UNIVERSITY OF SOUTHAMPTON

FACULTY OF MEDICINE HEALTH AND LIFE SCIENCES

THE CARDIORENAL CONTINUUM: A FOCUS ON IRON DEFICIENCY, ARRHYTHMIA BURDEN AND TREATMENT.

By

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Thesis for the degree of DM

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ABSTRACT

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ABSTRACT

Introduction:

Heart failure remains a leading cause of hospitalisation in the developed world. Comorbidities such as iron deficiency and chronic kidney disease (CKD) pose management challenges and have been proven to have prognostic impact. The link between cardiac and renal disease is complex and is now understood to begin before abnormalities in either system become manifest. This gradual transition from coexistence of risk factors to target organ damage and death is often referred to as the cardiorenal continuum. When cardiac and renal dysfunction co-exist, further challenges arise due to the high burden of cardiovascular risk factors that characterise these populations and the lack of a robust evidence base specific for these patients. Management can therefore be biased and sub optimal.

This thesis focuses on two major comorbidities of heart failure- iron deficiency and chronic kidney disease, with the former being a common comorbidity to both pathologies. The first part of this thesis aims to understand the role of iron deficiency in acute heart failure, its prevalence and its potential links to inflammation and renal dysfunction. The second part of this thesis focuses on cardiorenal disease and aims to define arrhythmic burdens and incidence of sudden cardiac death in end stage kidney disease patients. This thesis also aims to establish the safety and feasibility of using evidence-based therapies in the presence of CVD and renal dysfunction and compares prescribing practices, complication rates and outcomes in those with and without significant CKD.

Methods:

To answer the above questions, I carried out 5 studies focusing on the different aspects of the cardiorenal continuum.

1. The Iron status in decompensated heart failure (IRON-STATS –DHF) study focused on iron deficiency, an important risk factor at one end of the cardiorenal continuum with prognostic implications in chronic heart failure. This was a prospective observational study of 100 acute decompensated heart failure (ADHF) patients and 30 patients with stable chronic heart failure (CHF) and 20 healthy subjects. The aim of the study was to assess the prevalence of iron deficiency in ADHF and to understand the relationship between serum hepcidin, renal function and inflammatory markers and iron status.
2. The second part of IRON STATS-DHF aimed to define the changes in iron status following decompensation and evaluate whether this is associated with markers of inflammation and renal function. 1-year cardiovascular (hospitalisation and death) outcomes as well as all cause mortality were also assessed.
3. Arrhythmias are thought to make a significant contribution to the adverse prognosis in cardiorenal disease. The CRASH-ILR Study aimed to characterize the arrhythmia burden in patients on haemodialysis. 30 patients established on haemodialysis received a Reveal XT implantable loop recorder (Medtronic, USA) and ECG data were transmitted at each haemodialysis session using a remote monitoring system. Primary outcome was sudden cardiac death (SCD) or implantation of a (tachy or bradyarrhythmia controlling) device and secondary outcome, the development of arrhythmia necessitating medical intervention.

4. Suboptimal use of evidence-based therapies in cardiorenal disease has been proposed as one of the reasons for poorer outcomes in this population. The aim of the cardiac resynchronisation therapy in chronic kidney disease (CRT in CKD) study was to assess the safety and feasibility of implanting cardiac resynchronization therapy (CRT) devices in patients with severe CKD and to evaluate whether device implantation in those with CKD was associated with greater risks and/ or worsening renal dysfunction. To this end, 429 patients who had CRT devices implanted for advanced heart failure in two high volume centres in the UK, were retrospectively evaluated {characterised according to implant estimated glomerular filtration rate (eGFR)} for complications related to device implant, change in eGFR over time following CRT implantation and impact of eGFR on mortality.
5. The primary percutaneous coronary intervention (PPCI) secondary prevention study aimed to evaluate real life prescribing practices in the UK in those with and without CKD. The study evaluated whether early treatment with PCI translated to more aggressive use of evidence based secondary prevention in those with significant CKD. 1169 consecutive patients from 5 UK centres receiving PPCI for ST elevation MI were assessed based on their eGFR for the use of evidence based secondary prevention at discharge; 567 of these patients were followed up at 6 weeks to assess titration practices for these drugs. Follow-up prescribing practice was assessed in 567 patients.

Results:

The main findings of each study were:

1. Iron deficiency in ADHF is a common finding; 83% of patients admitted with ADHF had iron deficiency at admission to hospital while 63% of stable CHF patients were iron deficient. Absolute iron deficiency was seen more frequently than functional iron deficiency. Hepcidin was significantly lower in patients with absolute iron deficiency as compared to those with functional iron deficiency (mean 20.30 ± 29.77 ng/mL in the absolute iron deficiency group vs 67.09 ± 39.38 ng/mL in the functional iron deficiency group, p = 0.001). There were no significant relationships between iron status and renal function (eGFR or Cystatin C).

2. In those with iron deficiency at baseline, there was a significant increase in serum iron, transferrin saturation (TSAT) and serum ferritin following discharge from hospital. TSAT and serum iron plateaued at 1-month post discharge while serum ferritin continued to increase to 3 months post discharge.

3. During 379 512 hours of continuous ECG monitoring (mean 12648 ± 9024 hours/patient), there were 8 deaths - 2 SCD and 6 due to generalised deterioration/sepsis. 5 (20%) patients had a primary outcome event (2 SCD and 3 pacemaker implantations for bradyarrhythmia). 10 (33%) patients reached an arrhythmic primary or secondary end point. Median event free survival for any arrhythmia was 2.6 years (95% confidence intervals 1.6 – 3.6 years).

4. 26% of the patients in the 'CRT in CKD' study had significant CKD, defined as an eGFR < 45 ml/min/1.73 m^2. There was no significant difference in the occurrence of

complications in the presence of significant CKD. Improvement in symptomatic wellbeing was consistent in patients with or without significant CKD. At 6 months post implant, 33.3% of the population (n = 89) had a decline in eGFR of ≥ 5 ml/min/1.73 m^2 compared to implant eGFR. 40.8% (n = 109) remained stable with an eGFR change between -4 to +4 ml/min/1.73 m^2 and 25.8% (n = 69) demonstrated an improvement in eGFR of 5 ml/min/1.73 m^2. At 12 months post implant compared to implant eGFR, these figures were 37.4%, 40.5% and 22% respectively. 1-year mortality was greatest in those with the lowest eGFR at implant. Change in eGFR in the 6 months prior to implant did not appear to predict mortality.

5. CKD (eGFR< 60 ml/min/1.73 m^2) was seen in 17.6% of patients receiving PPCI. One fifth of patients were older than 75 years. Reduced renal function was associated with age, female sex, and lower use of angiotensin converting enzyme (ACE) inhibitor/ angiotensin receptor blockers (ARB). At discharge 83.5% of patients with eGFR < 60 ml/min/1.73 m^2 were receiving ACE inhibitors/ARB, dropping to 77.5% at 6 weeks (compared to 95% and 92% respectively in patients with eGFR > 60 ml/min/1.73 m^2).

Conclusions:

Iron deficiency is common in ADHF and iron status seems to improve after discharge from hospital (i.e. recompensation). Serum TSAT and iron levels plateau by 1-month post discharge and appear to be associated with CV outcomes. The findings of CRASH-ILR study confirm the high mortality rate seen in haemodialysis populations however in this study, death was secondary

to terminal illness rather than arrhythmias in most cases. Bradyarrhythmias emerged as a common and potentially significant arrhythmic event and will need further assessment in larger ILR based studies. Thus while the benefits of implanting defibrillators in a haemodialysis population remain questionable, CRT therapy was proven to be safe and of symptomatic benefit in those with significant CKD.

Even in those with established CKD, secondary prevention medication such as ACE inhibitors, beta-blockers and mineralocorticoid antagonists (MRA) were tolerated well post PPCI and would benefit from aggressive uptake and up titration where possible.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABBREVIATIONS

ACE (i)	Angiotensin converting enzyme inhibitor
ADHF	Acute decompensated heart failure
AKI	Acute kidney injury
BNP	B-natriuretic peptide
CAD	Coronary artery disease
CHF	Chronic heart failure
CI	Confidence interval
CKD	Chronic kidney disease
CRS	Cardiorenal syndrome
CV	Cardiovascular
CVD	Cardiovascular disease
eGFR	Estimated glomerular filtration rate
ESKD	End stage kidney disease
ESC	European Society of cardiology
EPO	Erythropoietin
GFR	Glomerular filtration rate
HR	Hazard ratio
Hb	Haemoglobin
LV	Left ventricular
LVSD	Left ventricular systolic dysfunction
LVEF	Left ventricular ejection fraction
NICE	National Institute of clinical excellence
NYHA	New York Heart Association
RAAS	Renin angiotensin aldosterone system
TIBC	Total iron binding capacity
TSAT	Transferrin saturation
VF	Ventricular fibrillation
VT	Ventricular tachycardia

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Completion of this work has only been possible because of the contributions of many people. I am indebted to Dr. Paul Kalra my primary supervisor, for the opportunities that he has given me, his unwavering support and encouragement, and in particular for being my mentor for as long as I can remember. I am also indebted to Professor Mark Hanson for his enthusiasm, guidance and grounded advice.

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I undertook a number of specific collaborations during the course of this thesis, and I would especially like to thank Dr. Paul Roberts (Southampton University hospital), for the CRASH-ILR project. I would also like to thank Dr. Bartosz Olechowski, Dr. Rebecca Sands, Dr. Mike Pope, (Wessex deanery), Professor Jan Shute (Portsmouth University) as well as the teams who helped with data collection for the collaborative work on the impact of chronic kidney disease on secondary prevention post primary percutaneous coronary intervention (PPCI).

I am grateful to my husband Ashok and my daughter Shreya who have endured my absences and pressures during this entire period and have stuck by me nevertheless, providing me the strength and the freedom to make this possible.

Above all, I am extremely grateful to the patients and volunteers for their altruistic participation in my studies.

STATEMENT OF ROLES AND RESPONSIBILITIES

For studies 1, 2, 4 and 5, I produced original study paperwork, obtained ethics committee and research and development department approval where applicable. I recruited all participants and conducted the majority of visits. I collated all the results, follow up data and carried out statistical analysis of findings. Blood sample analysis was conducted in collaboration with the research laboratory at Queen Alexandra hospital, Portsmouth.

Some sample paper work is included in the appendix accompanying this thesis. All of the work presented here is my own original work. The work presented in chapter 4 – The CardioRenal Arrhythmia Study in Haemodialysis patients using Implantable Loop Recorders (CRASH ILR) was performed in collaboration with Dr. Paul Roberts (Southampton University hospital). I recruited all of the participants to this study, performed their baseline investigations and implanted their loop recorders. I also analysed several thousand hours of ECG downloads from these patients. Study 4 was carried out in collaboration with Royal Bournemouth hospital and Study 5 on the impact of chronic kidney disease on secondary prevention post PPCI was conducted in collaboration with 4 other PPCI centres across the UK. For collaborative studies, I conducted all the local data

collection, compilation of results and analysis of findings. These tasks were conducted under the supervision and guidance of my supervisor Dr. Paul Kalra.

DECLARATION OF AUTHORSHIP

I, DONAH ELIZA ZACHARIAH declare that this thesis and the work presented in it are my own and have been generated by me as the result of my own original research.

THE CARDIORENAL CONTINUUM: A FOCUS ON IRON DEFICIENCY, ARRHYTHMIA BURDEN AND TREATMENT.

I confirm that:

1. This work was done wholly or mainly while in candidature for a research degree at this University;
2. Where any part of this thesis has previously been submitted for a degree or any other qualification at this University or any other institution, this has been clearly stated;
3. Where I have consulted the published work of others, this is always clearly attributed;
4. Where I have quoted from the work of others, the source is always given. With the exception of such quotations, this thesis is entirely my own work;
5. I have acknowledged all main sources of help;
6. Where the thesis is based on work done by myself jointly with others, I have made clear exactly what was done by others and what I have contributed myself;
7. Parts of this work have been published as listed in the publications section.

Signed: .....

Date: 31 MAY 2018 .....

PUBLICATIONS (related to thesis)

1. Roberts PR and **Zachariah D,** Morgan JM, Yue AM, Greenwood EF, Phillips PC, Kalra PA, Green D, Lewis RL, Paul R Kalra. Monitoring of arrhythmia and sudden death in a haemodialysis population: the CRASH-ILR study (undergoing revisions following peer review PLoS One).
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ABSTRACTS AND ORAL PRESENTATIONS

1. European Society of Cardiology Heart failure, Rome August 2016: Dosing and tolerability of Ivabradine in the LIVE: LIFE study, an evaluation of Ivabradine given to older patients with heart failure across the UK
2. European Society of Cardiology Heart failure, Rome August 2016: Improvement in quality of

life in older patients with heart failure initiated on Ivabradine across the UK: results from the multi-centre LIVE: LIFE prospective cohort study

3. European Society of Cardiology Heart Failure, Florence May 2016: Baseline characteristics of the LIVE: LIFE study and presentation of results at 'Late breaking trial session'
4. European Society of Cardiology Congress Amsterdam September 2013: Should cardiac resynchronization therapy be considered for patients with severe chronic kidney disease?
5. BCS Annual conference London June 2013: Characteristics and treatment of 2346 Patients with stable coronary artery disease in UK Primary Care
6. European Society of Cardiology Congress Amsterdam September 2013: Is cardiac resynchronisation therapy (CRT) feasible, safe and beneficial in the very elderly?
7. 'Safety and feasibility of CRT implantation in patients with chronic kidney disease'. Abstract presentation at BSH annual conference London, Nov 2013. **Runner up for young investigator award.**
8. 'Secondary prevention in the elderly post myocardial infarction'. Abstract presentation at BSH annual conference London, Nov 2012. **Runner up for young investigator award.**

CHAPTER 1- INTRODUCTION

Heart failure is a syndrome of debilitating symptoms resulting from impaired cardiac function. It is the commonest cause of hospitalisation in patients aged more than 65 years (1) and despite the availability of several drugs with proven prognostic benefit, increasing utilisation of biventricular pacing and active involvement of specialist heart failure teams in the management of these patients, prognosis generally remains poor. (2, 3) Various pathophysiological systems are thought to occur in response to a myocardial insult, (hormonal, immunological, inflammatory and metabolic), and all of these systems are thought to propagate and sustain the progression of heart failure.

This thesis will focus on two major comorbidities of heart failure- iron deficiency and chronic kidney disease. Both of these entities, which are known to impact on outcomes are common and can occur independently. In the presence of cardiorenal disease, the presence of iron deficiency may be potentially linked to the underlying pathophysiology of coexistent renal and cardiac dysfunction.

Iron deficiency has been the subject of recent research and may potentially offer a prognostically beneficial therapy in heart failure patients. Many aetiologies have been proposed for the development of iron deficiency; one such is the role of inflammation. A key player in the role of inflammation in influencing iron status could be via the hepatic hormone hepcidin.
The first section of this thesis is comprised of studies designed to understand the mechanism of iron deficiency in heart failure, and potential links with renal dysfunction. The second section

will focus on cardiorenal disease and is comprised of studies designed to define the burden of arrhythmias in end stage kidney disease (ESKD) as well as understand the utilisation, impact and feasibility of evidence-based therapies in the presence of cardiorenal disease.

HEART FAILURE

Heart failure is commonly described as 'a pathophysiological state in which an abnormality of cardiac function is responsible for the failure of the heart to pump blood at a rate commensurate with the requirements of metabolising tissues'. For a diagnosis of heart failure to be made, the European Society of Cardiology (ESC) guidelines stipulate that there should be the presence of symptoms such as dyspnoea (exertional dyspnoea, orthopnoea and / or paroxysmal nocturnal dyspnoea), swollen ankles, lethargy, or fatigue - as well as an objective evidence of resting cardiac dysfunction. (4) Presentation can be acute (such as in acute pulmonary oedema or cardiogenic shock) or chronic.

Heart failure can be caused by 4 major pathologies. The first includes traditional risk factors such as myocardial ischaemia, hypertension, alcohol, metabolic syndrome (diabetes mellitus, hyperlipidaemia, obesity). (5) The second and third categories include genetic cardiomyopathies (such as hypertrophic cardiomyopathy) and mechanical aetiologies (such as valvular disease). The final category consists of autoimmune and infectious (viral and bacterial) triggers. Irrespective of the primary trigger, an insult to the myocardium triggers a series of events initially aimed at maintaining circulatory homeostasis. One of the key responses is immune activation. With the first 3 pathologies resulting in heart failure, immune activation is a

secondary response whereas in heart failure secondary to autoimmune or infectious causes, the innate and adaptive immune systems are activated to coordinate a primary response. (6, 7)

Heart failure can occur across the spectrum of left ventricular (LV) function- from severe systolic dysfunction through to preserved systolic function. Left ventricular systolic dysfunction (LVSD) is much better understood with regards to pathophysiology and interventional studies in this population form the basis of majority of current evidence based therapies.

Pathophysiology of heart failure

Following a reduction in LV stroke volume, as seen in the presence of reduced systolic function, a number of responses aimed at maintaining circulatory volume and vital organ perfusion come into play (Figure1.1). These can be broadly classified as intrinsic and extrinsic and are discussed in more detail below. Although these mechanisms attempt to compensate reduced LV function and do so acutely, in the long term, they can be deleterious.

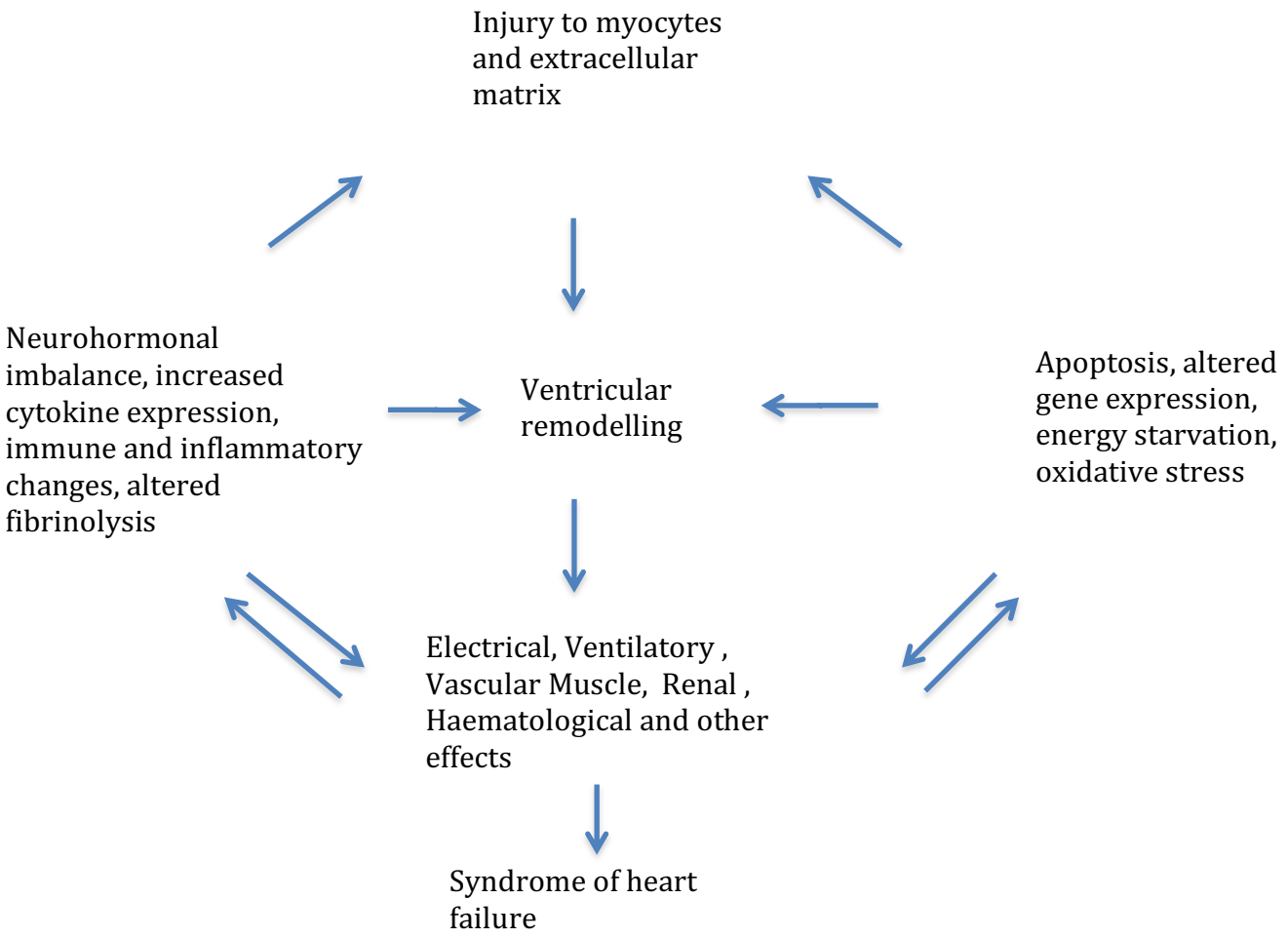


Figure 1.1-Pathophysiology of heart failure as a result of LV systolic dysfunction.

Damage to myocytes and extracellular matrix leads to remodelling (changes in size, shape and function of the left ventricle and heart). These changes in turn may lead to electrical instability and other systemic processes resulting in many effects on other organs and tissues and further damage to the heart. (8)

Intrinsic responses

1. Frank-Starling mechanism

   As per Frank-Starling law, a reduction in the volume of blood ejected from the ventricle (stroke volume) leads to a rise in LV end diastolic volume and pressure. This rise in preload leads to increased force of contraction, in an attempt to restore stroke volume. In the chronic setting, sodium retention, water retention and vasoconstriction may all

represent on-going attempts to utilise the Frank- Starling mechanism to increase LV filling pressure and pre-load. These, over time, however lead to arterial stiffness and constriction, increasing afterload and cause the injured left ventricle to fail further.

2. Ventricular remodelling such as development of left ventricular hypertrophy (LVH) in pressure overload and LV dilatation in volume overload, are further intrinsic cardiac mechanisms to compensate for the failing heart. Over time however these mechanisms fail and result in further depression of myocardial contractility.

Extrinsic/ Systemic responses

These include the activation of the renin angiotensin aldosterone system, inflammatory immunological as well as metabolic pathways.

Renin angiotensin aldosterone system (RAAS)

In response to low circulating volumes (or poor renal perfusion from reduced cardiac output) and subsequent decreased stretch of the glomerular afferent arteriole, there is an increase in renin release. This can also be independently stimulated by the sympathetic system.
Increased renin leads to increased production of angiotensin II, which directly as well as via aldosterone, induces vasoconstriction and salt and water retention.

Angiotensin II also has prothrombotic actions and can increase the release of arginine vasopressin. It can also cause myocardial cell hypertrophy and fibrosis leading to maladaptive remodelling and progressive reduction in myocardial function. It can also independently activate the sympathetic nervous system.

Aldosterone independently contributes to sodium and water retention and potassium loss. This in combination with autonomic dysfunction and myocardial fibrosis (also caused by aldosterone) is associated with increased risk of ventricular arrhythmias and contributes to vascular fibrosis seen in heart failure.

Vasopressin (antidiuretic hormone) is a neurohypophysial peptide involved in the regulation of free water resorption, body fluid osmolality, blood volume, blood pressure, cell contraction, cell proliferation and adrenocorticotropin secretion. It is a very powerful vasoconstrictor and stimulates blood platelet aggregation, coagulation factor release and cellular proliferation.

Inflammation and heart failure

Elevated pro-inflammatory mediators have been demonstrated in acute myocarditis and acute myocardial infarction and hinting towards the tissue injury hypothesis. (9) Studies have also consistently demonstrated elevated pro-inflammatory cytokines in association with heart failure progression in both human and animal cohorts, supporting the hypothesis that inflammation may contribute to heart failure. (10)

Cytokines form a vast array of low molecular weight, pharmacologically active proteins secreted by different cell types for the purpose of altering either their own function (autocrine) or that of adjacent cells (paracrine). The most important cytokines implicated in the progression of CHF are tumour necrosis factor α (TNFα), interleukin (IL) 1, and IL-6. The production of pro-inflammatory cytokines has mostly been attributed to secretion by mononuclear cells. The myocardium via catecholamine stimulation may also contribute to the production of pro

inflammatory cytokines. The triggers for increased production of pro-inflammatory mediators are thought to be myocardial injury (9) and under-perfusion of peripheral tissues (11). Endotoxin, also known as lipopolysaccharide (LPS), is one of the strongest inducers of TNFα and other pro-inflammatory mediators. LPS can be triggered by increased bowel oedema for example. (12) LPS concentrations are raised in CHF patients with peripheral oedema and diuretic treatment appears to reduce LPS levels. (13) Peripheral IL-6 spillover, indicating peripheral cytokine production, has also been demonstrated in patients with CHF on comparing arterial and venous plasma concentrations. (11)

Tumour necrosis factor α

TNF alpha was first described in 1975 and termed cachectin. In 1990 Levine *et al* demonstrated higher mean serum concentrations of TNFα in CHF patients than in healthy subjects (115 (25) vs. 9 (3) U/ml, $p = 0.001$) and patients with high concentrations of TNFα were more often suffering from cardiac cachexia. (14) TNFα has been implicated in the development of left ventricular dysfunction, left ventricular remodelling, increased cardiac myocyte apoptosis, the development of anorexia and cachexia, reduced skeletal muscle blood flow and endothelial dysfunction, severity of insulin resistance, activation of the inducible form of nitric oxide synthase (iNOS), β receptor uncoupling from adenylate cyclase, and other effects. (10, 15) (16) TNFα exerts its effects via TNFα receptors (TNFR), expressed by almost all nucleated cells. There are two types of TNFR (TNFR-1 and TNFR-2) and of these, TNFR-1 is more abundantly expressed and appears to be the main signaling receptor (15, 16, 17) and long term (18) prognosis better than TNFα in CHF patients.

Interleukin 6

Cytokines exacerbate haemodynamic abnormalities or exert direct toxic effects on the heart resulting in the progression of heart failure. (19) IL-6 is also found to be increased in CHF and can produce myocyte hypertrophy, myocardial dysfunction, and muscle wasting. IL-6 is thought to be released in direct response to TNFα and a linear correlation between the two has been observed. (19) Unlike TNFR, which is linked to prognosis, IL-6 receptor (IL-6R) is not. A trans membrane glycoprotein termed gp130, rather than IL-6R, renders cells susceptible to IL-6. Both gp130 and IL-6R are always required for signaling and the soluble form of gp130 inactivates the soluble IL-6/IL-6R complex. Both the concentrations of gp130 and the overall level of bioactivity of IL-6 are increased in CHF.

Interleukin 1

IL-1 along with TNFα is generally thought of as prototypical pro-inflammatory cytokines. IL-1 has been demonstrated in the myocardium of patients with idiopathic dilated cardiomyopathy. Depression of myocardial contractility occurs in a dose dependent fashion. This effect is synergistic with that of TNFα, and the stimulation of iNOS seems to be involved. IL-1 has also been shown to be involved in myocardial apoptosis, hypertrophy, and arrhythmogenesis. (20)

C-reactive protein

C-reactive protein (CRP) was first discovered in 1930; the name comes from its ability to react with the somatic C polysaccharide of Streptococcus pneumoniae. CRP specifically binds to specific microbial polysaccharides (phosphocholine moieties), which gives this substance a host defensive role. Upon binding to these structures, CRP activates the classical complement

pathway and opsonises ligands for phagocytosis. CRP is exclusively produced in the liver. It is secreted in increased amounts within 6 hours of an inflammatory stimulus and is thus regarded as a marker of acute inflammation.

The first observation of raised concentrations of CRP in CHF was published in 1990. More recently studies have linked higher CRP with poorer prognosis. (21) CRP has been observed to is to augment IL-1β induced production of iNOS however it is still not clear if CRP is merely a marker of inflammation with no particular role in the development of cardiac disease or if it directly contributes to disease progression.

Interleukin 10

IL-10 is one of the most important anti-inflammatory cytokines and is known to down regulate the production of TNFα, IL-1, and IL-6, respectively. This has been confirmed in LPS stimulated peripheral blood mononuclear cells from CHF patients. (22) IL-10 limits the production of macrophage-derived nitric oxide (NO) and oxygen-free radicals. IL-10 also enhances the release of soluble TNFR, which contributes to the reduction of TNFα activity. Similar to pro-inflammatory cytokines, IL-10 mRNA has also been detected in the failing myocardium. Circulating IL-10 concentrations have been reported to be either increased or decreased in CHF patients compared to healthy, age matched controls.

Sympathetic nervous system

Elevated levels of plasma norepinephrine (noradrenaline) are commonly seen following myocardial insult, partially driven by reduced stimulation of stretch activated baro receptors in the carotid arteries and aorta. This enhanced sympathetic activity initially increases myocardial

contractility and heart rate leading to an increase in cardiac output. This also promotes renin release, sodium retention and vasoconstriction thereby increasing preload and activating Frank-Starling mechanism. Chronic activation over time becomes detrimental. Sympathetic activation can also be directly toxic to cardiomyocytes and can increase the electrical instability of myocytes.

Natriuretic peptides

These are usually released in response to atrial and ventricular wall stretch and serve to maintain sodium and volume homeostasis by enhancing sodium and water excretion. The haemodynamic effects include vasodilation. They suppress the RAAS and possibly the sympathetic nervous system. As a consequence of increased synthesis and release, circulating levels of ANP and BNP are greatly increased in heart failure. BNP has a precursor pro-BNP, which is stored in granules in myocytes. Pro-BNP is activated by a protease to form its biologically active form BNP and N-terminal pro BNP.

Vasoconstriction and sodium retention are mediated by the renin angiotensin aldosterone system (RAAS), the sympathetic nervous system, non-osmotic release of vasopressin, endothelin-1 and thromboxane. Vasodilatation and natriuresis on the other hand are mediated by atrial natriuretic peptide (primarily released from atria), brain natriuretic peptide (BNP released from atria and ventricles, predominantly from the latter), nitric oxide as well as vasodilatory prostaglandins.

COMORBIDITIES OF HEART FAILURE

Despite the various evidence based treatment modalities available for CHF, prognosis remains poor and the number of admissions to hospital with heart failure continues to grow. (23)

The mean age of patients admitted to hospital with heart failure in the UK is 77.8 years (75.9 years for men and 80.1 years for women). (23) For patients admitted with heart failure, in-hospital mortality in the UK was 9.6% in 2014-15 and mortality rate at one year was 29.6%; these figures have remained relatively static over the last few years. (23)

Therapeutics gaps have been proposed as a reason for the continued poor prognosis, i.e. inability of currently available evidence based drugs to adequately block all of the pathophysiological mechanisms driving heart failure. A number of studies have therefore been aimed at modifying immune activation in heart failure, but without much success. The RECOVER, RENNAISSANCE and ATTACH trials all targeting TNFα were stopped prematurely due to the absence of any clinical benefit. (24) More recently, proof-of-concept studies tested the feasibility of IL-1 blockade with anakinra for 14 days in patients with stable heart failure, and although there is demonstration of a significant reduction in CRP levels and a significant improvement in peak oxygen consumption, with favourable safety and tolerability profiles, it remains to be seen how this translates to clinical practice. (25)

Other reasons proposed for the poor prognosis associated with heart failure include variations in the use of evidence-based drugs, degree of specialist input, as well as the presence of comorbidities. Traditional comorbidities such as hypertension, diabetes, atrial fibrillation and ischemic heart disease continue to be the most prevalent, however other comorbidities are frequently identified in heart failure cohorts. The presence of comorbidities such as chronic kidney disease (CKD) and anaemia pose particular challenges to the management of heart failure and will form the focus of this thesis. Renal dysfunction is extremely common in CHF with

studies suggesting that an estimated glomerular filtration rate (eGFR) of 60 ml/min/1.73 m^2 or less (i.e. CKD stage 3 or worse) is present in 35 – 50% of CHF patients. (26) Similarly, up to 50% of all patients with CHF have anaemia, with the prevalence of anaemia varying widely between studies mainly due to the variations in the definitions of anaemia.

ANAEMIA IN HEART FAILURE

The World Health Organisation (WHO) defines anaemia as haemoglobin < 130 g/L for males and haemoglobin < 120 g/L for females. (27) Anaemia is an independent risk factor for mortality in CHF patients (28) and also forms a potential link with renal dysfunction. The prevalence of anaemia increases with the severity of CHF, approaching 80% in those with New York Heart Association (NYHA) class IV (Table 1.1 describes NYHA classification of symptoms in CHF). (29)

NYHA class	Patient Symptoms
I	No limitation of physical activity.
II	Slight limitation of physical activity. Ordinary physical activity results in fatigue, palpitation, and dyspnoea.
III	Marked limitation of physical activity. Less than ordinary activity causes fatigue, palpitation, or dyspnoea.
IV	Unable to carry on any physical activity without discomfort. Symptoms of heart failure at rest.

Table 1.1- New York Heart Association (NYHA) classification of heart failure symptoms.
The NYHA classification is a universally used tool for assessment of functional status in heart failure patients.

Anaemia is associated with elevated bio-markers (reflecting the severity of heart failure) and inflammatory markers such as elevated N-terminal pro BNP and CRP and an increase in LV mass. Anaemia is also associated with impaired functional capacity, greater incidence of hospitalisation as well as increased mortality. (30-36)

Anaemia is common in the acute decompensated state. Acute decompensated heart failure (ADHF) is defined as a sudden worsening of symptoms of heart failure, i.e. breathlessness, peripheral oedema or fatigue, in persons with objective evidence of a structural or functional abnormality of the heart. When anaemia is present, it is associated with higher morbidity and mortality. The OPTIMIZE-HF (Organized Program to Initiate Lifesaving Treatment in Hospitalized Patients With Heart Failure) registry, for example, with data on 48 612 patients hospitalised for heart failure in the U.S., demonstrated that 51.2% of the cohort had haemoglobin levels of < 121 g/l and 25% had moderate to severe anaemia with haemoglobin levels of 50 to 107 g/l). (37). Anaemic patients had higher in-hospital mortality (4.8% vs 3.0%, lowest vs highest quartile), longer hospital length of stay (6.5 vs 5.3 days), and more readmissions by 90 days (33.1% vs. 24.2%) (all $p < 0.001$).

Several aetiologies have been proposed for anaemia in heart failure (Table 1.2). Opasich *et al* in their 2005 paper on mechanisms of anaemia in heart failure concluded that half of anaemic CHF patients showed anaemia of chronic disease with blunted endogenous erythropoietin (EPO) production and/or a defective iron supply for erythropoiesis. (38) EPO is a glycoprotein hormone produced primarily in the kidney by specialized peritubular fibroblasts. It regulates erythroid cell proliferation in the bone marrow in response to tissue hypoxia. The primary stimulus for EPO

production is reduced oxygen tension that induces the transcription of the EPO gene. This in turn stimulates erythroid cell proliferation and differentiation. In the above study by Opasich, of 148 consecutive CHF patients with haemoglobin concentration < 130g/L (males) or 120 g/L (females) recruited to the study, 85 patients had anaemia of chronic disease (defined as: reduced concentrations of serum iron, transferrin, and total iron binding capacity; normal or raised ferritin; normal or slightly increased soluble transferrin (sTNFR) receptor). All 85 patients had normal ferritin values and 78 patients (92%) showed iron deficiency for erythropoiesis {high soluble transferrin receptor and/or low transferrin saturation (TSAT) and/or defective EPO production}. Haemoglobin was related to gender (females 104 + 9 g/L vs. males 111 + 10 g/L; $p = 0.002$) and inversely correlated with serum creatinine ($r = -0.42$; $p = 0.001$), urea ($r = -20.29$; $p = 0.008$), sTNFR ($r = -0.44$; $p < 0.001$), IL-6 ($r = -0.41$; $p < 0.001$) and EPO levels ($r = -0.21$; $p = 0.04$).

Tissue hypoxia aside, CKD per se is associated with reduced production of EPO in the kidney and as CKD is very common in CHF, defective EPO could be driven by the presence of concomitant CKD. Oxygen delivery into the kidney is determined by renal blood flow, hematocrit, and pO_2 of the haemoglobin oxygen-dissociation curve. Conversely, oxygen consumption is determined by proximal tubular sodium reabsorption and the eGFR. The haemodynamic and parenchymal distortion characteristic of CKD in CHF also contributes to reduction in EPO production.

Other causes for anaemia in heart failure include iron deficiency due to nutritional deficiencies, occult gastrointestinal bleeding owing to concomitant use of antiplatelet drugs or chronic

disease; haemodilution and EPO resistance secondary to use of drugs such as ACE inhibitors or ARBs.

Relative EPO deficiency/ EPO resistance
Iron deficiency due to nutritional deficiency or malabsorption secondary to oedema of the gastrointestinal mucosa
Limited availability of iron for erythropoiesis
Chronic kidney disease
Elevated levels of inflammatory cytokines
Haemodilution due to fluid retention
Drugs (e.g., ACE (i), ARB, warfarin, and aspirin)

Table 1.2- Causes of anaemia in heart failure.
In clinical practice, anaemia is often the result of a combination of factors.
ACE (i)- angiotensin-converting enzyme inhibitor; ARB- angiotensin-II receptor blocker. EPO- erythropoietin.

IRON DEFICIENCY AND CHRONIC HEART FAILURE

Iron deficiency is well recognised in CHF and is known to occur in anaemic as well as non-anaemic patients. Iron deficiency can be absolute or functional and does not always translate to a reduction in haemoglobin levels. Absolute iron deficiency is characterised by deficiency of iron within the body and is diagnosed by the presence of a low ferritin and TSAT < 20%. Low ferritin (< 20 μg/L) identifies iron deficiency in patients without chronic inflammatory disease. However, since ferritin is increased in inflammatory states in the presence of chronic disease, a cut-off for iron deficiency is usually considered as < 100 μg/L. Functional iron deficiency is said to be present when iron stored within the reticulo-endothelial system cannot be

mobilised effectively to where it is required e.g. bone marrow resulting in deficient circulating iron levels. Serum ferritin would therefore be normal or elevated but TSAT would be < 20%. The generally accepted definition of iron deficiency in patients with a chronic disease is ferritin < 100 μg/L (absolute) or 100-300 μg/L with TSAT < 20% (functional).

Iron staining of bone marrow biopsy is widely quoted as the gold standard measure of iron status, but the invasive nature of this procedure severely limits its use. The diagnosis of iron deficiency is therefore made based on measures of serum iron, serum transferrin or iron binding capacity and serum ferritin. These parameters need to be interpreted in conjunction with clinical status, since inflammation or chronic illness can influence these markers individually.

Several studies have outlined the prevalence of iron deficiency in heart failure (Table 1.3). In their historic 2006 study Nanas *et al* performed bone marrow biopsies in 37 patients with advanced heart failure and iron deficiency anaemia was found to be present in 73%. (39) In Jankowska's study of 546 patients with advanced (but not decompensated) CHF and LVSD from 2 tertiary centres in Poland, iron deficiency was prevalent in 57% of anaemic and 32% of non-anaemic patients. (40) Multivariable analysis in this study highlighted that the increased risk of death or heart transplantation associated with iron deficiency was independent of the presence of anaemia (adjusted HR 1.58, 95% CI 1.14-2.17, p < 0.001). John Cleland's outpatient heart failure clinic study has the largest number of patients enrolled and demonstrates an absolute iron deficiency prevalence of 43.8%. (39)

Okonko *et al*'s study of 157 patients with CHF characterised anaemia and iron status in greater detail. (41) Iron deficiency was identified in 43% of patients and disordered iron homeostasis was independently related to worsening inflammation, disease severity and lower haemoglobin. The prevalence of iron deficiency increased with progression of NYHA functional class (16%, 72% and 100% in anaemic NYHA class I/II, III and IV patients respectively) and using the criteria described above, iron deficiency was present in 69%, 78% and 65% of all, anaemic and non-anaemic patients respectively. This also translated to lower peak oxygen consumption and the presence of an iron deficient state identified those at an enhanced risk of death.

Subsequent studies have continued to explore the associations between iron deficiency, anaemia and outcomes. Parikh *et al* evaluated the associations between iron deficiency, haemoglobin, CRP, and all-cause and CV mortality in 574 adults with self-reported heart failure. (42) Iron deficiency was present in 61.3% of participants and was associated with reduced mean haemoglobin (136 vs. 142 g/L, $p = 0.007$) and increased mean CRP (0.95 versus 0.63 mg/dl, $p = 0.04$). Follow up was for a median of 6.7 years and in this time although CRP, and TSAT were significantly associated with all-cause and CV mortality, iron deficiency was not. In multivariate models, haemoglobin remained an independent predictor of CV mortality, whereas CRP remained an independent predictor of both all-cause and CV mortality.

Majority of the above studies suggest that iron deficiency is common in CHF (Table 1.3) and appears to be a powerful independent predictor of adverse outcome irrespective of haemoglobin levels. (43) Iron deficiency by virtue of iron having roles far beyond oxygen transport and erythropoiesis, may potentially have greater prognostic importance than anaemia in isolation.

There is also a need to understand how decompensated heart failure influences iron status and how this impacts on subsequent iron status and CV outcomes.

Study	Population	n	Type of study	Definition of iron deficiency	Prevalence of iron deficiency	Follow up	Outcome
Opasich *et al* (2005) (38)	Stable CHF patients with LV systolic or diastolic dysfunction (71% anaemic)	148	Observational	Combination of low serum iron (<60 mg/dL), high soluble transferrin receptor (>1.76 g/L) and/ or low TSAT (<15%)	54/148 (36.5%)	Not applicable	Not applicable
Nanas *et al* (2006) (39)	Patients hospitalized for advanced CHF were recruited after initial treatment for stabilization.	37	Prospective observational study	Absence of iron stores on bone marrow biopsy	27 patients (73%)	3 months	No difference in survival in iron deficient vs non–iron-deficient patients (44.4% vs. 50.0%, respectively, p = 0.83).
Jankowska e*t al* (2010) (44)	Systolic heart failure	546	Prospective observational study	Ferritin <100 µg/L, or 100-300 µg/L with TSAT <20% Anaemia was defined as Hb <12 g/dL in women and <13 g/dL in men	37 ± 4% (95% CI) (32 ± 4 vs. 57 ± 10%-in subjects without vs. with anaemia p < 0.001)	731 ± 350 days	Iron deficiency (but not anaemia) was related to an increased risk of death or heart transplant (adjusted HR 1.58, 95% CI 1.14-2.17, P < 0.01)
Parikh *et al* (2011) (45)	Data from the Third National Health and Nutrition Examination Survey, USA consisting of self-reported heart failure patients	574	Prospective observational study	Absolute iron deficiency- ferritin level <100 µg/L, functional iron deficiency- ferritin level between 100 and 299 µg/L if the TSAT <20%.	61.3%	6.7 years	Hb but not iron deficiency was associated with CV mortality in multivariate analysis.

