227$; $P<0.0001$). This was not true in the other group who had no significant change in their SBP during HD over 12 months ($F(16,820) = 0.25$; $MSE = 655$; $P>0.9$), see figure 7.4.5c.

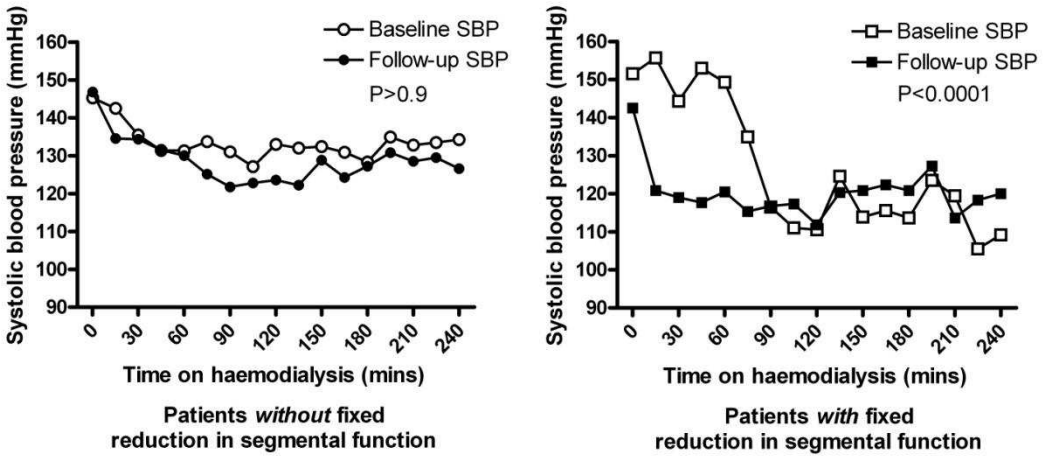


Figure 7.4.5c: Changes in SBP during HD over 12 months - after 12 months there was a significant difference in SBP during HD in those patients with new fixed reductions of >60% in previously stunned myocardial segments.

In this sub group, haematological, biochemical and dialysis details gave the following results: both at baseline and follow-up, 83% of patients had a significantly raised plasma cTnT concentration (≥0.03 µg/L), consistent with the presence of repetitive acute myocardial injury. However, there were no significant differences among any of the haematological or biochemical measurements (including cTnT) within or between either of the groups at baseline or over the 12 month follow-up period. There were also no significant differences between the groups with respect to dialysis adequacy (Kt/V_{urea}) or ultrafiltration volumes. These data are summarised in table 7.4.5b.

Parameter	Patients *without* fixed reduction in segmental systolic reduction				Patients *with* fixed reduction in segmental systolic reduction			
	Baseline		Follow-up		Baseline		Follow-up	
	Pre-HD	Post-HD	Pre-HD	Post-HD	Pre-HD	Post-HD	Pre-HD	Post-HD
Haemoglobin (g/dl)	10.8±1.1	11.3±1.0	11.8±1.7	12.2±1.8	11.2±1.5	11.7±1.6	11.9±1.6	12.2±1.7
Na^+ (mmol/L)	137±3	137±2	136±4	137±2	138±3	138±2	137±4	137±3
K^+ (mmol/L)	4.3±0.5	2.8±0.5	4.4±0.8	2.8±0.4	4.9±1.0	2.9±0.4	4.6±0.1	3.0±0.7
Urea (mmol/L)	19.6±4.7	5.8±2.1	17.8±5.6	5.4±2.4	21±4.6	6.5±2.2	23.3±8.7	7.0±2.9
Creatinine (µmol/L)	665±165	258±74	618±165	250±87	658±213	267±107	725±257	300±148
Phosphate (mmol/L)	1.8±0.4	0.8±0.1	1.7±0.5	0.7±0.2	1.5±0.5	0.8±0.3	1.8±0.6	0.8±0.3
Bicarbonate (mmol/L)	23.8±1.9	27.1±4.1	22.8±2.2	27±1.6	23.1±2.5	27.9±2.4	24.2±2.6	26.8±1.7
Corrected Ca^{2+} (mmol/L)	2.42±0.2	2.34±0.1	2.43±0.13	2.31±0.1	2.4±0.1	2.4±0.1	2.4±0.19	2.3±0.14
Albumin (g/L)	34.4±2.9	35.8±3.8	30.6±6.2	32.3±7.4	35.3±4.7	36.6±5.5	35.2±4.4	37.1±4.9
cTnT (µg/L)	0.07±0.05	-	0.12±0.12	-	0.09±0.06	-	0.08±0.05	-
Kt/V urea	1.3±0.2	-	1.4±0.2	-	1.3±0.3	-	1.3±0.4	-

Table 7.4.5b: Pre- and Post-HD haematological and biochemical values for both groups at baseline and follow-up - there were no significant differences between different or within the same groups at either time point (P>0.1).

7.5 Discussion

We have previously demonstrated that HD can directly induce RWMAs and that these are associated with a corresponding decrease in segmental MBF with recovery post-HD consistent with myocardial stunning. This chapter demonstrates for the first time that repetitive haemodialysis induced cardiac injury can lead to chronic reduction in LV function and also, that LV segments which develop myocardial stunning during HD can progress over time into areas of fixed systolic dysfunction that have distinct systemic haemodynamic consequences. These manifest as reductions in LVEF at rest and a reduced ability to increase LV contractile function to maintain BP during HD with fluid removal. This could potentially create a vicious cycle for further HD-induced cardiac injury.

At baseline, both groups of patients had similar $LVEF_{rest}$. During HD, patients with myocardial stunning were unable to maintain cardiac contractile performance, and had a significant drop in their $LVEF_{HD}$. After 12 months, $LVEF_{rest}$ had fallen in patients with HD induced myocardial stunning and their ability to respond to the challenge of HD was even further compromised. Patients without myocardial stunning at baseline maintained their $LVEF_{rest,}$ but $LVEF_{HD}$ was significantly reduced. This is entirely consistent with the pattern of injury associated with ischaemic cardiomyopathy in the non-dialysis population. Repetitive ischaemic injury initially causes a loss of contractile reserve that may lead to myocardial fibrosis and resting dysfunction [194]. In HD

patients, repetitive HD-induced myocardial stunning led to a reduction in contractile reserve and then resting LV systolic dysfunction. This process of deterioration has already begun in patients unaffected by HD-induced myocardial stunning at baseline, as almost half now have evidence of HD-induced RWMAs and reduced contractile reserve. This also has major potential clinical implications. In the non-dialysis population timely revascularisation can lead to an improvement in LVEF, heart failure and prognosis [195]. In addition to modifications to the HD procedure, treatment of underlying coronary artery disease may slow progression of heart failure and improve outcome.

Looking specifically at patients with myocardial stunning at baseline, over the period of 12 months, a number of these abnormal regions had progressed to areas of fixed systolic dysfunction. That is to say that not only was there a significant reduction in resting shortening fraction in that region after 12 months compared to baseline (>60% reduction in %SF) but that this did not improve during the haemodynamic stress associated with haemodialysis. This would be wholly consistent with progression to segmental myocardial hibernation or even fibrosis.

Within the cohort of patients that developed myocardial stunning at baseline, there was no significant association between the progression of LV regional wall dysfunction and demographic variables. It is not possible to identify patients experiencing HD induced cardiac injury on the basis of known co-morbidities. In the HD population decline in LVEF is usually progressive over time and often accelerated [163] but there is

increasing evidence that this is not due to traditional cardiac risk factors or conventional atherosclerotic coronary artery disease [165]. It is therefore not surprising that the presence or absence of IHD and diabetes mellitus were not different between the two groups. Similarly, no significant difference in dialysis vintage (time on dialysis in months) was observed, however both groups had been receiving HD for a considerable time (>2 years). There is currently no evidence as to whether a critical time period on dialysis exists after which the process of irreversible myocyte damage begins and the potential for improvement in LVEF is lost. In reality this is likely to differ considerably from patient to patient and be determined by a complex interrelationship between a number of underlying factors that affect cardio-renal performance. Some of these pathological processes associated with HD that predispose to demand cardiac ischaemia (coronary and peripheral arterial calcification [196], impaired microcirculation and increased pulse wave velocity [22]) are now more clearly understood but there are an equal number of humoral and genetic factors whose roles are very poorly defined [197].

At baseline within patients who developed myocardial stunning, around half of all LV regions developed RWMAs on HD. At rest these regions had a significantly higher regional SF than those unaffected by HD. This may reflect vulnerability to demand ischaemia in more kinetic areas of the LV in response to haemodynamic stress in HD patients with reduced CFR. At follow up, these regions had significantly reduced their SF in comparison to unaffected regions at baseline. The magnitude of

that reduction varied but a considerable number (32.2%) showed a reduction of >60% that remained fixed during HD.

As a result of these areas of fixed systolic dysfunction, affected patients showed a decline in both resting and demand EF (on HD) after 12 months, which was not seen in the unaffected group. This is in keeping with other published work looking at the effect of chronic myocardial stunning and myocardial hibernation on LV function in patients with IHD. In our dialysis patients however, this reduction in EF seems to have a direct impact on intradialytic haemodynamics.

This sub-group as a whole represents patients with HD-induced cardiac dysfunction. Both individual study groups had some reduction in absolute SBP during HD. However, the only patients that had a significant deterioration in their SBP during HD after 12 months were in the group that developed regional fixed systolic reductions of >60%. This is not attributable simply to volume overload. A small subset of patients with below average UF volumes had significant reductions in SBP. In addition, SBP at the end of HD is primarily determined by cardiac output [198] and therefore it is likely that the absolute reduction in SBP seen here is secondary to the reduction in LVEF. Intradialytic hypotension (IDH) is known to be associated with increased mortality. Given the underlying vulnerability of hibernating myocardium to increases in demand [78] coupled with decreased CFR in HD patients it may be that this adaptive process actually leads to further segmental injury by exacerbating intradialytic instability. This may be one of the

reasons that prevalence of heart failure is so high and survival so poor in HD patients.

This hypothesis is supported by evidence that a continuation of HD results in a significant increase in cardiovascular events as well as myocyte fibrosis and death compared to uraemic patients not on HD [199]. A study by Wali et al [84] looking at the effects of renal transplantation in patients with heart failure demonstrated that a longer duration of dialysis in months prior to transplantation was the only significant factor associated with a decreased likelihood of achieving a normal LVEF in the post-transplantation period. They attributed this to prolonged exposure to potentially negatively inotropic factors and other toxins that are present in uraemic plasma, which may be decreased after transplantation [200]. Whilst these potential uraemic toxins may play a part we assert that the observed changes related to the removal of dialytic stress.

Previous studies in our centre have shown that myocardial segments which develop stunning during HD have normal resting blood flow but a reduction in blood flow during treatment. Conventional definitions of myocardial hibernation suggest that resting MBF in these areas would be reduced, but this was beyond the scope of this current study. Although MBF has not been measured, repeated myocardial stunning has been shown in animal models to precede myocardial hibernation and remodeling [201]. Such fixed reductions in systolic function in those regions undergoing chronic myocardial stunning would be consistent

with myocardial hibernation but may also potentially represent areas that have already undergone fibrosis and remodeling. Therefore, these chronically stunned segments may well have deteriorated over 12 months and lie somewhere along the continuum of disease classified as myocardial hibernation and remodeling.