Table 1.3- Prevalence of iron deficiency in heart failure- some key studies.

CHF- chronic heart failure; CV- cardiovascular; Hb- haemoglobin; TSAT- transferrin saturation; HR- hazard ratio; CI- confidence interval.

Study	Population	n	Type of study	Definition of iron deficiency	Prevalence of iron deficiency	Follow up	Outcome
Okonko *et al* (2011) (41)	Recruited from heart failure clinics with prior diagnosis of CHF	157	Prospective observational study	TSAT ≤ 20%	43% of entire cohort Iron deficiency was present in 16%, 72% and 100% of anaemic NYHA class I/II, III and IV patients respectively	743 days	Iron deficiency was associated with lower pO2 and identified those at enhanced risk for death (HR: 3.38; 95% CI: 1.48 to 7.72; p = 0.004) independently of haemoglobin. Non-anemic iron-deficient patients had a 2-fold greater risk for death than anaemic iron-replete subjects.
Cleland *et al* (2016) (39)	Single outpatient clinic	4456		Definitions used: serum ferritin less than 30 ng/mL and less than 100 ng/mL; serum iron concentration < 45 µg/dL and < 67 µg/dL.	Serum ferritin < 30 ng/mL in 478 (10.7%) and < 100 ng/mL in 1951 (43.8%) Serum iron < 45 µg/dL in 497 (14.0%) and < 67 µg/dL in 1296 (36.6%).	Up to 10 years	Lower Hb (HR 0.92; 95% CI, 0.89 - 0.95; p < 0.001) and serum iron (HR 0.98; 95% CI, 0.97 - 0.99; p = 0.007) were independently associated with higher all-cause and CV mortality in multivariable analyses.

Table 1.3 (continued)- Prevalence of iron deficiency in heart failure- some key studies.

Most studies have shown a prevalence of iron deficiency in CHF to be between 37-44%, the definition of an iron deficient state being based on serum ferritin and TSAT levels. CHF- chronic heart failure; CV- cardiovascular; Hb- haemoglobin; TSAT- transferrin saturation; HR- hazard ratio; CI- confidence interval; pO2- peak oxygen consumption.
Nanas' study based on bone marrow biopsy for iron staining (gold standard diagnostic test) demonstrated a 73% prevalence of iron deficiency. Patients recruited to this study were in hospital for decompensated heart failure and the prevalences may therefore reflect not just better diagnosis due to the method used but also increased prevalence following decompensation of heart failure.

Iron metabolism

Iron is an essential constituent of haemoglobin in red blood cells, myoglobin in muscle and several cytochrome enzyme systems in mitochondria. (46, 47) It plays a fundamental role in erythropoiesis and is involved in the crucial biologic functions of oxygen binding via haemoglobin, electron transfer (via cytochromes), oxygen metabolism, oxidative phosphorylation via oxidases, peroxidases, catalases and the Krebs cycle. (48) It is a direct trigger of erythroid proliferation, enhances globin synthesis and is also the strongest inducer of aminole-vulinic acid synthase, the rate-limiting enzyme of haeme production. Similarly, as a constituent of non-haeme iron containing proteins, iron is involved in energy metabolism and DNA synthesis. Iron containing proteins are also required for the metabolism of collagen, tyrosine and catecholamines. (49)

A 70 kg male would have approximately 3.7 g of iron. The distribution of iron is outlined in Table 1.4. (50). There are two major iron pools in the body: stored iron (in hepatocytes, bone marrow sideroblasts, and reticulo-endothelial cells) and iron available for utilisation (circulating iron bound mainly to transferrin and intracellular iron in haematopoietic and extra-haematopoietic cells). The description of iron status should ideally include both pools, as they closely interact with each other and iron can be transferred between these compartments. (51-53)

	Grams (g)	%
Haemoglobin	2.5	68
Myoglobin	0.15	4
Transferrin	0.003	0.1
Ferritin (tissue)	1.0	27
Ferritin (serum)	0.0001	0.004
Enzymes	0.02	0.6
Total	3.7	100

Table 1.4- Distribution of iron in a 70 kg male.

The usefulness of iron in various enzyme systems is attributed to its ability to donate electrons in its ferrous form (Fe^{2+}) and accept electrons in its ferric form (Fe^{3+}). However, in its free form, it is potentially toxic and can promote the generation of free radicals. For this reason, it is bound to proteins and homeostasis is mostly regulated by absorption in the duodenum and proximal jejunum. Post absorption iron circulates in plasma bound to the iron transport protein known as transferrin (or apotransferrin when not transporting iron). This iron-transferrin complex circulates in plasma until it comes into contact with specific transferrin receptors on the surface of marrow erythroid cells. This complex is internalized and iron is made available for haeme synthesis and the transferrin-receptor complex is recycled back to the surface of the cell. Ferrous iron in excess of haemoglobin synthesis requirements binds to a storage protein apoferritin in its ferric form forming ferritin. (54)

The process described above takes place in all the cells that require iron. Iron can also be stored within the gut cell as ferritin. Under steady-state conditions therefore, serum ferritin level correlates with total body iron stores. Stored iron can be extracted for release by reticulo-endothelial cells. However, being an acute phase reactant, in chronic disease ferritin levels do not correlate accurately with iron stores.

The main cells responsible for releasing iron into plasma include enterocytes, macrophages and hepatocytes, their specific roles being absorption, recycling and storage respectively. Reticuloendothelial macrophages located in bone marrow, hepatic Kupffer cells and the spleen degrade senescent red cells and constitute the major iron recycling pathway. It is essential for the body to regulate the amount of iron absorbed as a deficiency can lead to iron deficiency and an excess to iron overload and associated disorders such as anaemia and haemochromatosis respectively. This regulation is mediated by the iron-regulatory hormone hepcidin. Hepcidin binds to the only known iron export protein, ferroportin, inducing its internalization and degradation, thus limiting the amount of iron released into the bloodstream. (55)

Role of hepcidin in iron homeostasis

Hepcidin is a 25-amino acid peptide primarily synthesized by the liver, initially identified as part of a search for novel antimicrobial peptides. (56) In 2001, mouse studies demonstrated that hepatic hepcidin mRNA synthesis was induced by iron loading, pointing towards a potential link between hepcidin and iron homeostasis. (57)

Hepcidin was first isolated from human urine and named on the basis of its site of synthesis (hep-) and its in-vitro antibacterial properties (-cidin). (56) In human urine, the predominant

form contains 25 amino acids, although shorter 22 and 20 amino acid peptides are also present. Only hepcidin-25 has been shown to participate in the regulation of iron metabolism. The main peptide is notable for containing 8 cysteine residues linked as 4 disulphide bridges resulting in a molecule with a simple hairpin structure and the bridges in a ladder-like configuration. This structure is characteristic of peptides capable of disrupting bacterial membranes and is similar to other antimicrobial peptides.

Hepatocytes act as sensors of body iron status, either releasing or down-regulating hepcidin, which then interacts with ferroportin to modulate the release of cellular iron. Hepcidin directly binds to ferroportin and decreases its functional activity by causing it to be internalized from the cell surface and degraded. (58)

The physiological response to infection and inflammation (via cytokine mediation as described above) is thought to be similar to that seen in iron overload, i.e. there would be hepcidin mediated shut down of iron absorption (enterocyte), recycling (macrophage) and storage (hepatocyte) (Figure 1.2). (59) The restricted bio-availability of iron leads to the classic features of the anaemia of chronic disease, low TSAT, iron-restricted erythropoeisis and mild to moderate anaemia. (60) Liver congestion in animal models has also been linked to increased hepcidin production, which then leads to anaemia and functional iron deficiency. (61, 62) The nature of the hepcidin receptor is presently unknown and could present a potential therapeutic target in treating iron deficiency seen in chronic disease such as heart failure.

Animal models have demonstrated reductions in hepcidin levels in the presence of anaemia and hypoxia. (63) This down-regulation of liver hepcidin synthesis results in an increase iron export from absorptive cells (enterocytes), recycling cells (macrophages) and storage cells (hepatocytes). As shown in Figure 1.2, anaemia and hypoxia both trigger a decrease in hepcidin levels. This allows for the rapid mobilisation of iron from macrophages and enterocytes necessary to allow for the increased erythropoietic activity triggered by EPO release. Up regulation of hepcidin appears to be influenced by two main factors- iron status and inflammation. Cytokines have been proposed as the link between infection and inflammation on hepcidin up regulation. Down regulation on the other hand appears to be driven by erythropoiesis and hypoxia. The molecular mechanisms for these are explored in further detail in the next section.

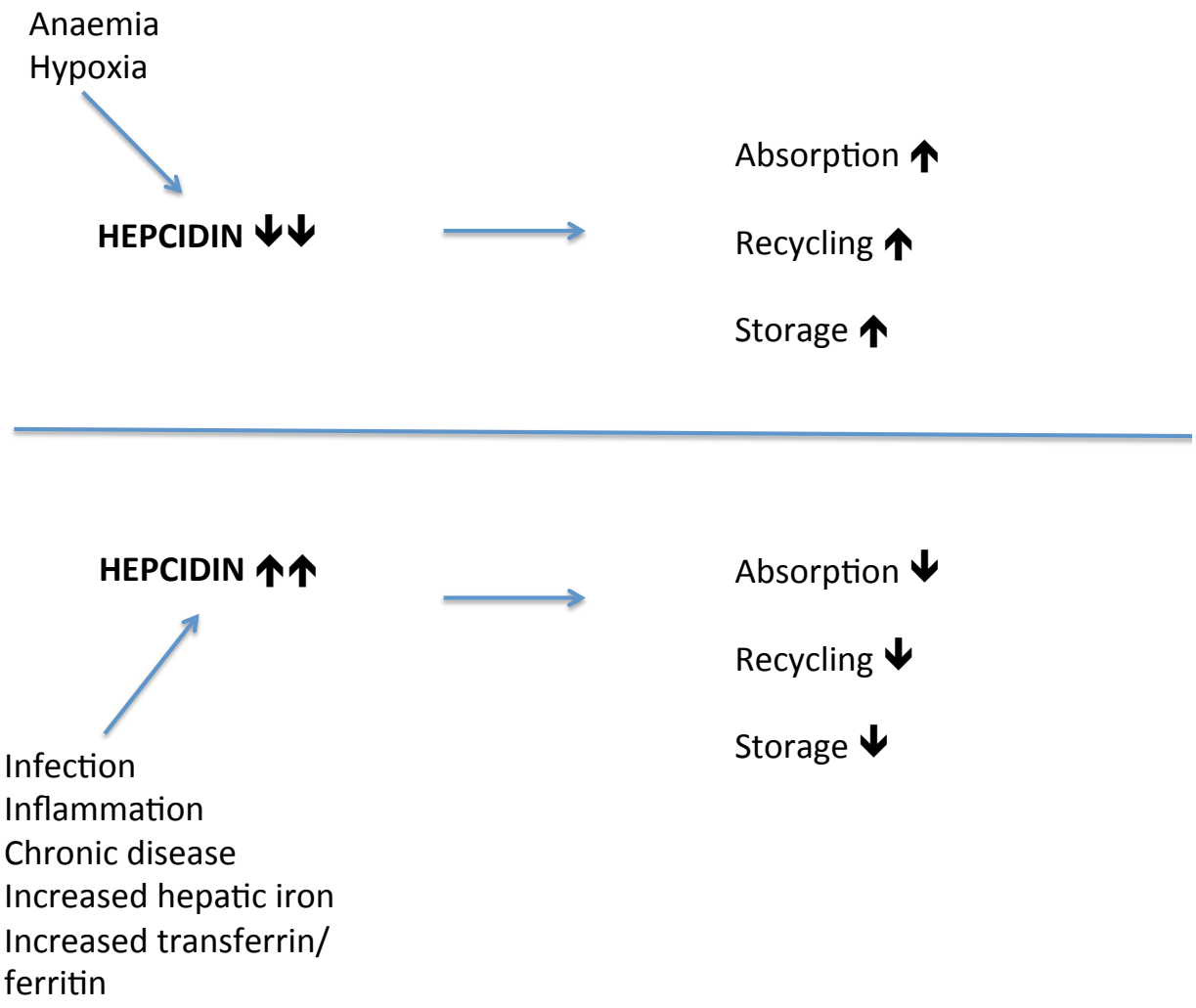


Figure 1.2- Mechanisms of up regulation and down regulation of hepcidin within the liver.

Iron mediated hepcidin regulation

It is now well established that the bone morphogenetic protein (BMP)– sma and mother against decapentaplegic (SMAD) pathway has a central role in the regulation of hepcidin in response to body iron levels. BMPs belong to the transforming growth factor-β (TGF-β) superfamily of cytokines and have been shown to play crucial roles in development, cell proliferation, cell differentiation and apoptosis.(64)

Hepatocyte- specific deletion of SMAD4 in mice models, have been associated with the development of severe iron overload. (65) The livers of these mice had a hundred-fold suppression of HAMP mRNA levels, suggesting that SMAD4 is involved in the transcriptional regulation of HAMP. HAMP mRNA levels increase in response to treatment with TGF-β or BMP4 with the opposite effect in SMAD4 $^{-/-}$ hepatocytes, suggesting that the TGF-β–BMP pathway is involved in hepcidin regulation. (65) In the animal model, BMP6 is the predominant BMP ligand responsible for hepcidin regulation *in vivo*. (66 - 68) Mice treated with BMP6 have shown dose- dependent reduction in serum iron and transferrin saturation along with an increase in hepatic HAMP expression, further supporting the concept that BMP6 is required for proper HAMP regulation. (68)

The liver is thought to be the centre for systemic iron regulation and by modulating the amount of HAMP produced in response to body iron status. The control of hepcidin expression involves molecules that can sense the circulating levels of iron and relay these messages through signal transduction pathways to the nucleus to regulate hepcidin transcription. This iron sensing mechanism depends on the HH-related proteins haemochromatosis protein (HFE) and transferrin receptor 2 (TFR2) and the classical transferrin receptor 1 (TFR1). It has been suggested that the interactions between these molecules are important for cells to sense the levels of transferrin. With increasing levels of transferrin, HFE is displaced from the HFE – TFR1 complex, a step thought to be important for initiating a signalling cascade that results in hepcidin transcription. (69)

Inflammatory regulation of hepcidin

The second major signalling pathway known to play a role in the regulation of HAMP transcription is the JAK (Janus kinase) – STAT3 (signal transducer and activator of transcription 3) pathway and the stimulus for this regulation comes mainly through inflammatory cytokines. HAMP is a type II acute phase protein; animal models have shown it to be induced by treatment with IL6 but not with cytokines involved in the type I response (TNF-α or IL1α). The increase in hepcidin levels in response to infection seems to have evolved as a defence mechanism to protect the host from infections. Most micro-organisms require iron for their growth and proliferation, hence limiting the release of iron into the blood by increasing the levels of hepcidin would result in restricted iron availability to the infectious agent. It has been suggested that there is some interaction/cross-talk between the BMP – SMAD and the JAK – STAT pathway. (70) Mice lacking SMAD4 in the hepatocytes do not show an increase in hepcidin mRNA levels when treated with IL6, suggesting that the inflammation- and iron-mediated pathways of hepcidin regulation may intersect at SMAD4.

Negative regulators of hepcidin

EPO is one of the main signalling molecules which mediate erythropoiesis; it is produced mainly in the kidney and it helps in the maturation and development of erythroblasts in the later developmental stages. When human subjects were treated with EPO the levels of circulating hepcidin decreased abruptly within 24 hours with the maximal suppression at 72 hours, this showed that EPO can influence hepcidin. (71)

Two potential erythroid regulators, growth differentiation factor 15 (GDF15) (72) and twisted gastrulation BMP signalling modulator 1 (TWSG1) (73) have been identified. High

concentrations of GDF15 (similar to those found in patients with thalassaemia) inhibits hepcidin transcription. TWSG1 inhibits hepcidin synthesis in primary human hepatocytes by inhibiting BMP – SMAD signalling. (73) Based on these 2 studies and the expression pattern of the 2 molecules a model was proposed for the inappropriate erythroid regulation of hepcidin in thalassaemia, where TWSG1 (produced in the early erythroblasts) acts indirectly by inhibiting the BMP – SMAD pathway and GDF15 which is produced in late erythroblasts acts directly to inhibit hepcidin, although the signalling mechanism is unknown. (73)

The most recent addition to the list of potential erythroid regulators is the product of the Fam132b gene, referred to as erythroferrone (ERFE). (74) ERFE has been proposed to be a stress-erythropoeisis specific erythroid regulator of hepcidin. Similar to GDF15 and TWSG1, ERFE is expressed in erythroblasts and after EPO treatment, the mRNA levels of Fam132b increase only in the erythropoietic organs of adult mice (bone marrow and spleen). (74)

The molecular mechanisms underlying the action of ERFE are still not known, although it appears that its regulation of HAMP is not related to the iron sensing or BMP–SMAD signalling pathway. (74)

Hypoxia and hepcidin

HAMP levels have been shown to decrease in hypoxic conditions. (63)

The main mediator of the hypoxic regulation of genes is the transcription factor hypoxia-inducible factor (HIF). There are 3 known subunits of HIF: HIF1α, HIF2α and HIF3α.

Recent studies have suggested that in addition to the 4 major stimuli (iron, inflammation, erythropoiesis and hypoxia) other factors could regulate HAMP levels as well. These include growth factors like hepatocyte growth factor (HGF) and epidermal growth factor (EGF) (both of

which inhibit iron and BMP6 mediated induction of HAMP) (75), and hormones like oestrogen and testosterone which affect HAMP. (76)

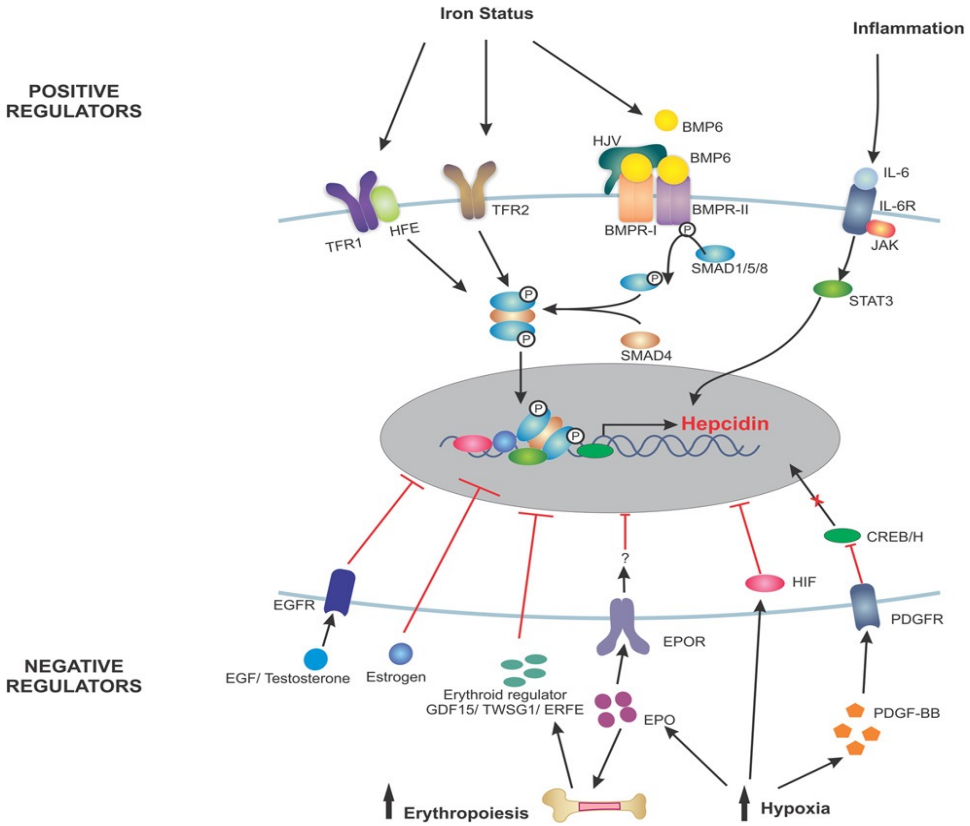


Figure 1.3 Positive and negative regulators of hepcidin (Figure taken from Rishi *et al*) (77)
BMP6, bone morphogenetic protein 6; BMPR-I, bone morphogenetic protein receptor-I; BMPR-II, bone morphogenetic protein receptor-II; CREB/H, cAMP response-element binding protein/H; EGF, epidermal growth factor; EGFR, epidermal growth factor receptor; EPO, erythropoietin; EPOR, erythropoietin receptor; ERFE, erythroferrone; GDF15, growth differentiation factor 15; HFE, hemochromatosis protein; HIF, hypoxia-inducible factor; HJV, hemojuvelin; IL6, interleukin 6; IL-6R, inter- leukin 6 receptor; JAK, Janus kinase; PDGF-BB, platelet-derived growth factor-BB; PDGFR, platelet-derived growth factor receptor; SMAD1/5/8, sma and mothers against decapentaplegic homologue 1/5/8 complex; SMAD4, sma and mothers against decapentaplegic homologue 4; STAT3, signal transducer and activator of transcription 3; TFR1, transferrin receptor 1; TFR2, transferrin receptor 2; TWSG1, twisted gastrulation BMP signaling modulator 1.

Heart failure and iron deficiency.

Heart failure is often associated with inflammatory responses as well as the presence of anaemia. How this complex interplay influences hepcidin and the role hepcidin thereon plays on iron status in heart failure is yet to be fully elucidated. Studies have documented high hepcidin levels in heart failure in the presence of functional iron deficiency suggesting hepcidin induced poor availability of iron for metabolism. (78) Hepcidin reduces the export of iron absorbed from the intestinal mucosa to blood and also reduces the release of iron from macrophages recycling iron from senescent erythrocytes. (63) This in turn further deprives erythroid progenitor cells of iron necessary for erythropoiesis. It would therefore be plausible that with continued stimulus for hepcidin up regulation such as in a chronic inflammatory condition like CHF and decompensated heart failure in particular, abnormal iron metabolism would result in functional iron deficiency. It remains unclear whether heart failure (ADHF in particular) influences hepcidin levels and therefore iron metabolism. There is also little information regarding potential links between hepcidin level and outcomes. A recent study by Jankowska *et al* explored the usefulness of measuring hepcidin levels in combination with serum transferrin receptor in patients with ADHF. (79) They postulated that while serum hepcidin may increase due to either inflammation and/or excessive iron stores, low-circulating hepcidin reflects specifically depleted body iron stores with or without concomitant anaemia.(53, 80, 81) Low hepcidin in combination with high-serum soluble transferrin receptor would therefore reflect unmet cellular iron requirements (37% of cohort) and would constitute the most profound form of iron deficiency. Isolated high serum transferrin receptor or low hepcidin in their study cohort was demonstrated in 29% and 9% of

patients respectively. 25% of subjects demonstrated preserved iron status. The presence of concomitant low hepcidin and high serum soluble transferrin receptor had the highest 12-month mortality [(41% (95% CI: 29 - 53%)] when compared with those with isolated high serum transferrin receptor [15% (5 - 25%)], isolated low hepcidin [7% (0 - 19%)] and preserved iron status (0%) ($p < 0.001$).

Hepcidin can also be produced by cardiac cells; a recent animal model by van Breda *et al* studied the differential and local regulation of hepcidin gene expression (hepatic and cardiac) in response to myocardial infarction and/or CKD. (82) In this study rats were subjected to coronary ligation or sham procedure 9 weeks after subtotal nephrectomy or sham surgery. This created a model of chronic renocardiac failure by subtotal nephrectomy followed by coronary ligation. Coronary ligation superimposed on subtotal nephrectomy led to more severe heart failure. At week 16, the gene expression of hepcidin, iron and damage markers in cardiac and liver tissues was assessed by quantitative polymerase chain reaction and ferritin protein expression was studied by immunohistochemistry. Cardiac hepcidin gene expression was significantly induced in both local (2-fold increase in coronary ligation leading to myocardial infarction, $p = 0.03$) and remote (3-fold increase in subtotal nephrectomy leading to CKD, $p = 0.01$) injury. Conversely, liver hepcidin gene expression was decreased (50% reduction, $p < 0.05$) in subtotal nephrectomy and coronary ligation compared to either procedure alone. However cardiac iron content in non-infarcted tissue remained unchanged in all experimental groups. Thus, cardiac hepcidin expression was increased in response to injury, but no evidence of an association with local iron accumulation was observed. The conclusion was that injury rather than iron could be the driving force for cardiac hepcidin expression in renocardiac failure.

Reliable assays to measure hepcidin in blood and urine by use of immunochemical and mass spectrometry methods have been developed and results of preliminary studies have highlighted hepcidin as a promising diagnostic tool and therapeutic target for iron disorders. (83)

There is thus emerging interest in the role of hepcidin in iron metabolism in heart failure. Studies in humans with heart failure have suggested it may play a role but more work is needed to elucidate this relationship in more detail. In a study of 320 heart failure patients by Ohno *et al* (62), log-serum hepcidin concentration of patients with liver congestion was found to be higher than those without liver congestion ($p = 0.032$) in the absence of active infection/cancer. Anaemia where present, was associated with the higher serum hepcidin concentrations. The authors also produced a rat model of heart failure with liver congestion by injecting monocrotaline, an agent that causes pulmonary hypertension. These rats displayed liver congestion with increase of hepcidin expression 4 weeks post monocrotaline injection, followed by anaemia and functional iron deficiency at 5 weeks. It is therefore plausible that liver congestion arising from decompensation in heart failure for example, may influence hepcidin production and subsequent functional iron deficiency.

Causes of iron deficiency in heart failure

There are a number of potential contributory causes of iron deficiency in CHF. This could be precipitated by the syndrome of heart failure itself as well as associated comorbidities like CKD where iron deficiency is common. (84) In advanced CKD, iron deficiency is felt to be secondary to reduced oral intake, small gastro intestinal losses secondary to uraemia, gastritis and the use of drugs (antiplatelet agents and anticoagulants due to direct impact on bleeding; phosphate binders

by potentially binding iron and proton pump inhibitors by potentially reducing iron absorption). (85, 86) Some of these mechanisms can be extrapolated to CHF.

CHF is a pro-inflammatory state and it is plausible that this could result in up regulation of hepcidin and subsequent impaired gut absorption of iron. Overproduction of hepcidin also results in macrophage iron retention, consequently making less iron available for erythropoiesis. Although synthesised in the liver, hepcidin is eliminated via the kidney and therefore accumulation in the presence of impaired kidney function may present another potential mechanism for iron deficiency in CHF complicated by CKD.

CHF is associated with abnormal gastric mucosa and increase in intestinal wall thickness and collagen tissue, proportional to the severity of heart failure (87, 88); intestinal permeability also appears to be altered as a consequence of local ischemia. (89) All of these factors could contribute to impaired iron absorption.

Diagnosing iron deficiency in chronic heart failure in clinical practice

There are a number of ways of assessing iron deficiency, each offering some benefits and pitfalls. In routine clinical practice, serum ferritin needs to be assessed in conjunction with TSAT and serum iron. (4, 90) Iron deficiency in CHF can be absolute or functional. An absolute deficiency would be characterised by diminished iron stores (serum ferritin < 100 μg/L with decreased transferrin levels and TSAT. With functional iron deficiency, stores appear normal (normal ferritin, normal transferrin and normal iron) but availability of iron for its physiological functions is impaired. This often manifests as anaemia of chronic disease and the only clue may

be the presence of low TSAT in the presence of ferritin levels of 100 - 300 μg/L (Table 1.5). It must also be mentioned in this context that there is increasing interest in the prognostic value of low TSAT independent of other parameters. (91)

PARAMETER	NORMAL	FUNCTIONAL IRON DEFICIENCY	TRUE IRON DEFICIENCY
IRON	6 - 27 mmol/L	Normal/ decreased	Decreased
TRANSFERRIN	25 - 45 mmol/L	Normal/decreased	Decreased
TRANSFERRIN SATURATION	20 - 45 %	Decreased	Decreased
FERRITIN	100 - 300 μg/L	Normal	Decreased

Table 1.5- Diagnosing functional and absolute iron deficiency using a combination of markers.

Impact of iron deficiency in chronic heart failure

Iron deficiency impairs aerobic performance and results in fatigue and exercise intolerance. (92) Okonko *et al* performed cardiopulmonary exercise testing in 157 patients with heart failure and found peak oxygen consumption to be lower in iron-deficient than in iron-replete (11.4 ± 2.1 ml/kg/min vs. 14.9 ± 1.7 ml/kg/min, p = 0.03) patients. This positively correlated to TSAT (r = 0.71, p = 0.001) and ferritin (r = 0.48, p = 0.01) independently of NYHA functional class and haemoglobin. (41)

Lower serum iron levels are associated with an increased risk of coronary artery disease, cardiovascular disease (CVD), and all-cause mortality. (93) This inverse association between serum iron levels and risk of mortality is more pronounced when iron deficiency is associated with a reduction in haemoglobin levels, as demonstrated by Okonko's study of CHF patients described above. (41) Here, patients with iron deficiency anaemia had a 2-fold greater risk for death than those with non-anaemic iron deficiency and a 4-fold greater risk for death than iron-replete patients with or without anaemia.

The actual mechanism by which iron deficiency impacts cardiac function has not been fully elucidated. On a cellular level, iron deficiency may be associated with myocardial iron depletion (more marked depletion in the presence of concomitant anaemia (94, 95) and subsequent reduction in serum EPO and cardiac STAT3 phosphorylation. (96) Experimental studies have shown iron deficiency to induce cardiac hypertrophy in rat hearts characterized by mitochondrial swelling, abnormal sarcomere structure along with increase in cytochrome C. (97) Severe iron deficiency anaemia can lead to LVH, ventricular dilatation, cardiac fibrosis resulting in diastolic dysfunction and heart failure.

Potential treatments of anaemia and iron deficiency in chronic heart failure

The different aetiologies for anaemia postulated over the years have sparked a great deal of interest in the various treatment options, i.e. iron replacement (oral and intravenous), recombinant human EPO and even blood transfusion. However large scale reported randomised control trials are lacking. Smaller studies of EPO have demonstrated an improvement in haemoglobin, exercise capacity and reduction in hospitalisation in the short

term. There have been concerns regarding its side effects (hypertension and thromboembolic risks being the prominent ones), especially in the presence of severe renal dysfunction. (98) The Reduction of Events by Darbepoetin Alpha in Heart Failure (RED-HF) trial has been the largest EPO randomized, double-blind trial to date, recruiting 2278 patients with systolic heart failure and mild-to-moderate anaemia (haemoglobin level, 90 to 120 g/L) to receive either darbepoetin alpha (to achieve a haemoglobin target of 130 g/L) or placebo. (99) There was no significant difference in the occurrence of the primary outcome, which was a composite of all-cause mortality or hospitalisation for worsening heart failure (HR in the darbepoetin alpha group, 1.01; 95% CI vs 0.90 to 1.13 in the placebo group; $p = 0.87$). Secondary outcomes were not significantly different either; these included death from any cause, the composite of death from CV causes or first hospitalisation for worsening heart failure and change from baseline to 6 months in quality of life assessment scores {Overall Summary Score and Symptom Frequency Score on the Kansas City Cardiomyopathy Questionnaire (KCCQ)}. While there was no difference in the occurrence of fatal or non fatal stroke, thromboembolic adverse events were greater in the darbepoetin alpha group (13.5% vs 10.0% in the placebo group, $p = 0.01$). This would suggest that recombinant EPO does not appear to play a beneficial role in the treatment of anaemia in heart failure and in fact appears to be associated with greater adverse events.

Blood transfusions in the acute setting of anaemia and renal dysfunction have also been less beneficial. Mortality outcomes have been demonstrated to be worse (100) and if indeed iron deficiency is the primary aetiology for anaemia in heart failure, none of these treatments would be correcting the cause in the long term.

Oral iron supplementation in iron deficiency of heart failure has not been proven to be beneficial. The IRONOUT HF randomised clinical trial by Lewis *et al* in 225 iron deficient patients with reduced LVEF is a recent illustration of this. (101) Patients were followed up for 16 weeks after the administration of oral iron or placebo and the primary end point was change in exercise capacity measured by peak VO2. 6-minute walk distance, NT-proBNP levels and KCCQ scores were also assessed. None of the parameters showed a significant difference between the treatment and placebo arms over time.

There have been 3 placebo controlled double blind studies to date where intravenous iron was administered to iron deficient CHF patients (Table 1.6). FAIR-HF (90) and CONFIRM-HF (102) studies form the largest contributors to what we know today on iron administration in patients with CHF. There have been no concerns to date about the safety or administration of intravenous iron and irrespective of haemoglobin levels its use has been associated with beneficial effects. All indices of iron status (ferritin, TSAT) as well as haemoglobin have been shown to improve. Improvements in physical functioning were seen following administration of intravenous iron in iron-deficient patients with CHF even in those without anaemia and in whom haemoglobin levels did not change following i.v. iron administration. None of the individual studies were powered for assessment of mortality or CV outcome, yet meta-analyses such as the one by Kapoor *et al* (103) using pooled individual patient data demonstrated significant reductions in hospitalisations (OR 0.26, 95% CI 0.08 - 0.80) and improvements in NYHA class (mean improvement 1.2 classes, 95% CI 0.69 - 1.78, and LVEF (mean improvement 5.0%, 95% CI 0.13 - 9.80) but no relationship was found on mortality in this study (OR) 0.66, 95% CI 0.30 - 1.44). A more recent meta-analysis by Anker et al in

2017 extracted individual patient data from 4 randomised control trials (n = 839 of whom 504 were randomised to receive intravenous iron). (104) Compared with those taking placebo, patients on iron had lower rates of recurrent CV hospitalisations and CV mortality (rate ratio 0.59, 95% CI 0.40 - 0.88; p = 0.009]. Treatment with intravenous iron also reduced recurrent heart failure hospitalisations and CV mortality (rate ratio 0.53, 95% CI 0.33 - 0.86; p = 0.011) and recurrent CV hospitalisations and all-cause mortality (rate ratio 0.60, 95% CI 0.41 - 0.88; p = 0.009). Analyses were not carried out for mortality alone.

A major landmark study of iron replacement in heart failure with long-term outcomes and CV events is awaited. The recently published ESC guidelines on heart failure have however highlighted anaemia as being common in heart failure, especially among hospitalised patients or those who are elderly or with renal impairment, but do not routinely recommend its treatment. Quoting the FAIR-HF trial, ESC suggests that the treatment of iron deficiency with intravenous iron could be considered. (4)

A number of on-going trials powered to evaluate mortality and heart failure hospitalisation are on-going. In the UK, IRONMAN, a randomised, open-label multicentre trial assessing the effectiveness of intravenous iron treatment vs. standard care in patients with heart failure and iron deficiency is aiming to recruit 1300 patients. (105) The primary end point is CV mortality or hospitalisation for worsening heart failure.

STUDY/ AUTHOR	NUMBER	NYHA CLASS	TREATMENT	STUDY DESIGN	STUDY DURATION	OUTCOME
Silverberg *et al* (2001) (106)	N=32	III/IV	Erythropoietin and i.v. iron or no treatment	Randomized control study	8.2 months	Significant improvement in Hb, NYHA class, mean EF, reduction in diuretic requirements and duration of hospitalisation in treated group.
Bolger *et al* (2006) (107)	N = 16	II/III	i.v. iron sucrose only over 12 days	Uncontrolled	12 weeks	Significant increase in Hb level, NYHA class, MLHFQ score and 6MWD. Trend towards improved renal function with iv iron.
Tobbli *et al* (2007) (108)	N = 40	II-IV	i.v. iron sucrose or saline over 5 weeks	**Double blind placebo controlled study**	24 weeks	Improvement in Hb, NYHA class, LVEF, 6MWD, hospitalisation rate, creatinine clearance, C-reactive protein, N-t pro BNP, and lower diuretic requirements.
FERRIC-HF (Okonko *et al*, 2008) (109)	N = 35	II/III	i.v. iron sucrose or no treatment over 16 weeks	Randomized open-label, observer blind	16 weeks	No significant improvement in Hb levels, but significant improvements in NYHA class, PGA, MLHFQ and fatigue score in treated group, with more significant changes in anaemic group.

Table 1.6- Studies using intravenous iron in CHF.

FCM- ferric carboxy maltose; HF- heart failure; NYHA- New York Heart Association; i.v. - intravenous; 6MWD- 6 minute walk distance; QoL- quality of life; EF- ejection fraction

AUTHOR		CLASS		DESIGN	DURATION	
STUDY/ AUTHOR	**NUMBER**	**NYHA CLASS**	**TREATMENT**	**STUDY DESIGN**	**STUDY DURATION**	**OUTCOME**
Usmanov, Silverberg *et al* (2008) (110)	N = 32	III/IV	i.v. iron sucrose over 29 weeks		26 weeks	Significant improvement in Hb, NYHA in class III group but not in IV.
FAIR HF (Anker *et al*, 2009) (90)	N = 459	II/ III	i.v. ferric carboxymaltose or saline over 24 weeks	**Double blind placebo controlled study**	24 weeks	Significant improvement in NYHA class, 6MWD, EQ - 5D scale, Kansas City QoL scale, renal function. No difference in first CV hospitalisation or death but trend to improvement in both in the treated group.
CONFIRM-HF (Ponikowski *et al* 2014) (102)	N = 304		i.v. ferric carboxymaltose vs normal saline	**Double blind placebo controlled study**	52 weeks	Significant reduction in hospitalisation for worsening HF [HR (95% CI): 0.39 (0.19 – 0.82), p = 0.009] following i.v. FCM. Significant improvement in 6MWD at week 24, sustained to week 52. Improvement in NYHA class, markers of QoL, and Fatigue Score from Week 24.

Table 1.6 (continued)- Studies using intravenous iron in CHF. FAIR-HF and CONFIRM-HF were the largest of the 3 double blind placebo controlled studies and showed significant improvement in exercise tolerance and quality of life. FCM- ferric carboxy maltose; HF- heart failure; NYHA- New York Heart Association; i.v- intravenous; 6MWD- 6 minute walk distance; QoL- quality of life; EF- ejection fraction

IRON DEFICIENCY AND DECOMPENSATED HEART FAILURE

Most studies relating to anaemia, iron deficiency and heart failure have been conducted in chronic stable heart failure patients. Decompensated heart failure (i.e. an acute presentation of heart failure with fluid retention) is associated with marked inflammatory immune activation (13) and frequently worsening renal function (111); how this impacts on iron status is poorly understood. Significantly increased cardiac filling pressures and venous congestion have been observed days or weeks before overt clinical decompensation. (112-114) Tissue oedema results from transudation of fluid from capillaries into the interstitium. This is a consequence of increased transcapillary hydrostatic pressure gradient, decreased transcapillary oncotic pressure gradient and increased interstitial compliance. (115, 116) Glycosaminoglycan networks may also become dysfunctional resulting in increased interstitial compliance and storage of sodium and water. This in effect bypasses renal clearance and consequently, even mild elevation in venous pressure can lead to pulmonary and peripheral oedema. (117)

Decompensated heart failure currently represents a major therapeutic challenge, with patients spending a median of 9 days in hospital in the UK. (118) The pathophysiology and optimal management of this aspect of the heart failure syndrome is relatively poorly understood and there is undoubtedly an associated inflammatory milieu. Up regulation of inflammatory mediators such as IL-1 and IL-6 are thought to contribute to disease progression and have also been demonstrated to be independent predictors of adverse outcome. (119) This is of particular interest in the context of cardiorenal iron deficiency syndrome as up regulation of inflammatory mediators such as IL-1 and IL-6 can also stimulate hepcidin production in the liver. Hepcidin is a

key mediator of iron absorption from the gut and mobilisation from reticulo-endothelial stores. (120) It is thus plausible that decompensation in patients with CHF, via hepcidin up regulation can also impact on iron homeostasis.

Unanswered questions

It is important to understand the burden of iron deficiency in clinically high risk and challenging groups, such as the decompensated heart failure cohort. If present, iron replacement could be a potential therapeutic target alongside intravenous diuretics and standard prognostically beneficial drugs once the patient is stabilised. Improved understanding of the mechanism of developing iron deficiency in this group as well as the relationships between cardiac and renal dysfunction, inflammatory immune activation and iron status is vital to help ensure we target the most appropriate treatment strategy. The role of hepcidin in acute decompensated heart failure is of particular interest. It remains to be seen whether inflammatory cytokines driven by decompensated heart failure override the suppression of hepcidin secondary to pre-existing anaemia for example. This is of clinical importance as it would suggest that oral iron would be of no therapeutic benefit in iron deficient anaemic decompensated heart failure patients. Similarly if the prevalence of iron deficiency in the acute setting is linked to inflammation and likely to reverse with recompensation, then iron therapy (intravenous or oral) in the acute setting may not be warranted.

With these questions in mind, I designed the Iron status in decompensated heart failure study (IRON-STATS-DHF), a prospective observational study of patients admitted with ADHF. By performing a cross sectional and prospective evaluation of patients admitted with decompensated

heart failure, my objective was to define the prevalence of iron deficiency in this group and outline the natural history of an iron deficient state following discharge from hospital.

CHRONIC KIDNEY DISEASE AND HEART FAILURE

The relationship between the heart and kidney goes back a long way in history. William Withering, in his *"An Account of the Foxglove and Some of its Medical Uses"* published in 1785, described many cases of "dropsy," which responded well to digitalis. Fluid loss of nearly 7 liters was described in one patient and was associated with dramatic symptomatic relief. Withering recounts that those who responded best to digitalis had a "weak, often intermittent pulse" suggestive of atrial fibrillation and a cardiac cause for the widespread oedema. (121)

Richard Bright one of the founders of Guy's Hospital in19th century London, described the clinical features of nephritis, which in many patients also included dropsy, demonstrating that "dropsy" was not universally attributable to heart failure. Some of these patients had cardiac hypertrophy, which often, but not always, was associated with hypertension.