Dialysis sessions that are complicated by episodes of IDH are associated with a significant rise in cardiac troponin I and T levels [100] suggesting underlying sub-clinical myocardial damage. A significant amount of work has been done looking at the clinical significance of biochemical markers of cardiac injury including cardiac troponins but their value as long-term prognostic markers is as yet undetermined. We observed no difference in cTnT levels between the two groups of patients however this may in part be due to the fact that a number of the most potentially unstable patients died before 12 months and were not included in the analysis. More work is needed to assess the use of biochemical markers as a screening tool for the identification of 'at risk' patients.

The process of myocardial stunning in HD has been shown to be modifiable in a number of ways (apart from transplantation) that improve intradialytic haemodynamics. These include biofeedback mechanisms and cooled dialysate [70,71] although there are as yet no long term data looking at improvements in LVEF as a result of the longer term applications of these interventions. Other dialysis treatment such as nocturnal haemodialysis have been shown to result in an

improvement in LVEF [180] which may also be in part due to abrogation of HD induced myocardial stunning, through modifications to ultrafiltration rates and IDH. Given the current shortage of donor organs for kidney transplantation these other dialysis options may offer treatment strategies that can minimise / avoid HD induced myocardial stunning, myocardial hibernation and remodeling, improve LV function and thereby reduce heart failure and mortality.

7.5.1 Study limitations

Echocardiography was the only method used to quantify functional decline however this method is repeatable and quantitative. Also, the use of other imaging techniques such as magnetic resonance imaging to assess functional change while the patient was receiving HD would be technically impossible.

There was no angiography or perfusion imaging available to quantify epicardial CAD in these patients. However, HD induces myocardial ischaemia even in the absence of epicardial CAD and myocardial scintigraphy is an imperfect technique for the evaluation of CAD in patients with end-stage renal disease [202]. Although the mechanism of HD driven myocardial stunning and hibernation would still apply in this case, it may be that coronary revascularisation is a potential intervention that will improve LVEF and heart failure in this group. That question is outside the scope of this study.

7.5.2 Conclusions

The results in this chapter demonstrate that HD-induced cardiac injury is associated with a reduction in segmental and global LV function. The cycle of intra-dialytic instability resulting in repetitive cardiac injury leads to a potential for yet more dialysis induced hypotension. This fits well with the observation that heart failure is associated with very poor outcome in HD patients. However, haemodynamic instability during HD is a potentially avoidable phenomenon. This raises the possibility that addressing HD-induced repetitive injury might prevent the development of myocardial stunning, hibernation and fibrosis which lead to LV systolic dysfunction in these patients and potentially prevent the development of heart failure. How much such interventions may improve outcome is not yet known and the next chapter will investigate any potential associations between haemodialysis induced cardiac injury and mortality.

Chapter 8

Results:

Haemodialysis induced repetitive myocardial stunning and the associations with increased hazard of death and time to first cardiovascular event.

8 Results: Haemodialysis induced repetitive myocardial stunning is associated with an increased hazard of death and time to first cardiovascular event

8.1 Introduction

In the previous chapters, results have shown that haemodialysis induced myocardial stunning is common and associated with a number of negative predictors of outcome including intradialytic hypotension, ventricular arrhythmias and elevated serum cardiac troponin-T concentrations. Potentially more serious however is the observation that, over time, this phenomenon leads to a reduction in global and segmental left ventricular function, both at rest and during haemodialysis.

Records from the US Renal Data System have shown that HD is an independent risk factor for the development of both *de novo* and recurrent heart failure with a two-year mortality after a diagnosis of congestive heart failure as high as 51% [124], making it one of the most common causes of cardiovascular mortality in this patient group.

The aim of this chapter was to evaluate the impact of chronic haemodialysis induced myocardial stunning on patient survival and time to first cardiovascular event.

8.2 Methods

8.2.1 Study protocol

Recruitment of the 70 initial patients is covered in chapter 5 section 5.2.1 and is not repeated here.

After 12 months, mortality data was collected for all patients, as well as time to first cardiovascular event. Cause of death was obtained from patients' hospital notes. In the event that a patient died in the community, their General Practitioner was contacted and asked to provide a cause of death. In one case where the patient died out-of-hospital, a post mortem examination was conducted and the final cause of death was obtained from the Coroner's Office.

Defined cardiovascular events included: a new diagnosis of coronary artery or cerebrovascular disease; myocardial infarction; cerebrovascular and peripheral vascular events. All hospital admissions were identified using the PAS system and subsequently confirmed using patients' hospital records. Any of the above conditions were recorded including date of first diagnosis.

At this final stage, the primary endpoints were to understand the longer term consequences of this phenomenon with respect to mortality and cardiovascular morbidity. Secondary endpoints included: the potential role of haematological and biochemical variables (cardiac troponin-T) as a potential marker to identify the most at risk patients.

8.2.2 Haemodialysis details

Dialysis was performed as described in chapter 3 using Hospal Integra monitors (Hospal, Mirandola, Italy) using low-flux polysulfone dialysers, either 1.8 or 2.0 m^2, per individual patients' usual prescriptions (LOPS 18/20; Braun Medical Ltd, Sheffield, UK). Exact prescriptions and treatment regimes for these 70 patients are detailed in section 5.2.3 and not repeated here.

8.2.3 Collection of study data

Measurement of regional and global LV function using echocardiography (to define the presence of myocardial stunning), blood pressure, haematological and biochemical variables is outlined in full in sections 5.2.4 – 5.2.6 and not repeated here.

8.3 Statistical analysis

The Wilcoxon rank sum test was used to investigate potential associations between variables of interest (cTnT) over time. Descriptive analyses are presented as median (interquartile range) unless otherwise stated. Significant deviations from a normal distribution were evaluated with the Kolmogorov-Smirnov test. Survival data was analysed using a logrank test to create Kaplan-Meier curves for both mortality and the combined endpoint of mortality and time to first cardiovascular event. Cox regression analysis was used to investigate

the association between variables of interest. All statistical tests were two-tailed with a *P* value less than 0.05 taken to indicate significance.

8.4 Results

8.4.1 Myocardial stunning is associated with a decreased 12 month survival

Of the 70 patients who were consented for the study, nine had died within the 12 month follow-up period. Of these nine patients, all of them (100%) had evidence of haemodialysis induced myocardial stunning during the baseline study session. Causes of death were: six from cardiac disease, two from sepsis and one stroke. As a result of this the presence of haemodialysis induced myocardial stunning was associated with increased relative mortality at 12 months (P=0.019), see figure 8.4.1a.

8.4.2 Myocardial stunning is associated with an increased cardiovascular event rate

Similarly, a composite end point of mortality and time to first cardiovascular event confirmed the association between myocardial stunning and such events.

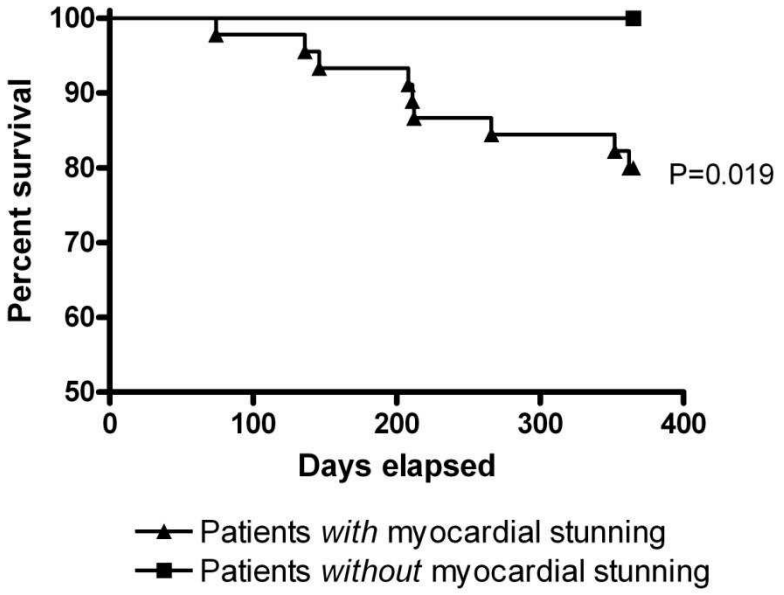


Figure 8.4.1a: The association of haemodialysis induced myocardial stunning with mortality. The development of HD-induced RWMAs was associated with increased relative mortality at 12 months.

Of the 70 patients who were consented for the study (and in addition to the nine who had died), a further five had a non-fatal cardiovascular event within the 12 month follow-up period. These events consisted of: two strokes, two peripheral vascular events and one new diagnosis of ischaemic heart disease confirmed by angiography. Of these additional five patients, four had evidence of haemodialysis induced myocardial stunning at baseline and only one did not. So, survival to a composite end point of mortality and first cardiovascular event also demonstrated almost complete separation in events, and mortality, between patients with and without HD induced myocardial injury; with only one patient in

the unaffected group, compared to 13 in the myocardial stunning group (P=0.017, hazards ratio of 8, 95% CI 1.264 to 10.99), see figure 8.4.2a.

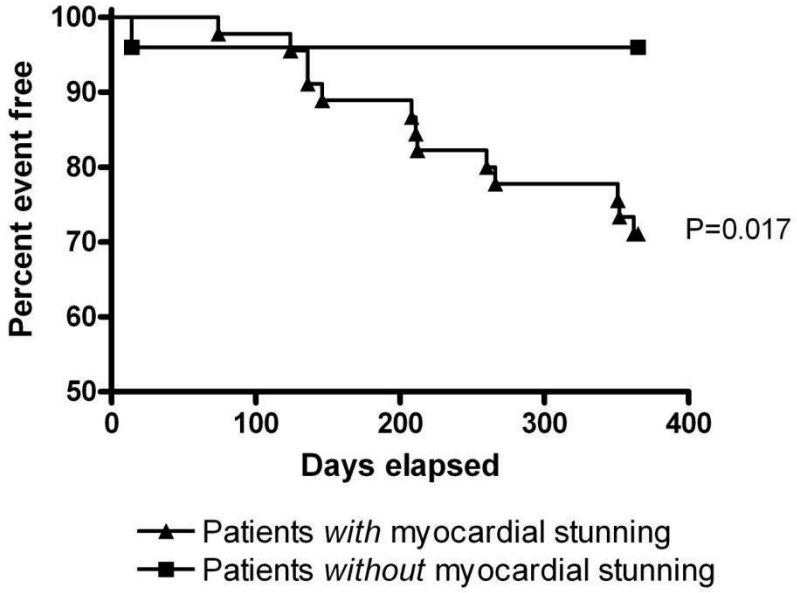


Figure 8.4.2a: The association of haemodialysis induced myocardial stunning with mortality and time to first cardiovascular event. The development of HD-induced RWMAs was associated with reduced survival to a composite end point of mortality and time to first cardiovascular event.

8.4.3 Baseline troponin-T concentrations were associated with an increased mortality

As reported, baseline cTnT levels were associated with the presence and severity of myocardial stunning on univariate and multivariate analyses (sections 5.4.4 and 5.4.6-7). After 12 months, the mean cTnT levels for the cohort as a whole had increased significantly (0.06 µg/L [IQR 0.02 to 0.1] vs. 0.07 µg/L [IQR 0.03 to 0.1], P<0.03) and there remained a significant separation in cTnT levels between patients who developed haemodialysis induced myocardial stunning and those who did not (0.04 µg/L [IQR 0.02 to 0.07] vs. 0.08 µg/L [IQR 0.05 to 0.14], P<0.002) figure 8.4.3a).