Withering, therefore, in his description of what the heart could do to the kidney, and Bright, on what the kidney could do to the heart, were early commentators on coexistent heart failure and renal dysfunction, what is now known as the cardiorenal syndrome.

Measures of renal dysfunction

Current measurements of renal function are based on evaluation of glomerular filtration rate (GFR). This can be achieved by directly measuring renal clearance of specific substances like

creatinine and inulin or radioactive markers (99mTc-DTPA) or alternatively can be estimated by application of validated formulae. Traditionally, eGFR is calculated using the Modification of Diet in Renal Disease (MDRD) (122) or Cockcroft-Gault formulae (National Kidney Foundation 2002) (123) incorporating serum creatinine and demographic parameters.

Serum creatinine which is routinely used as a measure of renal function in clinical practice is affected by many factors like age, gender, muscle mass and metabolism, medications, sepsis and level of hydration and may not necessarily be an accurate reflection of kidney injury. Creatinine reflects loss of glomerular filtration, not tubular injury and only increases when half of renal function is lost in patients with previously normal function. When it does increase, the pathophysiological processes driving tubular deterioration may have irreversibly progressed suggesting it is a 'late' marker of acute kidney injury (AKI). Intuitively preventative and restorative measures for kidney injury are likely to be successful if instituted early after the initiating event occurs, and waiting to act on a rise in creatinine may be a futile activity.

Other biomarkers may enable an early and accurate diagnosis that might help predict outcomes at a stage where timely intervention might halt deterioration.

Biomarkers of renal injury

A number of biomarkers specific for renal tubular damage are outlined in Table 1.7.

Biomarker	Source	Pathology causing abnormal levels
Cystatin C	Protein found in all tissues	Acute kidney injury, Myocardial infarction, Stroke, Heart failure, Peripheral vascular disease, Metabolic syndrome, Alzheimer`s disease
NGAL	Protein in neutrophils, kidney, prostate, respiratory tract	Acute kidney injury, Heart failure, Sepsis, Multi-organ failure
KIM-1	Membrane protein	Ischaemic and nephrotoxic proximal, tubular, acute kidney injury
NAG	Active glycosidase found in proximal tubular epithelial cells lysosomes	Acute kidney injury, Diabetic nephropathy, Chronic lithium exposure
NHE3	Protein found in nephrons and enterocytes	Acute kidney injury
IL-6, IL-8, IL-18	Proteins (cytokines) produced by macrophages and other cells	Acute kidney injury, Inflammatory reactions, Adenomyosis, Hashimoto`s thyroiditis, Alzheimer`s disease
GST	Proteins enzymes	Acute kidney injury
L-FABP	Protein found in liver	Acute kidney injury

Table 1.7- Renal biomarkers.

Although produced in different parts of the body, elevated levels serve as markers of renal injury, becoming abnormal before changes in measured serum creatinine levels become apparent.

KIM- Kidney Injury Molecule-1; NAG- N-acetyl- (D) glucosaminidase; NHE3 - sodium hydrogen exchanger, IL-6, IL-8, IL-18 – interleukins; GST- glutathione S-transferase; and L-FABP- L-type fatty acid binding protein

Cystatin C

An endogenous cysteine proteinase inhibitor, Cystatin C is helpful in detecting AKI and is a marker of proximal tubule injury. (124 - 126) As it is not influenced by age, gender, muscle mass or inflammation, it may offer superiority over other conventional markers of renal function (i.e. creatinine and eGFR). It remains unbound to protein and is freely filtered across glomeruli.

Renal dysfunction per se can be acute or chronic. AKI refers to a sudden onset (within 48 hours) of reduction in kidney function manifested as an increase in serum creatinine by 50% from baseline or >26.4 micromol/L in absolute values. In 2009-2010, there were an estimated 360 000 AKI events in UK hospital admissions, amounting to health care costs in the range of £434 million to £620 million per annum (NHS Kidney Care report). (127) This is not unique to the UK. A meta-analysis of 154 large cohort studies of hospitalised patients (between2004 - 2012, n = 49 147 878) predominantly from North America, Northern Europe, and Eastern Asia demonstrated a pooled incidence rate of AKI of 21.6% in adults (95% confidence interval [95% CI], 19.3 - 24.1) and a pooled AKI-associated mortality rate of 23.9% in adults (95% CI, 22.1 - 25.7). (128) The incidence of AKI is even greater in already high-risk patients (such as those hospitalised with acute myocardial infarction, acute heart failure, or sepsis) (129). Irrespective of the underlying pathology, when present, AKI is strongly associated with increased morbidity, mortality, and progression to end-stage kidney disease. (130) Management of these patients in the acute setting is complicated and a proportion of these patients progress to develop CKD. (For conversion of creatinine in mg/dL to μmol/L, see box below.)

Conversion factor for creatinine

1mg/dL = 88 μmol/L

CKD refers to more long-standing changes where there is a reduction in the GFR to < 60 ml/min/1.73 m^2 or where there is kidney damage evidenced by an abnormal urine sediment (proteinuria, haematuria and casts) or abnormalities in renal architecture (e.g. polycystic kidney disease) even if GFR is preserved within normal levels. CKD encompasses all renal disease

states from the earliest stages through to (ESKD) requiring renal replacement therapy. The classification of CKD based on GFR is shown in Table 1.8.

Stage	Estimated GFR (ml/min/1.73m^2)
1	>90
2	60-89
3a	45-59
3b	30-44
4	15-29
5	<15
ESKD	Dialysis

Table 1.8- Staging of chronic kidney disease (CKD). (131)

The KDOQI clinical practice guidelines for CKD defines as kidney damage or GFR < 60 ml/min/1.73 m^2 for ≥ 3 months. Kidney damage is defined as pathological abnormalities in blood or urine tests or in imaging studies.

ESKD = end-stage kidney disease; GFR = glomerular filtration rate.

Early-stage CKD affects 10% to 16% of adults in developed nations and the prevalence continues to increase rapidly as the population ages and as rates of obesity, diabetes, and hypertension continue to rise. Patients with CKD often develop CVD, more commonly myocardial infarction and heart failure. (132) Most of the original studies examining CVD in CKD focused on patients with ESKD and in dialysis patients, CVD accounted for 50% of all deaths. CVD (with CHF as a key component) is the leading cause of morbidity and mortality in this patient population. In a sample cohort study of the United States Medicare population in 1998 and 1999 (n = 1 091 201), Foley *et al.* found that 39.9% of patients with CKD had CHF on initial presentation (coded as per the International Classification of Diseases), and another 30.7% developed it over the next year. (133) The U.S. Renal Data System annual reports have also suggested that approximately two-thirds of incident dialysis patients develop CHF within 3

years. (134) This is a significant finding as CHF is an established finding despite the rigorous fluid management strategies adopted during dialysis.

More recent large studies have demonstrated that CV risk increases early in the natural history of CKD, at an eGFR level of approximately 75 ml/min/1.73 m^2. (26) In an analysis of the Candesartan in Heart Failure: Assessment of Reduction in Mortality and Morbidity (CHARM) database (a series of randomised studies of candesartan in CHF) by Hillege *et al*, the risk for CV death or hospitalisation for worsening CHF (as a combined primary end-point) as well as the risk for all-cause mortality increased significantly below an eGFR of 60 ml/min/1.73 m^2. When compared to the individuals with eGFR > 60 ml/min/1.73 m^2, the adjusted hazard ratio (HR) for the primary end point was 1.54 for patients with eGFR 45 – 60 ml/min/1.73 m^2 and 1.86 for those with eGFR < 45 ml/min/1.73 m^2 (both $p < 0.001$). For all-cause mortality, the respective HRs were 1.50 ($p = 0.006$) and 1.91 ($p = 0.001$). The HR for CV events was 2.8 in patients with a GFR < 30 ml/min/1.73 m^2. Other studies have observed up to 40% higher adjusted risk for adverse CV outcomes or death in those with relatively minor degrees of renal dysfunction. (135) Although all stages of CKD are considered in the highest risk group for the development of CVD, an independent stepwise relation is seen with a marked increase in the risk in those with eGFR < 60 ml/min/1.73 m^2. This is suggestive of a direct pathophysiological role for renal dysfunction in the disease process.

The converse is also true and concomitant renal dysfunction in heart failure has been consistently associated with worse prognosis. (136, 137) Meta analyses have documented prevalences of CKD between 32 to 63% with up to a third presenting with moderate to severe CKD (creatinine

1.5 mg/dL, eGFR 53 ml/min/1.73 m^2 or cystatin-C 1.56 mg/dL). (138) A recent meta-analysis by Damman *et al* (139) including more than 1 million patients showed the prevalence of CKD in heart failure to be 32%. CKD in this analysis was defined as an eGFR < 60 mL/min/1.73 m^2.

Studies have consistently demonstrated renal dysfunction to be an independent marker of adverse outcome (138, 140) and an even stronger predictor of mortality than left ventricular ejection fraction (LVEF) or NYHA functional class. (136) In a meta-analysis of 8 studies (n = 18 634) worsening renal function in itself was demonstrated to be an adverse predictor of clinical outcome. (141) Worsening renal failure (defined as an increase in serum creatinine ≥ 0.2 mg/dl (17.6 μmol/L) or a corresponding decrease in eGFR ≥ 5 ml/min/1.73 m^2) developed in 4734 (25%) patients and was associated with a higher risk for mortality (odds ratio [OR] 1.62; 95% CI 1.45–1.82, p < 0.001) and hospitalisation (OR 1.30, 95% CI 1.04–1.62, p < 0.022). The severity of worsening renal function was also associated with greater mortality.

In-hospital mortality has been noted to increase from 2% in those with normal kidney function (eGFR > 90 mL/min/1.73 m^2) to 30% in those with ESRD (eGFR < 15 mL/min/1.73 m^2). (142) Similar prognostic implications have been noted for 5-year mortality.

Stage	**Estimated GFR (ml/min/1.73m^2)**	**Cardiovascular Risk (Odds Ratio)**
1	>90	Dependent on degree of proteinuria
2	60-89	1.5
3	30-59	2-4
4	15-29	4-10
5	<15	10-50
ESKD	Dialysis	20-1000

Table 1.9- Cardiovascular risk Odds ratio as per stage of chronic kidney disease.
Adapted from Schiffrin *et al* (Circulation 2007). (132)

Although shared aetiological risks factors for the development of CHF and CKD may play a role (e.g. hypertension, diabetes, smoking and resultant atherosclerosis), renal dysfunction in the context of CHF would appear to be more than just a sequelae of reduced renal perfusion and is more likely a reflection of a complex clinical syndrome in which haemodynamic abnormalities, neurohormonal activation, inflammation and oxidative stress all play a role.

The risk of CV death in early-stage CKD far exceeds the risk of progressing to dialysis; therefore, treatment should be focused at least as much on reducing this risk as on reducing the rate of progression of renal disease. Optimal and careful management of cardio renal disease is crucial, and there remains a need to tailor all available evidence towards managing this high-risk population. In the subsequent chapters of this thesis, I will be exploring potential causes of CV mortality in the presence of ESKD and evaluating the management of CV conditions in the presence of CKD.

CARDIORENAL DISEASE

The heart-kidney association has been formalised with the term 'cardiorenal syndrome'. Cardiorenal syndrome (CRS) includes a broad spectrum of diseases in which the heart and kidney are both involved. A classification proposed by Ronco and colleagues describes two main groups, cardiorenal and renocardiac CRS, on the basis of the primary pathology (cardiac or renal); it is then divided into acute and chronic according to the disease onset (Table 1.10). (143)

Acute CRS (Type 1)
Acute worsening of cardiac function leading to renal dysfunction

Chronic CRS (Type 2)
Chronic abnormalities in cardiac function leading to renal dysfunction

Acute Renocardiac Syndrome (Type 3)
Acute worsening of renal function causing cardiac dysfunction

Chronic Renocardiac syndrome (Type 4)
Chronic abnormalities in renal function leading to cardiac disease

Secondary CRS (Type 5)
Systemic conditions causing simultaneous dysfunction of the heart and kidney

Table 1.10- Classification of Cardiorenal syndrome by Ronco *et al*, based on the primary pathology in cardiorenal disease.

The above classification offers a pathophysiological classification for cardiorenal syndrome. Often however, the precise pathophysiology and the timing of diagnosis in different clinical scenarios are not clear enough to allow for compartmentalisation based on primary aetiology and thus there can be a significant overlap between the different types of CRS. Indeed an individual patient may exhibit different types at different times of the disease course.

Pathophysiology of cardiorenal syndrome

A number of potential mechanisms have been proposed to explain the pathophysiological relationship between CVD and CKD. These include shared risk factors, metabolic abnormalities, haemodynamic abnormalities, neurohormonal and inflammatory activation, anaemia, iron deficiency and even differential treatment strategies for patients with co-morbid CKD. The CV continuum first described by Eugene Braunwald and Victor Dzau two decades ago (Figure 1.3) was based on the theory of shared risk factors. (144 - 146) Diabetes and hypertension, two well-

established risk factors for CVD for example constitute two of the largest population-attributable causes of CKD. Similarly, established CV risk factors (age, body mass index, diabetes, smoking, hypertension, hypercholesterolemia, metabolic syndrome) have been shown to be associated with the development of new onset kidney disease by several studies including the Framingham study. (147) There is also a significant overlap between CV manifestations secondary to the risk factors above and those seen in cardiorenal disease. For example, LVH, a frequent finding in hypertensive patients is also the commonest cardiac structural abnormality in CKD patients, with up to 75% patients reaching dialysis, having evidence of LVH on echocardiography.

Chronic kidney disease is associated with specific haemodynamic and metabolic alterations such as inflammatory up regulation, hyperparathyroidism, anaemia, hyper phosphatemia, hyper homocysteinemia, hyper calcaemia and overtreatment with calcium and vitamin D, all of which can accelerate and predispose to CVD. Excessive parathyroid hormone is cardiotoxic and is associated with increased myocardial fibrosis as well as the development of dilated cardiomyopathy. Hyper homocysteinemia seen in ESKD is a risk factor for atherogenesis, causing endothelial injury and platelet aggregation. Uraemia induced increased oxidative stress are thought to contribute to accelerated atherosclerosis.

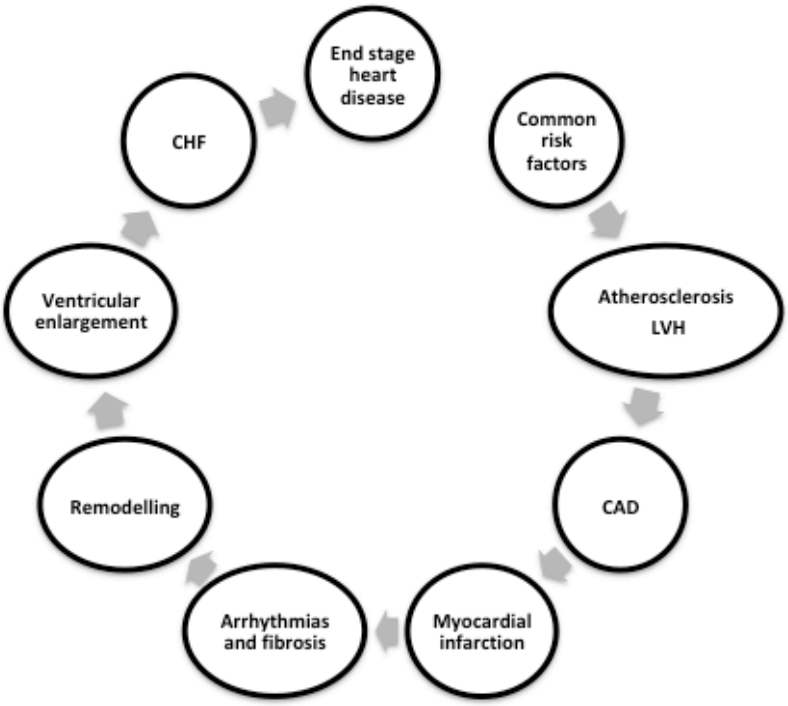


Figure 1.3- Cardiovascular continuum

This depicts the link between cardiovascular disease and renal dysfunction was thought to stem from the presence of shared risk factors, leading on to the development of atherosclerosis, cardiac structural changes, ischemia, heart failure and eventually end stage heart disease.

Common origins to some extent explain why CV and renal disease are so frequently seen together, however the clinical impact of one on the other as well as the independent nature of the effect of CKD on CV outcomes would suggest a relationship more complex than the mere presence of shared risk factors (Figure 1.4).

Studies of renal haemodynamics, GFR and the severity of heart failure have demonstrated that the reduction in renal blood flow in ACE inhibitor naïve patients is out of proportion to the reduction in cardiac index, while GFR is relatively maintained. When renal blood flow drops further, GFR declines as the auto regulatory capacity of the kidney is exhausted. In

patients on ACE inhibitors renal blood flow and GFR decline in parallel since compensatory efferent arteriolar vasoconstriction is reduced by ACE inhibitors. (148) There is also now evidence of an epidemiologic association between increased central venous pressure or venous congestion and reduced GFR in the chronic setting. (149) (150, 151) In acute heart failure however the relationship between high central venous pressure and GFR appears to be complex. (152) There have in fact been suggestions that lower central venous pressure predisposes to WRF. (153) (154) (155) Renal blood flow remains the most important determinant of GFR in heart failure as GFR is simply the product of renal plasma flow times the filtration fraction. Although there can be a modest dynamic range of filtration fraction, a high value for filtration fraction multiplied by a very low renal blood flow will still result in a low GFR, as is true of the opposite analogy. The relative contribution of venous congestion in these circumstances is therefore likely to be limited.

Cardiorenal connectors, inflammation, endothelial dysfunction and anaemia are known to influence the association between haemodynamics and GFR. The 4 cardiorenal connectors via their respective yet closely linked mechanisms can augment each other with their deleterious effects and result in severe cardiorenal syndrome. RAAS, nitric oxide and reactive oxygen species (NO - ROS) balance, the sympathetic nervous system, and inflammation are the key players implicated in this proposed interactive network of cardiorenal connectors. (156) Inappropriate activation of RAAS in renal and cardiac failure causes dysregulation of extracellular fluid volume and vasoconstriction, resulting in vascular inflammation and increased sympathetic activity. (157) Angiotensin II has a direct effect on renal perfusion and GFR, promotes renal fibrosis, induces hypo responsiveness to natriuretic peptide and mediates

sympathetic nervous system activation. Sympathetic nervous system activation can result in tubular injury and the formation of reactive oxygen species (as well as RAAS activation). (156) (158) (159) The effect of oxidative stress and endothelial dysfunction also seems to be modulated by angiotensin II. Through NADP(H) activation, angiotensin II promotes the formation of reactive oxygen species, which in turn can cause intra renal (proximal tubular) damage. (156)

Finally, anaemia is an important factor in heart failure patients with renal impairment. Anaemia as discussed previously has diverse causes in heart failure, including reduced renal function with lower erythropoietin production and blunted response, bone marrow suppression in heart failure, iron deficiency, and haemodilution.

Albuminuria is seen in up to 30% of CHF patients. It commonly occurs due to increased glomerular permeability seen with reduced GFR but can also be seen with normal GFR where it is a consequence of decreased re-absorption in the tubules due to tubular damage. Tubular damage is now increasingly recognized in patients with acute and CHF and can occur both due to congestion as well as increased oxygen requirements in the context of reduced renal blood flow. (160) In a retrospective analysis of the GISSI-HF trial, tubular damage assessed by urinary markers such as N-acetyl-b-D-glucosaminidase (NAG), neutrophil gelatinase-associated lipocalin (NGAL), and kidney injury molecule 1 (KIM-1) was frequently demonstrated among patients with CHF and was strongly associated with mortality. (161)

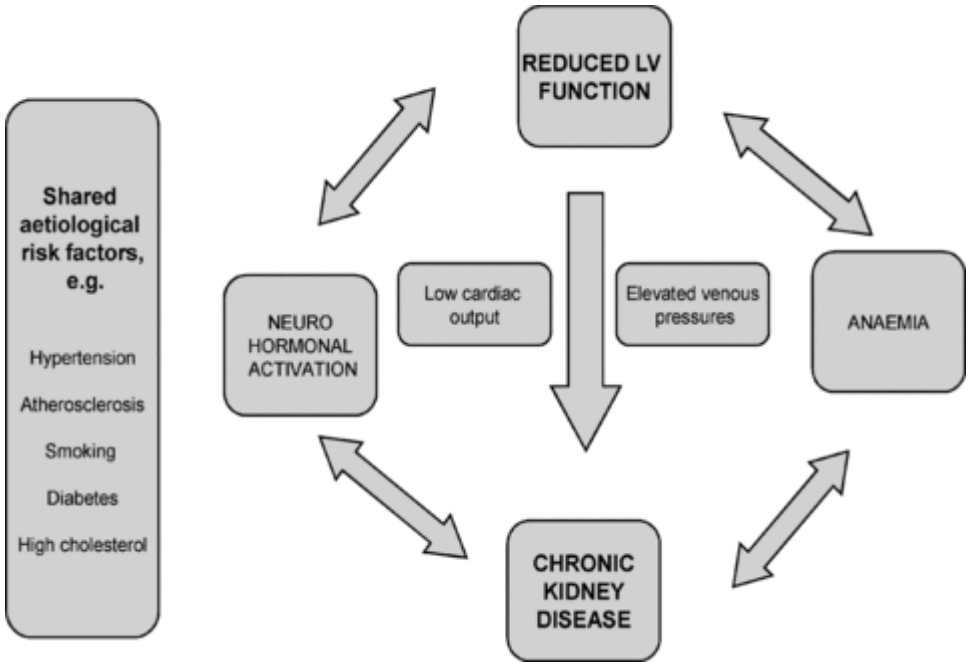


Figure 1.4- Schematic overview of some of the pathophysiological relations between left ventricular impairment and chronic kidney disease.

Alongside the presence of shared risk factors, neuro hormonal activation and anaemia are two of the major players in the link between the two entities. (162)

In the natural history of this complex relationship between the heart and the kidney (Figure 1.4), it is essential to understand that the risk of developing CAD or arrhythmias or heart failure in the presence of CKD is not a time dependent progression. Sudden cardiac death (SCD) could be the first manifestation in someone with CKD and no overt CV manifestations. Figure 1.5 below depicts a revamped and renamed version of the previously described CV continuum. This schematic representation of the cardiorenal continuum focuses on the transition from an asymptomatic phase of target organ damage where CKD and CVD may coexist, to manifest disease and finally ESKD or death. It highlights the potential role for early and aggressive risk management in slowing progression of CKD and other target organ damage. (163) Even where

target organ damage is present, adequate control of risk factors such as blood pressure can facilitate the regression of target organ damage. For example, studies have suggested that regression of albuminuria (164, 165) and LVH (166, 167) is associated with a significant reduction in number of CV events. Increase in urinary albumin excretion has also been demonstrated to predict the development of CV events or death. (165)

Once symptomatic target organ damage has set in however, risk factor modification and therapies specifically directed at overt CV and renal disease may only delay the appearance of new CV or renal events. Identification of patients at high risk of developing CV and renal events in the first two stages described above is therefore crucial, as this is when the great majority of CV and renal events take place.

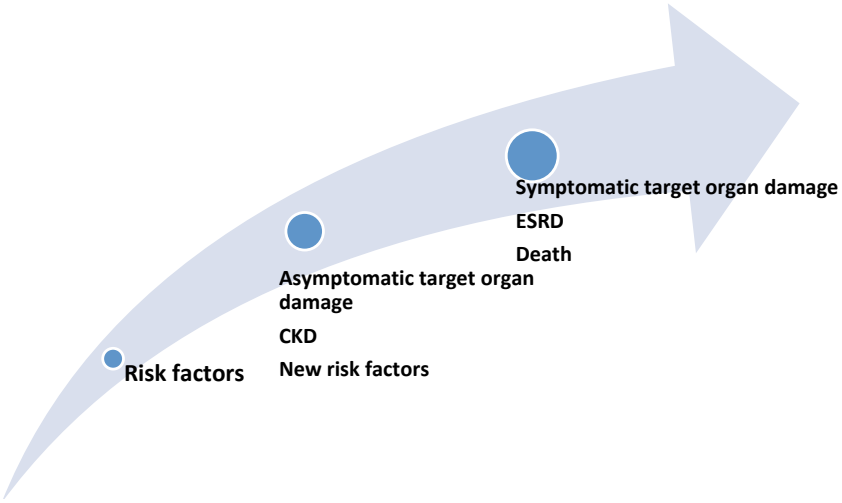


Figure 1.5 – The cardiorenal continuum illustrating the sequential occurrence of the atherosclerotic process from the first stages of CKD to end stage kidney disease (ESKD).
The first stage is characterized by the presence of CVD risk factors without any target organ damage; atherosclerosis often begins at this stage and progress with time. Aggressive detection and management of these risk factors can prevent progression of cardiorenal disease. The second stage is characterized by asymptomatic target organ damage such as atherosclerosis, left ventricular systolic dysfunction, heart failure as well as the presence of CKD. Here too, strict risk factor control may regress target organ damage. The third stage is clinically overt CVD and /or advanced renal failure due to the progression of target organ damage, eventually resulting in CV mortality or ESKD.

Anaemia in chronic kidney disease

Anaemia is identified as a modifiable risk factor in the cardiorenal continuum and has generated a lot of interest in recent studies as a potential therapeutic target with potential prognostic implications. Declining renal function is associated with anaemia with up to 90% prevalence being reported in patients with a GFR < 30 ml/min/1.73 m^2. (168) The coexistence of heart failure, renal failure and anaemia has been referred to as the cardiac-renal-anaemia syndrome (CRAS).

In CKD, anaemia occurs early in the course of the disease and worsens as kidney function declines. In a study by McClellan *et al*, the prevalence of haemoglobin ≤ 12 g/dl was documented to be up to 48% in patients with CKD with evidence of a strong association with declining GFR. In this study, the percentage of patients with haemoglobin levels ≤ 12 g/dl increased from 26.7% to 75.5% when GFR decreased from ≥ 60 to <1 5 ml/min/1.73 m^2. (169)

The aetiologies for development of anaemia in CKD include decreased EPO production, iron deficiency, decreased red blood cell life span due to uraemic toxins, chronic blood loss secondary to platelet dysfunction, nutritional (e.g. folate) deficiencies and elevated inflammatory cytokine levels that suppress the bone marrow (Table 1.11). EPO production typically declines in renal failure and reduction in EPO sensitivity in patients with cardiorenal syndrome is also thought to be a major factor leading to the frequent occurrence of anaemia with CKD and CHF. (170)

Relative erythropoietin deficiency
Resistance to erythropoietin
Reduced expression of erythropoietin receptors
Perturbed erythropoietin signal transduction
Impaired proliferation of erythroid precursor cells
Limited availability of iron for erythropoiesis
Nutrition deficiencies (e.g., iron, folate, and vitamin B12)
Elevated levels of inflammatory cytokines
Platelet dysfunction resulting in blood loss (e.g., occult gastrointestinal bleeding)
Retained blood in extracorporeal circuits for patients with ESKD
Hyperparathyroidism
Drugs (e.g., ACE (i), ARB, and aspirin)

Table 1.11- Causes of anaemia in chronic kidney disease (CKD).

The above list highlights the similarity between the aetiologies for anaemia in heart failure and anaemia in CKD.

ESKD = end-stage kidney disease, ACE (i) = angiotensin-converting enzyme inhibitor; ARB = angiotensin-II receptor blocker.

More recently, there have been suggestions that CKD per se could be linked to hepcidin up regulation. (171) In mice experiments using HepG2 cells, indoxyl sulphate, a uraemic toxin seen in CKD, increased hepcidin expression in a dose-dependent manner. Adenine-induced CKD mice demonstrated an increase in hepcidin concentration in association with renal anemia, decreased plasma iron concentration, increased plasma ferritin and increased iron content in the spleen. Ferroportin was decreased in the duodenum and increased in the spleen. These changes were ameliorated by AST-120 treatment (n oral adsorbent of the uremic toxin). Hepcidin could

therefore form another potential link between heart failure iron deficiency and chronic kidney disease.

Unanswered questions

Having established the prevalence of iron deficiency in an acute decompensated heart failure cohort, (IRON STATS DHF), the question arises, as inflammation and neuro hormonal responses settle following an episode of ADHF, does iron status improve? The second part of my IRON STATS-DHF study will aim to follow the progression of iron status over time. By utilizing measures of renal function such as serum creatinine, and Cystatin C, serum hepcidin and inflammatory markers in conjunction with measures of iron status, I will aim to tease out some of the pathophysiological links between decompensation, inflammation, renal dysfunction and iron status.

SUDDEN CARDIAC DEATH AND CKD

The third stage of the cardiorenal continuum is characterised by symptomatic target organ damage. Cardiovascular target organ damage can range from heart failure to SCD. SCD is defined as an unexpected natural death from a cardiac cause within 1 hour of onset of symptoms in a person not known to have a potentially fatal condition. (172) It has been suggested to contribute to 70% of CV mortality and up to 29% of all-cause mortality in haemodialysis patients. (173)

Our understanding of SCD has evolved over the years, yet the mechanism remains poorly understood. In the true sense of the word, SCD would refer to death due to a ventricular arrhythmia occurring suddenly and without warning. However the definition of SCD has been misrepresented in many ways resulting in possible over estimation in CKD populations. The recent UK Renal Registry annual report revamped the coding system for classification of cause of death, differentiating 'Uncertain' and 'Other' causes of death and as a result, there was a substantial reduction in the proportion of cases attributed to uncertain causes of death. (174) In the 2010 report, cardiac disease was responsible for 22% of deaths in prevalent dialysis patients, with 'uncertain' and 'other' causes of death contributing to 30%. It is therefore possible that other aetiologies such as intra-cerebral pathology, pulmonary emboli or aneurysms might be contributing to so called 'SCD'. If this is the case, the role of an implantable cardioverter defibrillator (ICD) in this population becomes questionable.

Nevertheless SCD remains a troublesome matter; the precise mode of death or the ability to predict it is not well understood. Patients on haemodialysis are at potential risk of brady and tachy arrhythmias, and both of these might be preventable if a patient was identified as being of high risk (e.g. by use of prophylactic device therapy).

Risk factors and pathogenesis for sudden cardiac death

It is likely that the existence of shared risk factors contributes to the link between arrhythmias and CKD. Diabetes for example is the aetiology of ESKD in 20% of new dialysis cases (175) and is itself associated with a risk of SCD as a consequence of nocturnal hypoglycaemia causing ventricular arrhythmias via QT interval prolongation. (176) Similarly, coronary artery disease (CAD), documented in up to 50% of post mortem studies of SCD in the general population (177) is found in 40% of dialysis patients. (178) However coronary artery bypass grafting (CABG) or revascularisation in the haemodialysis population does not appear to remove all risk of CV mortality. In one study, all-cause and arrhythmia-related mortality in dialysis patients treated with coronary revascularisation remained high at 290 and 76 deaths per 1000 patient years respectively, suggesting that the link between the two was more complex than a cause and effect phenomenon secondary to myocardial ischaemia. (179)

Haemodialysis induces changes in cardiac structure such as LVH and LV dilatation (documented in 75% and 34% of patients respectively (173)). LVSD as well as diffuse myocardial fibrosis altering conduction and repolarization have also been proposed as possible precipitants for poor CV outcome. (180, 181) Independent associations have been demonstrated even between moderate renal impairment and abnormal LV geometry in the absence of clinical heart failure.

(182) This may be partly driven by uraemic toxins such as indoxyl suplhate and p-cresyl sulphate via activation of renal inflammatory and fibrotic processes seen in CV disease related to renal dysfunction. (183) These novel pathways may offer potential early intervention strategies for pre-dialysis patients by targeting the site of toxin production (colon in this case).

In dialysis patients, the various haemodynamic and biochemical factors (electrolyte imbalances), rapid fluid and electrolyte shifts (184), interstitial fibrosis, autonomic neuropathy (sympathetic over activity), alterations to the renin-angiotensin-aldosterone axis and importantly changes to their cardiac structure are potential triggers for arrhythmias. (180, 181, 185) In addition to the above LV changes described, atrial dilation/ stretch due to chronic fluid overload, long standing hypertension and scars of previous myocardial infarctions are also felt to be potential substrates for arrhythmias. These haemodynamic, metabolic, cellular, and molecular mediators of myocardial hypertrophy, fibrosis, apoptosis, and capillary degeneration, by promotion of electrical instability, re-entry arrhythmias and CHF may therefore be important drivers of SCD.

Arrhythmias and dialysis

There is limited literature evaluating the actual arrhythmic burden in patients with ESKD. This may occur in the subclinical or asymptomatic target organ damage phase of the cardio renal continuum and can therefore be difficult to characterise. Studies have shown the presence of repolarization abnormalities (QT prolongation, QT dispersion and abnormal T wave axis deviation) in the presence of renal impairment (186, 187) and a significant burden of high-grade ventricular ectopy including non-sustained ventricular arrhythmias (188, 189) (Table 1.12). Ventricular ectopy from available studies do not appear to contribute to increased risk of SCD.

Thus whilst various ECG and echocardiographic criteria have been identified in association with SCD, definite risk markers are yet to be identified.

ECG parameter		Author (Year)	N	Method of ECG evaluation	Findings
Heart rate	Heart rate (HR)	Cice *et al* (2008) (190)	407 ESKD patients	48 hour holter monitoring	HR > 85 beats per minute was an independent predictor of global and CV risk (ROC curve and Cox regression analysis $p < 0.001$)
	Heart rate variability (HRV)	Suzuki *et al* (2012) (191)	281 HD patients	24 hour ECG	Decreased scaling exponent α (1), a non-linear measure of HRV reflecting fractal organisation remained significant after adjusting for clinical risk factors (HR per 0.25 decrement of 1.46, 95% CI 1.16 - 1.85) and in a prediction model composed of clinical risk factors, increased the C statistic from 0.84 to 0.87 ($p = 0.03$), with 50.8% (95% CI, 20.2 - 83.7) continuous net reclassification improvement for 5-year mortality.
	HRV	Oikawa *et a*l (2009) (192)	383 HD patients	24 hour ECG	Decreased HRV parameters in the time- and frequency-domain were identified as predictors of all-cause and CV death in a Cox univariate and multivariate analysis respectively {HR 0.988 (95% CI 0.982 - 0.994) and 0.984 (95% CI 0.974 - 0.993)}
	HRV	Fukuta *et al* (2003) (193)	120 chronic HD patients	24 hour ECG	HRV measures were significantly reduced in HD patients. During a follow-up period of 26 +/- 10 months, 17.5% patients died (47% from cardiac causes). Decreases in HRV measures such as triangular index, very-low-frequency (0.0033-0.04 Hz) power, ultra-low-frequency (< 0.003 Hz) power (ULF) and the ratio of low-frequency (0.04 - 0.15 Hz) power to high-frequency (0.15 - 0.4 Hz) power had significant predictive value for cardiac death but not for non-cardiac death.

Table 1.12- ECG parameters in advanced CKD/ haemodialysis patients linked to arrhythmias and sudden cardiac death.

CI-Confidence interval; CV- cardiovascular; CVD- cardiovascular disease; ESKD- end stage kidney disease; HD- haemodialysis; HR- hazard ratio; LV- left ventricular; LVH- left ventricular hypertrophy; LVMI- left ventricular mass index; LVSD- left ventricular systolic dysfunction; QTc- corrected QT interval; ROC- receiver operating characteristic; RR- relative risk; SCD- sudden cardiac death.

ECG parameter		Author (Year)	N	Method of ECG evaluation	Findings
QT interval	QT duration	Thomson *et al* (2014) (194)	39 dialysis patients	Holter monitoring during dialysis	Frequent nocturnal HD was associated with a decrease in QTc interval for all patients (from 436.5 to 421.3 ms, p = 0.019) and for patients with prolonged QTc at baseline (468.2 to 438.2 ms, p = 0.013). Dialysis duration predicted a decrease in QTc better than dialysis frequency and prevalence of borderline or prolonged QTc increased in patients who dialysed for less than 4 hours per session (12/39 to 22/39, p = 0.039).
	QT duration	Genovesi *et al* (2013) (195)	122 HD patients	Holter recording	44 patients (36.0%) had a prolonged QTc (450 ms in men and 460 ms in women.). Median follow-up was 3.9 years. In multivariate analysis age at recruitment [HR = 1.07, 95% CI: 1.03-1.11, p < 0.001], prolonged QTc (HR = 2.16, 95% CI: 1.20-3.91, p = 0.011) and presence of dilated cardiomyopathy (HR = 3.75, 95% CI: 1.01-7.00, p < 0.001) were independently associated with total mortality, while only prolonged QTc (HR = 8.33, 95% CI: 1.71 - 40.48, p = 0.009) and increasing LVMI (HR = 1.01, 95% CI: 1.00 - 1.02, p = 0.022) were associated with SCD.
	QT dispersion (QTd)	Oktavia *et al* (2013) (196)	61 dialysis patients	12 lead ECG	No correlation between increased QTd and clinical factors assessed (hypertension, pulse pressure, intradialytic hypotension, LVH, old myocardial infarct, diabetes mellitus, and nutritional status). The means of QT interval and QTd increased after HD session (from 382 ± 29 to 444 ± 26 ms, p < 0.05; and from 74 ± 21 to 114 ± 53 ms, respectively, p < 0.05).

Table 1.12 (continued)- ECG parameters in advanced CKD/ haemodialysis patients linked to arrhythmias and sudden cardiac death.

CI-Confidence interval; CV- cardiovascular; CVD- cardiovascular disease; ESKD- end stage kidney disease; HD- haemodialysis; HR- hazard ratio; LV- left ventricular; LVH- left ventricular hypertrophy; LVMI- left ventricular mass index; LVSD- left ventricular systolic dysfunction; QTc- corrected QT interval; ROC- receiver operating characteristic; RR- relative risk; SCD- sudden cardiac death

ECG parameter		Author (Year)	N	Method of ECG evaluation	Findings
Left ventricular hypertrophy (LVH)	LVH as per 14 different ECG criteria.	Covic *et al* (2013) (197)	418 dialysis patients	Interval ECGs during dialysis	An independent association between LVH and CV mortality using Novacode method for LVH assessment was demonstrated (HR = 3.04; 95% [CI] = 1.11 - 8.28, $p < 0.05$). Patients with persistent ECG changes of LVH had increased risk of CV mortality compared to those with new LVH, LVH regression and no LVH ($p = 0.044$).
QRS/ T	QRS-to-T angle (TCRT) and T wave morphology dispersion (TMD)	Poulikakos *et al* (2014) (198)	81 dialysis patients	Holter monitor during dialysis	Patients with major arrhythmic events exhibited extreme TCRT and TMD values and minimal intradialytic changes.
	QRS/T	De Bie *et al* (2013) (199)	277 haemodialysis patients	12 lead ECG	An abnormal spatial QRS-T angle (≥ 130° in men, ≥ 116° in women) was associated with a higher risk of death from all causes (HR 2.33; 95% CI 1.46 - 3.70) and SCD (HR 2.99; 95% CI 1.04 - 8.60). It was also of incremental prognostic value when added to a risk model consisting of known risk factors.
T wave morphology	T-wave residuum (TWR)	Lin *et al* (2007) (200)	325 HD patients	12 lead ECG	Direct comparison between CV death and non-CV death patients showed that relative TWR predicted CV mortality (0.20+/-0.21% vs. 0.24 +/- 0.17%, $p = 0.005$). In a Cox model, relative TWR was an independent predictor of CV (RR=1.86; $p = 0.013$) and arrhythmia-related mortality (RR=2.10; $p = 0.012$).

Table 1.12 (continued)- ECG parameters in advanced CKD/ haemodialysis patients linked to arrhythmias and sudden cardiac death. Although various ECG, ambulatory or echocardiographic criteria have been analysed and associations have been seen, there still remains uncertainty as to the mode of death in these studies and subsequent lacking of a reliable risk marker for SCD. CI-Confidence interval; CV- cardiovascular; CVD- cardiovascular disease; HR- hazard ratio; LV- left ventricular; LVH- left ventricular hypertrophy; LVMI- left ventricular mass index; LVSD- left ventricular systolic dysfunction; QTc- corrected QT interval; ROC- receiver operating characteristic; RR- relative risk; SCD- sudden cardiac death

Haemodialysis per se is thought to be arrhythmogenic, yet intradialytic cardiac arrest is a relatively rare phenomenon. Factors such as inter-dialytic interval (SCD being most common following a long inter-dialytic period, mode of dialysis (haemodialysis carries greater risk compared to peritoneal dialysis), composition of dialysate fluid and dialysis induced rapid fluid and electrolyte shifts {potassium, magnesium, phosphate and calcium (184)} have been identified as potential risk factors for SCD, yet the relative importance of the various pathophysiological mechanisms that drive this phenomenon remain largely mysterious. ECG findings such as micro T wave alternans, abnormal ventricular repolarization manifesting as QT prolongation and QT dispersion, secondary to the above, have been linked to SCD.

Studies have demonstrated intra-dialytic abnormal ventricular repolarization (increased QT dispersion (201) and QT prolongation) but not evidence of a direct relationship between intradialytic arrhythmias and SCD. The risk of SCD also appears to be most significant immediately before and immediately after the first weekly haemodialysis session. (202, 203) A bimodal distribution of death occurrences, with a 1.7-fold increased death risk occurring in the 12 hour period starting with the dialysis procedure and a 3 fold increased risk of death in the 12 hours before haemodialysis at the end of the weekend interval was demonstrated by Bleyer *et al.* (182) These daily variations seem to be less in patients on peritoneal dialysis or home haemodialysis or even haemodialysis patients receiving more than 3 sessions per week. It is thus plausible that the bimodal peak in risk of arrhythmias may be driven by two different types of underlying arrhythmic phenomena, such as tachy arrhythmias and brady arrhythmias. Brady arrhythmias secondary to conducting system disease or electrolyte

abnormalities may prove detrimental either via profound bradycardia or asystole, or via bradycardia-induced tachyarrhythmia.