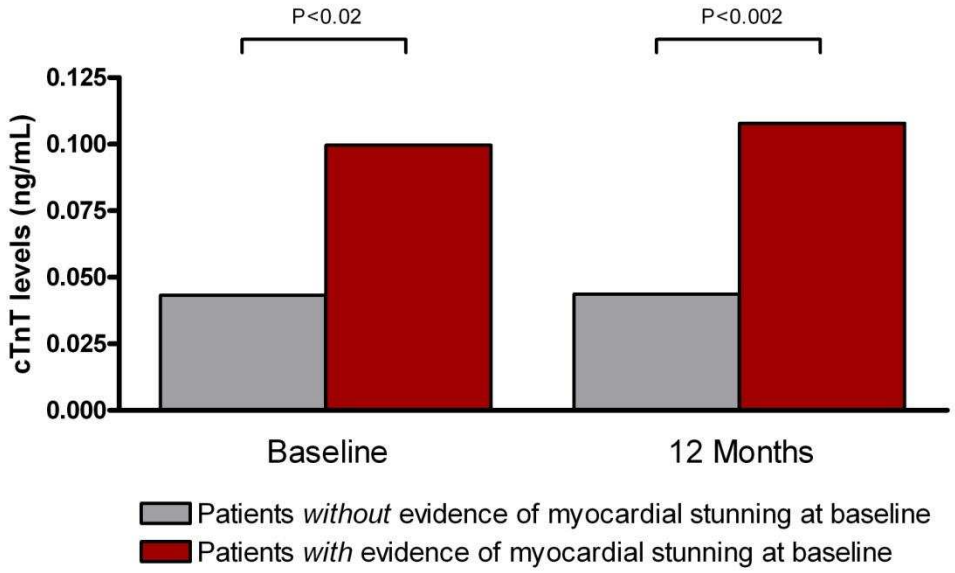


Figure 8.4.3a: Cardiac troponin-T concentrations in patients with and without myocardial stunning at both baseline and after 12 months. A significant difference remained between the groups at both timepoints.

There was no significant increase in cTnT within groups (those with myocardial stunning or those without, P=ns). Baseline cTnT levels were significantly higher in those patients who died compare to those who survived to 12 months (0.14 µg/L [IQR 0.1 TO 0.16] vs. 0.05 µg/L [0.02 TO 0.09], P<0.001, figure 8.4.3b).

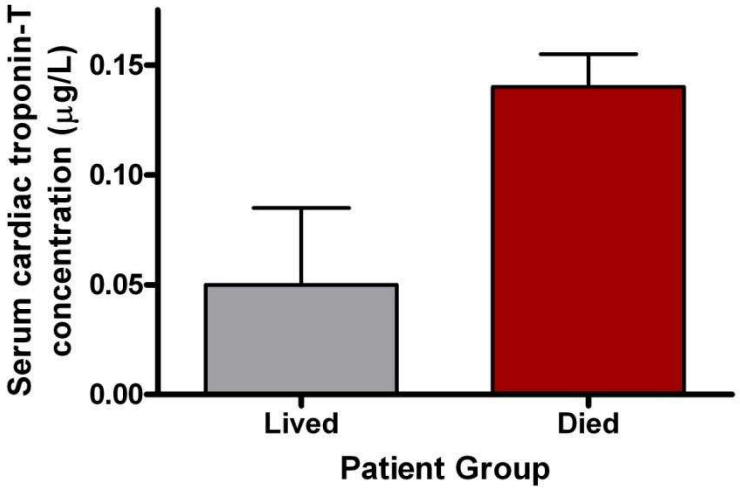


Figure 8.4.3b: Baseline cardiac troponin-T levels were significantly higher in patients who died within 12 months (P<0.001).

Cox regression analysis showed an increased hazard of death in patients with myocardial stunning and an elevated cTnT than in patients with elevated cTnT alone (P<0.02).

8.5 Discussion

These data demonstrates for the first time that dialysis induced recurrent cardiac injury is associated with not only with reductions in myocardial contractile function but also with patient survival.

To date, only surrogate markers of myocardial ischaemia in haemodialysis patients have been used to investigate the long term effects of myocardial ischaemia precipitated by the dialysis process. Studies looking at silent myocardial ischaemia using 12-lead Holter monitoring have yielded conflicting results. One study of 67 dialysis patients who underwent a single 12-hour period of Holter monitoring reported that 16 (23%) displayed evidence of silent ST depression. After two years, there was no difference between in mortality and cardiac event rates between those who did and did not display ST depression at baseline [58]. In a separate study of 62 haemodialysis patients who were studied for six months, of the two patients who died, both had experienced ST depression at baseline leading the authors to believe that silent ST depression was associated with a negative prognosis [62]. In reality, it is most likely that these studies were inadequately powered to detect a difference in mortality and in any case, relying solely on Holter monitoring alone has been shown to underestimate the true incidence of silent ischaemia which would have patients being wrongly classified at the outset [65]. This chapter has shown that myocardial stunning, which is known to be associated with reductions in myocardial blood flow consistent with ischaemia, is

associated with increased mortality. Given that myocardial stunning in present in over 60 per cent of haemodialysis patients, it is likely that the true incidence of haemodialysis induced ischaemia has, up until this point, been greatly underappreciated. Although the mortality observations are derived from a small number of patient deaths and the study is clearly underpowered in this respect. However statistical significance was achieved and the clear separation for cardiovascular events and mortality between the two groups is compelling.

The association between myocardial stunning and the combined endpoint of all cause mortality and first cardiovascular event is also of importance. Studies have shown that increased levels of biomarkers which indicate cardiac damage are associated with not just cardiovascular death but also from other causes (e.g. sepsis) [90] Specifically, it is well recognised that cTnT levels are often elevated in dialysis patients and that elevated levels predict mortality [89]. This would suggest that patients with repetitive cardiac injury and leak of such biomarkers into the plasma are less capable of mounting a response to the increased cardiovascular demands at the time of systemic insult from other causes. This is entirely consistent with the results in this thesis. Myocardial stunning results in a fall in systolic cardiac function, firstly during the systemic stress of haemodialysis but then at rest causing a reduction in LVEF. This is associated with a significantly higher troponin-T level, both at baseline and after 12 months. Such patients are at continuous risk from the repetitive damage caused by myocardial ischaemia and over time become less and less able to cope

with the haemodynamic demands put upon them from systemic illness of any cause.

So, in addition to the association with presence and severity of myocardial stunning, elevated plasma cTnT levels were also associated with an increased hazard of death. A recent study of cardiac troponins and outcome in acute heart failure revealed associations with lower SBP, lower LVEF and higher mortality [88].

The fact the Cox regression analysis showed that elevated cTnT concentrations in patients with haemodialysis induced myocardial stunning is more predictive of mortality than elevated cTnT alone shows that it is those patients with functional abnormalities who are most vulnerable. This may help to develop strategies to identify those patients most at risk. Individuals who display any evidence of cardiac dysfunction (e.g. intradialytic hypotension or cardiac abnormalities on routine clinical echocardiography) in conjunction with a raised random cTnT level (not done in response to a clinical episode) may represent an at risk section of the haemodialysis population. As such cTnT and other biochemical markers of cardiac damage (e.g. BNP) may provide useful tools to identify vulnerable patients who might benefit from modified dialysis therapies.

8.6 Conclusions

The results summarised within this chapter demonstrate for the first time that HD-induced myocardial stunning is associated with poor

outcomes. Despite the small numbers, these findings are consistent with evidence linking other cardiovascular abnormalities with increased mortality (e.g. left ventricular hypertrophy, intradialytic hypotension and left ventricular systolic dysfunction). This supports the hypothesis that haemodialysis induced myocardial ischaemia and myocardial stunning is intrinsically linked with these pathological conditions and may in fact represent a unifying pathophysiological process. If so, then dialysis based interventions aimed at reducing the incidence and prevalence of myocardial stunning may reduce the impact on the cardiovascular system and help to improve outcomes.

Chapter 9

Conclusions

9 Conclusions

Cardiovascular death remains grossly elevated in haemodialysis patients with very little change in mortality over the last decade despite extensive research into the field of cardio-renal medicine. In the future, as the dialysis population continues to expand (and whilst donor kidneys remain scarce), this excess of mortality is becoming an increasingly important issue. Haemodialysis patients are subject to a grossly elevated rate of cardiovascular attrition and it is becoming apparent that the current prevailing paradigm of considering this as a result of a particularly severe form of the cardiovascular risk factors seen in the general population is not true. This results in the well appreciated failure of Framingham risk models to predict events in this group, and the almost universal failure of attempts to successfully apply interventions that are well proven to be effective in the general population.

There are many different factors that impact negatively on the cardiovascular system in haemodialysis patients and it is likely that any solution to this growing problem will have to be multi-faceted in nature. However, our results suggest that the haemodialysis procedure itself may be central to this process by inducing a reduction in myocardial blood flow sufficient to induce subclinical myocardial ischaemia. Previous small studies have demonstrated the development of dialysis induced LV regional wall motion abnormalities [70,71] and now these

results have confirmed that such abnormalities are associated with changes in myocardial blood flow consistent with current definitions of myocardial stunning. Although there are other reports of silent ischaemia occurring during dialysis [43,56-65] our work is the first to shown a direct association between the process of haemodialysis and changes in myocardial blood flow (which has subsequently been reproduced in other studies [134]). Although it was inadequately powered to show a true difference between the two dialysis modalities (standard haemodialysis and biofeedback dialysis) our study suggested that biofeedback dialysis was associated with an improvement in myocardial blood flow at rest and better recovery in the post-dialysis period. Cooled dialysate has also been shown to reduce the number of stunned myocardial segments [70] and these two widely available treatment options may represent a simple, cost effective therapeutic intervention aimed at reducing myocardial stunning and ischaemia. Other modifications to the dialysis treatment regime have shown to have benefit. Short daily dialysis treatments were associated with a reduction in LVMI, extracellular fluid volume and better control of blood pressure [203]. Similarly, frequent nocturnal haemodialysis has been shown to improve left ventricular ejection fraction, LVMI and cause regression of LVH [179,180,204] with a reduction in biochemical markers of cardiac damage [205]. Unfortunately, the estimated reduction in cost needed to make these therapies cost neutral with current thrice weekly practises is likely to be prohibitive at present [206], making other dialysis modifications mentioned above more practicable.

In a large group of haemodialysis patients, we have now demonstrated that the prevalence of myocardial stunning is significantly higher than previously thought. This is important as repeated episodes of ischaemia and myocardial stunning may be cumulative and contribute to the genesis of chronic heart failure in patients with ischaemic heart disease [77]. Therefore, we postulated that thrice weekly haemodialysis and the resulting repetitive dialysis induced myocardial stunning may contribute to chronic cardiac dysfunction. It is perhaps logical that myocardial stunning would be associated with an increased troponin-T concentration given that the mechanism underlying this phenomenon is ischaemic cardiac injury. However, the observation that increased intradialytic ultrafiltration volumes and poor intradialytic haemodynamics are associated with myocardial stunning is of crucial importance as these both represent potential modifiable risk factors and therapeutic targets.