The burden of supraventricular tachyarrhythmias in dialysis patients also remains largely undefined. Atrial ectopics and atrial fibrillation (AF) have been documented in routine ECGs and 24 hour ECG recordings, with a recent meta-analysis suggesting AF prevalence of about 12% (204) in patients on haemodialysis. AF appears to be more common in the intra dialytic period. (205) This is important as AF not only increases the risk of stroke 9.8 fold (204) in patients receiving haemodialysis but has also been identified as an independent risk factor for sudden death. Stroke prevention utilising oral anticoagulants in this population remains controversial, warranting careful consideration of risks and benefits. All of the above therefore call for a better understanding of the burden of AF and paroxysmal AF in haemodialysis patients.

Unanswered questions

The true burden of arrhythmias in a haemodialysis population has not been defined. It is not clear for example, whether it is solely a tachyarrhythmia that contributes to SCD or whether there is a role for bradyarrhythmias (conducting system disease, electrolyte abnormalities). If life threatening tachyarrhythmias are truly the reason for SCD in this population, a study based on ICDs would be appropriate prior to embarking on interventional trials such as those involving ICDs, it would be fundamental to gain an understanding of the true burden of arrhythmias in the presence of ESKD. Documenting arrhythmias and potential mechanisms of SCD in the ESKD population is fraught with challenges. Like any patient with transient arrhythmias, there are issues around cardiac monitoring in the presence of few or no

symptoms. A 12 lead ECG would only represent a snapshot of cardiac electrical activity and may be recorded when the patient is asymptomatic or between periods of abnormal arrhythmias. Conventional Holter monitoring (an ambulatory ECG recording device) is more favourable as it provides data for 1-10 days at a time but again this could be an asymptomatic period. Event monitors require patients to have symptoms, have sufficient warning to activate the monitor and to be able to activate or attach the monitor when symptomatic. All of these limitations would appear to be addressed by the use of Implantable loop recorders (ILR) as the next logical step in the investigation of SCD in the presence of ESKD.

The CardioRenal Arrhythmia Study in Haemodialysis patients using Implantable Loop Recorders (CRASH ILR) has been designed with the above questions in mind. By implanting loop recorders in a typical cohort of patients receiving haemodialysis, I would aim to define the prevalence of SCD as well as understand the nature of arrhythmias.

POOR PROGNOSIS IN CARDIORENAL DISEASE- CAN THERAPEUTIC NIHILISM CONTRIBUTE?

Pathophysiological reasons aside, the question arises whether poor prognosis in cardiorenal disease is partly driven by the under-utilisation of potentially beneficial therapies. This stems not just from the lack of evidence base (most trials exclude participants with severe CKD) but also from possible excess caution on the part of the treating physician.

Cardiac resynchronisation pacemakers (CRTP) in heart failure

Paucity of evidence regarding benefits of established therapies in those with significant CKD and potential harm from them may contribute to decision making in management of cardiorenal disease. Take for example the use of cardiac resynchronisation therapy (CRT), this is well established as a prognostically beneficial intervention in selected patients with heart failure. By restoring synchronous contraction of the inter-ventricular septum and LV free wall with resultant improvement in LV geometry and function, cardiac resynchronisation pacemakers offer a therapy that beneficially impacts on both of these haemodynamic parameters. Current ESC and NICE guidelines recommend implantation of a CRT device in all symptomatic heart failure patients with broad QRS complexes on ECG and an EF < 35%. (4, 206)

The CARE-HF study was one of the landmark trials that demonstrated the positive impact of CRT on mortality and hospitalisation for CHF. (207) The median value of serum creatinine in this study was 106μmol/l with one fourth of patients having values ≥ 133 μmol/l, indicating a high prevalence of some degree of renal dysfunction. (208) Several subsequent studies have reiterated this benefit however there remains an uncertainty regarding the benefit of these therapies in those with severe renal dysfunction. Table 1.13 highlights the key trials supporting CRT use in heart failure with special mention of renal function in these studies. In addition to the impact on prognosis by the mere presence of CKD, there are also additional concerns regarding the safety of device implantation in this high-risk group (infection risk, contrast induced nephropathy, problems with vascular access and bleeding risks).

Trial	n	Inclusion criteria	Intervention	Renal function at baseline	Renal subgroup analysis
CARE-HF(207)	813	NYHA III/IV, QRS ≥ 120 msec on standard heart failure treatment	Medical treatment ± CRT	Median eGFR 61 ml/min/1.73m^2 (IQR 467 - 73 ml/min/1.73 m^2)	Yes(209) No change in the benefit of CRT for primary outcome if GFR≥ 60 or <60 ml/min/1.73m^2
COMPANION (210)	1520	NYHA III/IV, ischaemic/non ischaemic cardiomyopathy, QRS ≥ 120 msec	Medical treatment vs. medical treatment + ICD vs. medical treatment + ICD/CRT	Not reported, 22% described as having 'renal dysfunction'	Yes(211) HR 1.69 for SCD in patients with renal dysfunction
MADIT-CRT (212)	1820	NYHA I/II, ischaemic/non ischaemic cardiomyopathy, EF ≤30%, QRS ≥130 msec	ICD ± CRT	24% with baseline BUN ≥ 26 mg/dl. ESKD excluded	No
MIRACLE (213)	453	NYHA III/IV, EF ≤35%, QRS≥130 msec	CRT vs. control subjects	Not reported, Creatinine >3 mg/dl excluded	Yes(214) CRT improved GFR if baseline GFR 30 - 60 ml/min/1.73m^2.

Table 1.13- Trials Supporting CRT Use in Patients With Heart Failure and Reported Renal Characteristics
(adapted from Cannizzaro *et al*. Device Therapy in HF, CKD, and ESRD) (218)

Trial	n	Inclusion criteria	Intervention	Renal function at baseline	Renal subgroup analysis
MIRACLE ICD (215)	369	NYHA II/IV, EF ≤35%, QRS ≥130 msec	All received ICD/CRT, C RT inactivated in 182	Not reported, Creatinine >3 mg/dl excluded	No
RAFT (216)	1798	NYHA II/III, EF ≤30%, QRS ≥120 msec or paced QRS ≥200 msec	ICD ± CRT	GFR<30 ml/min/1.73m^2 GFR 30 - 59ml/min/1.73m^2 ; 781 (43%) GFR ≥ 60ml/min/1.73m^2: 881 (49%)	No significant difference between GFR <60 or ≥60 ml/min/1.73m^2
REVERSE (217)	610	NYHA I/II, EF ≤ 40%, QRS ≥120 msec	All received CRT device (± defibrillator) CRT inactive in 191	CRT inactive: GFR 89.6 ±36.4 ml/min/1.73m^2 CRT active: GFR 84.2 ±31.3 ml/min/1.73m^2 CRT active: GFR 84.2 ± 31.3 ml/min/1.73m^2	No significant difference between GFR < 82.7 or 82.7 ml/min/1.72m^2

Table 1.13 (continued)- Trials Supporting CRT Use in Patients With Heart Failure and Reported Renal Characteristics
(adapted from Cannizzaro *et al*. Device Therapy in HF, CKD, and ESRD) (218)
CARE-HF -Cardiac Resynchronization Therapy in Heart Failure; COMPANION - Comparison of Medical Therapy, Pacing, and Defibrillation in Heart Failure; CRT - cardiac resynchronization therapy; EF- Ejection fraction; HR - hazard ratio; GFR- glomerular filtration rate; MADIT-CRT- Multicenter Automatic Defibrillator Implantation Trial With Cardiac Resynchronization Therapy; MIRACLE- Multicenter InSync Randomized Clinical Evaluation study; NYHA- New York Heart Association; RAFT - Resynchronization/Defibrillation for Ambulatory Heart Failure Trial; REVERSE- Resynchronization Reverses Remodeling in Systolic Left Ventricular Dysfunction trial; SCD- sudden cardiac death.

Unanswered questions

Data on the complication rates of CRT implantation in those with CKD are limited. There are also very limited data in this population regarding the impact of implanting a CRT device on subsequent decline of renal function. A retrospective analysis of data from the MIRACLE trial (which excluded patients with serum creatinine > 264 mmol/l or 3.0 mg/dL) showed CRT was associated with improved eGFR in patients with moderately reduced eGFR ($30 \leq eGFR \leq 60$ mL/min/1.73 m^2) compared to controls. (214) It however had no effect on renal function in patients with normal/ increased (≥ 90 mL/min/1.73 m^2) or with mildly reduced eGFR ($60 \leq eGFR <$ 90 mL/min/1.73 m^2). A few other studies have also suggested that while the presence of severe CKD translates to poorer outcomes (219-221) (heart failure hospitalisations and death), if renal function does not deteriorate post device implantation, there appears to be significantly greater benefit with CRT (greater reduction in LV end-systolic volume, lower all-cause mortality) than in those whose renal function declined. (222)

A real-life analysis of device related complications in CKD and change in renal function following CRT therapy implanted as per current guidelines is lacking. In my CRT-CKD study, I therefore conducted a two centre retrospective study of 459 heart failure patients to assess the safety and feasibility of implanting biventricular pacemakers in the presence of significant CKD (eGFR cut off of 45 ml/min/1.73 m^2). I also assessed the change in progression of renal function before and after device therapy comparing renal function 6 months pre-device to 6 and 12 months post device implantation.

Renal dysfunction and management of coronary artery disease

40% of patients with CKD have evidence of coronary artery disease (223) and historical studies have shown that these high-risk patients with CKD receive sub-optimal therapy post myocardial infarction. Reasons attributed to this include the apprehension that drugs such as ACE inhibitors may result in detrimental effects on renal function or are poorly tolerated, the failure of healthcare systems to provide specialist cardiology care to older patients or those with co-morbidity or even a belief that these patients are beyond help ('therapeutic nihilism'). (179)

Unanswered questions

The decision to prescribe thrombolysis for acute ST segment elevation myocardial infarction (STEMI) and the subsequent utilization of secondary prevention or revascularization was historically driven by patient characteristics. Treating acute myocardial infarction with primary percutaneous coronary intervention (PPCI) irrespective of age or renal function (the latter is usually unknown when taken to the catheterization laboratory) may have reduced the possible bias in healthcare professionals against prescribing in high-risk groups. National audit databases do not differentiate drug utilisation based on renal function hence it would be useful to test the above hypothesis in a real world ischaemic heart disease population.

My study of secondary prevention post primary PCI in patients with renal dysfunction study has been designed to answer this. I hypothesised that by reducing the possible bias in healthcare professionals against prescribing in high-risk groups, PPCI would be associated a high uptake of secondary prevention medication. By performing a multi centre retrospective study of all patients undergoing PPCI in 5 UK centres, I assessed

the influence of renal function on the utilisation of secondary prevention medication post myocardial infarction.

AIMS OF THESIS AND SUMMARY OF STUDIES

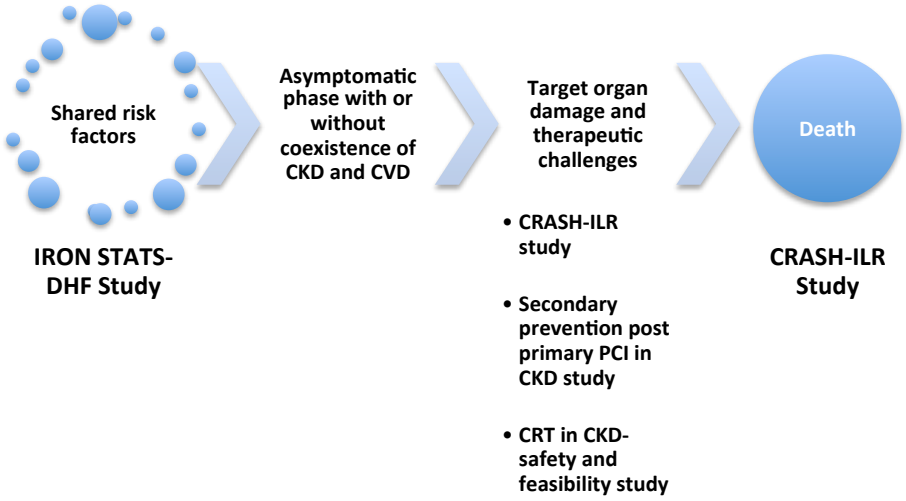


Figure 1.6- Modified cardiorenal continuum from a physician's perspective and summary of studies

CKD- Chronic kidney disease; CRASH-ILR- Cardiorenal arrhythmia study in haemodialysis; CRT- Cardiac resynchronisation therapy; IRON STATS- DHF- Iron status in decompensated heart failure; PCI- Percutaneous coronary intervention

This thesis will focus on two major comorbidities of heart failure- iron deficiency and chronic kidney disease and the studies will focus on various aspects of the cardiorenal continuum (Figure 1.6).

Studies 1 & 2 - IRON STATS- DHF (iron status in decompensated heart failure) study is, a cross sectional and prospective evaluation of patients admitted with decompensated heart failure. The aims of my study were to define the prevalence of iron deficiency in this cohort and the natural history of an iron deficient state following discharge from hospital.

My hypotheses were that-iron deficiency would be common in acute decompensated heart failure (ADHF) and would be driven by inflammatory immune activation and up regulation of hepcidin. I expected iron deficiency at discharge from hospital to be a predictor of death and cardiovascular hospitalisation at 12 month follow up.

Study 3:

My **CRASH-ILR** study is a novel study of implanting loop recorders in patients on haemodialysis patients. The aims of the study were to evaluate the prevalence of asymptomatic arrhythmias (brady or tachy) and to characterise the symptomatic and asymptomatic arrhythmias in this high-risk population. Silent arrhythmias could be the first manifestation of cardiorenal disease and my expectation was that a novel study of this nature, which offers continuous ECG monitoring, would shed more light on the presumed 'sudden cardiac deaths' characteristic of this population..

Study 4:

Following target organ damage such as the development of heart failure in the setting of CKD, utilisation of therapies is of paramount importance. My 4th study focuses on

the use of cardiac resynchronisation therapy in advanced heart failure with concomitant renal dysfunction. This study was designed across two sites, Portsmouth hospitals NHS Trust and Royal Bournemouth Hospital. My aim was to ascertain how safe and feasible CRT implantation was in the presence of CKD, in an unselected cohort of all patients who underwent CRT implantation at these 2 centres. A comparison was made between those with and without significant renal dysfunction for immediate complications as well as 1-year mortality. Cut off for eGFR was 45 ml/min/1.73 m^2. In addition, I assessed the relationship between CRT implantation and progression of renal dysfunction over time. For this, historic and post implant variations in renal function in the above cohort was studied and analysed.

Study 5:

My final study was also based on the utilisation of evidence-based therapies in cardiorenal disease. The aim was to take a snapshot of current prescribing practice in the UK for patients with renal dysfunction who undergo primary PCI for STEMI. I conducted an analysis of retrospective data on 1159 patients who underwent PPCI across UK. Data collected centred around their index admission to hospital as well as subsequent follow up. The hypothesis was that following the widespread use of primary PCI for STEMIs, there would be a high uptake of secondary prevention medication even in the presence of comorbidities such as renal dysfunction. The specific aim of this study was to assess the influence of renal function on secondary prevention medication in 5 UK sites offering PPCI. Significant renal dysfunction was defined as an eGFR < 45 ml/min/1.73 m^2.

CHAPTER 2- IRON STATUS IN DECOMPENSATED HEART FAILURE- A STUDY OF PREVALENCES.

BACKGROUND

Iron deficiency is increasingly recognised as an important comorbidity in CHF. Both anaemia and iron deficiency are associated with an increase in all-cause and CV mortality. (43) Studies have suggested that the association of iron deficiency with poor therapeutic response (224) as well as poor prognosis (all cause mortality and CV events), independent of the presence of anaemia. (225)

Replenishing iron stores by intravenous iron has therefore been proposed as a therapeutic target. Intravenous iron therapy in iron-deficient patients with heart failure and reduced ejection fraction has been demonstrated to improve exercise capacity, quality of life, and alleviate heart failure symptoms over 24 to 52 weeks. (44) Although none of the trials published to date have been powered to detect differences in CV hospitalisation/ mortality, CONFIRM-HF demonstrated a significant reduction in the risk of hospitalisations for worsening HF in those treated with intravenous iron. (102)

The majority of studies of iron status in patients with heart failure have been in stable outpatient cohorts. The relationship of a heart failure decompensation to iron status is less well known. Decompensated heart failure is associated with marked derangement of haemodynamic and metabolic homoeostasis, and may be linked to impaired efficiency of energy production and inflammatory immune activation.

(226) As such, decompensation per se might contribute to derangement in iron metabolism.

AIMS

Iron status in decompensated heart failure **(IRON STATS- DHF)** is a prospective observational study of 100 patients with heart failure and acute decompensation. The aims of the study are:

- To estimate the prevalence of iron deficiency in this group.
- To understand the factors determining iron status in heart failure with particular interest in hepcidin levels in those with and without iron deficiency.

METHODS

The study is comprised of 2 parts:

1. Cross sectional assessment of prevalence of iron deficiency in decompensated heart failure and the association with markers of renal dysfunction and inflammatory immune activation **(IRON STATS-xs)**.
2. Prospective evaluation of iron status (i.e. change in iron parameters moving through to stable heart failure) and its relationship with changes in renal dysfunction and inflammatory immune activation **(IRON STATS-p)**.

The main study cohort was comprised of 100 near consecutive patients with a clinical diagnosis of acute decompensated heart failure admitted to the Department of Cardiology, Queen Alexandra Hospital, Portsmouth. Based on an estimated prevalence of iron deficiency of 43 ± 10% derived from studies on stable heart failure cohorts, the expectation was that in ADHF, the prevalence of iron deficiency

will not be below 50%. The sample size of 100 was therefore arrived at, for the resulting prevalence estimate to fall within 10% points of the true prevalence with 95% confidence.

To permit appropriate comparisons we also recruited 30 patients with stable CHF and 20 age-matched healthy patients with no history of CVD. The healthy subjects permit a comparison of parameters collected in the study cohort, i.e. blood tests and functional status assessment with a 6-minute walk test (6MWT) with a healthy individual of similar age. Participants for this group were recruited either from healthy relatives of the patients in the study or from the community.

The 30 patients with stable CHF were recruited from the heart failure specialist nurse clinic at Portsmouth Hospitals NHS Trust. This group was recruited to allow an accurate assessment of the impact of decompensation vis-à-vis stable CHF patients over time.

The study was registered on the Integrated research application system (IRAS) and received ethics approval from the NRES Committee South Central- ref 12/SC/0536. IRON STATS-DHF was also added to the NIHR CRN portfolio.

Inclusion criteria

Study arm- ADHF patients

Patients admitted with acute decompensated heart failure (defined as symptoms and signs secondary to abnormal cardiac function with weight gain of more than 3 kg above target weight if known CHF or requiring intravenous diuretics for > 48 hours.)

Comparator arm- Stable heart failure patients

Patients recruited to this arm of the study had a prior diagnosis of CHF based on the presence of signs and symptoms of heart failure with objective evidence of structural or functional abnormality of the heart at rest and were already established on therapy (ACE inhibitors/ ARBs, beta blockers, aldosterone antagonists +/- diuretics). They should also not have had a history of hospitalisation in the preceding 3 months.

Comparator arm- Healthy age matched participants

Healthy participants eligible to take part in the study were those with no known cardiac abnormality or history of anaemia or iron deficiency. Exclusion criteria included any chronic conditions requiring aspirin, beta-blockers, ACE inhibitors, ARBs or aldosterone antagonist therapy in the preceding 3 months.

Exclusion criteria

Patients with overt blood loss, i.e. leading to haemodynamic compromise or drop in haemoglobin >20 g/L or requiring blood transfusion during this admission.

Sepsis or the need for antibiotics during treatment for acute heart failure.

Patients less than 18 years of age.

Ongoing participation in another research study.

Any hospitalisation in the last 3 months for those in the stable heart failure or healthy participant groups.

Pregnancy.

Data Collection

I identified potential ADHF study participants from the cardiology wards, with the help of the clinical team responsible for their care.

Once consent was obtained, I collected clinical data and blood samples for baseline assessment as soon as possible after admission and repeated these 1 day prior to discharge. 15mls of venous blood was collected on each occasion. Where discharge was delayed for social reasons, I collected discharge data at the point the medical team deemed the subject 'medically fit for discharge'. Follow up for the study patients occurred at 4 and 12 weeks post-discharge and the visit would be conducted by myself or the research nurses.

The cohort of stable heart failure patients had a baseline assessment. I performed blood tests as described below at enrolment and at 12 weeks. I conducted a single assessment on the healthy age matched comparator group and this visit included blood tests.

All patients had a detailed baseline transthoracic echocardiogram performed at recruitment; if not requested by the clinical team, I would carry them out as part of the research visit. Details regarding recruitment and follow up visits of each of the participants in the IRON STATS- DHF study are outlined in Figures 2.1 and 2.2 below. I carried out all of these visits wherever possible and when I was unable to carry out follow up visits, they were carried out by the research nurses.

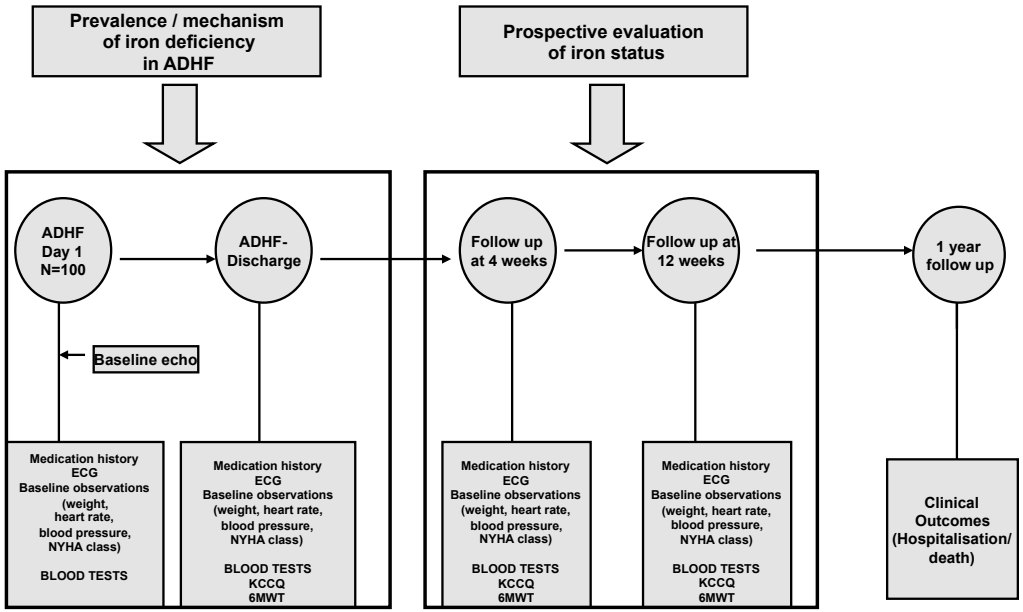


Figure 2.1- Overview of IRON STATS- DHF: ADHF arm.

Every patient recruited would expect to have 4 visits, 1 at recruitment, 1 pre discharge and subsequently at 4 and 12 weeks post discharge. The tests carried out at each visit are outlined above.

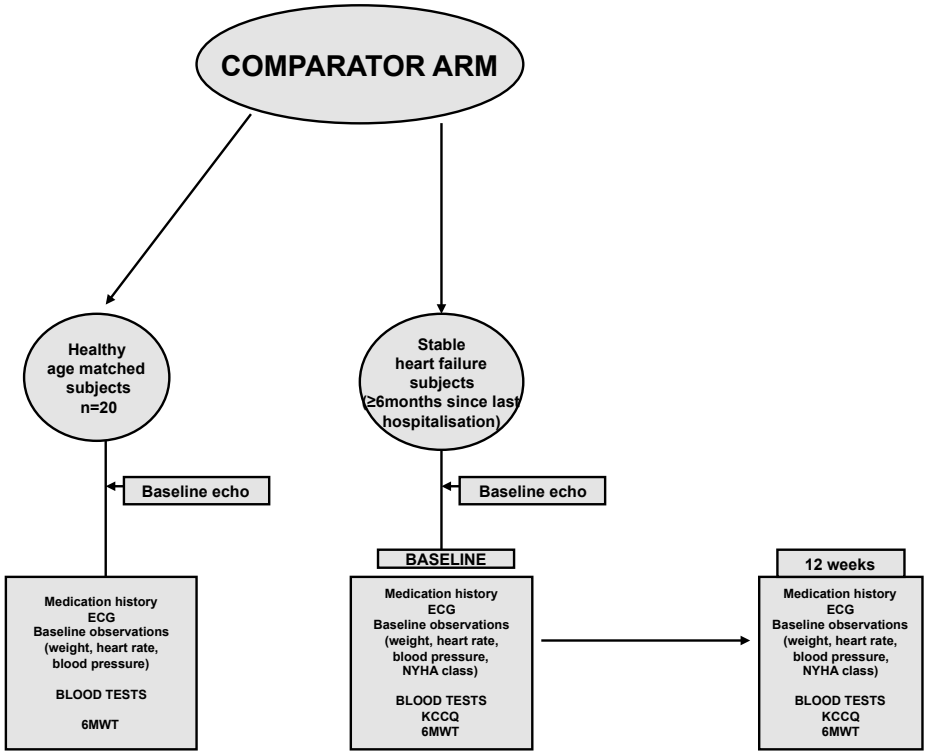


Figure 2.2- Overview of IRON STATS- DHF: Comparator arm.

Stable heart failure patients had a recruitment visit and a follow up visit at 3 months later. Healthy controls had a single recruitment visit. Tests carried out at each visit are outlined above.

Baseline visit:

Subject demographics, medication history, NYHA class, observations (heart rate, blood pressure, weight) and 12 lead ECG were recorded. Blood tests were carried out in all patients at all visits (Table 2.1; B12 and folate levels were measured in all patients only at baseline visit).

Medication history focused on cardiac drugs, i.e. aspirin, warfarin, ACE inhibitors, ARBs, MRAs, beta blockers and other anti arrhythmic agents), duration of intravenous therapy where applicable.

A baseline echocardiogram was performed on all patients specifically looking at LVEF, LV diastolic function, right ventricular function, right ventricular systolic pressures (RVSP) and inferior vena cava (IVC) measurements using standardised methodology as per British Society of Echocardiography (BSE) guidelines.

Kansas City Cardiomyopathy questionnaire (KCCQ) data was collected on stable CHF patients at baseline and follow up. For the ADHF cohort, KCCQ data was collected only at discharge 4 week and 12 weeks post discharge. The KCCQ is a 23- item, self-administered instrument that quantifies physical function, symptoms (frequency, severity and recent change), social function, self-efficacy and knowledge, and quality of life. An overall summary score is derived from the physical function, symptom (frequency and severity), social function and quality of life domains. For each domain, the validity, reproducibility, responsiveness and interpretability have been independently established. Scores are transformed to a range of 0-100, in which higher scores reflect better health status.

Follow up visits:

For ADHF patients, subsequent visits were at discharge, 4 weeks and 12 weeks post discharge. Visits were arranged for mutually convenient dates as close as possible to these pre-specified visits and taking into consideration any follow up clinical visits planned around this time.

For the control cohort comprised of stable heart failure patients, a single follow up was carried out at 12 weeks post enrolment.

No follow up visits were carried out on the healthy controls.

At each follow up visit, data were collected on medication history and NYHA class. Observations recorded included body weight, heart rate and blood pressure. A 12 lead ECG and a 6MWT {as per American Thoracic Society guidelines (227)} were performed and a KCCQ filled in. Blood tests as outlined in Table 2.1 were carried out in all patients.

PARAMETERS		
Serum Hb (g/L)		Analysis performed by haematology lab as part of routine clinical care
Haematological indices	Haematocrit (%) Mean corpuscular volume (MCV, fL) Mean corpuscular haemoglobin (MCH, pg) Mean corpuscular haemoglobin concentration (MCHC, g/L).	Analysis performed by haematology lab as part of routine clinical care
Serum haematinics	Ferritin (microg/L), folate (microg/L) and B12 (ng/L))	Analysis performed by haematology lab as part of routine clinical care
Additional markers of iron status	Serum iron (micro mol/L) Transferrin saturation (%)	Analysis performed by haematology lab as part of routine clinical care
Serum hepcidin (ng/mL)		Samples spun and stored for later analysis
Renal function and electrolytes	Serum Urea (mmol/L) and Creatinine (μmol/L) Serum Na and K (mEq/L), Cystatin-C (mg/L)	Analysis performed by biochemistry lab as part of routine clinical care. Cystatin C spun and stored for later analysis.
Inflammatory markers	C- reactive protein (mg/L), TNF-α (pg/mL), IL-6 (pg/mL)	C-reactive protein was analysed routinely while other samples were spun and stored for later analysis
Serum urate (mmol/L)		Analysis performed by biochemistry lab as part of routine clinical care
Serum erythropoietin (IU/L)		Analysis performed by biochemistry lab as part of routine clinical care

Table 2.1- Blood tests carried out at each visit. B12 and folate were only measured once, at baseline visit.

At 12 months post enrolment, data on CV hospitalisation, CV mortality and all-cause mortality were collected by scanning hospital records/ contacting the patient and patient's GP via telephone.

Hospitalisation noted at any of the visits rendered the patient ineligible to continue the study, as they would no longer be in the phase of recompensation following their admission at the time of recruitment. In the case of stable CHF controls, an admission with decompensated heart failure rendered them 'unstable' and hence would not be eligible to continue in the study. Oral iron therapy was not an exclusion criterion. 12 month follow up data on CV outcomes (composite of CV hospitalisation and death) and all cause mortality was however collected on all patients.

Blood tests

Laboratory measurements were made in venous blood with EDTA (3.7mls) or clotted blood (3 x 3.7 mls). Haemoglobin, haematinics, markers of iron status, renal function, electrolytes and CRP were measured via standard clinical pathways through the departments of haematology and biochemistry at Queen Alexandra Hospital, Portsmouth. 1x 3.7 ml purple topped EDTA sample and 1 x 3.7 ml yellow-topped serum sample were used for this. The 4-variable MDRD equation was used to eGFR from measured creatinine values.

Processing of samples for storage

2 yellow-topped serum samples (3.7 ml x2) were collected for storage in the research laboratory at Queen Alexandra Hospital, Portsmouth. A serum separator

tube (SST) was used and samples allowed to clot for 30 minutes at room temperature before centrifugation for 15 minutes at 1000 x g. After centrifuging, the plasma and serum were collected and frozen at −70°C until further laboratory analyses.

Hepcidin, Cystatin C, IL-6 and TNF-α were measured using the sandwich ELISA method with commercially available kits. All samples were thawed to room temperature, analysed together and measured in duplicate. Tests were carried out in collaboration with Portsmouth University by myself and the research team at the research laboratory in Queen Alexandra Hospital, Portsmouth.

Principle of ELISA test (Figure 2.3)

Samples, including a standard containing protein of interest, control specimens, and unknowns, are pipetted into wells. During the first incubation, the protein antigen binds to the capture antibody. After washing, a detection antibody is added to the wells, and this antibody binds to the immobilized protein captured during the first incubation. After removal of excess detection antibody, an HRP conjugate (secondary antibody or streptavidin) is added and binds to the detection antibody. After a third incubation and washing to remove the excess HRP conjugate, a substrate solution is added and is converted by the enzyme to a detectable form (colour signal). The intensity of this coloured product is directly proportional to the concentration of antigen present in the original specimen.

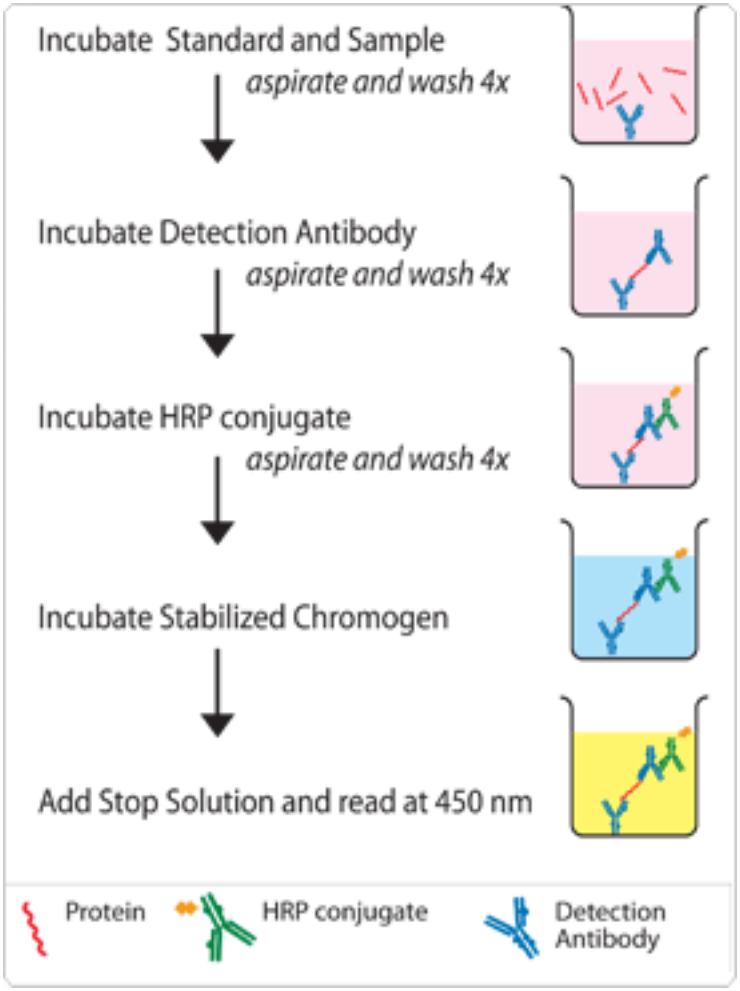


Figure 2.3- Principle of the sandwich ELISA method.

Reagents used were:

- 10x PBS (without Ca/ Mg)
- 1x PBS (without Ca/ Mg, optional - can dilute from 10x)
- Tween 20
- Deionised Water
- Bovine Serum Albumin (BSA) Powder
- Sodium Acetate Trihydrate
- Glacial Acetic Acid
- Tetramethylbenzidine (TMB)
- DMSO
- 30% Hydrogen Peroxide

Reagents provided with the kit were capture antibody x1, detection antibody x1 and streptavidin-HRP x 1 (secondary antibody). The standard varied with each test.

Streptavidin-HRP was available as 3x 2 ml per vials with working concentration of 1:40 diluted with reagent diluent. 10 ml was required per plate.

To dilute streptavidin-HRP 1:40 with reagent diluent			
Number of 96 well plates	Vol. of streptavidin-HRP	Vol. of reagent diluent	Total vol. of 1:200 streptavidin HRP with reagent diluent
1	250 µl	9750 µl	10000 µl (10 ml)
2	500 µl	19500 µl	20000 µl (20 ml)
3	750 µl	29250 µl	30000 µl (30 ml)
4	1000 µl	39000 µl	40000 µl (40 ml)

Table 2.2- Process of dilution for streptavidin- HRP

Plate preparation:

The capture antibody was diluted to the working concentration. A 96-well microplate was immediately coated with 100 µL per well of the diluted capture antibody. The plate was then sealed and incubated overnight at room temperature. Each well was aspirated and washed with wash buffer and the process repeated for a total of 3 washes. After the last wash, any remaining wash buffer was removed by aspirating/ inverting the plate and blotting it against clean paper towels. 300 µL of reagent diluent was added to each well and the plates incubated for a minimum of 1 hour. The aspiration/wash was repeated and the samples were now ready for sample addition.

Assay procedure:

100 µL of sample or standards in reagent diluent was added to each well. This was incubated for 2 hours at room temperature. The aspiration/wash step was repeated and 100 µL of detection antibody was added in reagent diluent to each well and incubated for another 2 hours. 100 µL of the working dilution of streptavidin-HRP (secondary antibody) was added to each well and the plate covered and incubated

for another 20 minutes at room temperature. Contact with direct light was avoided. The aspiration/ wash step was repeated. 100 μL of the substrate solution was then added to each well and incubated for another 20 minutes at room temperature, again avoiding contact with direct light. 50 μL of stop solution was added to each well and the optical density of each well determined immediately. A microplate reader set at 450 nm was used for this and wavelength correction used appropriately to minimize errors.

Hepcidin

The Quantikine Human Hepcidin Immunoassay (R&D Systems Europe, Ltd.), a 4.5 hour solid-phase ELISA designed to measure human hepcidin employing the quantitative sandwich enzyme immunoassay technique was used. A monoclonal antibody specific for human hepcidin was pre-coated onto a microplate. Standards and samples were pipetted into the wells and any hepcidin present was bound by the immobilised antibody. After washing away any unbound substances, an enzyme-linked monoclonal antibody specific for human hepcidin was added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution was added to the wells and color developed in proportion to the amount of hepcidin bound in the initial step. The color development was stopped and the intensity of the color is measured within 30 minutes using a microplate reader set to 450 nm.

Calculation of results

The duplicate readings for each standard, control, and sample were averaged and the average zero standard optical density subtracted. A standard curve was created

by reducing the data using computer software capable of generating a 4 parameter logistic curve-fit. The data was linearized by plotting the log of the human hepcidin concentrations versus the log of the optical density and the best-fit line determined by regression analysis.

Hepcidin concentrations were expressed in nanogram/ml (1 ng/ml = 1 µg/L)
(1 nanomoles per liter (nM) serum hepcidin equals 2.79 µg/L).

Unlike hepcidin where the monoclonal antibody specific for hepcidin was pre-coated to the microplate, for Cystatin C, IL-6 and TNF α, the detection antibody had to be prepared and added to the plates. The stepwise process for this is briefly detailed below.

Cystatin C (R&D Systems Europe, Ltd.)

Standard (recombinant human Cystatin C)

One vial was reconstituted (47.5 ng) in 0.5 ml reagent diluent to give 95 ng/ml. This was divided into 15x 30 µl aliquots and could be stored at -20 °C for up to two months until needed. Each 30 µl aliquot (130 ng/ml) was diluted in 1.425 ml reagent diluent to give 1.425 ml top standard (2000 pg/ml).

Dilution was as below:

- 500 µl top standard + 500 µl reagent diluent = 1000 pg/ml
- 500 µl of 1000 pg/ml + 500 µl reagent diluent = 500 pg/ml
- 500 µl of 500 pg/ml + 500 µl reagent diluent = 250 pg/ml
- 500 µl of 250 pg/ml + 500 µl reagent diluent = 125 pg/ml
- 500 µl of 125 pg/ml + 500 µl reagent diluent = 62.5 pg/ml
- The final standard was a blank containing only reagent diluent.

Protocol (for 1 plate)

Plate preparation:

The wash buffer, reagent diluent, TMB-DMSO stock and substrate buffer stocks were made up. Standards and aliquots were thawed on ice. Capture antibody was removed from the freezer and thawed on ice (or beads). Capture antibody was diluted to working concentration (66.6 µl aliquot in 12 ml 1x PBS). A maxisorp 96 well high protein-binding affinity plate was used and 100 µl of working concentration capture antibody pipetted into each well of the plate. The plate was then sealed and incubated overnight at room temperature.

After 24 hours, the plate was unsealed and contents flicked into sink, dabbing upside down onto paper towels. Plates were washed using 200 µl wash buffer per well, flicked and dabbed. The wash, flick/ dab process was repeated twice more (total of 3 applications of wash buffer per wash step). To block the plate, 200 µl of reagent diluent was added to each well. The plate was resealed and incubated at room temperature for minimum of 1 hour. After at least 1 hour of blocking the wash step was repeated.

Sample assay:

100 µl of standard/sample was pipetted as per plate map (in duplicate). The plate was sealed and incubated at room temperature for 2 hours. Wash step was repeated after 2 hours of sample incubation.

Detection antibody was diluted to working concentration as outlined above (66.6µl in 12ml reagent diluent). 100 µl of detection antibody was pipetted into each well. The plate was resealed and incubated for 2 hours at room temperature. Wash step was repeated after 2 hours of detection antibody incubation. Streptavidin-HRP was diluted to working concentration as outlined above (50 µl in 9.95 ml reagent diluent). 100 µl

of working concentration streptavidin-HRP was pipetted into each well, the plate resealed and incubated for 20 minutes. The plate had to be protected from direct light during incubation. After 20 minutes streptavidin-HRP incubation, the wash step was repeated. Substrate solution was comprised of 12 ml substrate buffer + 200 µl TMB stock solution + 1.2 µl H_2O_2 30% solution. 100 µl substrate solution was added to each well and incubated at room temperature for up to 20 minutes. The plate had to be obscured from direct light during incubation. 50 µl stop solution (5ml 2M H_2SO_4) was added to each well after 20 minutes substrate incubation (or sooner if deemed appropriate). The plate was tapped to ensure thorough mixing. Optical densities were immediately measured using a plate reader (450 nm wavelength (570 nm wavelength was used if correction was available).

Interleukin-6 (R&D Systems Europe, Ltd.)

Standard (recombinant human IL-6)

One vial (65 ng) was reconstituted in 0.5 ml deionised water to give 130 ng/ml. This was then divided into 15x 20 µl aliquots and stored at -20 °C until needed.
10 µl was diluted from each aliquot (130 ng/ml) in 2.167 ml reagent diluent to give 2.167 ml top standard (600 pg/ml). Dilution was as below:

- 500 µl top standard + 500 µl reagent diluent = 300 pg/ml
- 500 µl of 300 pg/ml + 500 µl reagent diluent = 150 pg/ml
- 500 µl of 150 pg/ml + 500µl reagent diluent = 75 pg/ml
- 500 µl of 75 pg/ml + 500 µl reagent diluent = 37.5 pg/ml
- 500 µl of 37.5 pg/ml + 500 µl reagent diluent = 18.75 pg/ml
- 500 µl of 18.75 pg/ml + 500 µl reagent diluent = 9.375 pg/ml
- The final standard was a blank containing only reagent diluent

Protocol (for 1 plate)

Plate preparation:

The wash buffer, reagent diluent, TMB-DMSO stock and substrate buffer stocks were made up as above. Standards and samples were thawed on ice. Capture antibody was diluted to working concentration (100 µl aliquot in 12 ml 1x PBS) and used immediately. A maxisorp 96 well high protein-binding affinity plate was used.

100 µl of working concentration capture antibody was pipetted per well of the plate; the plate was then sealed and incubated overnight at room temperature. Samples were run neat and therefore no dilution was required. After 24 hours the plates were unsealed and contents flicked into sink, dabbing upside down onto paper towels.

A plate washer was used to wash plates {if done manually, each well was filled with 200 µl wash buffer (use multi-channel pipette) (modified from 400 µl)} and following this was flicked/dabbed. The wash, flick/ dab was repeated twice more (total of 3 applications of wash buffer per wash step). To block the plate, 200 µl of reagent diluent was added to each well, the plate resealed and incubated at room temperature for minimum of 1 hour. After at least 1 hour of blocking, the wash step was repeated.

Sample assay:

100 µl of standard/sample was pipetted, in duplicate, as per plate map. The plate was sealed and incubated at room temperature for 2 hours.

Detection antibody was diluted to working concentration (200 µl in 12 ml reagent diluent). 100 µl of detection antibody was pipetted into each well, the plate was resealed and incubated for 2 hours at room temperature. The wash step was repeated after 2 hours of sample incubation. 100 µl of detection antibody was pipetted into each well, the plate was resealed and incubated for 2 hours at room temperature. The wash step was repeated after 2 hours of detection antibody incubation Streptavidin-

HRP was diluted to working concentration as outlined above (250 µl in 9.75 ml reagent diluent) and pipetted into each well, the plate was then resealed and incubated for 20 minutes. The plate was obscured from direct light during this incubation. After 20 minute streptavidin-HRP incubation, the wash step was repeated. Substrate solution was constituted using 12 ml substrate buffer + 200 µl TMB stock solution + 1.2 µl H_2O_2 30% solution and had to be used promptly. 100 µl of this substrate solution was added to each well and incubated at room temperature for up to 20 minutes, again avoiding direct light. 50 µl stop solution (5 ml 2M H_2SO_4) was added to each well after 20 minutes of substrate incubation. Optical densities were measured immediately using a plate reader (450 nm wavelength).

TNF-alpha (R&D Systems Europe, Ltd.)

Standard (recombinant human TNFα)

One vial (67.5 ng) was reconstituted in 0.5 ml reagent diluent to give 135 ng/ml. This was then divided into 25x 20 µl aliquots and stored at -20 °C until needed. This could last up to 3 months.

Each 20 µl aliquot (130 ng/ml) was diluted in 2.7 ml reagent diluent to give 2.7 ml top standard (1000 pg/ml). Dilution was carried out as below:

- 500 µl top standard + 500 µl reagent diluent = 500 pg/ml
- 500 µl of 500 pg/ml + 500 µl reagent diluent = 250 pg/ml
- 500 µl of 250 pg/ml + 500 µl reagent diluent = 125 pg/ml
- 500 µl of 125 pg/ml + 500 µl reagent diluent = 62.5 pg/ml
- 500 µl of 62.5 pg/ml + 500 µl reagent diluent = 31.3 pg/ml
- 500 µl of 31.3 pg/ml + 500 µl reagent diluent = 15.6 pg/ml
- The final standard is a blank containing only reagent diluent

Protocol (for 1 plate)

Plate preparation:

The wash buffer, reagent diluent, TMB-DMSO stock and substrate buffer stocks were made up as shown above. Standards and samples were thawed on ice. Capture antibody was diluted to working concentration as described above (100 µl aliquot in 12 ml 1x PBS) and used immediately. Maxisorp 96 well high protein-binding affinity plates were used and 100 µl of working concentration capture antibody pipetted out into each well of the plate. The plate was sealed and incubated overnight at room temperature. After 24 hours, samples were removed from the freezer and defrosted on ice (40 samples, in duplicate, per plate). Samples were run neat and therefore no dilution was required. The plate was unsealed and the contents flicked contents into the sink, dabbing upside down onto paper towels.

A plate washer was used to wash plates {if done manually, each well was filled with 200 µl wash buffer (use multi-channel pipette) (modified from 400 µl)} and following this, was flicked/dabbed. The wash, flick/dab was repeated twice more (total of 3 applications of wash buffer per wash step). The plates were blocked using 200 µl of reagent diluent was added to each well, the plate resealed and incubated at room temperature for minimum of 1 hour. The wash step was repeated after at least 1 hour of blocking.

Sample assay:

100 µl of standard/sample was pipetted as per plate map, in duplicate. The plate was sealed and incubated at room temperature for 2 hours. Wash step was repeated after 2 hours of sample incubation.100 µl of detection antibody {diluted to working concentration (200 µl in 12 ml reagent diluent)} was pipetted into each well, the plate resealed and incubated for 2 hours at room temperature. The wash step was repeated

after 2 hours of detection antibody incubation. 100 µl of working concentration streptavidin-HRP was pipetted into each well. The plate was resealed and incubated for 20 minutes. Direct light was obscured from the plate during this step. After 20 minute of streptavidin-HRP incubation, the wash step was repeated. 100 µl of substrate solution (12 ml substrate buffer + 200 µl TMB stock solution + 1.2 µl H_2O_2 30% solution prepared just before use) was added to each well and incubated at room temperature for up to 20 minutes. The plate had to be obscured from direct light during this process. 50 µl stop solution (5 ml 2 M H_2SO_4) was added to each well after 20 minutes of substrate incubation. The plate was tapped to ensure thorough mixing. Optical densities were measured immediately using a plate reader (450 nm wavelength).

Biomarkers of iron metabolism

The following standard blood biomarkers reflecting iron metabolism were measured directly: serum concentrations of ferritin (µg/L), iron (µg/L) and transferrin (mg/dL). TSAT was calculated based on iron and transferrin levels and expressed as a percentage.

Serum iron

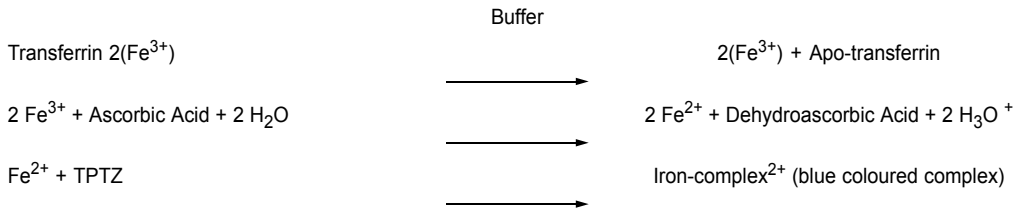
A photometric colour test for the quantitative determination of iron in human serum and plasma was carried out on AU Beckman Coulter analysers.

Methodology

Serum samples were used. TPTZ [2,4,6-Tri-(2-pyridyl)-5-triazine] acted as the chromogen and utilized TPTZ was measured. In an acidic medium, transferrin-bound iron dissociates into free ferric ions and apo-transferrin. Hydrochloric acid

and sodium ascorbate reduce the ferric ions to the ferrous state. The ferrous ions then react with TPTZ to form a blue coloured complex, which can be measured bichromatically at 600/800 nm. The increase in absorbance is directly proportional to the amount of iron present.

Chemical reaction scheme:



Buffer

2(Fe3+) + Apo-transferrin 2 Fe2+ + Dehydroascorbic Acid + 2 H3O + Iron-complex2+ (blue coloured complex).

Normal range of serum iron in adults:

Male 12.5-32.2 μmol/L (70-180 μg/dL)

Female 10.7-32.2 μmol/L (60-180 μg/dL)

Ferritin, transferrin and transferrin saturation

An immuno-turbidimetric test was used for the quantitative determination of ferritin and transferrin in human serum and plasma on Beckman Coulter analysers.

Latex agglutination reactions occur as a result of antibody-coated latex beads aggregating if antigen is present in sufficient quantity. Immune complexes formed in solution scatter light in proportion to their size, shape and concentration. Under

conditions of antibody excess, increasing amounts of antigen result in higher scatter. Turbidimeters measure the reduction of incident light due to reflection, absorption, or scatter.

Transferrin was used in conjunction with iron to calculate TSAT.

Methodology for transferrin

When a plasma sample is mixed with R1 buffer and R2 antiserum solution, human transferrin reacts specifically with anti-human transferrin antibodies to yield insoluble aggregates. The absorbance of these aggregates is proportional to the transferrin concentration in the sample.

Calculations

The Beckman Coulter analysers automatically compute the transferrin concentration of each sample.

Normal range of serum transferrin (Adults): 2.0 – 3.6 g /L (200 – 360 mg/dL)

Methodology for ferritin

In the Beckman Coulter procedure, the measurement of the decrease in light intensity transmitted (increase in absorbance) through particles suspended in solution as a result of complexes formed during the antigen-antibody reaction, is the basis of this assay. The anti-ferritin reagent is a suspension of polystyrene latex particles, of uniform size, coated with polyclonal rabbit anti-ferritin antibody. When serum, containing ferritin, is mixed with the anti-ferritin reagent, an agglutination mixture occurs. This is measured spectrophotometrically on Beckman Coulter Chemistry Analyzers.

For patients with heart failure, iron deficiency was defined as serum ferritin < 100 μg/L (absolute iron deficiency), or serum ferritin between 100 and 299 μg/L with TSAT < 20% (functional iron deficiency), according to the criteria used in the FAIR-HF study, as reported in the ESC heart failure guidelines 2012. Anaemia was defined according to the World Health Organization as haemoglobin < 130 g/L for men and < 120 g/L for women. In healthy age-matched controls, serum ferritin < 20 μg/L was considered as iron deficient (as per local biochemistry laboratory protocols).

Transferrin saturation was calculated as (Iron in μg/dL / Transferrin in mg/dL) x 71.24. An alternative calculation is (Ferritin/ Total iron binding capacity) x 100. TIBC reflects transferrin, the protein to which virtually all iron in the blood is bound.

STATISTICAL ANALYSIS

The primary endpoint was the prevalence of iron deficiency in patients hospitalized for decompensation of CHF. Secondary endpoints included the prevalence of iron deficiency in non-anaemic and anaemic patients, the prevalence of absolute and functional iron deficiency and evaluation of factors associated with iron deficiency. Statistical analyses were performed using SPSS software version 24.
Summary statistics (e.g., mean with standard deviation if normally distributed variables, medians with upper and lower quartiles if not normally distributed and proportions where applicable) were calculated for all variables. T- tests were carried out for normally distributed variables and repeated measures that were not normally distributed were compared using non-parametric tests (Mann-Whitney U test for continuous variables and McNemar for categorical variables). Pearson's

correlation coefficients were calculated to assess the relationships between the various parameters and serum ferritin and TSAT. Where the relationship was non parametric, the Spearman correlation coefficient was used instead of the Pearson. A p value of < 0.05 was considered to be statistically significant. Serum hepcidin, TNF alpha, Cystatin C and IL-6 were log transformed for comparison purposes.

RESULTS

Recruitment took place from February 2014 to November 2015. The pace of recruitment slowed after January 2015 to completion, due to my return to clinical commitments. The study came to a close in November 2016 following collection of 1-year follow up data on the last recruited patient.

Patient characteristics

Acute heart failure cohort

100 near consecutive consenting patients with a clinical diagnosis of heart failure were recruited to the ADHF arm of the study. One patient's diagnosis was subsequently not felt to be heart failure during the course of the admission hence all data pertaining to this patient was removed from data analysis. 2 patients withdrew from the study prior to discharge from hospital. 2 patients were lost to follow up and 10 patients withdrew from the study following discharge from hospital citing mobility reasons and comorbidities. All of these patients were agreeable to have 1-year follow up data collected using the predetermined methods. Figure 2.4 illustrates the follow up rates among all patients recruited. Discharge visits were planned by keeping track of every patient recruited on a daily basis. 26 patients left

hospital without having a discharge visit as per protocol. All patients were however contacted regarding subsequent follow up visits.

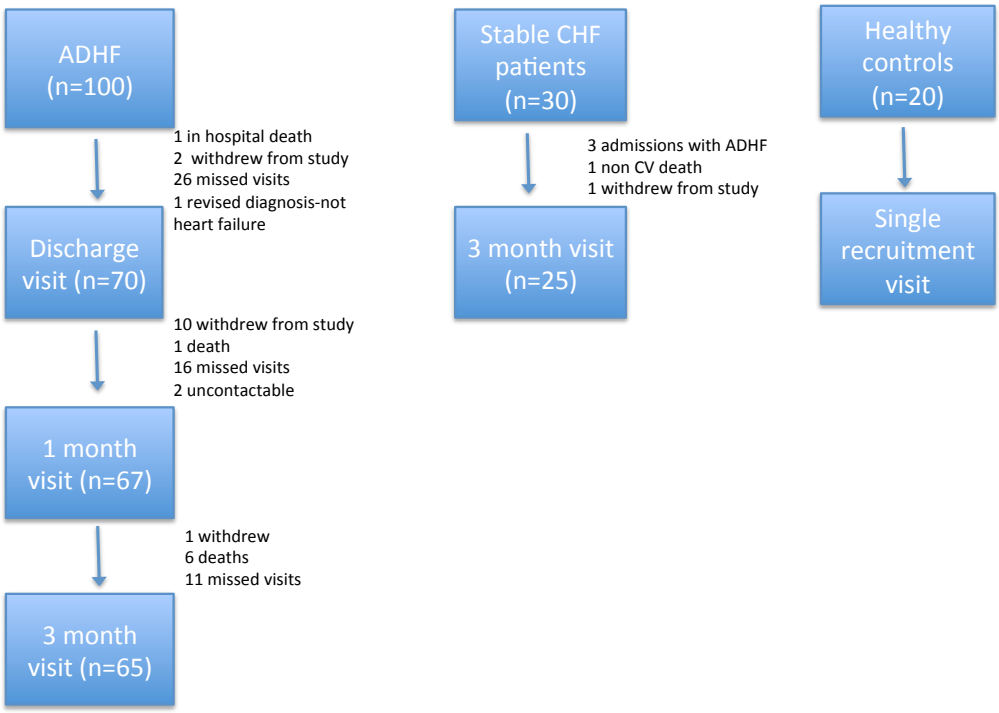


Figure 2.4- Flow diagram depicting recruitment and follow up of patients to each arm of the IRON STATS DHF Study.

Patients who missed visits were invited for subsequent follow up visits at all stages in the study. Patients who received intravenous iron were excluded from further analysis.

Mean age of the ADHF cohort was 74 ± 10 years with 55% being males. Mean length of hospital stay was 12 ± 6 days, with 3 patients receiving intravenous iron during their in-patient stay. The patients who received intravenous iron were invited for follow up visits but were excluded from repeated measures analysis and outcome models. 6 patients were on oral iron preparations at the time of admission to hospital; data on these patients were included in all analyses. Majority of patients

were in NYHA class III or IV. 61% of patients were anaemic and 80% of anaemic patients were iron deficient. 73% of those who were not anaemic as per WHO guidelines were iron deficient. (27) 41% of patients had heart failure with LVEF > 50%. Baseline characteristics are depicted in Table 2.3.

Resting heart rate was higher at the time of admission to hospital. Mean weight loss during inpatient stay was 6 ± 2 kg. CRP was significantly greater in the ADHF cohort.

3 patients had biventricular pacemakers, 2 had defibrillators and 10 had simple pacemakers in situ at the time of admission. Following discharge, one of these patients had an upgrade to a CRTP, 4 patients received de novo CRT devices (of which 2 were defibrillators) and two patients received pacemakers (VVI and DDD for slow AF and complete heart block respectively; both of these patients had heart failure with preserved LVEF).

Comparator arm

The stable CHF cohort was comprised of 30 patients; mean age was 71 ± 11 years with 70% being males. 50% had biventricular pacemakers and 17% had defibrillators. 3 patients had CRTPs implanted during the course of the study.

The 20 age-matched participants comprising the healthy cohort had no history of any CVD and were not on regular medication. Mean age was 71± 6 years and 80% were female.

	ADHF (N=99)	Stable CHF (N=30)	p value (comparing ADHF with stable CHF)	Healthy control (N = 20)
Age (mean ± SD in years)	74 ± 10	71 ± 11	0.632	72 ± 6
Males (%)	55	70	0.043	20
NYHA Functional class (%)				
I	0	27		N/A
II	0	60		N/A
III	82	13		N/A
IV	18	0		N/A
Systolic BP (mm Hg)	118 (107 - 130)	119 (100 - 140)	0.951	134 ± 18
Diastolic BP (mmHg)	70 (60 - 80)	70 (60 - 80)	0.797	82 ± 11
Heart rate (bpm)	84 (69 - 96)	65 (59 - 75)	0.001	69 ± 10
Mean 6MWT (m)	236 ± 107 (discharge)	318 ± 88		441 ± 87
Co-morbidities				
Hypertension (%)	39	37	0.716	0
Diabetes (%)	34	37	0.744	0
IHD (%)	39	37	0.791	0
Atrial fibrillation (%)	50	57	0596	0
Mean LVEF (%)	45 (30 - 55)	35 (27 - 42)	0.033	60 (55 - 64)
LVEF ≤35% (%)	45	57	0.041	0
LVEF ≥50% (%)	41	10	0.023	100
Treatment of heart failure				
Beta blockers (%)	73	90		0
ACE inhibitors/ ARB (%)	68	93		0
MRA (%)	58	80		0
Treatment of iron deficiency				
Oral iron (%)	6	10		0
Parenteral iron (%)	3	0		0

Table 2.3a- Baseline characteristics of the Iron Stats DHF participants.

Baseline characteristics where relevant, were compared for statistical significance between those with ADHF and stable CHF (p value). Mean ± SD for normally distributed variables and median (with lower and upper quartiles) for variables that are not normally distributed. Heart rate was significantly higher while LVEF and utilisation of evidence-based therapies were lower on admission in the acute heart failure (ADHF) cohort. Values highlighted in red denote statistically significant values.

Laboratory parameters	ADHF (N = 99)	Stable CHF (N = 30)	p value (comparing ADHF with stable CHF)	Healthy control (N = 20)
Haemoglobin (g/L)	122 ± 19	130 ± 14	0.033	135 ± 11
Serum iron (µmol/L)	7.8 (5.7 - 11.5)	12 (9.5 - 16)	0.001	15.7 (11.4 - 18.5)
Serum ferritin (µg/L)	72 (36 - 166)	67 (36 - 226)	0.958	51 (29 - 115)
Transferrin saturation (%)	13 (9 - 18)	19 (13 - 23)	0.005	23 (19 - 34)
Serum erythropoietin (IU/L)	16.8 (8.5 - 27.1)	12 (9.3 - 17.3)	0.181	8.8 (7.1 - 13.0)
Serum sodium (mmol/L)	137 (134 - 139)	137 (134 - 139)	0.523	138 (137 - 139)
Serum urea (mmol/L)	9.8 (7.0 - 15.6)	9.6 (7.3 - 17.2)	0.907	5.6 (4.6 - 6.2)
Serum creatinine (µmol/L)	107 (79 - 140)	103.5 (81 - 154.8)	0.835	65 (61 - 77)
eGFR (ml/min/1.73m^2)	49 (36 - 66)	61 (48 - 68)	0.382	72 ± 15
C-reactive protein (mg/L)	11 (6 - 29)	4 (1 - 8)	0.001	2 (1 - 6)
Serum hepcidin (ng/mL)	40.03 (7.8 - 69.0))	26.4 (18.0 - 52.2)	0.460	25.62 (17.9 - 49.7)
Serum IL-6 (pg/mL)	9.71 (4.1 - 23.1)	4.02 (1.3 - 15.1)	0.145	1.50 (0.5 - 8.5)
Serum Cystatin-C (mg/L)	2.23 (1.7 - 3.0)	2.08 (1.7 - 3.1)	0.962	1.42 (1.1 - 1.7)
Serum TNF-alpha (pg/mL)	4.76 (1.4 - 13.1)	11.57 (2.9 - 73.5)	0.532	0.001(0.0 - 8.5)

Table 2.3b- Baseline characteristics of the Iron Stats DHF participants (laboratory parameters).

Baseline characteristics were compared for statistical significance between those with ADHF and stable CHF (p value). Mean ± SD for normally distributed variables and median (with lower and upper quartiles) for variables that are not normally distributed. CRP was significantly greater in the acute heart failure (ADHF) cohort while serum iron was significantly lower when compared to the stable CHF patients. Values highlighted in red denote statistically significant values

Comparing patients recruited to the ADHF and control arms, haemoglobin, serum iron, ferritin, TSAT and CRP were lower in patients with ADHF. Patients in the stable CHF arm also had a significantly lower LVEF.

Prevalence of iron deficiency (Table 2.4)

83% of patients admitted with ADHF had iron deficiency at admission to hospital. 63% of patients with chronic stable heart failure were iron deficient while 10% of healthy age-matched controls were iron deficient, i.e. ferritin < 20 µg/L (67% had ferritin < 100 µg/L and no patients had TSAT < 20%).

Iron deficiency was seen to be absolute as well as functional, the proportion of functional iron deficiency being significantly greater in the ADHF cohort (p = 0.023). None of the healthy controls had functional iron deficiency. 6 patients in the ADHF cohort had ferritin ≥ 300 µg/L with TSAT < 20%. As per pre-defined protocol these patients were not considered as iron deficient; mean hepcidin in this population was 88.52 ng/ml. 6 patients in this cohort who had a ferritin < 100 µg/L had a TSAT > 20%.

	ADHF (admission)	Stable CHF	p value	Healthy control
Prevalence of iron deficiency (%)	83 (n = 83)	63 (n = 19)	0.042	10 (n = 2)
Absolute iron deficiency (%)	81 (n = 67)	95 (n = 16)	0.023	100 (n = 2)
Functional iron deficiency (%)	19 (n = 16)	5 (n = 3)	0.003	0 (n = 0)

Table 2.4- Prevalence of iron deficiency, classified as absolute and functional.

Absolute and functional iron deficiency percentages are expressed as a proportion of total iron deficiency. Absolute iron deficiency in healthy control patients was defined as ferritin < 30 µg/L.

Where iron deficiency was present at baseline, serum hepcidin was lower in the presence of ADHF compared to those with stable CHF and iron deficiency at baseline (14.14 vs. 19.51 ng/mL; $p = 0.050$). Comparing patients with and without iron deficiency at baseline, in the ADHF cohort, serum ferritin, TSAT, hepcidin, C-reactive protein and IL-6 were significantly lower in those with iron deficiency (Table 2.5).

Biological parameters	ADHF (Without iron deficiency at baseline) N = 17	ADHF (With iron deficiency at baseline) N = 82	p value
Haemoglobin (g/dL)	127 ± 22	120 ± 18	0.218
Serum ferritin (µg/L)	323 (189 - 468)	61 (31 - 95)	0.001
Serum iron (µmol/L)	11.5 (6.5 - 20.4)	7.4 (5.5 - 10.9)	0.141
Transferrin saturation (%)	19 (13 - 34)	12 (9 - 16)	0.042
Serum hepcidin (ng/mL)	72.97 (47.9 - 98.2)	14.14 (2.2 - 53.4)	0.003
Serum erythropoietin (IU/L)	12 (5.5 - 20.7)	18 (9.84 - 29.10)	0.617
C-reactive protein (mg/dL)	15.5 (10.3 – 44.0)	10.0 (5 - 21.5)	0.018
Serum creatinine (µmol/L)	111(80 - 200)	105 (79 - 139)	0.802
eGFR (1.73ml/min/m^2)	50 (40 - 72)	45 (34 - 66)	0.739
Serum urate (mmol/L)	0.56 ± 0.23	0.52 ± 0.13	0.850
Cystatin C (mg/L)	2.19 (1.6 - 3.3)	2.35 (1.7 - 3.1)	0.784
IL-6 (pg/mL)	9.85 (1.5 - 44.7)	5.92 (1.4 – 16.0)	0.038
TNFα (pg/mL)	2.41 (0.0 - 6.9)	0.997 (0.0 - 5.1)	0.784

Table 2.5- Baseline characteristics differentiating ADHF patients into those with and without iron deficiency at admission to hospital.

Mean ± SD for normally distributed variables and median (with lower and upper quartiles) for variables that are not normally distributed. All statistically significant values, i.e. p < 0.05 are shown in red font.

Factors determining iron deficiency in the acute heart failure cohort

The population was categorised based on absolute versus functional iron deficiency at baseline and there was a significant difference in hepcidin levels (mean 20.30 ± 29.77 ng/mL in the absolute iron deficiency group vs. 67.09 ± 39.38 ng/mL in the functional iron deficiency group, p = 0.001)

Relationships between TSAT, serum ferritin and each of the other markers were evaluated by drawing correlation plots and calculating Pearson's correlation coefficients. Baseline ferritin weakly correlated with baseline CRP levels (p = 0.05, r = 0.266). Serum hepcidin correlated strongly with serum ferritin (p = 0.001, r = 0.699) and weakly with the presence of iron deficiency (p = 0.001, r = -0.384). IL6 and TNF alpha did not demonstrate any significant correlations with markers of iron status.

To evaluate the impact of inflammation on iron status, the acute heart failure cohort was categorized into two, with the median CRP value of 11 mg/L as cut off. In those with raised CRP, there was a strong correlation between ferritin and hepcidin (p = 0.001, r = 0.663) as well as serum iron and TSAT (p = 0.001, r = 0.913).

Factors determining iron deficiency in the stable CHF cohort.

Baseline ferritin and iron levels correlated with baseline TSAT (p = 0.001, r = 0.753 and p = 0.001, r = 0.591 respectively). Baseline iron levels and eGFR correlated with baseline ferritin levels (r = 0.759, p = 0.001and r = - 0.918, p = 0.028 respectively).

On categorizing the stable CHF population as per median CRP (cut off 4 mg/L), in those with CRP < 4 mg/L, ferritin correlated with iron and TSAT at baseline ($p = 0.002$, $r = 0.807$ and $p = 0.002$, $r = 0.793$ respectively). Serum hepcidin at baseline correlated with baseline ferritin ($p < 0.001$, $r = 0.883$) and iron at baseline ($p = 0.042$, $r = 0.594$). Serum iron correlated with TSAT at baseline and ($p < 0.001$, $r = 0.981$) and hepcidin at baseline ($p = 0.042$, $r = 0.594$).

In those with CRP > 4 mg/L, ferritin correlated with hepcidin ($p = 0.001$, $r = 0.787$) and serum iron correlated with TSAT ($p = 0.033$, $r = 0.615$). Hepcidin correlated with TSAT ($p = 0.013$, $r = 0.688$).

The proportion of functional iron deficiency in this cohort was too small to compute differences between those with absolute and functional iron deficiencies.

DISCUSSION

Iron deficiency is very common in ADHF and is seen more frequently in ADHF than in stable CHF in our study. Functional iron deficiency is more commonly seen in decompensated heart failure compared to the stable CHF population. None of the healthy patients in this study had functional iron deficiency. Absolute iron deficiency in the ADHF cohort was associated with lower hepcidin levels. Hepcidin levels in those with iron deficiency at baseline were significantly lower than those without iron deficiency. The role of hepcidin and inflammatory cytokines appeared to be superseded by the presence of absolute iron deficiency

Ferritin is an acute phase reactant as evidenced by its correlation with CRP levels at admission in ADHF patients. Ferritin can therefore be artificially elevated in the setting of acute decompensation. There nevertheless remains a correlation between hepcidin and ferritin in this population. This was demonstrated even after categorising patients as per median CRP values suggesting that serum hepcidin values in this cohort is likely subsequent to the development of iron deficiency. It does not appear to be driving the iron deficiency as initially thought.

Hepcidin is emerging as a central regulatory molecule in systemic iron homeostasis. The role of hepcidin in heart failure is of interest. Anaemia in CHF has been linked with lower hepcidin levels. A study by Matsumoto *et al* in 61 stable CHF patients and 16 healthy control subjects demonstrated this and also showed that EPO and ferritin but not IL-6 were independent predictors of serum hepcidin in multivariable regression analysis. (228) Another study by Divakaran *et al* in 97 patients with CHF and 38 control subjects showed serum hepcidin to be reduced in heart failure but not significantly lower than in controls. (229) The presence of anaemia did not appear to drive hepcidin levels in this study.

Both of these studies would suggest that hepcidin might not drive the development of iron deficiency in patients with heart failure.

The findings of IRON STATS- DHF would also suggest that in this very sick population, any potential impact hepcidin may have secondary to inflammation appears to be outweighed by its response to serum iron and the presence of absolute iron deficiency. Despite the on-going inflammatory activation that accompanies

decompensation (elevated CRP), the prevalence of absolute iron deficiency appears to override any potential influence that hepcidin may have on iron status. Functional iron deficiency, although more frequent than in stable CHF, appears to be a minor cause of iron deficiency once ADHF sets in. The findings of this study cannot however exclude the role hepcidin may play in the days leading up to decompensation. There were no significant differences in hepcidin levels between those with decompensated and stable heart failure, even after categorising patients according to CRP levels. Levels of hepcidin were however lower in the presence of iron deficiency in the decompensated patients, reflecting the greater prevalence of absolute iron deficiency in this cohort.

The healthy age-matched cohort had a 10% prevalence of iron deficiency, of note this cohort comprised mainly of females. All patients with iron deficiency had absolute iron deficiency. Studies of reference values have suggested that females in the general population have higher hepcidin levels and amongst females, increasing age is associated with increased hepcidin levels. (230) This may explain the reason for higher than normal values for hepcidin in the healthy cohort and the absence of a significant difference in hepcidin levels between the 3 groups of participants.

A similar study of ADHF patients showed a similar prevalence of 74% for iron deficiency with absolute iron deficiency being twice as common. (231) Patients were evaluated pre-discharge from hospital and those with absolute iron deficiency showed an increased rate of 30-day readmission compared with those with functional iron deficiency and no iron deficiency (19.9, 13, and 13.5%, respectively, $p = 0.005$). In a multivariate model absolute iron deficiency and not

functional iron deficiency remained associated with higher risk of readmission [HR1.72; 95% CI 1.13 - 2.60, p = 0.011].

The findings of this study also raise the question as to what role the development of iron deficiency may play in the process of decompensation.

Limitations

The actual impact of decompensation could not be measured with a measure of iron status pre decompensation; this would not be practical in an acute heart failure population. A measure of hepcidin and iron status markers in newly diagnosed patients with heart failure and subsequent follow up to a potential decompensation would be a more accurate reflection of the role of hepcidin in iron status in the presence of decompensation. As majority of the stable CHF cohort patients were recruited from the heart failure nurse clinics, these patients as per current clinic protocols had LVSD and therefore the LVEF in this cohort was less. Gender differences and the older age of the cohort may also have contributed to baseline iron status.

CONCLUSIONS

Iron deficiency is very common in ADHF with absolute iron deficiency being the common finding. The impact of developing an acute iron deficient state in an otherwise stable heart failure patient remains unclear. Whether there is a potential role for the development of iron deficiency in causing subsequent decompensation needs to be elucidated in larger prospective studies.

CHAPTER 3- IRON STATUS IN ACUTE HEART FAILURE FOLLOWING DECOMPENSATION.

BACKGROUND

Iron deficiency in heart failure has been linked to poor exercise tolerance and poor CV outcome. Iron deficiency is significantly associated with an increased risk for heart failure admission or all-cause mortality irrespective of the presence of anaemia. The utility of prognostic therapies has also been demonstrated to be affected by the presence of iron deficiency. A recent CRT study demonstrated less symptomatic improvement 6 months after implantation as well as significantly lower reduction in LV end-diastolic diameter (-3.1 vs. -6.2 mm; $p = 0.011$) and improvement in ejection fraction (+11% vs. +15%, $p = 0.001$) in the presence of iron deficiency. The results of the IRON STATS-DHF confirmed the high prevalence of iron deficiency in decompensated heart failure. During the course of clinical recovery, a reversal of inflammatory over activity and down regulation of neurohormonal activity would be anticipated. How this translates to changes in iron status is not known. This chapter explores these changes in iron status over time following an episode of decompensation as well as the subsequent impact on outcomes.

AIMS

- To outline the change in iron status over time following discharge from hospital through to a stable state.
- To understand the relationship between iron deficiency, CKD and inflammatory immune activation.

METHODS

Data obtained from the patients recruited to the ADHF and stable CHF arms were used for this part of the IRON STATS-DHF study.

Follow up in the ADHF cohort occurred at discharge (or as soon as deemed medically fit, whichever occurred first), 1month post discharge and 3 months post discharge. Details of follow up visits have been outlined in chapter 2. At 12 months post enrolment, data on CV hospitalisation, CV mortality and all-cause mortality were collected by scanning hospital records/ contacting the patient and patient's GP via telephone.

Quality of life assessments were carried out using KCCQ questionnaires. An overall summary score was derived from the physical function, symptom (frequency and severity), social function and quality of life domains. Clinical summary score was calculated from the physical function and symptom (mean of frequency and severity) domains. Scores are transformed to a range of 0 - 100, in which higher scores reflect better health status.

Patients were categorised as per the presence or absence of iron deficiency at admission (recruitment) for further analysis. Analysis was also carried out in the uncategorised population.

STATISTICAL ANALYSIS

Statistical analyses were performed using SPSS software version 24. Summary statistics (e.g., mean, standard deviation, minimum, maximum, proportions) were

calculated for all variables. A repeated measures ANOVA was used for assessing the changes in parameters of iron deficiency over time. Where Mauchly's test of sphericity was not satisfied, Greenhouse Gaisser corrections were applied. Repeated measures that were not normally distributed were compared following logarithmic transformation or using non-parametric tests (Wilcoxon-rank for paired continuous variables, Friedman's test for >2 samples). Normality was tested for using Shapiro-Wilk tests. Pearson's correlation coefficients were calculated to assess the relationships between the various parameters and serum ferritin and TSAT. Where the relationship was non parametric, the Spearman correlation coefficient was used instead of the Pearson. Predictive values of various variables were compared using binomial logistic regression models. A p value of < 0.05 was considered to be statistically significant.

RESULTS

ACUTE HEART FAILURE COHORT

Baseline characteristics (Table 3.1)

In the acute heart failure cohort, during follow up from admission to 3 months post discharge, there was a significant reduction in heart rate, body weight, systolic and diastolic blood pressure. Mean weight loss during admission was 6 ± 2 kg. Exercise tolerance steadily improved from discharge to 3 months post discharge, 6MWT distances were 236 ± 107 m, 278 ± 93.4 m, 283.2 ± 139.5 m at discharge, 1 and 3 months respectively ($p = 0.001$).

Changes in iron status over time

83% of patients admitted to hospital with ADHF were noted to be iron deficient. At 3 months post discharge, 65% remained iron deficient with 17% having functional iron deficiency. There was no significant difference in the proportion of absolute vs. functional iron deficiency at the 4 time points measured. There were no significant variations in haemoglobin levels in the study cohort; this was irrespective of whether there was iron deficiency at baseline (Figure 3.1a and 3.1b).

Mean serum iron and TSAT in the population improved over time (Table 3.1). There were non-significant variations over time with serum ferritin. Repeated measures analysis to evaluate within subject variations demonstrated a statistically significant improvement in serum iron and TSAT, both of which steadily increased from admission to 3 months post discharge reaching a plateau at 1-month post discharge from hospital. Serum hepcidin demonstrated significant variations across the acute heart failure cohort.

Biological parameters	ADHF (admission) N = 99	ADHF (discharge) N = 60	ADHF (1 month) N = 67	ADHF (3 months) N = 65	p value
Heart rate (bpm)	84 (69 - 96)	77 (67 - 88)	69 (62 - 88)	70 (62 - 79)	0.001
Systolic BP (mm Hg)	118 (107-130)	110 (100-120)	113 (100-129)	113 (100 - 130)	0.328
Diastolic BP (mm Hg)	70 (60 - 80)	68 (60 - 77)	68 (58 - 78)	68 (60 - 74)	0.206
Body weight (kg)	84.9 ± 21.3	82.0 ± 19.1	79.42 ± 19.44	79.83 ± 19.14	0.002
6MWT (m)	-	236 ± 107	278 ± 93.43	283.20 ± 139.51	0.001
Haemoglobin (g/L)	122 ± 19	124 ± 20	124 ± 28	123 ± 14	0.277
Mean corpuscular volume (μm^3)	93.8 (89.5 - 97.3)	93.2 (89.7 - 97.7)	92.44 (88.1 - 95)	92.7 (89.7 - 96.0)	0.060
Serum ferritin (µg/L)	72 (36 - 166)	93 (50 - 189)	111 (39 - 220)	95 (55 - 191)	0.573
Serum iron (µmol/L)	7.8 (5.7 - 11.5)	10.4 (6.7 - 17.0)	13.9 (9.7 - 19.2)	14.5 (10.3 - 19.1)	0.001
Transferrin saturation (%)	13 (9 - 18)	14 (12 - 24)	21 (15 - 33)	23 (16 - 28)	0.001
Serum hepcidin ng/mL	40.0 (7.8 - 69.0)	41.6 (15.2 - 85.4)	50.2 (27.2 - 78.3)	44.4 (19.8 - 68.3)	0.010
Serum erythropoietin (IU/L)	16.8 (8.5 - 27.1)	18.8 (11.6 - 31.2)	11.2 (7.4 - 21.9)	13.65 (9.1 - 19.8)	0.089
C-reactive protein (mg/L)	11 (6 - 29)	10 (7 - 20)	6 (2- 15)	4 (2 - 8)	0.001
Serum sodium (mEq/L)	137 (134 - 139)	6 (133 - 137)	136 (133 - 138)	136 (135 - 138)	0.402
Serum creatinine (µmol/L)	107 (79 - 140)	108 (80 - 141)	105 (82 - 147)	103 (79.5 - 132.5)	0.159
eGFR (ml/min/1.73m^2)	49 (36 - 66)	46 (34 - 62)	53 (35 - 70)	49 (37 - 65)	0.308
Serum urate (mmol/L)	0.53 ± 0.15	0.58 ± 0.19	0.53 ± 0.18	0.51 ± 0.15	0.042
Cystatin C (mg/L)	2.23 (1.7 - 3.0)	2.91 (1.9 - 3.5)	2.60 (1.8 - 3.3)	2.37 (1.8 - 3.1)	0.049
IL-6 (pg/mL)	9.71 (4.1 - 23.1)	9.42 (4.3 - 36.3)	7.83 (2.5 - 36.8)	2.57 (0.0 - 13.4)	0.254
TNFα (pg/mL)	4.76 (1.4 - 13.1)	4.35 (1.8 – 15.0)	5.44 (2.1 - 21.0)	4.74 (2.4 - 18.9)	0.90

Table 3.1- Comparison of parameters within subjects over period of follow up.

Mean ± SD for normally distributed variables and median (with lower and upper quartiles) for variables that are not normally distributed. Heart rate, body weight and walk distances improved with time following admission with ADHF. Significant variations were seen in serum iron, transferrin saturation and hepcidin levels. Red font represents values that reached statistical significance. Comparisons were made following logarithmic transformation of variables that were not distributed normally.

Trends over time- iron deficiency at baseline vs no iron deficiency at baseline

In those with no iron deficiency at baseline, a significant reduction in ferritin levels was noted over time, implying the role of ferritin as an acute phase reactant in this population (Table 3.2b). The converse was also seen, i.e. a steady increase in serum ferritin levels in those with iron deficiency at baseline. There were no significant variations in haemoglobin (Figure 3.4a and 3.4b).

Where iron deficiency was present at baseline, serum ferritin, serum iron and TSAT significantly improved over time (Table 3.2b,). Serum EPO, urea creatinine, Cystatin C, CRP were also seen to have significant variations over time.

On classifying subjects as per the presence or absence of iron deficiency at baseline, a significant variation in hepcidin levels was only seen in those with iron deficiency at baseline (Table 3.2a).

The patients who received intravenous iron during their hospital admission had ferritin levels of 31, 16 and 104 μg/L with TSAT of 7, 3 and 11%. At 3 months, ferritin levels were 90 and 33 μg/L while TSAT was 22 and 23% for the first two patients. The 3rd patient withdrew from the study and the first patient died 299 days after the hospital admission.

Biological parameters	ADHF (admission)	ADHF (1 month)	ADHF (3 months)	p value
Without iron deficiency at admission (N = 17)				
Haemoglobin (g/L)	127 ± 22	135 ± 23	126 ± 17	0.772
Mean corpuscular volume (μm^3)	96.63 ± 4.12	93.03± 5.11	94.8 ± 5.2	0.053
Serum ferritin (µg/L)	323 (189-468)	243 (87-375)	125 (63-208)	0.002
Serum iron (µmol/L)	12.12 ± 6.34	15.73 ± 5.40	13.42 ± 5.23	0.636
Transferrin saturation (%)	21 ± 10	26 ± 9.42	21 ± 6	0.875
Serum hepcidin (ng/mL)	76.65 (49.47-98.38)	64.05 (24.73-99.85)	47.66 (22.45-94.03)	0.424
Serum erythropoietin (IU/L)	12 (5.51-20.7)	14.3 (4.24-56.81)	16.5 (11.81-19.62)	0.223
C-reactive protein (mg/L)	15.5 (10.32-44)	7 (2-17)	7 (1-12)	0.034
Serum sodium (mEq/L)	135 (132-140)	136 (132-139)	136 (133-138)	0.991
Serum urea (mmol/L)	9.51 (7.31-17.92)	8.7 (7.21- 15.82)	12.32 ± 4.84	0.734
Serum creatinine (µmol/L)	111(80-200)	120 (73-174)	126 (73-174)	0.565
eGFR (ml/min/1.73m^2)	50 (40-72)	53 (46-78)	49 (32-65)	0.635
Serum urate (mmol/L)	0.56 ± 0.23	0.61 ± 0.23	0.55 ± 0.19	0.120
Cystatin C (mg/L)	2.19 (1.62-3.28)	2.28 (1.63-3.25)	2.37 (1.67-2.58)	0.282
IL-6 (pg/mL)	13.36 (9.21-56.15)	5.7 (1.79-29.72)	4.83 (1.15-14.22)	0.472
TNF alpha (pg/mL)	6.24 (3.40- 36.50)	0.01 (0.01-4.42)	1.31 (0.01-3.86)	0.560

Table 3.2a. Comparison of parameters within subjects with iron deficiency at admission.

3 time points were considered, i.e. admission, 1 month post discharge and 3 months post discharge. Mean ± SD for normally distributed variables and median (with lower and upper quartiles) for variables that are not normally distributed. Statistically significant values are highlighted in red font.