Mirroring the published literature, we found an increase in ventricular ectopy and complexity during haemodialysis compared to the post dialysis period however, these ventricular abnormalities were significantly higher in patients with haemodialysis induced regional left ventricular dysfunction. Also, these electrophysiological abnormalities were higher in those patients in whom there were additional factors associated with demand ischaemia (left ventricular hypertrophy and ischaemic heart disease). These results demonstrated for the first time that haemodialysis induced myocardial ischaemia may lead to the development of both functional LV abnormalities and ventricular

arrhythmias, potentially playing a role in the development of the two commonest causes of cardiovascular death in chronic HD patients. Therapeutic strategies targeting the mechanisms of myocardial stunning during dialysis may reduce HD induced ischaemia and the life-threatening arrhythmias that go alongside it. This in turn may reduce the incidence of sudden death in our HD population as well as any long-term benefit seen with respect to improvements in left ventricular function.

After 12-months, these results confirmed the hypothesis that repetitive dialysis induced cardiac injury leads to a reduction in left ventricular function and may be a driving force behind the development and progression of heart failure in this group. Those patients with myocardial stunning at baseline got worse, developing areas of fixed systolic dysfunction consistent with myocardial hibernation and scarring with a corresponding reduction in left ventricular function on and off dialysis. Those patients without myocardial stunning at baseline had evidence of regional wall motion abnormalities and had also developed evidence of demand associated systolic decline. All of this was in the context of elevated biochemical markers of myocardial injury (cardiac troponin-T levels).

Mortality was significantly higher in patients with dialysis induced myocardial stunning, as was time to mortality combined with cardiovascular events. This demonstrates for the first time that dialysis induced myocardial stunning is associated with poor outcomes. Despite

the small numbers, these findings are consistent with evidence linking other cardiovascular abnormalities with increased mortality (e.g. left ventricular hypertrophy, intradialytic hypotension and left ventricular systolic dysfunction). This supports the hypothesis that haemodialysis induced myocardial ischaemia and myocardial stunning is intrinsically linked with these pathological conditions and may in fact represent a unifying pathophysiological process. Raised troponin-T levels were also associated with an increased mortality at 12-months which is entirely in keeping with current literature [90]. However, there was an increased hazard of death in those patients with a raised troponin-T and myocardial stunning compared to those with myocardial stunning alone. It could be speculated that these patients with myocardial stunning and a troponin-T leak represent those patients with the most clinically significant reductions in myocardial blood flow during dialysis and hence are the most at risk. This would also fit with our observation that troponin-T level is an independent determinant of both the presence and severity of myocardial stunning and provides an exciting possibility for the early detection of the most vulnerable patients in whom dialysis based interventions may have the most positive outcomes.

In final conclusion, the results of this thesis describe a potentially highly significant, common and previously unappreciated direct effect of the dialysis process itself in producing recurrent cumulative myocardial injury. Furthermore, this the studies contained within this thesis define the characteristics of conventional thrice weekly haemodialysis treatment that drive this process, and the longer term severe effects on

cardiac function and overall patient survival. All of the findings within it are consistent with recently published small scale, short term mechanistic studies of this phenomenon, confirming this process is related to dialysis induced changes in myocardial blood flow and can be abrogated acutely by modification of the dialysis technique. The findings of this thesis suggest several novel, potentially modifiable mechanisms related to the short and long-term effects of dialysis that are potentially implicated in the development of uraemic cardiomyopathy. It remains to be seen whether improving the haemodynamic tolerability of dialysis would be an effective intervention to reduce the development of heart failure in this patient group.

9.1 Limitations

The principle limitation of these results (aside from the limitations of each individual aspect which are mentioned above) is the use of echocardiography as the sole imaging technique for the detection of myocardial stunning. Echocardiography is the predominant technique used for evaluation of left ventricular function and for the assessment and quantification of valvular heart lesions and in conjunction with flow Doppler is well established as a safe, non-invasive, and versatile diagnostic modality. However, assessment of regional cardiac dysfunction at rest and during stress remains subjective and semi-quantitative, with high inter-observer variability [207-209].

The limitations of the conventional echocardiographical evaluation of LV function have been attributed to its two-dimensional nature, which reveals only partial information about cardiac anatomy and function contained in specific cross sectional planes. Alternative techniques based on three-dimensional reconstruction from multiple two-dimensional planes (as we have done) are time consuming and therefore not clinically practical. To minimise these factors, a single operator acquired all echocardiographical images to minimise inter-observer variability.

Cardiac magnetic resonance (CMR) imaging, whilst an extremely powerful tool for looking at myocardial structure and would have been superior in measuring indices such as LVMI and LVH as well as cardiac dimension. However, CMR is increasingly recognised as a less than ideal reference for LV functional measurements because of several limitations that include poor endocardial definition near the apex and the use of gadolinium as a contrast medium is now extremely limited in the CKD population. In any case, the use of intradialytic CMR would be impossible. However, the use of real-time three-dimensional echocardiography has been shown to be as accurate as using CMR images for measuring volumetric indices of regional LV function and for the detection of regional wall motion abnormalities [210] and this technique would be available to use during haemodialysis. Other more up-to-date forms of cardiac imaging include tissue Doppler and strain Doppler imaging which depict local myocardial motion and may enable

quantification of myocardial function and measurement of regional myocardial dysfunction [211,212].

Unfortunately, we had no data pertaining to myocardial blood flow, coronary flow reserve or coronary artery anatomy in any significant number of the long term cohort of patients. The use of PET to assess these would have been impracticable and the use of non-routine angiography would have been a challenge both ethically and for the purposes of recruitment. Ideally, another surrogate marker for myocardial perfusion or coronary anatomy would have been used as angiographic data for coronary anatomy was only available for patients in the study investigating myocardial perfusion (chapter 4). High resolution CT scanning is available to image significant proximal coronary vessel lesions and two-dimensional speckle tracking is also validated for the detection of significantly diseased coronary arteries using altered diastolic deformation at rest [213]. All of these techniques are becoming more widely available and would have made the results more robust.