Biological parameters	ADHF (admission)	ADHF (1 month)	ADHF (3 months)	p value
With iron deficiency at admission (N= 82)				
Haemoglobin (g/L)	120 ± 18	121 ± 29	123 ± 13	0.197
Mean corpuscular volume (μm^3)	92.7 (89.10-96.34)	91.7 (88-95.12)	92.5 (89.42-95.73)	0.076
Serum ferritin (µg/L)	61 (31-95)	95 (38-178)	90 (46-181)	0.007
Serum iron (µmol/L)	7.4 (5.52-10.92)	12.7(9.31-19.62)	14.5 (10.33-19.42)	0.001
Transferrin saturation (%)	12 (9-16)	20 (14-33.34)	23 (16-30)	0.001
Serum hepcidin (ng/mL)	14.14 (2.18-53.39)	45.88 (13.14-68.79)	41.6 (17.12-64.82)	0.002
Serum erythropoietin (IU/L)	18 (9.48-29.10)	11.6 (7.61-22.10)	13.41 (8.52-19.25)	0.034
C-reactive protein (mg/L)	10.0 (5-21.52)	5 (2-12)	3.5 (2-7)	0.001
Serum sodium (mEq/L)	137 (134-139)	135 (133-138)	136 (135-138)	0.727
Serum urea (mmol/L)	9.83 (6.82-15.63)	11.2 (7.21-19.62)	10.6 (7.6-17.3)	0.001
Serum creatinine (µmol/L)	105 (79-139)	104 (83-146)	102 (80-133)	0.007
eGFR (ml/min/1.73m^2)	45 (34-66)	49 (32-66)	49 (37-64)	0.359
Serum urate (mmol/L)	0.52 ± 0.13	0.52±0.17	0.50 ± 0.14	0.086
Cystatin C (mg/L)	2.35 (1.73-3.06)	2.73 (1.85-3.38)	2.49 (1.82-3.26)	0.048
IL-6 (pg/mL)	5.92 (1.35-15.99)	5.58 (0.57-20.34)	1.96 (0.01-13.3)	0.343
TNF alpha (pg/mL)	0.997 (0.01-5.10)	2.31(0.01-12.52)	1.71 (0.01-7.04)	0.066

Table 3.2b. Comparison of parameters within subjects with no iron deficiency at admission.

3 time points were considered, i.e. admission, 1 month post discharge and 3 months post discharge. Mean ± SD for normally distributed variables and median (with lower and upper quartiles) for variables that are not normally distributed. Statistically significant values are highlighted in red font.

Trends over time- absolute vs functional iron deficiency at baseline.

The prevalence of iron deficiency declined over time reaching its lowest at 1 month post discharge. At all time points, the proportion of all iron deficient patients with functional iron deficiency was approximately 20%. (Table 3.3)

	ADHF admission N = 100	**ADHF discharge N = 60**	**ADHF 1 month N = 67**	**ADHF 3 months N = 65**
Prevalence of iron deficiency (%)	83 (n = 83)	70 (n = 42)	59 (n = 39)	65 (n = 42)
Absolute iron deficiency (%)	81 (n = 67)	81 (n = 34)	79 (n = 31)	83 (n = 35)
Functional iron deficiency (%)	19 (n = 16)	19 (n = 8)	21 (n = 8)	17 (n = 7)

Table 3.3- Prevalence of iron deficiency at the different time points.
Absolute and functional iron deficiency percentages were expressed as a proportion of total iron deficiency.

A steady increase was demonstrated in serum TSAT in those with functional iron deficiency at admission. Patients with absolute iron deficiency also had a significant increase in TSAT over time (Figure 3.1). In Chapter 2, a significant difference was demonstrated in baseline hepcidin levels between those with absolute and functional iron deficiency at baseline. The variations of hepcidin level within these two groups were only significant for those with absolute iron deficiency at admission (Figure 3.2).

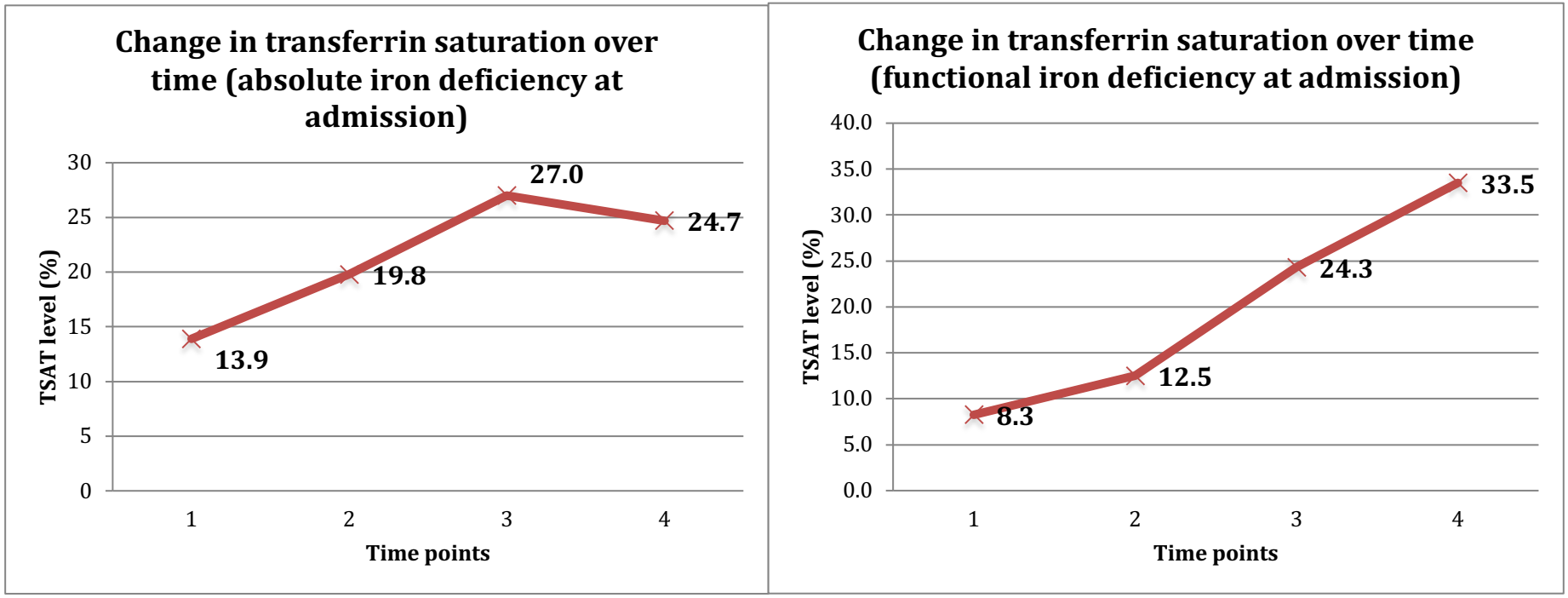


Figure 3.1- Change in transferrin saturation over time in ADHF patients, absolute vs. functional iron deficiency at admission.

Transferrin saturation was seen to change significantly within both categories of patients (p = 0.004 for those with absolute iron deficiency and p = 0.045 for those with functional iron deficiency at admission).

Time on x-axis (1=admission, 2=discharge, 3=1month post discharge, 4=3 months post discharge)

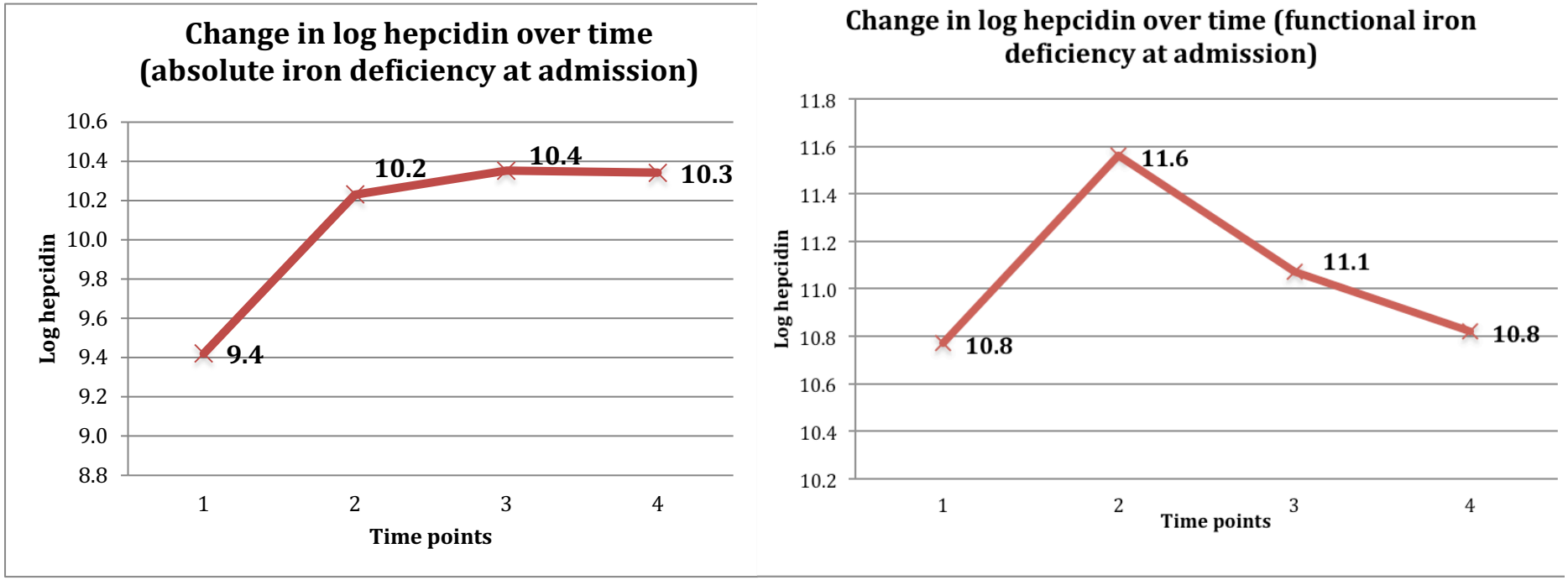


Figure 3.2- Variation in serum hepcidin within subjects, classified as absolute vs. functional deficiency.

Serum hepcidin levels (ng/mL) were compared between admission, discharge, 1 month and 3 months post discharge after categorising the population according to the type of iron deficiency seen at admission. There was significant variation in hepcidin levels in those with absolute iron deficiency at admission (p = 0.001) but not in those with functional iron deficiency at admission (p = 0.886). Logarithmic transformations of hepcidin levels were carried out prior to analysis.

Time on x-axis (1= admission, 2= discharge, 3= 1month post discharge, 4= 3 months post discharge)

On categorizing the ADHF population with the admission median value of 11 mg/L as cut off, in those with raised CRP, there was a strong correlation between ferritin and hepcidin ($p < 0.001$, $r = 0.663$), ferritin and TNF alpha ($p = 0.031$, $r = 0.031$), serum iron and TSAT ($p < 0.001$, $r = 0.913$).

There were no significant correlations between change in TSAT between admission and 3 months and hepcidin in any of these groups.

Quality Of Life Assessments

In the ADHF cohort, QOL was assessed using the KCCQ questionnaire. There was a significant improvement in all parameters of the KCCQ except symptom stability (Table 3.4). Overall summary score and clinical summary score at discharge correlated with the presence of iron deficiency at 3 months post discharge ($p = 0.025$, $r = 0.357$ and $p = 0.045$, $r = 0.323$ respectively). Overall summary score at 1 month and 3 months correlated with all cause mortality and CV events ($p < 0.001$, $r = -0.434$ and $p = 0.002$, $r = -0.381$ for all cause mortality; $p = 0.001$, $r = -0.392$ and $p = 0.001$, $r = -0.396$ for CV events). Clinical summary score at 1 month and 3 months also correlated with all cause mortality and CV events ($p = 0.001$, $r = -0.392$ and $p = 0.003$, $r = -0.363$ for all cause mortality; $p = 0.001$, $r = -0.392$ and $p = 0.002$, $r = -0.377$ for CV events).

	Discharge	1 month	3 months	p value
Physical limitation score	25 (15-54)	58 (34-83)	54 (25-85)	0.011
Symptom stability score	75 (50-100)	75 (50-100)	50 (50-100)	0.106
Total symptom score	27 (46-57)	63 (44-84)	71 (42-88)	0.001
Self efficacy score	75 (50-88)	88 (53-100)	88 (63-100)	0.023
Quality of life score	33 (17-42)	50 (33-75)	58 (33-83)	0.001
Social limitation score	19 (3-38)	44 (19-75)	50 (25-75)	0.003
Overall summary score	30 (20-41)	56 (35-72)	63 (34-81	0.001
Clinical summary score	32 (18-50)	59 (45-78)	64 (33-84)	0.001

Table 3.3- Quality of life (QoL) measurements at various time points in the acute heart failure cohort.

QoL was measured using KCCQ scores from discharge, 1 month and 3 months post discharge visits. All parameters improved over time except symptom stability score. Red font highlights the values that are statistically significant. p values ≤ 0.05 were considered to be significant variations between the three time points and are highlighted in red.

One year cardiovascular outcome

There were 21 deaths in the acute heart failure group during the 1-year of follow up. 3 of these were non-CV whilst all the other deaths were due to CV causes. 1 patient died while in hospital and one more patient died within 30 days of admission. Mean time to death in the ADHF population was 177 ± 129 days. 1 of the patients who died (CV death) had received intravenous iron whilst in hospital.

During the 1 year of follow up, 8 patients required readmissions to hospital with heart failure requiring intravenous diuretics. 1 patient had 3 admissions over 3 months, the first admission occurring within 30 days of the index admission. 1 patient had a readmission at 2 months and one patient required admission for intravenous diuretics following clinical

assessment at the 3-month research visit. The other readmissions occurred after the 3-month research visit.

Cardiovascular outcomes (composite of CV hospitalisation and death) occurred more frequently among patients with iron deficiency at baseline (88%, $p < 0.001$).

Logistic regression analysis was carried out at discharge, 1 month and 3 months post discharge utilizing all measures of iron status ferritin, serum iron, TSAT and hepcidin). In a binary logistic regression model, serum TSAT and serum iron at 1 month predicted CV outcome ($p = 0.002$ and 0.017 respectively). Increasing TSAT at 1 month was less likely to be associated with CV outcome. The model explained 37.1% (Nagelkerke R^2) of the variance in CV outcome and correctly classified 74.6% of cases. Only TSAT at 1 month predicted all cause mortality ($p = 0.027$).

Transferrin saturation and serum iron at 1 month post discharge were significantly different between patients who had an all cause mortality or a CV outcome and those who did not ($p = 0.043$ and 0.005 for TSAT and $p = 0.029$ and 0.005 for iron respectively). Haemoglobin on admission ($p = 0.031$), CRP at 1-month post discharge ($p = 0.021$) and heart rate at 3 months post discharge ($p = 0.028$) were also significantly different between those with and without an all cause mortality outcome. Haemoglobin at admission was significantly different in those with a CV outcome ($p = 0.016$) but there was no such association with heart rate at any time point. LVEF, hepcidin, Cystatin C, IL-6 and TNF alpha did not demonstrate any meaningful differences between those who had an all cause mortality or CV outcome and those who did not.

STABLE HEART FAILURE COHORT

Baseline characteristics

The baseline characteristics in the stable heart failure population remained unchanged from baseline visit to 3 month follow up (Table 3.4). In those patients who had iron deficiency at baseline, a significant increase was seen in serum ferritin between recruitment and 3 months (Table 3.5).

Biological parameters	Stable CHF Baseline	Stable CHF (3 months)	p value
Heart rate (bpm)	67 ± 11	69 ± 10	0.825
Systolic BP (mm Hg)	118 ± 22	123 ± 17	0.507
Diastolic BP (mm Hg)	70 (60 - 80)	70 (67 - 80)	0.477
Body weight (kg)	86 ± 21	84.3 ± 16.4	0.303
6MWT (m)	318 ± 88	321 ± 114	0.306
Haemoglobin (g/L)	130 ± 14	131± 13	0.670
Serum ferritin (µg/L)	67 (36 - 226)	89.5 (34.3 - 130.5)	0.670
Serum iron (µmol/L)	12 (9.5 - 16)	15.5 (13.2 - 19.1)	0.157
Transferrin saturation (%)	19 (13 - 23)	23 (19 - 31)	0.225
Serum hepcidin (ng/mL)	26.4 (18.0 - 52.2)	35.4 (18.8 - 49.8)	0.672
Cystatin C (mg/L)	2.08 (1.7 - 3.1)	2.05 (1.7 - 2.8)	0.547
IL-6 (pg/mL)	4.02 (1.3 - 15.1)	8.22 (1.7 - 36.4)	0.670
TNF alpha (pg/mL)	11.57 (2.9- 73.5)	5.31 (1.5-15.5)	0.197
Serum erythropoietin (IU/L)	12 (9.3 - 17.3)	13 (9.1 - 19.9)	0.910
C-reactive protein (mg/L)	4 (1 - 8)	2 (1 - 8)	0.285
Serum urea (mmol/L)	9.6 (7.3 - 17.2)	8.5 (6.3 - 10.9)	0.627
Serum sodium (mEq/L)	136 ± 4	136 ± 3	0.179
Serum creatinine (µmol/L)	103.5 (81 - 154.8)	98 (72 - 131)	0.221
eGFR (ml/min/1.73m^2)	61 (48 - 68)	61 (54 - 73)	0.465
Serum urate (mmol/L)	0.50 ± 0.13	0.45 ± 0.14	0.135

Table 3.4- Comparison of parameters within stable CHF subjects over period of follow up.
Mean ± SD for normally distributed variables and median (with lower and upper quartiles) for variables that are not normally distributed

Biological parameters	Stable CHF (Baseline)	Stable CHF (3 month)	p value
Without iron deficiency at baseline (n=11)			
Haemoglobin (g/L)	135 ± 17.8	137 ± 17	0.607
Mean corpuscular volume (μm^3)	94 ± 6.1	95.8 ± 4.4	0.334
Serum iron (µmol/L)	27.8 (15.3 - 29.8)	19.1 (16.1 - 19.9)	0.414
Serum ferritin (µg/L)	272 ± 156	214 ± 114	0.169
Transferrin saturation (%)	23 (21 - 57)	30 (24 - 35)	0.655
Serum hepcidin (ng/mL)	56.26 (49.2 - 68.4)	45.08 (26.0 - 63.4)	0.132
Serum erythropoietin	10.25 (8.5 - 12.6)	10 (7.3 - 14.2)	0.249
C-reactive protein (mg/L)	5 (3 - 12)	1 (1 - 6)	0.027
Serum sodium (mEq/L)	136 ± 2	137 ± 2	0.456
Serum urea (mEq/L)	11.4 (7.1 - 18.7)	8.6 (6.4 - 16.7)	0.739
Serum creatinine (µmol/L)	131 ± 37	128 ± 55	0.889
eGFR (ml/min/1.73m^2)	53 ± 16	59 ± 3	0.506
Serum urate (mmol/L)	0.56 ± 0.11	0.51 ± 0.12	0.144
Cystatin C (mg/L)	2.38 (1.7 -3.7)	2.90 (2.0 - 3.8)	0.763
IL-6 (pg/mL)	8.48 (2.7 - 16.6)	8.22 (1.9 - 35.4)	0.333
TNF alpha (pg/mL)	1.95 (0.0 -8.2)	5.13 (1.1 - 12.5)	0.866
With iron deficiency at baseline (n=19)			
Haemoglobin (g/L)	126 ± 10.4	128 ± 8.8	0.372
Mean corpuscular volume (μm^3)	92.8 (89.8 - 94.7)	93.6 ± 4.4	0.670
Serum iron (µmol/L)	11.2 ± 4.0	13.8 ± 4.9	0.273
Serum ferritin (µg/L)	40.5 (27 - 65)	47 (32 - 91)	0.048
Transferrin saturation (%)	17 (12 - 21)	22 (18 - 25)	0.204
Serum hepcidin (ng/mL)	19.51 (12.0 - 25.4)	19.81 (12.6 - 48.8)	0.109
Serum erythropoietin (IU/L)	15.9 (10.6 - 31.7)	14.1 (9.3 - 20.2)	0.461
C-reactive protein (mg/L)	3 (1 - 6)	3 (1 - 11)	0.160
Serum sodium (mEq/L)	137 ± 5	136 ± 4	0.057
Serum urea (mEq/L)	9.1 (7.3 - 17.2)	7.5 (6.3 - 11.1)	0.796
Serum creatinine (µmol/L)	88 (78 - 142)	83 (66 - 127)	0.378
Serum eGFR (ml/min/1.73m^2)	67 ± 8	68 ± 19	0.601
Serum urate (mmol/L)	0.47 ± 0.12	0.41 ± 0.14	0.482
Cystatin C (mg/L)	1.93 (1.6 - 2.9)	1.84 (1.2 - 2.3)	0.077
IL-6 (pg/mL)	3.26 (0.6 – 16.0)	7.82 (1.2 - 142.7)	0.814
TNF alpha (pg/mL)	58.45 (2.71 - 91.85)	5.49 (0.97 - 68.45)	0.575

Table 3.5- Comparison of parameters in stable CHF patients with and without iron deficiency at recruitment.

Mean ± SD for normally distributed variables and median (with lower and upper quartiles) for variables that are not normally distributed.

In the stable cohort group, there was no significant difference within patients in the prevalence of iron deficiency over the 3-month follow up (Table 3.6).

	STABLE CHF		
	RECRUITMENT	3 MONTHS	p VALUE
Prevalence of iron deficiency (%)	63 (n = 19)	53 (n = 16)	0.16
Absolute iron deficiency (%)	95 (n = 18)	100 (n = 16)	0.08
Functional iron deficiency (%)	5 (n = 1)	0 (n = 0)	0.32

Table 3.6- Prevalences of iron deficiency at recruitment and follow up in the stable CHF cohort.

Baseline serum ferritin and iron correlated with baseline TSAT with no further significant correlations seen at 3 months (see previous chapter). Serum iron and TSAT at 3 months however had significant correlations with baseline serum ferritin ($r = 0.508$, $p = 0.019$ and $r = 0.548$ and $p = 0.011$ respectively). Other correlations are outlined in Table 1 of the appendix.

On categorizing the stable CHF population as per median CRP (cut off 4 mg/L), in those with CRP <4 mg/L, change in TSAT from baseline to 3 months correlated with ferritin ($p = 0.002$, $r = -0.857$). Ferritin also correlated with iron and TSAT at 3 months ($p = 0.017$, $r = 0.698$ for iron and $p = 0.009$, $r = 0.738$ for TSAT respectively). Hepcidin also correlated with ferritin at 1 month ($p = 0.013$, $r = 0.714$). Serum iron correlated with TSAT at 1 month ($p < 0.001$, $r = 0.981$ and $p = 0.001$, $r = 0.881$ respectively), ferritin at 1 month ($p = 0.020$, $r = 0.911$).

In those with CRP > 4 mg/L, change in TSAT correlated with serum iron ($p = 0.005$, $r = 0.942$). Hepcidin correlated with ferritin at 1 month ($p < 0.001$, $r = 0.948$).

On categorizing the stable CHF cohort as per median hepcidin, there were strong correlations between serum iron at baseline and TSAT at 3 months ($p = 0.029$ and $r = 0.758$) in those with hepcidin < 26 ng/mL. In those with hepcidin > 26 ng/mL, change in TSAT from baseline to 3 months correlated with serum ferritin at baseline ($p = 0.009$, $r = -0.774$). There were no significant correlations between change in TSAT and hepcidin in this population either.

One year cardiovascular outcomes.

In the stable heart failure cohort, 3 patients had admissions to hospital with ADHF, 1 of whom had intravenous iron during the hospital stay. These patients were therefore excluded from the 3-month follow up visit.

There was 1 CV death and 1 non-CV death at 1 year.

DISCUSSION

Iron deficiency is a common occurrence in decompensated heart failure with 20% being due to functional iron deficiency associated with a higher hepcidin than those with absolute iron deficiency. The prevalence of iron deficiency falls over time during the recovery period from decompensated heart failure and in this cohort reached its lowest 1-month post discharge from hospital. In those with iron deficiency at baseline, there was a significant improvement in serum iron, TSAT and serum ferritin following discharge from hospital. Serum hepcidin plateaued at 1 month post discharge while serum ferritin, iron and TSAT continued to improve to 3 months post discharge.

TSAT and serum ferritin at 1 month post discharge were predictive of CV outcome.

Studies have made distinctions between absolute and functional iron deficiency with increased risk of rehospitalisation in those with absolute iron deficiency. (231) Other studies have reported prevalences of iron deficiency in ADHF differentiating between genders and demonstrating independent associations with antiplatelet treatment, low CRP and anaemia. (232) IRON STATS DHF however would be the first study of its kind to study the sequential variations in markers of iron status following decompensation.

The overwhelming message of this study remains that an iron deficient state is extremely common with decompensation and that this improves with time. It does not appear to be driven by hepcidin levels or inflammation or renal function (measured traditionally with serum creatinine or with Cystatin C). The relationship between hepcidin and CRP does appear stronger following recompensation. The increase in hepcidin following discharge highlights the improvement in iron status and could imply that hepcidin could play a greater role in iron status in the more stable setting. Hypoxia and low iron appear to be driving hepcidin in the acute decompensated state rather than inflammation. It must be noted though that there was no obvious relationship between hepcidin and iron status in the small stable CHF control cohort.

The findings of this study would suggest that although iron deficiency is common in ADHF, as treatment effects set in and patients recover, parameters of iron status improve. This could have potential treatment implications as TSAT and ferritin levels at 1month post discharge appear to influence CV outcomes.

The question arises, why is acute decompensation associated with such profound iron deficiency? One could hypothesise that iron deficiency per se may have a role to play in the pathophysiology of decompensation however this will need to be tested in larger prospective studies with careful monitoring of hepcidin and inflammatory status in the run up to decompensation.

Limitations

There was a large drop out rate with the acute heart failure patients, a challenge typical of studies recruiting acutely unwell patients. Patients recruited were elderly and had a number of comorbidities. This, as well as poor mobility following discharge and inability to contact patients subsequently, were some of the reasons for missed visits. Discharge visits were missed despite rigorous daily ward visits to track patient journeys for every recruited patient. Patients discharged over the weekend could also not be assessed prior to leaving hospital.

Patients admitted with severe heart failure requiring coronary care unit admission or those who were unable to give informed consent within the first 24 hours of admission were excluded from the study. This reflects the relatively low mortality and readmission rates in the acute heart failure cohort.

CONCLUSIONS

Iron deficiency is very common in ADHF and variations in iron status are to be expected following decompensation. Iron status appears to progressively improve following a period of decompensation. However an iron deficient state persisting at 1-month post discharge may

have prognostic implications. These findings as well as the role of hepcidin in decompensation will need to be evaluated in larger studies of recent ADHF patients.

CHAPTER 4- MONITORING OF ARRHYTHMIA AND SUDDEN DEATH IN A HAEMODIALYSIS POPULATION: THE CRASH-ILR STUDY

BACKGROUND

Arrhythmias in the presence of CKD can be a manifestation of symptomatic target organ damage or asymptomatic target organ damage. In asymptomatic target organ damage (Figure 1.4), SCD could be the first sign of underlying CVD. SCD is defined as an unexpected natural death from a cardiac cause within 1 hour of onset of symptoms in a person not known to have a potentially fatal condition. (233) Based on this definition, it has been suggested that SCD contributes to 70% of CV mortality and up to 29% of all-cause mortality in haemodialysis patients. (173) The purpose of the above definition of SCD is to identify arrhythmic deaths in the absence of an immediate predisposing cause and is based on the key finding that a rapid time from onset of illness to death is a discriminator of arrhythmia as a cause of death rather than circulatory collapse. (234) However, identifying deaths that are truly sudden and cardiac can be challenging, particularly in ESKD. Deaths are often unwitnessed and even where witnessed, there are many reasons why sudden deaths in ESKD may be due to circulatory collapse and not arrhythmia. These include rapid fluid shifts of ultra filtration, diffuse arterial calcification, loss of autonomic tone, stroke and aortic rupture.

Prior to considering potential interventions to try and reduce SCD in these high-risk patients, such as implanting cardioverter defibrillators (ICD), a number of pertinent questions should be answered. Firstly, is truly arrhythmic SCD as common in this population as reported?

Studies have identified misrepresentation of SCD in registries (182) as well as differences in SCD proportions following improvement of classification systems in databases such as the UK renal registry. (174) Second, what proportion of SCD in ESKD is potentially reversible as opposed to occurring during multi system failure? The haemodynamic frailty of ESKD may obfuscate any therapeutic role an ICD might have to offer in this population, particularly during intercurrent acute illness.

ICD implantation will be beneficial for some patients, but justifying its cost effectiveness and identifying this high-risk sub-group within the haemodialysis population is difficult. Whilst wide QRS-T angle, QT duration, T wave residuum, lack of heart rate variability and the presence of LVH have been identified as potential risk predictors for SCD (191, 192, 195, 197, 200, 235), documenting the burden and nature of arrhythmias associated with these remains fundamental, and to do so is challenging. Ambulatory ECG recordings only give snapshots of potential arrhythmias and to date, data on the true burden of both tachy and bradyarrhythmias in this population are limited. Bradyarrhythmias may be of particular relevance in haemodialysis patients as concomitant fibrotic and calcific processes within the heart are commonplace. True SCD in a haemodialysis population needs to be characterised better to understand possible differences in phenotype from the general population with conventional SCD risk factors.

The CardioRenal Arrhythmia Study in Haemodialysis Patients using Implantable Loop Recorders (CRASH-ILR) is one of the first studies to comprehensively evaluate this high-risk population with continuous ECG monitoring without the limitations of external cardiac monitoring and spot ECGs in otherwise asymptomatic haemodialysis patients. Our study

offers the longest follow up period of continuous ECG monitoring in this population to date, and we now present our findings after > 379 000 hours of monitoring.

AIMS

1. To define the prevalence of SCD in the high-risk haemodialysis patient cohort and the need for tachy/ brady arrhythmia devices.
2. To define the incidence and prevalence of arrhythmic events in this population.

METHODS

CRASH-ILR is a prospective, loop recorder based, single centre study of 30 established haemodialysis patients. Inclusion criteria were that participants should be able to give informed consent, be more than 18 years of age, and have been receiving haemodialysis for at least 90 days. Exclusion criteria were pregnancy and participation in another research study. The study conformed with the principles of the declaration of Helsinki and was registered with the UK clinical research network (UKCRN ID 6356) and ISRCTN (study ID 35846572). Patients were recruited between 2011 and 2014 from a single tertiary nephrology centre (Portsmouth) in the United Kingdom, including its satellite dialysis units. Demographic information, medication details and details of primary renal disease were collected. Pre and post dialysis blood results (full blood count, electrolytes) and blood pressure measurements were collected from haemodialysis sessions close to the time of recruitment. A 12 lead electrocardiograph was performed. Detailed 2D echocardiograms were performed by a BSE accredited echocardiographer on a Phillips IE 33 machine on a non-dialysis day. LVEF was calculated by Simpson's biplane method and LV mass estimated by LV cavity dimension and wall thickness at end-diastole. (236) LV diastolic function was assessed using mitral valve inflow and tissue doppler as per BSE guidelines. (237)

After written informed consent was gained, Medtronic XT loop recorders (Reveal XT 9529, Medtronic, Minneapolis, USA) were implanted in the left parasternal region. These devices are routinely used in clinical practice for the diagnosis of arrhythmias, have around a 3 year battery life, and have magnetic resonance imaging (MRI) conditional labelling for safe patient management. A Reveal XT 9529 device weighs 15 g and measures 62x19x8mm (Figure 4.1). Electrocardiographic mapping was first performed to identify the best position to implant the device. A 3 cm incision was then made over this site under local anaesthesia and, following blunt dissection, the device was placed beneath this in a pre pectoral plane Figure 4.2). The incision was closed with absorbable sutures and the patient discharged home the same day. Device implantation was carried out on non-dialysis days. Patients were then reviewed at their respective dialysis units during a usual dialysis session by the research team around ten days post device implant to ensure adequate wound healing and to address any concerns the patient may have.

Figure 4.1- Implantable loop recorder.
The Reveal XT 9529 device weighs 15g and measures 62x19x8mm.

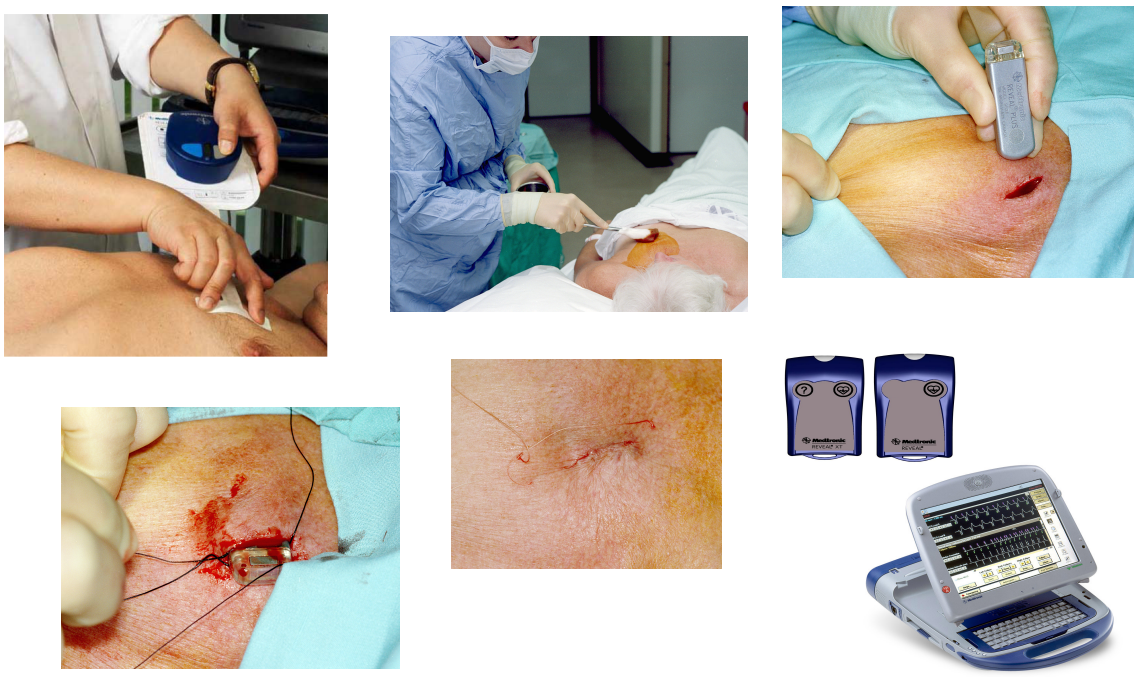


Figure 4.2- Implanting loop recorders.

The steps include identifying the ideal location using ECG mapping, cleaning the skin and making an incision in the parasternal area under aseptic precautions and local anaesthetic cover, placing the device in the subcutaneous layer and closing the wound with sutures. Patients are then educated on how to perform downloads from the device using remote monitoring.

A device typically stores 49.5 minutes of ECG data at a time (27 minutes of automatic activation triggered by pre-programmed parameters and 22.5 minutes of patient activation). Each device was remotely linked to a base unit on the dialysis unit thereby permitting transmissions up to 3 times a week where feasible (Figure 4.3). Patients were trained on how to transmit data from their device at each dialysis session via the remote monitoring CareLink® system (Medtronic, Medtronic, Minneapolis, USA). They were also educated on how to activate the device should they have a symptomatic episode (palpitations, dizziness or blackouts). Regular downloads were strongly encouraged to ensure availability of data memory on the devices at all times.

Figure 4.3- Remote monitoring from the reveal device.

The implantable loop recorders were all programmed in the same manner with no adjustments made to sensitivity or other parameters during the course of the study. The pre-programmed criteria for automatic detection of arrhythmias were: fast ventricular tachycardia (VT) - 12/16 beats with heart rate ≥ 200 beats per minute (bpm); VT - 12 beats with heart rate ≥ 162 bpm); asystole for 3 seconds or more; bradycardia - at least 2000msec between QRS complexes (≤ 30 bpm) for 4 beats. Symptom activation would trigger automatic recording irrespective of whether the above criteria were fulfilled and up to three 7.5 min episodes can be recorded before download is required to free up memory on the device. Reveal XTs are highly sensitive in recognising atrial fibrillation (AF), which was automatically recorded.

Follow up was from the day of implant to death, explant, or end of battery life of the device whichever came first. Follow up data collection was rigorously performed. A research link

nurse at each dialysis unit was responsible for ensuring patients were able to download from their devices, and for notifying the research team of hospital admissions, transplantation or other significant medical events.

For this study, the definition of SCD included patients who were found dead having been well at the last known point of contact, as well as those dying from as an unexpected natural death from a cardiac cause, within 1 hour of onset of symptoms in the absence of a known potentially fatal condition[1]. As soon as a death was notified, direct contact was made with the relevant medical institution/personnel to ascertain the circumstances of death. Devices were retrieved as soon as possible after death and analysed immediately.

Every device download was manually scrutinised independently by 2 members of the research team (PCP, DZ); occurrence of ectopic activity in each event was counted, as was the occurrence of noise/under sensing of R waves. The event adjudication panel consisted of named members of the research team (PCP, PRR, PRK). Any event felt to be of clinical significance was relayed to the nephrologist involved in the patient's care.

OUTCOME AND STATISTICAL ANALYSIS

The primary outcome measure was SCD or implantation of a pacing device (tachy/ bradyarrhythmia controlling device). The secondary outcome was the development of any significant arrhythmia necessitating medical intervention (SCD, new AF, atrial flutter, non sustained VT, and other tachy/bradyarrhythmia). Patients who were known to have AF or atrial flutter at recruitment were not considered to have developed secondary outcomes if they had a further arrhythmic event identical to previously documented arrhythmias.

Statistical analyses were performed using SPSS software version 24. Descriptive statistics are presented as mean ± standard deviation for normally distributed continuous variables, median (range) for non-parametrically distributed continuous variables, and percentage of study population for categorical variables. Between group comparisons of baseline clinical and echocardiographic parameters were made between patients who reached any primary or secondary outcome arrhythmic event versus those who did not. Comparisons of normally distributed continuous variables were made using unpaired t-tests, and for non-parametric variables using Mann Whitney U tests. Comparisons of categorical variables (occurrence of asystole and ectopic activity on dialysis days versus non dialysis days) were made using chi square tests.

Median survival estimates were calculated using a Kaplan-Meier method. Follow up was censored at time of death, explantation of device, or most recent data upload. The sample size of this study was not powered specifically for these analyses.

RESULTS

Based on the above inclusion and exclusion criteria, 120 patients were approached and 30 were recruited between 24 August 2011 and 23 October 2014. All of these patients were receiving standard thrice-weekly 4-hour dialysis sessions. 60% were male, and the mean age was 67 ± 12 years. Mean LV ejection fraction (LVEF) as per Simpson's biplane formula was 55 ± 8%; only 1 patient had LVEF <35%. Mean LV mass in females was 197 ± 30 g (severe LVH as per British Society of Echocardiography guidelines) and in males 236 ± 63 g (moderate LVH). A detailed outline of baseline characteristics in found in Table 4.1.

	Overall
Number	30
Follow up time (years)	1.5 ± 1.0
Clinical characteristics	
Age (years)	67.8 ± 12.1
Gender (% male)	60%
Diabetes (%)	37%
Coronary artery disease (%)	22%
CHA_2DS_2-VASc	2.2 ± 1.0
Beta blocker (%)	23%
Anti-coagulation (%)	7%
Dialysis parameters	
Time on dialysis (months)	45 ± 40
Pre-dialysis SBP (mmHg)	159 ± 32
Pre-dialysis DBP (mmHg)	66 ± 18
Intra-dialytic δSBP (mmHg)*	-19 (-99, +34)
Serum urea	17.3 ± 3.4
Serum creatinine	729 ± 187
Serum sodium (mmol/L)	137 ± 4
Serum potassium (mmol/L)	4.9 ± 0.6
Haemoglobin (g/L)	118 ± 14
Platelets ($x10^9$/L)	238 ± 74
ECG and echocardiography	
Resting heart rate (bpm)	73 ± 14
PR (m)	174 ± 31
QRS (ms)	102 ± 23
LVEF (%)	55 ± 8
Left atrial diameter (cm)	4.0 ± 0.4
Left ventricular mass (g)	224 ± 57
Diastolic dysfunction (%)	38%

Table 4.1- Baseline characteristics of study population.

SBP = systolic blood pressure, DBP = diastolic blood pressure, LVEF = left ventricular ejection fraction. Continuous variable data are expressed as mean ± standard deviation except * which indicates median (range). CHA_2DS_2-VASc- Risk factor scoring for AF stroke risk based on the presence of Congestive heart failure, Hypertension, Age, Diabetes mellitus, Stroke, Vascular disease, Sex/female

The study period entailed a total of 379 512 hours (15 813 days) of continuous ECG monitoring (mean 527 ± 376 days per patient, Figure 4.4). During follow up, 6 devices were

explanted. 2 were explanted due to persistent superficial infection, 2 as per patient requests following renal transplant, 1 device reached end of battery life, and 1 was explanted as the patient had a clinical indication for an MRI scan and the local radiology department were unwilling to perform the scan in the presence of an ILR. 8 patients had renal transplants during the study period. Full details of recruitment and outcomes are found in Figure 4.5 and Table 4.2.

There were 17 patient activations of the ILR in 5 patients, and all of these corresponded to sinus rhythm. The symptoms leading to these activations included light headedness and feeling generally unwell.

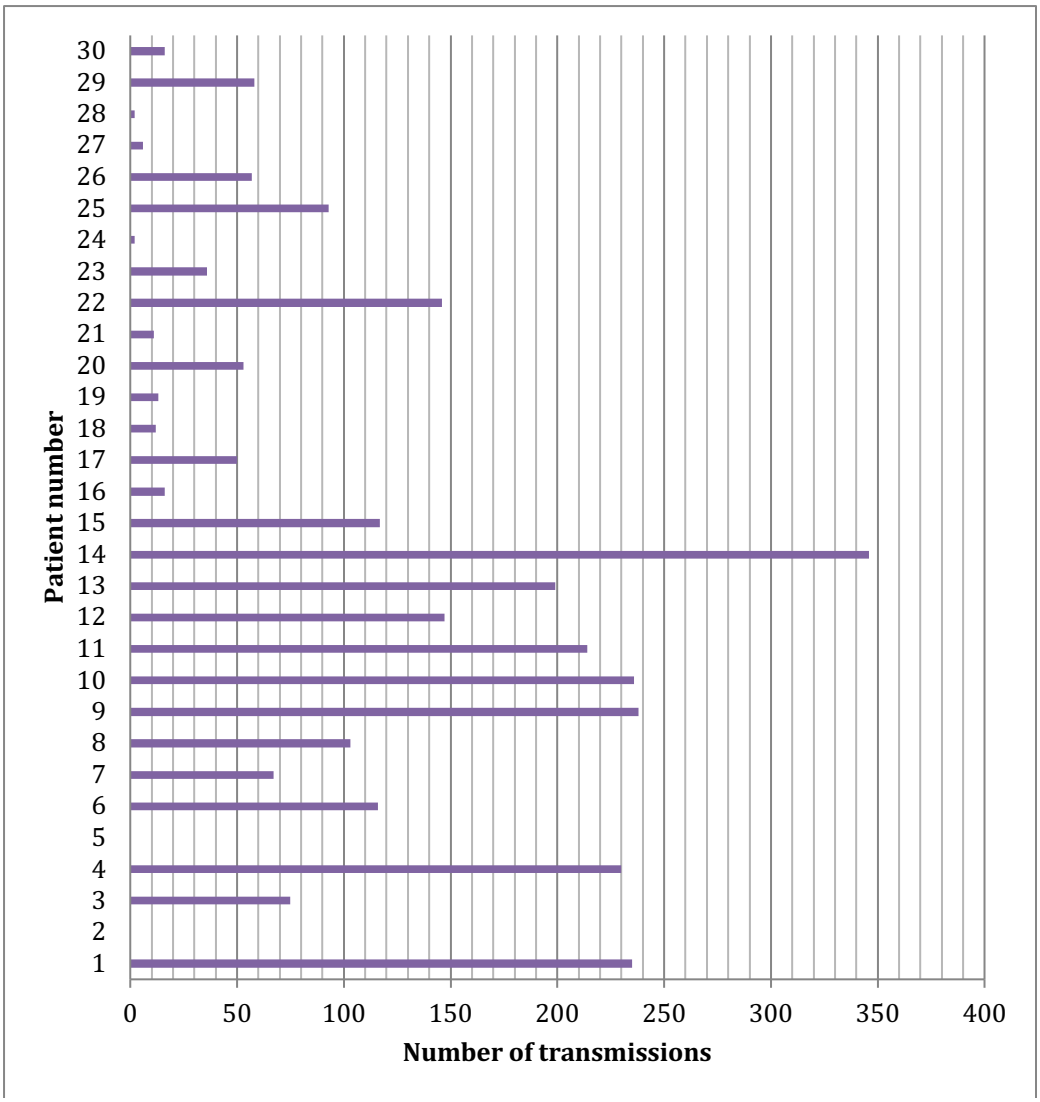


Figure 4.4- Number of transmissions per patient.

Patient 2 had ILR explant for infection soon after implant with no downloads prior, hence no data available. The device was misplaced following the death of patient 5 and the patient had not downloaded any data prior to death.

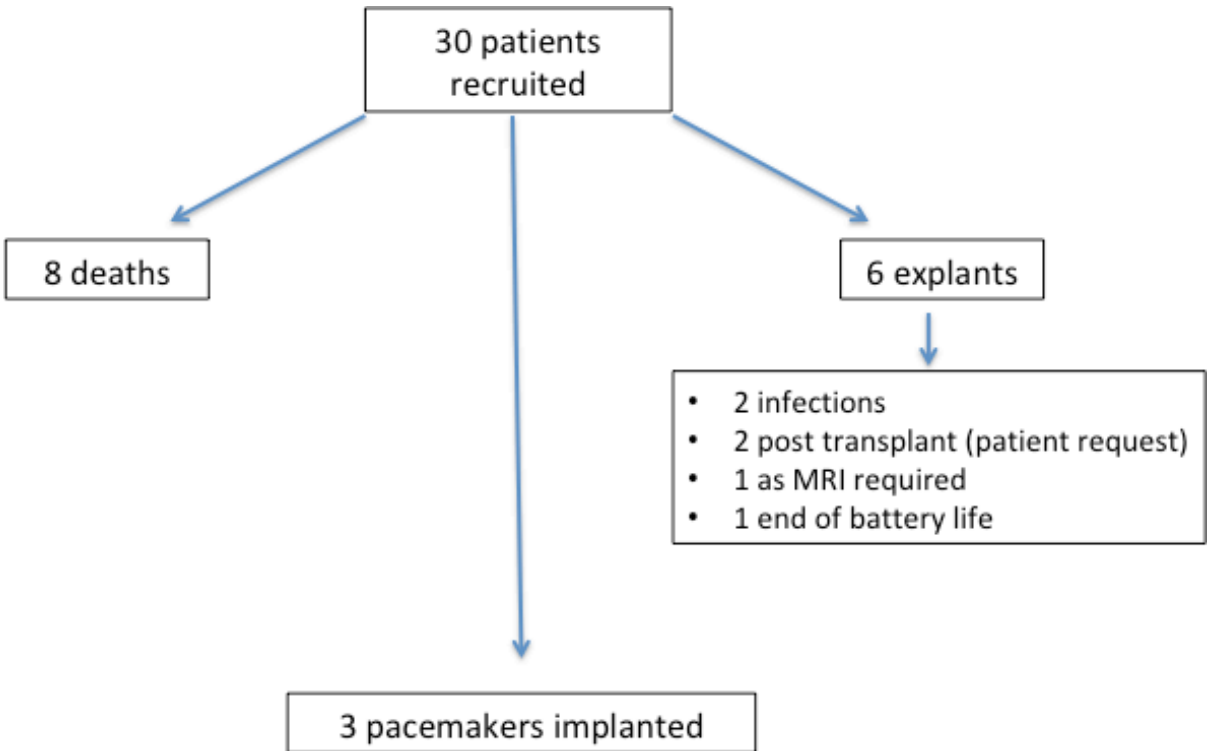


Figure 4.5- Overview of recruitment and outcomes.

Of 120 patients approached for the study, 30 agreed to participate. 6 devices had to be explanted during the course of the study follow up for reasons depicted above. 10% of the population received pacemakers for asymptomatic conduction system disease.

Table 4.2- Individual patient outcomes.

The outcome for every participant in the study and the presence of an arrhythmic end point where applicable and its timing in relation to dialysis sessions is described above.

SCD- Sudden cardiac death, ILR- Implantable loop recorder, AF- Atrial fibrillation, PAF- paroxysmal atrial fibrillation, SVT- Supraventricular tachycardia, VT- Ventricular tachycardia, PPM- Pacemaker

Study number	Age	Reason for device explant	Arrhythmic end point	Timing relative to dialysis	Outcome
1	64	-	-		ILR in situ
2	74	Infection	SCD	-	Death
3	63	Death	SCD	Mid-week	Death
4	73	-	-		ILR in situ
5	54	-	-	Weekend	Death
6	64	-	-		Death
7	83	PPM insertion	PPM	10 weekend 4 mid week	Bradycardia and PPM
8	64	End of battery life	-		Transplant
9	68	-	-		ILR in situ
10	72	-	-		ILR in situ
11	78	-	New PAF		Death
12	59	-	-		Transplant
13	56	-	-		Transplant
14	78	-	SVT	Dialysis days	ILR in situ
15	74	Transplant	-		Transplant
16	57	Death	-		Death
17	74	-	PPM		PPM
18	81	Death	-		Death
19	60	-	New PAF		ILR in situ
20	64	-	-		ILR in situ
21	36	-	-		Transplant
22	36	Transplant	VT	4 mid week 2 weekend	Transplant
23	88	-	PPM	2 mid week 1 weekend	ILR in situ
24	75	Death	Death		Death
25	75	-	-		ILR in situ
26	59	-	-		ILR in situ
27	79	MRI required	Pauses, AF	Mid week	Asymptomatic nocturnal pauses in AF
28	71	Infection	-		Infection
29	81	-	-		ILR in situ
30	74	-	-		ILR in situ

Primary outcome events

During the study period, there were eight deaths (details of deaths in Table 4.3). ILRs were not able to be explanted for post humous analysis in two of these patients as we were notified of their deaths only after burial. Of the other 6 deaths, two were attributable to SCD. Ventricular fibrillation (VF) was identified in 1 SCD patient's post humous ILR download with evidence of coronary artery disease (CAD) on post mortem. No arrhythmic events were recorded prior to the terminal arrhythmia. The other patient died from unrelated SCD, several weeks after ILR explantation for infection (no post mortem). (Although no downloads had been received from this patient, interrogation did not reveal any arrhythmias at the time of explant for local infection.)

Study number	Age	Device interrogated Y/N	Arrhythmia detected	Cause of death	Registered cause of death	
					Primary cause of death	Contributory causes of death
2	74	N (explanted before death)	No	Sudden cardiac death few weeks after device explanted for superficial infection. No post mortem.	ESKD	DM
3	63	Y	VF	Found dead at home.	IHD	DM. CHF
5	54	N	Device not retrieved.	Post mortem carried out.	MI	DM
6	64	Y	VF then PEA	Admitted to hospital with GI bleed, subsequently felt to be too sick for further investigations. Post mortem carried out.	Coronary artery thrombus	HTN, COPD
9	68	Y	PEA	Death, generalised deterioration following prolonged admission	ESKD	
11	78	Y	PEA	Death	ESKD	
18	81	Y	PEA -terminal event. Self-limiting VT also seen.	Death following palliative input. Prior admission with leg ulcers requiring limb amputation.	Sepsis	ESKD
24	75	N	Device not retrieved.	Death following generalised deterioration. Died in hospice after withdrawal of dialysis.	Sepsis	ESKD

Table 4.3- Deaths in CRASH –ILR; a breakdown of findings.

ESKD- End stage kidney disease, IHD- Ischaemic heart disease, CAD- Coronary artery disease, MI- myocardial infarction, DM- Diabetes mellitus, HTN- Hypertension, COPD-Chronic obstructive pulmonary disease, CHF-Chronic heart failure, PEA- pulseless electrical activity, VT-ventricular tachycardia.

Implantable loop recording demonstrated 2:1 atrio-ventricular (AV) block and significant sinus pauses (> 3 seconds) during the day in 3 asymptomatic patients that resulted in pacemaker implantation (2 dual chamber devices and 1 biventricular pacemaker). The decision to implant pacemakers was made after thorough clinical assessment of the patient in the light of ambulatory ECG fndings and international guidelines. Recruitment ECG showed left bundle branch block with QRS prolongation of 172 msec in the patient with AV block. The patients who received pacemakers for sinus pauses did not have any suggestions of impending heart block (PR interval prolongation, QRS widening or bundle branch block) in the run up to documentation of bradycardia. Bradycardic events were intermittent with pauses lasting between 3 to 7 seconds, and although predominantly nocturnal, all patients who had pacemakers implanted had also developed day time pauses. There did not appear to be an increased occurrence in the long inter-dialytic periods (Table 4.2) and there was no statistical difference in the occurrence of asystolic episodes on dialysis versus non-dialysis days ($p = 0.999$).

Five patients therefore reached a primary outcome event (2 SCD, 3 pacemakers). The overall event rate for primary end point was 116 per 1000 patient years. For SCD the event rate was 46 per 1000 patient years, and for device implantation 70 per 1000 patient years. Baseline characteristics that were significantly different between patients who did and did not reach a primary end point were mean age (76 ± 8 years vs. 66 ± 12 years respectively, $p = 0.04$), LVEF ($49 \pm 10\%$ vs. $57 \pm 6\%$, $p = 0.04$), and LV mass (272 ± 97 g vs, 213 ± 39 g, $p = 0.05$).

Secondary outcome events

There were 6 deaths not attributed to SCD. Two patients had confirmed CAD as cause of death following post mortem, (1 patient had non-sustained VF and PEA as the terminal event on ILR; this death occurred during hospitalisation with a severe gastro intestinal bleed; the patient was deemed too sick to have an endoscopy and death was expected. The other patient's was not retrieved). The final 4 patients had generalised deterioration requiring palliative care before death with evidence of sepsis in two of these patients. One of the septic patients (study number 18, Table 3) had confirmed non sustained VT and p-wave asystole during the period of progressive decline, the second patient had gradual bradycardia and asystole and the ILR was not retrieved in the third patient.

A total of 29 435 events were identified over the study period by the reveal device in the tachycardia log, but 99.7% were due to oversensing (device interprets noise/artefact as tachyarrhythmia). Automatic detection identified 1 387 bradycardic events of which 99.5% were due to device undersensing (failure to identify electrical activity that is present). The true events consisted of sinus bradycardia, sinus pauses and AV block.

Two patients were known to have persistent AF and 1 had atrial flutter at the start of the study. Three patients were detected to have new onset paroxysmal AF of which two required initiation of anti-arrhythmic drugs. Two of these patients were commenced on anticoagulant therapy by their nephrologist (CHA_2DS_2-VASc scores 3 and 1, respectively).

A further patient (study number 22, Table 2) had recurrent slowVT documented in the context of a structurally normal heart and was initiated on beta blockers (42% of events occurring on dialysis days). Another patient who had supraventricular tachycardia (all events on dialysis days) remains under monitoring due to low burden of events. Both patients were asymptomatic. As mentioned earlier, the patient who died of a VF arrest did not have any signifcant pre-morbid events recorded via the ILR prior to death.

Ten patients reached an arrhythmic primary or secondary end point. The overall event rate for any arrhythmic end point was 232 per 1000 patient years. The estimate of median event free survival for any arrhythmia was 2.6 years (95% CI 1.6 – 3.6 years). Event rate for VT/VF was 46 per 1000 patient years and for new AF 83 per 1000 patient years.

Event rate for bradycardia was 91 per 1000 patient years. Event rates for arrhythmias appeared to vary according to age: for example, the rate of any arrhythmic event in patients ≥ 65 yrs was 224 per 1000 pt years and 323 per 1000 patient years in those < 65 years (brady arrhythmia event rate: 106 per1000 pt years vs. 65 per 1000 pt years; VF/VT: 0 per 1000 pt years vs. 129 per 1000 pt years in patients ≥ 65 yrs vs patients < 65 years respectively).

A Kaplan-Meier curve of time to events is shown in Figure 4.6 (a-c). Baseline characteristics significantly different between patients who did and did not reach an arrhythmic end point were: LV mass (273 ± 70g vs. 200 ± 28g, $p < 0.01$), and LVEF

(52 ± 10% vs. 57 ± 5%, p = 0.03). Baseline characteristics between patients with and without arrhythmia are compared in Table 4.4a and b.

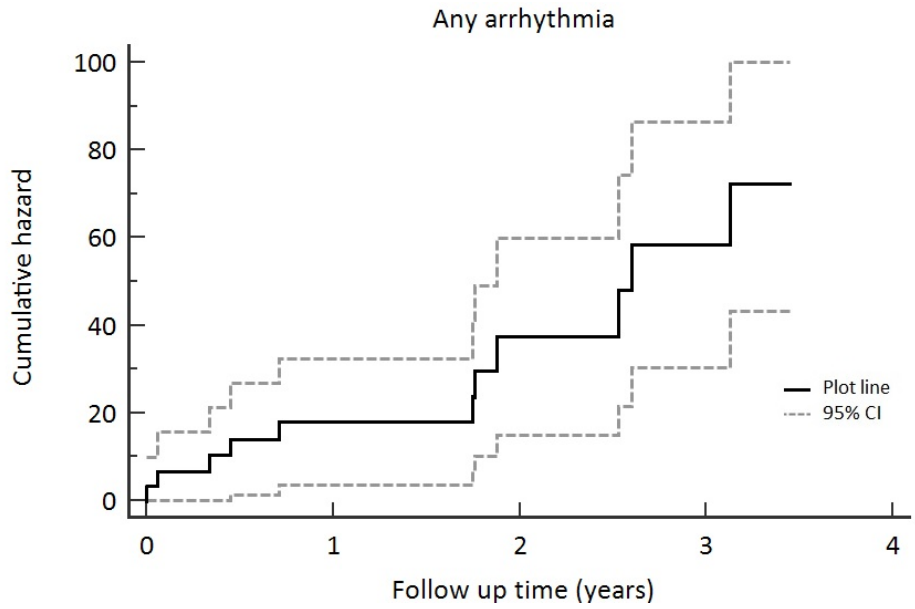


Figure 4.6a- Kaplan Meier hazard plots for any arrhythmia.
(95% confidence intervals represented as dotted lines).

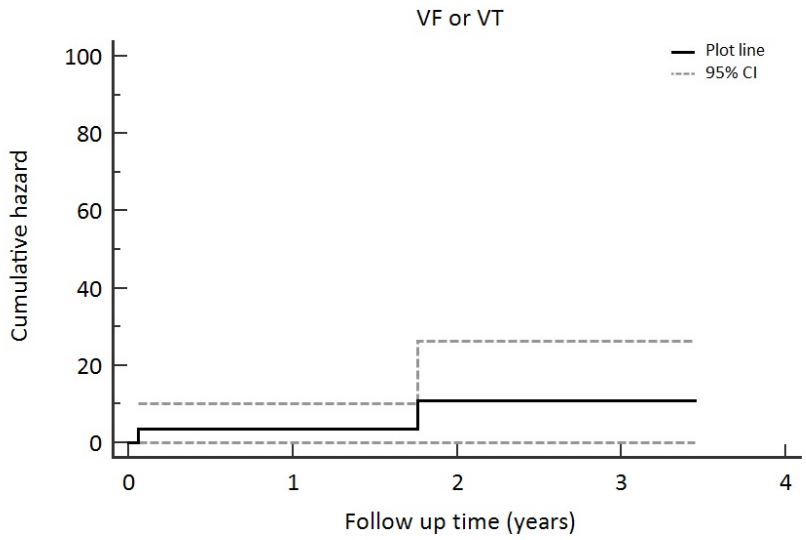


Figure 4.6b- Kaplan Meier hazard plots for ventricular arrhythmias.
(95% confidence intervals represented as dotted lines).

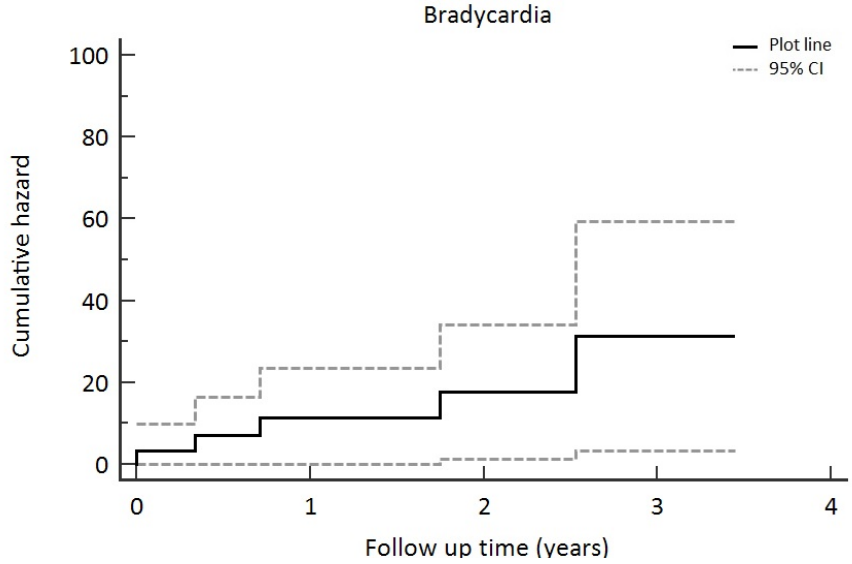


Figure 4.6c- Kaplan Meier hazard plots for brady arrhythmia.
(95% confidence intervals represented as dotted lines).

	Overall	Arrhythmia	No arrhythmia	p
Number	30	8	22	-
Follow up time (years)	1.5 ± 1.0	1.3 ± 1.0	1.5 ± 1.0	-
Clinical characteristics				
Age (years)	67.8 ± 12.1	66.5 ± 15.6	68.4 ± 10.7	0.699
Gender (% male)	60%	67%	57%	0.704
Diabetes (%)	37%	33%	38%	1.000
Coronary artery disease (%)	22%	33%	17%	0.815
CHA_2DS_2-VASc	2.2 ± 1.0	2.3 ± 1.2	2.1 ± 1.0	0.803
Beta blocker (%)	23%	22%	24%	1.000
Anti-coagulation (%)	7%	11%	5%	0.514
Dialysis parameters				
Time on dialysis (months)	45 ± 40	54.4 ± 46.7	41.4 ± 37.1	0.456
Pre-dialysis SBP (mmHg)	159 ± 32	168 ± 49	155 ± 22	0.332
Pre-dialysis DBP (mmHg)	66 ± 18	65 ± 27	67 ± 13	0.828
Intra-dialytic δSBP (mmHg)*	-19 (-99, +34)	-31 (-99, +21)	-14 (-61,+34)	0.157
Serum urea	17.3 ± 3.4	18.1 ± 3.0	16.9 ± 3.5	0.447
Serum creatinine	729 ± 187	730 ± 168	729 ± 19	0.987
Serum sodium (mmol/L)	137 ± 4	137 ± 2	137 ± 5	0.944
Serum potassium (mmol/L)	4.9 ± 0.6	5.1 ± 0.7	4.8 ± 0.5	0.221
Haemoglobin (g/L)	118 ± 14	126 ± 14	116 ± 13	0.104
Platelets ($x10^9$/L)	238 ± 74	205 ± 69	205 ± 74	0.195

Table 4.4a- Baseline characteristics of study population

SBP = systolic blood pressure, DBP = diastolic blood pressure, Continuous variable data are expressed as mean ± standard deviation except * which indicates median (range). CHA_2DS_2-VASc- Risk factor scoring for AF stroke risk based on the presence of Congestive heart failure, Hypertension, Age, Diabetes mellitus, Stroke, Vascular disease, Sex/female.

	Overall	Arrhythmia	No arrhythmia	p
ECG and echocardiography				
Resting heart rate (bpm)	73 ± 14	75 ± 11	72 ± 15	0.632
PR (m)	174 ± 31	178 ± 25	172 ± 34	0.644
QRS (ms)	102 ± 23	112 ± 32	98 ± 17	0.160
LVEF (%)	55 ± 8	52 ± 11	57 ± 5	0.028
Left atrial diameter (cm)	4.0 ± 0.4	4.0 ± 0.4	4.0 ± 0.4	0.829
Left ventricular mass (g)	224 ± 57	273 ± 70	200 ± 28	0.003
Diastolic dysfunction (%)	38%	25%	44%	0.477

Table 4.4b- Baseline characteristics of study population

The population has been analysed overall and divided into those who did and did not reach an arrhythmic end point (sudden cardiac death, pacing device implantation, new supraventricular or ventricular brady or tachyarrhythmia), and including between group comparison p values. Red font indicates a significance of $p < 0.05$.

PR- pulse rate, LVEF = left ventricular ejection fraction. Continuous variable data are expressed as mean ± standard deviation.

Ectopy (Figure 4.7)

Ectopic activity was defined as intrinsic cardiac activity occuring from a site other than the sino atrial node at a time before the next expected intrinsic elcctrical activity. It was classified as atrial or ventricular in origin depending on p/QRS morphology and deemed significant to be counted if more than 3 ectopics occurred in a 2 minute recording.

Nine patients were identified as having signifcant ectopic activity (2 atrial, 7 ventricular). One of the patients with atrial ectopic activity had > 50% of these events recorded on dialysis days. Four of the 7 patients with ventricular ectopy had >5 0% events on dialysis days. No statisitically significant difference was demonstrated between dialysis and non dialysis days for atrial or ventricular ectopy (p = 0.43 and 0.39, respectively).

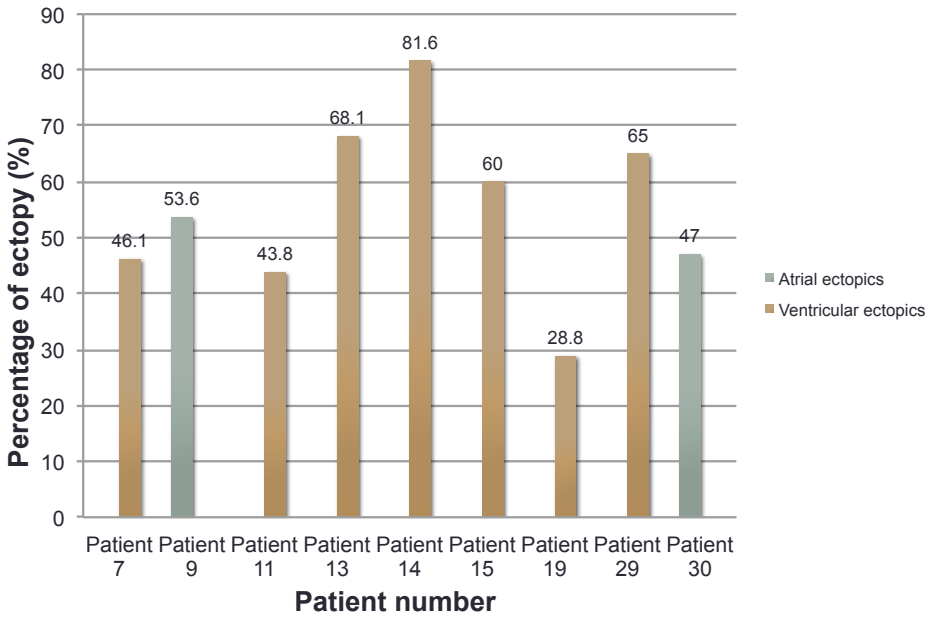


Figure 4.7- Proportion of ectopy occurring on dialysis days in patients with significant ectopy.

Undersensing and oversensing (Figure 4.8)

Undersensing (defined as failure of the device to sense intrinsic cardiac activity) was documented in 26 of the 30 patients, predominantly due to variations in R wave amplitudes. Four of the 26 patients had ≥ 50% undersensing episodes occuring on dialysis days. Undersensing was significantly more on non dialysis days ($p = 0.001$, 95% CI 34.2 to 78.5%).

Over sensing (defined as inappropriate recognition of signals as native cardiac activity) was documented in 24 of the 30 patients. This was predominantly due to T wave oversensing subsequent to sudden changes in T wave morphology or due to artefact/ noise. Five of the 24 patients had ≥ 50% oversensing episodes occuring on dialysis days. The tendency to oversensing was also significantly more on non dialysis days ($p < 0.001$, 95% CI 30.1 to 79%).

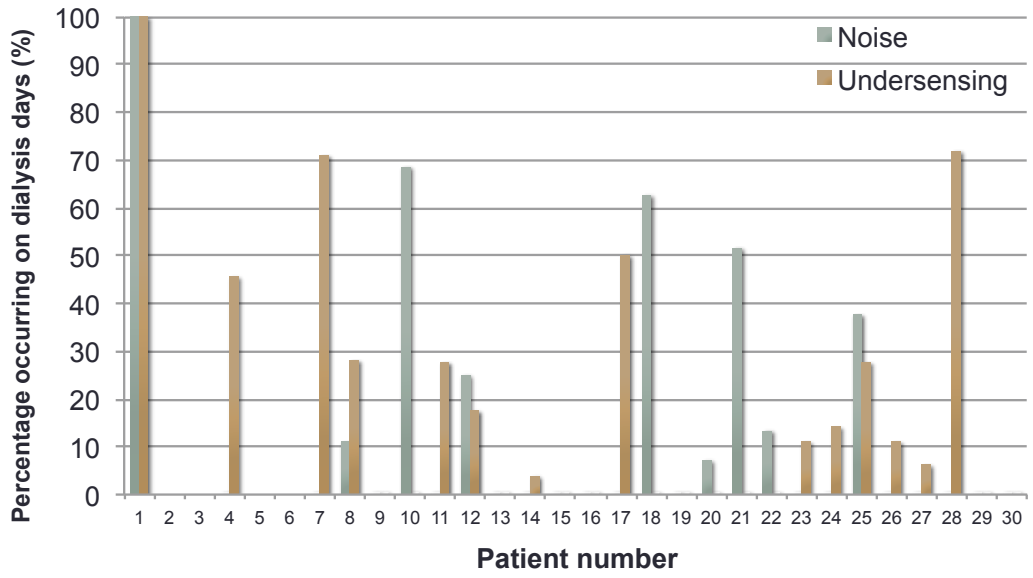


Figure 4.8- Individual patient data on percentage of noise/ under sensing occurring on dialysis days

DISCUSSION

Our findings confirm the high mortality rate seen in haemodialysis populations and contrary to initial expectations, bradyarrhythmias rather than tachyarrhythmias emerged as the commonest and most significant arrhythmic event. Analysis of 379,512 (median 13,356) hours of continuous ECG monitoring in our cohort suggests that, whilst arrhythmias are relatively common, a high proportion of fatal arrhythmia which may otherwise be classified as SCD occur at the end of natural life or in the context of a significant non-cardiac inter-current illness, and are therefore unlikely to benefit from preventative strategies such as ICD. Marked bradycardia, necessitating pacemaker implantation, was found in 10% of our cohort, all of whom were asymptomatic.

Supporting data towards the potential importance of bradycardia has come from a recent study by Wong *et al*. who evaluated 50 haemodialysis patients with ILRs for a mean of 12 ± 4 months. (238) In this study no VT/VF was documented and all 6 deaths occurred with severe bradycardia and ensuing asystole; the authors defined 83% of deaths in this study as SCD. CRASH-ILR with an even more prolonged period of monitoring and with attempts made to download data at every haemodialysis session, found asymptomatic episodes of bradyarrhythmia in 10% and that SCD accounted for 28% of all deaths, which is similar to that noted in the USRDS. (134)

These findings are fundamental when designing studies to evaluate interventions directed towards reducing the risk of SCD. Bradycardia in haemodialysis patients

remains largely undefined and could represent a manifestation of the accelerated calcific and fibrotic processes characteristic of the hearts of ESKD patients. The pacemaker implantation rate of 10% in this asymptomatic population is significantly more that the 2.5% prevalence rate seen in registry data of older persons. (239, 240) Implanting pacemakers is a cost effective and potentially lifesaving intervention and the influence of ESKD on the presence of conduction system disease need to be tested in larger studies.

Current guidelines for implantation of primary prevention ICDs require the presence of severe LV systolic dysfunction or the presence of high-risk congenital or inherited conditions. (4) Thus although up to 15% of patients have severely impaired LV systolic function at the initiation of chronic haemodialysis, (241) only around 6% of haemodialysis patients fulfil criteria for primary prevention ICDs after taking into consideration recommended factors such as life expectancy, comorbidities, functional status, presence of ischemic heart disease as well as psychological impact of having an ICD. (242) ESKD is not classified as a risk factor for SCD in its own right despite the presumed high risk. Also, the prognostic role of severely impaired LV function in ESKD has been questioned and studies have found it to be non-predictive of SCD. (243)

Where ICDs have been implanted in haemodialysis patients, there has not been clear demonstration of benefit. Pun *et al* identified 108 dialysis patients from the National CV Data Registry's ICD Registry who had received primary prevention ICDs and compared them with195 dialysis patients with similar characteristics who did not have ICDs. (30) One and 3 year mortality was 42.2% and 68.8%, respectively, in the ICD

registry cohort compared with 38.1% and 75.7% in the control cohort with no significant survival advantage associated with ICD [HR 0.87, 95% CI 0.66 - 1.13, log-rank p = 0.29] with or without propensity matching.

CRASH-ILR has highlighted a number of challenges when utilising ILR technology in a haemodialysis population. Despite strict aseptic precautions at implantation, two patients developed infection requiring explant, reflecting the general susceptibility of dialysis patients to procedure related infections. (244) The Reveal XT is particularly sensitive for the detection of AF. Yet AF was incorrectly identified in many transmissions due to T wave over sensing resulting from intermittent deep T wave inversion, under sensing of R waves following a drop in the size of recorded QRS complexes, as well as noise/interference. The false positive auto detected episodes in CRASH-ILR were greater than previously reported rates of 22.8% for Reveal XT devices. (245) Detailed analysis of our cohort suggested greater under-sensing and over-sensing on non-dialysis days, perhaps implying a role for increasing fluid volumes and reductions in thoracic impedances. As originally specified in the protocol, we did not make any adjustments to sensitivities of individual devices during the study. These findings need to be carefully considered when designing larger studies and are likely to necessitate the incorporation of re-programming protocols for the ILRs after initial data download.

Limitations

The patient number in this study was small making it difficult to draw generalizable conclusions about the haemodialysis population as a whole. In a number of cases data were lost where the device could not be retrieved following death, despite extensive

education of local mortuaries regarding the need to inform local pacing/device clinics when patients die with an implantable device in situ. However, we feel that the frequent downloads from the ILRs is a key strength of the study, ensuring that there was no overwriting of ECG traces. No events were missed due to lack of memory space on the device, such as seen in the recent loop recorder study in haemodialysis patients awaiting renal transplant by Silva *et al*. (246) Recruitment was slow primarily due to the challenges around recruiting patients with chronic disease; close working between cardiology and nephrology colleagues was key to the success of this study. The invasive nature of cardiac monitoring was a deterrent for many patients but the introduction of much smaller new generation injectable loop recorders, (Reveal linq-Medtronic, USA) would make this less of an issue. These devices are easier to implant with low risks of infection and are better equipped to deal with sensing issues. (247, 248)

Despite all of above the hurdles, the findings from our study appear representative of a typical UK dialysis population especially as the all cause mortality rate was similar to UK figures. (174)

CONCLUSIONS

This study has demonstrated that a study of ILRs with attempted downloads at every haemodialysis session is feasible in haemodialysis patients. Following almost 380 000 hours of continuous ECG monitoring in a sample cohort characteristic of a typical haemodialysis population, our findings raise pertinent issues. Undoubtedly there is a high mortality risk in patients receiving haemodialysis, yet SCD due to VT/VF is uncommon and tachyarrhythmias are more likely to reflect the final mode of death in

an otherwise complex downward spiral. Asymptomatic bradyarrhythmia requiring implantation of devices was common (10% of our cohort). The higher susceptibility to infection and potential risks of inappropriate shocks due to over-sensing for patients with ICD have been highlighted.

Further analysis is required to see if expanded use of ILRs in the haemodialysis population might help to risk stratify those at risk of life-threatening arrhythmias and whether detection and intervention towards asymptomatic bradyarrhythmias might translate into a life prolonging intervention. Data from the US based Monitoring in Dialysis (MD) study of ILRs in haemodialysis are also awaited.(249) Many lessons have already been learnt that will inform the design and conduct of larger scale studies to identify a phenotype of haemodialysis patients that might have a modifiable risk of SCD. An integrated approach between cardiologists and nephrologists is key.

CHAPTER 5- CARDIAC RESYNCHRONISATION THERAPY IN SEVERE CHRONIC KIDNEY DISEASE- IMPACT AND FEASIBILITY

BACKGROUND

Current guidelines recommend that patients with heart failure and LVEF < 35% despite an adequate trial of pharmacological therapy, who are expected to survive with a reasonable quality of life for ≥ 1 year should receive an ICD, and where QRS duration is prolonged, this should often be a CRTD. (4) Guidelines caution against implanting devices into patients with recently decompensated heart failure but make no specific caution or recommendation about renal dysfunction.

Many studies have highlighted that as one moves to the right of the cardiorenal continuum (Figure 1.5), i.e. symptomatic target organ damage and co-existence of CKD and CHF, although CV and mortality risks rise, the use of evidence based therapy remains suboptimal. A recent study by Pun *et al* examined associations between kidney function and guideline-recommended prescription of ICD/CRT in a heart failure registry by categorising patients according to eGFR; ≥ 60, 59 to 30, < 30 mL/min/1.73 m^2, and dialysis dependent. (250) Of the 26 286 patients eligible for a complex device, (61% of whom had an eGFR < 60 mL/min/1.73 m^2), only 45% received ICDs and 30.5% CRTs. Compared to patients with eGFR ≥ 60 mL/min/1.73 m^2, patients with eGFR 30 to 59 mL/min/1.73 m^2 were more likely to

receive an ICD (adjusted odds ratio [aOR] 1.08, 95% CI 1.01 to 1.14), whereas dialysis patients were less likely (aOR 0.61, 95% CI 0.5 to 0.76). Worse kidney function was associated with a decreased likelihood of CRT prescription (aOR 0.97 per 10 ml/min eGFR decrease, p = 0.03) and prescription of ICD/CRT was associated with greater 1-year survival in all eGFR groups.

Adelstein *et al* in their study of 787 heart failure patients who received CRT-D demonstrated that those with moderate renal insufficiency (GFR 30-59 ml/min/1.73 m^2) showed higher survival benefit compared to 80 control patients who received ICDs (Control patients were CRTD eligible but had failed LV lead implant). (251) Severe baseline renal dysfunction (GFR < 30 mL/min/1.73 m^2) predicted poor survival and limited echocardiographic improvement despite a modest GFR increase. CRT recipients with normal renal function in this study derived echocardiographic benefit but no overall survival advantage over those who had just ICDs.

Observational studies have also demonstrated CKD to be a strong and independent predictor of long-term mortality among patients undergoing CRT-D implantation. One study followed up 432 consecutive patients implanted with CRTDs for 4.3 ± 3.2 years. (252) Patients with normal and mild renal diseases (Stages 1 and 2 CKD) were found to have improved survival compared with those with moderate, severe, or end-stage (Stages 3 - 5) renal dysfunction. The estimated 5-year mortality in this cohort was 36.3% for stage 1, 33.4% for stage 2, 40.6% for stage 3, and 62.1% for stage 4 or 5 CKD (p = 0.004 by log-rank test).

There is limited data on the effect of CRT on renal function. It has been associated with stabilisation of renal function in patients with severe LV dysfunction and even improvement in stage 4 and 5 CKD. (253) Improved renal function following CRT has been associated with lower mortality. (253)

There thus remain significant CKD-based differences both in prescription of complex device therapy in CHF and their prognostic impact. Difference in uptake of device therapy in this population may be related to fear of procedural complications. The CRT-CKD study was thus designed to describe the incidence of procedural complication rates in a cohort of patients with significant CKD and to assess the impact of CRT on progression of renal dysfunction. Studies to date have looked at changes in mean eGFR in the entire cohort over time, following CRT implantation. In this CRT – CKD study, eGFR changes were assessed in individual patients and the repeated measures trend evaluated over time.

AIMS

1. To ascertain the post procedural complication rates and mortality risks in patients undergoing CRT implantation in the presence of significant CKF
2. To define the changes in renal function post device implantation and to compare variations pre and post device implantation.

METHODS

Data regarding CRT implantation (CRTP and CRTD) were rigorously collated after scanning patients' medical notes and comprehensive device databases maintained at 2 high volume implant centres in the UK (Royal Bournemouth hospital and Queen Alexandra hospital, Portsmouth). All consecutive device implants for the period of 1

January 2009 to 31 December 2011 were included in the study. The study was approved by the NHS health research authority (REC reference 14/NW/1523, IRAS ID reference 149782). I collected all the data at Queen Alexandra hospital Portsmouth, collated the information from both participating centres and carried out all the statistical analysis relevant to the study.

The primary outcome was to evaluate the difference in procedural complication rates (pneumothorax, lead displacement, lead repositioning, device related infection) and all-cause mortality post device implantation in patients with estimated glomerular filtration rate (eGFR) $\geq$ 45 ml/min/1.73 m^2 compared to patients with eGFR < 45 ml/min/1.73 m^2 (CKD class 3b-5). The secondary outcomes were to evaluate the difference in the symptomatic benefits of CRT (defined as change in NYHA class) and progression of renal function over a 1-year period post CRT implant between those patients with and without significant CKD (eGFR < 45 ml/min/1.73 m^2)

Demographic data, haematological and biochemical blood test results (haemoglobin, serum creatinine, LVEF (from echocardiogram), ECG findings, NYHA class and medication history were documented at the various time points. Data was collected by accessing electronic records including device databases, patient letters and pathology databases (for blood results). Chest Xrays post device implant were accessed and reviewed for each patient via the radiology online imaging database.

Evaluation of renal function

Renal function measured at various time points in the patient journey was collected for the study. Blood tests from 12, 6 and 3 months prior to CRT implant, implant and

3, 6 and 12 months post CRT implant were recorded. Renal function performed within 45 days of the time point of interest was considered adequate; for example, renal function at 6 months ± 40 days was accepted as the 6-month value. Significant CKD in this study was defined as the presence of an eGFR < 45 ml/min/1.73 m^2. eGFR was evaluated using the modification of diet in renal disease (MDRD) equation. (122)

Progression of renal function following CRT implant was evaluated by repeated measures analysis of eGFR at 6 months pre-implant, implant, 6 months and 12 months post implant. The change in eGFR was computed as the difference between implant eGFR and eGFR 6 months prior (time line1), 6 months post implant eGFR and implant eGFR (time line 2), 12 months post implant eGFR and implant eGFR (time line 3).

Patients were categorised into quartiles based on baseline (implant) eGFR and also based on the rate of change in eGFR 6 months prior to device implantation, the latter being to evaluate the impact of dramatic changes in eGFR in the weeks preceding device implant. At 12 months post implants, all-cause mortality was assessed for every individual patient included in the above categorisation.

STATISTICAL ANALYSIS

Statistical analyses were performed using SPSS software version 24. Summary statistics (e.g., mean, standard deviation, minimum, maximum, proportions) were calculated for all variables. Proportions were expressed in percentages. Differences in baseline, procedural, and follow-up characteristics between patients with and without CKD were compared using independent samples T test for variables with

normal distribution and Wilcoxon- Rank for the others. A repeated measures ANOVA was used for assessing the change in eGFR over time. Where Mauchly's Test of Sphericity was not satisfied, Greenhouse Gaisser corrections were applied. Mortality was assessed by Kaplan–Meier estimates and compared with the log-rank statistic (Mantel-Cox). A p value of < 0.05 was considered to be statistically significant.

RESULTS

Between January 2009 and December 2011, 458 patients underwent CRT implantation at the 2 selected centres. An LV lead was successfully placed in 448 patients. Patients who did not receive an LV lead (n = 10) and those with incomplete data entries at the time of device implant were excluded from further analysis (n = 19).