Unfortunately due to these factors, the observational nature of each of the studies and the lack of perfusion data for patients, we have extrapolated results that imply but do not implicitly prove myocardial stunning as the mechanism for long term injury. Whilst each of the steps outlined from one chapter to the next seems plausible the exact link remains unproven. However, the association with ischaemia, regional dysfunction and RWMA development is well established even if

the pathophysiological link with stunning and fibrosis remains controversial and efforts to reduce the mechanisms behind HD induced ischaemia should be a focus for future therapeutic targets.

9.2 Future work

This thesis explores the mechanisms, prevalence and consequences (both short and medium term) of haemodialysis induced myocardial stunning. However, there remain several unanswered questions.

Further work is required to prove the underlying pathophysiological model that has been proposed. Although the concept of myocardial stunning (persistent myocardial dysfunction after a return to normal underlying blood flow) has been demonstrated in a small number of patients on HD, the effect of repetitive HD induced ischaemia and associated myocardial hibernation and fibrosis has yet to be scientifically proven. The results in this thesis show a deterioration that would fit with a progression along the continuum of stunning-hibernation-fibrosis but in order for that link to be confirmed, prospective data would be needed to establish not only a reduction in function but also a corresponding reduction in myocardial blood flow.

With regard to the potential effect of modifications to the dialysis process on short and long term outcomes, a number of issues remain. To examine whether cooled temperature and biofeedback dialysis techniques would have the same positive effects on a larger scale, further work is now needed to investigate if such measures translate to

improved long term outcomes. Using grant monies awarded from the British Renal Society based on these results, data is already being collected looking at the effects of isothermic dialysis on intradialytic haemodynamic tolerability and ventricular ectopy. This pilot work has been used to design a study looking at the long term benefits on outcome (including survival, cardiovascular event rate, left ventricular geometry and function, troponin-T concentrations amongst others) using these dialysis based techniques.

The logistics of testing the hypothesis that longer dialysis regimes (either short daily or nocturnal dialysis) may improve outcome is difficult given the current pressures in haemodialysis units in the UK. However, despite this ethical approval has been granted for a collaborative study in the United States in a centre with an active daily dialysis programme to look at the positive associations of daily dialysis and improved haemodynamics, lower ultrafiltration requirements through smaller interdialytic weight gains and improved electrolyte homeostasis.

Similarly, timely renal transplantation has been shown to improve cardiac function in previously dialysis dependent patients. There exists the possibility of the future follow up of this cohort of patients for a longer period of time to look at not only the progression of cardiovascular decline but also for potential improvements in donor recipients.

Each of these may reveal mechanisms by which we can reduce acute haemodialysis induced cardiac injury. If so, then dialysis based interventions aimed at reducing the incidence and prevalence of

myocardial stunning may reduce the impact on the cardiovascular system and help to improve patient outcomes.

Chapter 10

Abbreviations

10 Abbreviations

ACEi	Angiotensin-II converting enzyme inhibitor
ANOVA	Analysis of variance
APKD	Adult polycystic disease
ARB	Angiotensin-II receptor blocker
BD	Twice daily
BFD	Biofeedback dialysis
BNP	Brain naturietic peptide
BP	Blood pressure
BV	Blood volume
CAD	Coronary artery disease
CAPD	Continuous ambulatory peritoneal dialysis
CFR	Coronary flow reserve
CKD	Chronic kidney disease
CO	Cardiac output
COREC	Central Office of Research Ethics Committees

CRP	C-reactive protein
cTnI	cardiac troponin-I
cTNT	cardiac troponin-T
CVA	Complex ventricular arrhythmia
Cx	Circumflex
DICOM	Digital imaging and communication in medicine
ECG	Electrocardiograph
EF	Ejection fraction
eGFR	Estimated glomerular filtration rate
ESRD	End stage renal disease
ETT	Exercise tolerance test
GFR	Glomerular filtration rate
GTN	Glyceryl trinitrate
Hct	Haematocrit
HD	Haemodialysis
HDF	Haemodiafiltration
HF	Haemofiltration

hsCRP	High sensitivity C-reactive protein
HT	Hypertension
IDH	Intradialytic hypotension
IHD	Ischaemic heart disease
IL-6	Interleukin-6
IQR	Interquartile range
ISMN	Isosorbide mononitrate
KDOQI	Kidney Disease Outcomes Quality Initiative
LMS	Left main stem
LV	Left ventricular
LVEF	Left ventricular ejection fraction
$LVEF_{HD}$	Left ventricular ejection fraction during haemodialysis
$LVEF_{rest}$	Left ventricular ejection fraction at rest
LVH	Left ventricular hypertrophy
LVMI	Left ventricular mass index
MAP	Mean arterial pressure
MBF	Myocardial blood flow

MICS	Malnutrition-inflammation complex syndrome
NHANES	National Health and Nutrition Examination Surveys
NYHA	New York Heart Association
OD	Once daily
PC	Personal computer
PET	Positron emission tomography
pmp	per million population
PVC	Premature ventricular complex
RCA	Right coronary artery
RPP	Rate pressure product
RR	Relative risk
RRT	Renal replacement therapy
RWMA	Regional wall motion abnormality
SBP	Systolic blood pressure
SD	Standard deviation
SF	Shortening fraction
Sf_{mean}	Mean shortening fraction

SF_{WMA}	Shortening fraction of wall motion abnormality
SPECT	Single photon emission computed tomography
SV	Stroke volume
TPR	Total peripheral resistance
UF	Ultrafiltration
US	United States
USRDS	United States Renal Data System
VE	Ventricular ectopic

Chapter 11

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