Baseline characteristics (Table 5.1)

Of the 429 patients evaluated, mean creatinine was 108 ± 58 μmol/L and mean eGFR 58 ± 20 ml/min/1.73m^2. 26% had an eGFR < 45 ml/min/1.73 m^2. Patients undergoing CRT implantation with an eGFR < 45ml/min/1.73 m2 were older with a mean age of 77.9 ± 6.5 years. The presence of comorbidities (AF and hypertension) was similar irrespective of the severity of kidney dysfunction. Ischaemic heart disease, previous MI and diabetes were significantly more common in those with an eGFR < 45 ml/min/1.73 m^2. Dilated cardiomyopathy was more commonly seen in those with an eGFR $\geq$ 45 ml/min/1.73 m^2. LBBB was documented in a greater proportion of patients with eGFR < 45 ml/min/1.73 m^2.

	eGFR ≥ 45 (N = 317)	eGFR < 45 (N = 112)	p value
Age (years)	70.3 ± 11.2	77.9 ± 6.5	0.001
Males (%)	74.2	72.7	0.780
Mean creatinine	88 ± 32	141 ± 73	0.001
Mean eGFR	68 ± 14	34 ± 8	0.001
LVEF (%)	31 ± 11	29 ± 9	0.350
QRS width (msec)	151 ± 30	157 ± 26	0.155
LBBB (%)	69	78	0.001
RBBB (%)	9.2	8.3	0.641
Primary indication heart failure (%)	81	92	0.007
Upgrade to CRT (%)	17	15	0.56
CRT-D (%)	43	37	0.31
Comorbidities			
AF (%)	48.4	50	0.057
Hypertension (%)	41.2	50.8	0.090
IHD (%)	52.6	69.7	0.001
Previous MI (%)	36.6	55.3	0.026
DM (%)	23.5	29.5	0.024
DCM (%)	31.4	17.4	0.001

Table 5.1- Baseline Characteristics: CRT in CKD Study. eGFR was expressed as ml/min/1.73m^2.

Post procedural complications (Figure 5.1)

There were no hematomas requiring surgical re-intervention. There was no incidence of acute renal failure or contrast induced nephropathy requiring intervention or warranting prolonged hospital stay. There was no significant difference in the occurrence of post procedural complications in the presence of significant CKD (eGFR cut off of 45 ml/min/1.73 m^2). Differences in the occurrence of complications were also evaluated after dividing patients as per implant eGFR quartile. This did not demonstrate any statistically significant differences. Variations in renal function in the months leading up to device implant were not associated with increased procedural risk either.

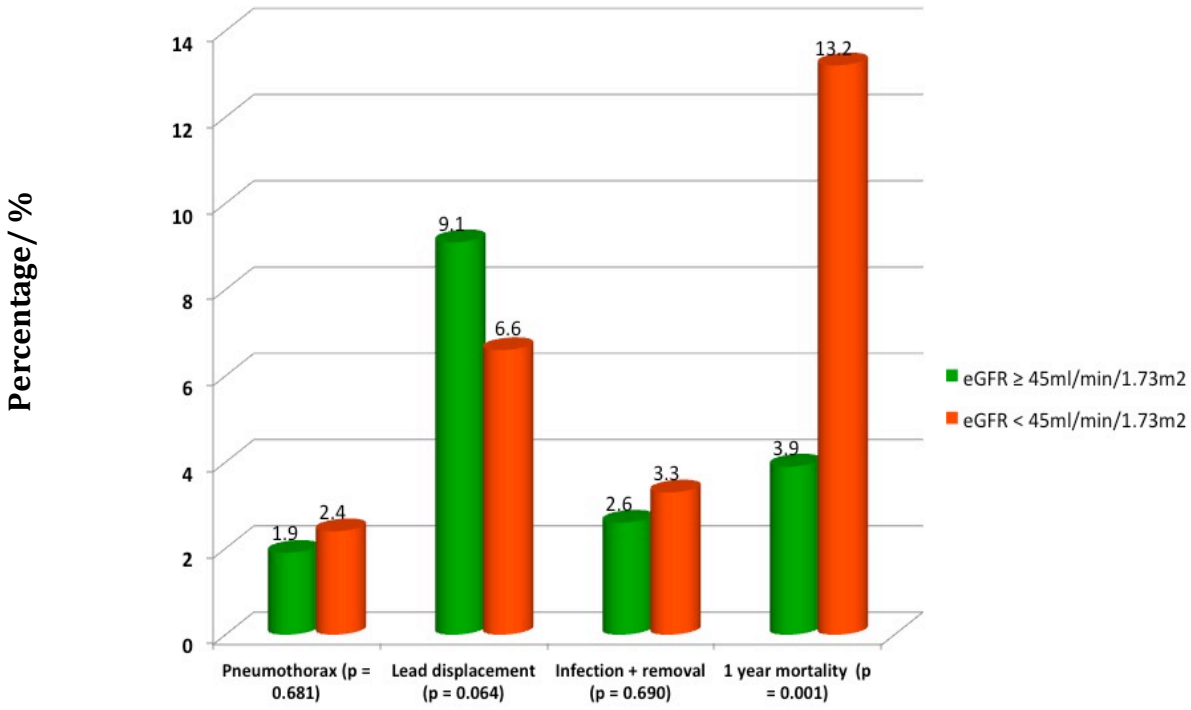


Figure 5.1: CRT-CKD study- procedural complications and 1-year mortality.

Complications are represented as percentages (y-axis). There was no statistically significant difference in the occurrence of pneumothorax, lead displacements and device infection necessitating removal, between those with and without significant CKD (denoted by p values). eGFR is expressed in ml/min/1.73 m²

Both patient groups exhibited symptomatic benefit. Symptomatic benefit was evaluated by the physician carrying out the follow up visit by assessing the change in NYHA class at 3 months post device implant. 65% of patients with eGFR ≥4 5 ml/min/1.73 m² and 71% with eGFR < 45 ml/min/1.73 m² improved by ≥ 1 NYHA class (p = 0.61).

Change in renal function and CRT implantation

All patients had blood tests within 10±4 days of CRT implant. 264 patients (60.2%) had bloods 6 months pre-implant, 429 at implant, 267 (60.9%) at 6 months post implant and 227 (51.8%) at 12 months post implant. Tests were carried out 181 ±

42 days pre implant, the 6 month post implant at 187 ± 45 days and the 12 month post implant at 350 ± 30 days post date of device implantation.

Compared to implant eGFR, at 6 months post implant, 33.3% of the population (n = 89) had a decline in eGFR of ≥ 5ml/min/1.73 m^2, 40.8% (n = 109) remained stable with an eGFR change between -4 to +4 ml/min/1.73 m^2. 25.8% (n = 69) demonstrated an improvement in eGFR of 5ml/min/1.73 m^2. At 12 months post implant, 37.4% (n = 85) had a decline of ≥ 5 ml/min/1.73 m^2 compared to implant eGFR. 40.5 % (n = 92) remained stable (-4 to +4 ml/min/1.73 m^2) and 22% (n = 50) had an improvement of eGFR by ≥ 5 ml/min/1.73 m^2.

In the overall population, there was a significant decline in eGFR over time as demonstrated by a repeated measures ANOVA with a Greenhouse-Geisser correction (F (2.618, 332.56) = 8.178, p < 0.001); Figure 5.2. Post hoc tests using the Bonferroni correction revealed that eGFR 6 months prior to implant was significantly different from the eGFR at 12 months post implant (51.67 ± 19.8 vs. 44.93 ± 18.605, respectively, p = 0.001). eGFR at implant was also significantly different from eGFR at 12 months post implant (50.11 ± 17.539 vs. 44.93 ± 18.605, respectively, p = 0.002). There was no significant difference between eGFRs 6 months pre implant and 6 months post implant (51.67 ± 19.85 vs. 48.07 ± 16.50, respectively, p = 0.055) or eGFR at implant and 6 months post implant (50.11 ± 17.54 vs. 48.07 ± 16.50 respectively, p = 0.449)

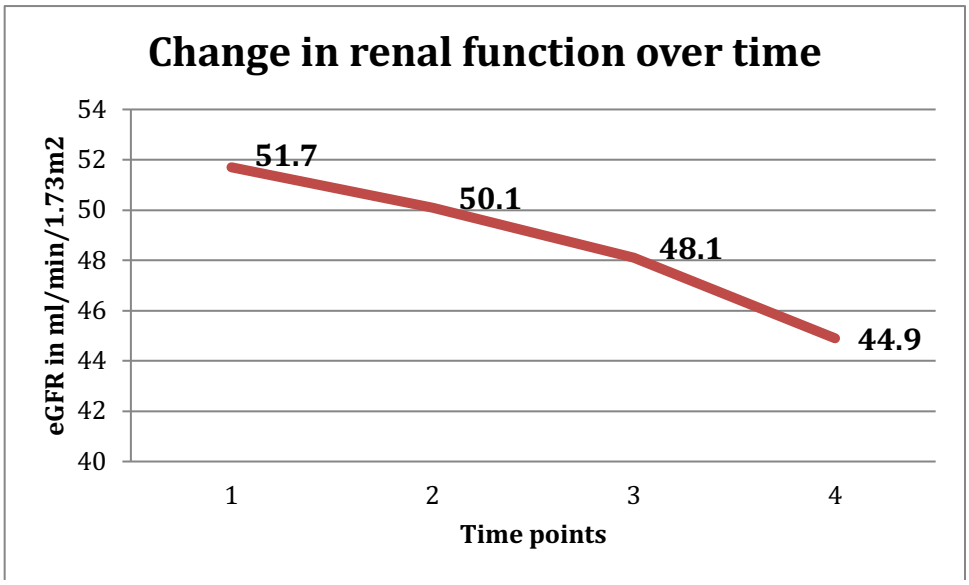


Figure 5.2- Change in eGFR over time (eGFR is expressed in ml/min/1.73m^2).
Time points are: 1= 6months pre implant, 2= at implant, 3= 6 months post implant, 4= 12 months post implant.

Median baseline eGFR was 57 ml/min/1.73 m^2 (lower and upper quartiles: 43-73 ml/min/1.73 m^2). When divided into quartiles as per baseline eGFR at implant, there was a significant difference in eGFR values over the 4 time points in quartiles 1 {F (2.455, 110.48)= 9.513, p < 0.001}, 2 {F (2.28, 89.07)= 3.201, p = 0.039} and 4 {F (2.03, 34.57) = 3.720, p = 0.034} but not in quartile 3 {F (1.83, 42.17)=1.672, p = 0.202} (Figure 5.3)

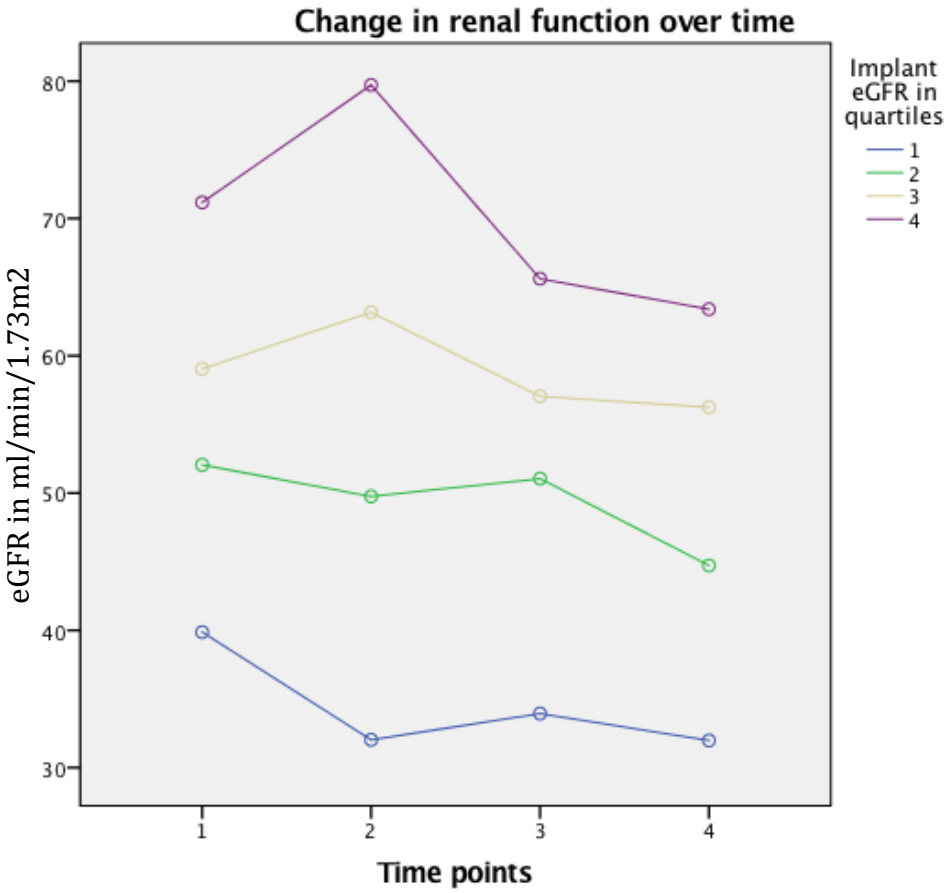


Figure 5.3- Change in eGFR over time, population categorised as per implant eGFR quartile (quartile 1 being the lowest)

Whilst the eGFR variation over time was significant in those with eGFR >73 ml/min/1.73m^2, eGFR 43-57 ml/min/1.73 m^2 and those with eGFR <43 ml/min/1.73 m^2, there was no significant variation in those with eGFR between 57-73 ml/min/1.73 m^2.

Time points are: 1= 6 months pre implant, 2= at implant, 3= 6 months post implant, 4= 12 months post implant.

Median change in eGFR over the 6 months prior to implant was -2 ml/min/1.73 m^2

(lower and upper quartiles were -12 and 4 ml/min/1.73 m^2 respectively)

Mortality

In the presence of significant CKD, mortality at 1 year was significantly greater ($p <$

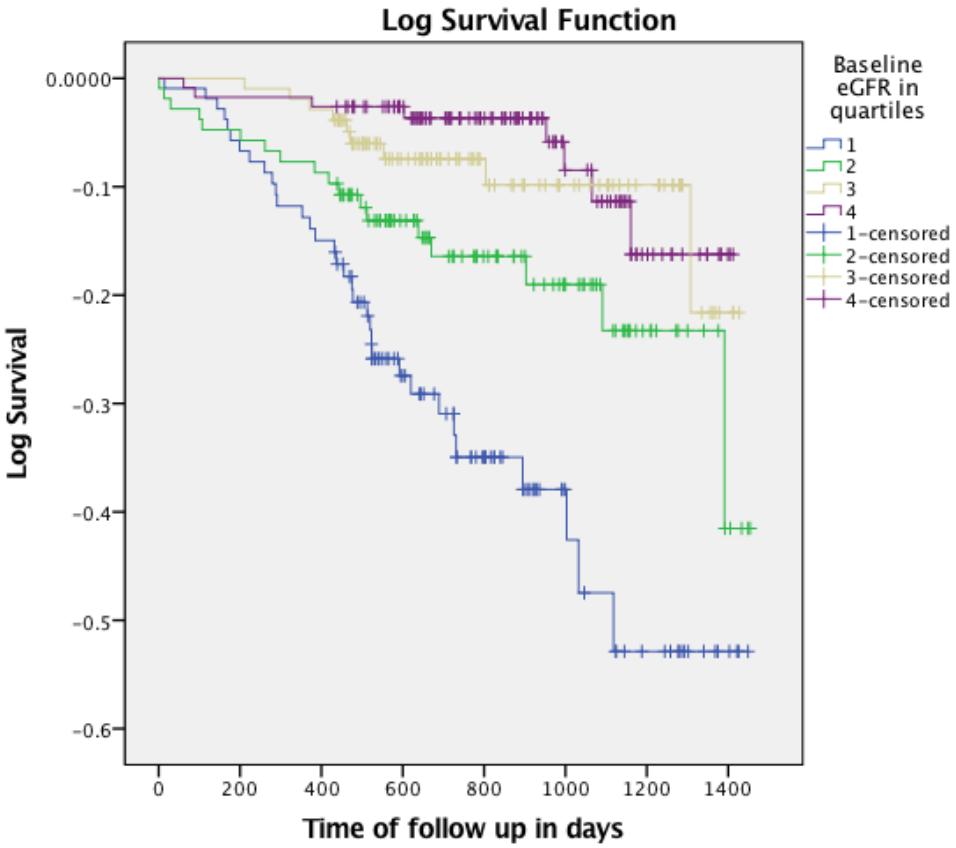
0.001, Figure 4.1).

This increased mortality risk was assessed in further detail by dividing the population into 4 quartiles based on

1. Baseline eGFR at implant.
2. Change in eGFR over 6 months prior to implant (Change in eGFR at implant- eGFR 6 months prior).

Quartile cut offs for baseline eGFR were 43, 57 and 73 ml/min/1.73 m^2 and for change in eGFR -12, -2 and 4 ml/min/1.73 m^2.

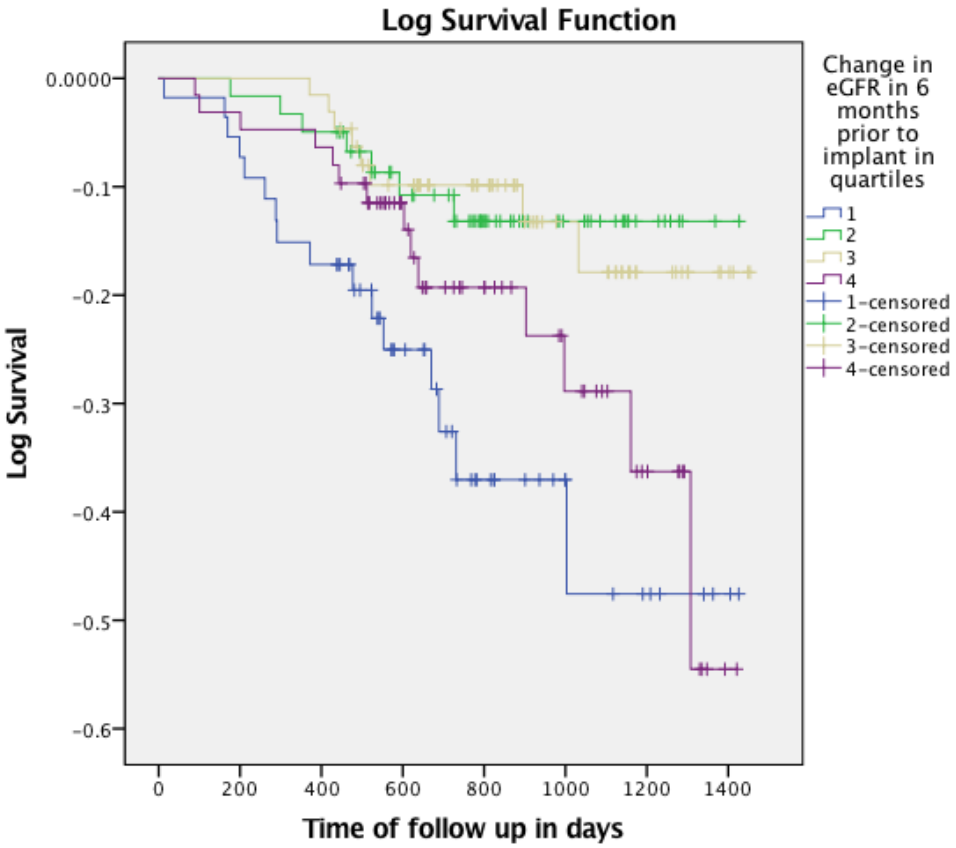
As expected, survival in the presence of severe CKD was significantly reduced as illustrated in the Kaplan-Meier curves below. Patients in the lowest quartile of eGFR had the greatest mortality (Figure 5.4). When categorised according to the change in eGFR in the 6 months pre-device implant, mortality was greatest in those who had the greatest fluctuations in eGFR (Figure 5.6). However change in eGFR pre implant did not seem to have any impact on survival once patients were divided into those with and without significant CKD.



Log-rank (Mantel Cox) p < 0.001. Plot 1- eGFR up to 43 ml/min/1.73 m^2, plot 2- eGFR between 43 ml/min/1.73 m^2 up to 57 ml/min/1.73 m^2, plot 3 – eGFR between 57- 73 ml/min/1.73 m^2, plot 4- eGFR of 73 ml/min/1.73 m^2 and above.

Figure 5.4- Kaplan- Meier hazard plots for the study cohort, a comparison based on implant eGFR.

The lowest quartile of eGFR, i.e., < 43 ml/min/1.73 m^2 was associated with the greatest mortality. Quartile cut-offs for baseline eGFR were: 43, 57 and 73 ml/min/1.73 m^2.



Log rank (Mantel Cox) p = 0.021. Plot 1- change in eGFR up to -12 ml/min/1.73 m^2, plot 2- change in eGFR between -12 up to -2 ml/min/1.73 m^2, plot 3- change in eGFR between -2 up to 4 ml/min/1.7 $3m^2$, plot 4- change in eGFR of 4 ml/min/1.73 m^2 and above.

Figure 5.6 – Kaplan- Meier survival curves for the study population, patients categorised as per change in eGFR in the 6 months leading up to device implant.
Cut-offs for quartiles change in were eGFR -12, -2 and 4 ml/min/1.73 m^2. Dramatic changes in eGFR pre- procedure appeared to be associated with poor survival.

DISCUSSION

Significant CKD is common in patients undergoing CRT implantation. Whilst 1-year mortality rates following CRT are greater in those with CKD class 3b - 5, a similar improvement in symptom status was seen in this cohort with no excess of implant related complications.

Changes in renal function (eGFR) in the period leading up to CRT implant in our study did not predict subsequent change in eGFR post implant. All patients demonstrated a significant decline in renal function over time. This decline only became significant by 12 months post implant and was independent of the change in eGFR in the months leading up to implant. Thus it would appear that CRT implant does not appear to worsen deterioration of renal function in those with severe renal dysfunction at baseline.

A similar study was conducted in 588 CRT patients where the effect of CRT on renal and cardiac function was studied at short term (≤ 6 months post implantation) and long term (> 6 months). (253) Here too, there was no significant deterioration in mean GFR during follow up and there appeared to be significant improvement of renal function in patients with advanced kidney disease. Multivariate logistic regression analysis demonstrated that stable GFR or an improvement in GFR independently predicted mortality after adjusting for co-morbidities. Another study of 238 patients undergoing CRTD implantation with eGFRs before implantation and 6 ± 3 months thereafter followed up patients for 4.3 years. (254) (ESKD patients were excluded.) Here eGFR decreased (78.5 ± 17.3 to 67.8 ± 26.8 $p < 0.001$) in patients with mild (stage I/II) CKD whereas eGFR did not change in patients with advanced (stage III/IV) CKD (45.6 ± 11.1 to 46.8 ± 17.0, $p = 0.46$). Patients with advanced CKD had higher mortality than those with mild CKD ($p < 0.002$) and increase in eGFR in both subgroups was associated with improved survival (HR = 0.79, $p < 0.001$).

Contrary to the above studies, our CRT-CKD study did not suggest significant improvements in renal function post CRT therapy. This could be because of our

longer follow up with regards to renal function. Heart failure with its inherently progressive nature is known to be associated with renal dysfunction over time; most patients with CHF older than 65 years of age have an eGFR < 60 ml/min/1.73 m^2 (prevalences of up to 57% have been reported). (255) In this study by de Silva *et al* (n = 1216), worsening renal function defined as an increase in serum creatinine of > 26.5 micromol/L (> 0.3 mg/dL) occurred in 13% patients during 6 months of follow up and this, along with baseline renal dysfunction, predicted a higher mortality ($p < 0.001$). An improvement in renal function over the first 6 months predicted a lower mortality (HR 0.8, 95% CI 0.6 - 1.0).

There were greater reductions in eGFR in our cohort (33% and 37% at 6 months and 12 months post implant respectively); this could reflect more advanced heart failure. Irrespective of the changes in eGFR, low eGFR predicted poorer long-term survival. These results demonstrate that complex devices can be implanted safely without excessive complications and with equal symptomatic benefit in patients with significant CKD, who otherwise fulfil the criteria for CRT implantation. Advanced CKD and labile changes in eGFR were associated with worse survival even following CRT implantation and further studies are required to ascertain the impact of CRT on mortality in those with significant CKD.

Limitations

This study by not being a randomised control trial limits its role in guiding therapies in heart failure patients with severe CKD. The absence of a control group makes it difficult to draw meaningful assumptions regarding the true impact of CRT on progression of renal function. As with all retrospective studies, there already exists a

selection bias as these patients were selected by their physicians as potential beneficiaries from CRT therapy despite their comorbidities. The frequency of blood tests might reflect how ill patients may be and variations in renal function may reflect hospitalisations/ decompensation episodes. This limits interpretations of findings especially when taking into account repeated measures analyses of eGFR and their impact on mortality. Also medication changes during the course of follow up were not taken into account and this may have had an additional impact on symptomatic states and changes in renal function. The conclusions are nonetheless based on a large number of participants reflecting current practice in England and therefore make a significant value addition to the limited repertoire of evidence for device therapy in CKD. Being a study based on retrospective data, minor complications like small wound hematomas were not recorded and hence not accounted for in comparing complication rates between those with and without severe CKD.

CONCLUSIONS

The presence of significant CKD in the advanced heart failure population participating in this study was not associated with increased post procedural complications. Irrespective of device therapy, worsening renal function is associated with increased mortality at 1 year. Nevertheless symptomatic benefit was seen in all patients, irrespective of baseline renal function. A prospective study comparing changes in renal function in those with and without CRT implantation would help understand the impact of cardiac resynchronisation per se on subsequent progression of renal function. However as CRT is a guideline recommended therapy irrespective of renal function, a meta analysis with pooled individual data and renal function from previous CRT based randomised control trials would be the way forward.

CHAPTER 6- THE IMPACT OF CHRONIC KIDNEY DISEASE ON SECONDARY PREVENTION POST PRIMARY PERCUTANEOUS CORONARY INTERVENTION

INTRODUCTION

Treating patients with CKD and CVD has historically been suboptimal despite major advances in therapies. Following a myocardial infarction for example, patients with co-morbidities such as CKD are less likely to be prescribed prognostically beneficial drugs. Such patients have a high absolute risk of an adverse outcome and thus have greater overall potential for benefit from therapy. Yet studies in post infarct patients in the nineties have shown the utilisation of secondary prevention to be as low as 8.6% (for the combination of aspirin, beta blockers and angiotensin-converting-enzyme (ACE) inhibitors) in the presence of severe CKD. (256) The Valsartan in Acute Myocardial Infarction Trial (VALIANT) with 14 527 acute myocardial infarction patients complicated by heart failure, highlighted this under-utilisation of aspirin, beta blockers and statins and additionally showed that declining eGFR was associated with increased risk of death and non-fatal CV outcomes. (257)

There are several plausible reasons as to why high-risk patients with CKD receive sub-optimal therapy post MI. These include the apprehension that drugs such as ACE inhibitors may result in detrimental effects on renal function or are poorly tolerated, the failure of healthcare systems to provide specialist cardiology care to those with co-

morbidity, or even a belief that these patients are beyond help ('therapeutic nihilism'). (258) Treating an acute myocardial infarction with PPCI, irrespective of renal function (the latter is usually unknown when taken to the catheterisation laboratory), may have reduced the possible bias in healthcare professionals against prescribing in high-risk groups.

AIM

My hypothesis was that PPCI would be associated a high uptake of secondary prevention medication and my study aim was to assess the influence of renal function on secondary prevention medication in 5 UK sites offering PPCI.

METHODS

This was a retrospective cohort review of 5 UK cardiac centres (Dundee, Hull, Leicester, Portsmouth, Stoke-on-Trent) providing primary PCI for acute STEMI. A cohort of consecutive patients undergoing PPCI (2010 - 11) who survived to discharge was included at each centre. Demographic data (age and sex) and results of local laboratory investigations at presentation (including haemoglobin, serum creatinine and estimated glomerular filtration rate) were recorded. Secondary prevention medications (statins, ACE inhibitors or ARB, beta-blockers as well as anti-platelet treatment) prescribed at discharge were obtained from electronic discharge summaries or case note evaluation.

Prescribing practice following discharge was carefully ascertained to assess the extent of drug withdrawal and titration in relation to renal dysfunction. Many of the included patients were followed up by their local non-PPCI centre. Only patients who attended

a post-myocardial infarction clinic at their PPCI centre were included in further analysis.

Medication prescription was compared according to renal function. Renal function was quantified as eGFR in ml/min/1.73 m^2 according to local laboratory calculations from the 4-component MDRD equation incorporating age, race, sex, and serum creatinine level (131): estimated GFR = 186 × (serum creatinine level [in mg/dL])$^{-1.154}$ × (age [in years])$^{-0.203}$. The population was divided into 3 groups based upon the Kidney Disease Outcomes Quality Initiative guidelines(131): eGFR ≥ 60 ml/min/1.73 m^2 (CKD class 1 and 2), eGFR 45-59 ml/min/1.73 m^2 (CKD class 3a) and eGFR < 45 ml/min/1.73 m^2 (CKD class 3b, 4 and 5). We anticipated that the unselected patient population would include many elderly individuals and therefore felt that it was important to separate CKD class 3 into 3a and 3b particularly since previous studies showed patients with eGFR < 45 ml/min/1.73 m^2 receive the poorest secondary prevention.

The doses of medication prescribed were considered. As there are several different drugs in each class, I used dose equivalence within each group. Bisoprolol was the most commonly prescribed beta-blocker and other beta blocker dosages were expressed as "bisoprolol equivalent doses" based on the proportion of maximal doses recommended in clinical guidelines (259) (2.5 mg bisoprolol OD = 6.25 mg carvedilol BD = 25 mg atenolol OD = 25 mg metoprolol BD = 2.5mg timolol BD). Similarly, ramipril was the most commonly used ACE inhibitor and other ACE inhibitor doses were expressed as "ramipril equivalent doses" based on the proportion of maximal doses recommended in the ESC ST elevation MI management guidelines (259) (2.5

mg ramipril= 2 mg perindopril = 5mg lisinopril). As the number of patients prescribed ARBs was very low (4.3%), I did not calculate equivalent ARB doses, but did include patients prescribed an ARB as being on an inhibitor of the renin-angiotensin system. Local audit committee approval was obtained for each participating site. The anonymous amalgamated data were analysed.

STATISTICAL ANALYSIS

Statistical analyses were performed using SPSS software version 19. Comparisons between different groups were performed by one-way analysis of variance (ANOVA). Cumulative values are presented as percentages and results for normally distributed data are presented as mean ± standard deviation (SD). All values are 2-tailed and $p < 0.05$ was considered statistically significant.

RESULTS

Complete drug history and demographics were available for 1169 of 1218 potential patients and these formed the final dataset. Two patients for whom no creatinine values were available were excluded from the analysis. The mean age of the study population was 63.7 ± 12.6 years, 73.3% were male and 20% aged > 75 years.

Discharge prescribing data

At discharge, 91.7% of the study population were prescribed an ACE inhibitor (90.4% of females and 92.3% of males), 93.6 % a beta blocker (92% of females and 94% of males), and 98.2% a statin (97.4% of females and 99% of males). There was no statistical difference in secondary prevention prescription between sexes.

Renal function and impact on discharge prescribing (Table 6.1)

Estimated GFR < 60 ml/min/1.73 m^2 was seen in 17.6%. The proportion of women increased with worsening renal function.

The proportion of patients prescribed beta blockers or statins was similar among the 3 eGFR groups. In contrast, the prescription of ACE inhibitors /ARB was lower in the group with the worst renal function: 76% compared to > 90% in patients with eGFR ≥ 45 ml/min/1.73 m^2 (Tables 6.1 and 6.2).

DISCHARGE	EGFR ≥ 60	EGFR 45-59	EGFR < 45	p VALUE
N (%)	962 (82)	138 (12)	67 (6)	
Mean eGFR (SD)	84 (17)	54(4)	33 (10)	
Male (%)	738 (77)	84 (61)	34 (51)	**0.025**
Age in years (SD)	62 (12)	73 (10)	75 (12)	0.108
% on beta-blocker BB	94	88	91	0.871
% on ACE(i) /ARB	95	91	76	**<0.001**
% on statin	98	96	96	0.914
Bisoprolol (mg)	3.4 (1.97)	2.9 (1.44)	3.6 (2.86)	0.300
Ramipril (mg)	3.8 (2.4)	4.0 (2.9)	4.0 (2.6)	**<0.001**

Table 6.1- Data from 1167 patients, classified according to eGFR (ml/min/1.73m2) showing demographics as well as utilisation of secondary prevention medication at discharge from hospital post primary angioplasty. ARB- Angiotensin receptor blocker, ACE (i)- angiotensin converting enzyme inhibitor, SD- Standard deviation. Values in red denote statistically significant values between groups (p < 0.05)

	DISCHARGE		FOLLOW UP	
	Number on ARB of total on ACE(i)+ARB	Percentage %	Number on ARB of total on ACE (i)+ARB	Percentage (%)
eGFR < 45	8 of 51	15.7	3 of 19	15.7
eGFR 45-59	6 of 126	4.8	6 of 66	9.1
eGFR ≥ 60	39 of 914	4.3	28 of 427	6.6

Table 6.2 - Utilisation of ARBs among different eGFR categories (eGFR in ml/min/1.73m^2). ARB- Angiotensin receptor blocker, ACE (i)- angiotensin converting enzyme inhibitor.

Follow-up

Follow-up data were available on 567 (49%) patients from 3 centres (Table 6.3). Most patients were followed up at 6 weeks post MI as per protocol of the hospital. Mean age was 62.3 ± 13.2 years and 18.2% of the follow up cohort had an eGFR < 60 ml/min/1.73 m^2. 73.6% were male and 20% of the follow up population was aged > 75 years. There was no statistical difference in proportions between the follow up cohort and the overall population. There was a similar pattern of prescribing at follow-up with the patients with a lower eGFR less likely to be prescribed ACE inhibitors. At 6 weeks post angioplasty, the mean bisoprolol dose was lower and use of ACE inhibitors was lower in patients > 75 years of age. There was a small decline in prescribing of all classes of secondary preventative drugs at 6 weeks, although this did not reach statistical significance (Tables 6.4). There was no significant difference in mean doses of ACE inhibitors or beta-blockers at follow up compared to discharge.

FOLLOW UP	EGFR ≥ 60	EGFR 45-59	EGFR < 45	p VALUE
N (%)	464 (82)	74 (13)	29 (5)	
Mean eGFR (SD)	82 (15)	54 (4)	35 (8)	
Male (%)	363 (78)	43 (58)	13 (45)	**<0.001**
% on beta-blocker	90	86	86	0.899
% on ACE (i)/ARB	92	89	66	**0.015**
% on statin	96	96	90	0.446
Bisoprolol	3.4 (1.99)	3.1 (1.80)	3.8 (3.13)	**0.002**
Ramipril	3.7 (2.32)	3.6 (2.14)	3.2 (2.08)	0.569

Table 6.3- Data from 567 patients, classified according to eGFR (ml/min/1.73 m^2) showing demographics as well as utilisation of secondary prevention medication at 6 weeks post primary angioplasty for STEMI

eGFR is expressed in ml/min/1.73m^2. Red font represents statistically significant values. ARB- Angiotensin receptor blocker, ACE (i)- angiotensin converting enzyme inhibitor, SD- standard deviation. Values in red denote statistically significant differences between the three groups, i.e. $p < 0.05$)

Renal function (n)	**Beta blocker use (%)**			**Mean Bisoprolol dose (mg)**			**ACE (i)/ ARB use (%)**			**Mean Ramipril dose (mg)**			**Statin use (%)**	
	D	F/U	p value	D	F/U	p value	D	F/U	p value	D	F/U	p value	D	F/U
eGFR ≥ 60 (464)	94	90	0.71	3.4	3.4	0.996	94	92	0.82	3.6	3.7	0.996	98	96
eGFR 45-59 (74)	87	86	0.93	3.1	3.1	0.995	92	89	0.74	3.6	3.6	1.0	95	96
eGFR <45 (29)	84	86	0.86	3.8	3.8	1.0	72	66	0.47	3.2	3.2	1.0	91	90

D- Discharge, F/U- Follow-up

Table 6.4- Data comparing secondary prevention utilisation in the same cohort of patients seen at discharge as well as at 6 week follow up post angioplasty

eGFR is expressed in ml/min/1.73 m^2. ARB- Angiotensin receptor blocker, ACE (i)- angiotensin converting enzyme inhibitor.

The impact of change in eGFR during the course of admission on ACE inhibitor/ ARB utilisation in 3 centres was also considered. Day 3 eGFR (when the potential effects of contrast nephropathy are likely to peak) was performed in 413 of 787 patients (52.4%). I divided this population (n = 413) into those with or without a clinically meaningful drop in eGFR (≥ 5 ml/min/1.73 m^2) during admission. A ≥ 5 ml/min/1.73 m^2 drop in eGFR on day 3 was seen in 34% (n = 140) and was associated with lower utilisation of ACE inhibitor /ARB (62 vs. 73%, $p < 0.001$) and a trend towards lower dose of ramipril equivalent (2.5 mg vs. 3.0 mg, $p = 0.058$).

DISCUSSION

This study shows that in the contemporary era of PPCI for acute STEMI, adherence to guidelines regarding secondary prevention pharmacological therapy is very high. In patients treated with PPCI in the UK, significant CKD (eGFR < 60 ml/min/1.73 m^2) is common (17.6%) and around 20% of the population are over 75 years of age. Older studies suggest that only 23% patients with eGFR < 45 ml/min/1.73 m^2 received ACE inhibitors and beta blockers post MI. (256, 260) This appeared to be the general trend irrespective of whether the population studied was from Europe or the United States. In our cohort, we found that > 90% of patients were prescribed beta blocker and statin at discharge, although there still appears to be a reluctance to prescribe ACE inhibitors/ARB (76% at discharge).

The hypothesis was that secondary prevention therapy would be more uniformly prescribed after PPCI, compared to historical datasets. Having delivered timely

revascularisation, the expectation was that there would be greater motivation to actively initiate and up titrate secondary prevention drugs irrespective of co-morbidity. The current data support this hypothesis, and overall utilization of evidence based secondary prevention is good.

However there remains some residual influence of age and renal function on prescribing practice and reductions in the overall utilisation of drugs is seen over time. With increasing age and falling eGFR, the proportion of patients prescribed each secondary prevention agent fell. Patients with eGFR < 45 ml/min/1.73 m^2 received a ramipril equivalent dose that was slightly higher than those with eGFR > 60 ml/min/1.73 m^2. Whilst it is possible that it reflects the presence of other risk factors like hypertension/diabetes, it also suggests that when used it appears to be tolerated to a similar extent to that seen for lesser stages of CKD.

Around half of the patients had renal function reassessed on day 3 post PPCI; there are a number of plausible reasons, such as the presence of co-morbidities, preserved baseline renal function, uncomplicated PPCI and early discharge, as to why patients did not have repeat renal function assessment. Complicating factors such as hypotension and more complex PCI requiring increased use of contrast may have influenced the prescribing patterns of ACE inhibitors and were not accounted for. Those who had renal function reassessed on day 3 likely represented a sicker cohort. The limited data on change in eGFR during hospital admission suggests that a reduction in renal function was associated with reduced ACE inhibitor/ ARB utilisation and emphasizes the need to individualise therapy in this population.

Post discharge monitoring of renal function following initiation and during up-titration of ACE inhibitor therapy is important to identify acute deterioration in renal function. In our study, while the withdrawal of ACE inhibitor was infrequent across the population as a whole, the proportion of patients with eGFR < 45 ml/min/1.73 m^2 prescribed ACE inhibitor /ARB had fallen to 66% at follow-up, compared to 76% at discharge. The specific reasons for this were beyond the scope of our current study. In patients continuing on treatment, mean dosages were similar at discharge and follow-up suggesting that little (if any) attempt has been made to up titrate the drugs. As the risk of adverse event after STEMI is greatest in the early post MI period, and in the context of early up-titration of therapy in clinical trials of ACEI/ARB or beta blocker, our observations have implications for the delivery of optimal secondary prevention after STEMI.

Gale *et al* (261) analysed data on 616 011 patients hospitalised with acute coronary syndrome (ACS) from the Myocardial Ischaemia National Audit Project (MINAP) dataset (1 January 2003 to 2 October 2010) and in this study, only 53% of the 75-84 year old and 46% of the > 85 year old patients were discharged on beta blockers. A similar pattern was seen for ACE inhibitors at 59% and 49%, respectively. There seemed to be slightly less inhibition in prescribing statins; 70% of the 75-84 year old and 59% of the >85 year old patients were discharged on statins. In contrast in the 65-74 year age group (which formed the bulk of the study population) 60% received beta blockers, 64.5% ACE inhibitors and 75% statins. The dataset in this case includes all ACS patients, many of whom would not have received timely angioplasty and again there were no follow up data available. We speculate that timely angioplasty in our patients, regardless of co morbidities,

removed the fear of initiating drug therapy and translated to better utilisation of secondary prevention. Patients post angioplasty would generally be under the care of specialists.

The data on ARB use are interesting but limited due to small numbers. There appears to be more confidence in using these agents over ACE inhibitors in the elderly or in those with worst renal function. One might hypothesise that enhanced tolerability plays a part, although a misguided belief that they are more 'renal friendly' might also be relevant.

I did not assess the routine use of mineralocorticoid receptor antagonists post myocardial infarction as current guidelines recommend it routinely in the presence of reduced LVEF or heart failure. A recent study in fact demonstrated no benefit in instituting early mineralocorticoid antagonist therapy irrespective of heart failure or LVEF. (262)

Limitations

My observations are limited by the restrictions inherent in any registry-based analysis. However, to minimise bias and to portray a representative overview of UK practice, consecutive patients across 5 geographically disparate PPCI centres in the UK were recruited. Historical data regarding this population (STEMI) is likely to be biased - the decision not to thrombolyse was often made based on frailty, co-morbidities or other issues. As such we chose to not explore historic data from STEMI patients receiving thrombolysis as a comparator group. Furthermore, the key aim of this paper was to establish a current UK benchmark.

Comprehensive co-morbidities, previous drug history and reasons why patients did not receive prognostically beneficial drugs e.g. blood pressure, pulse rate, and change in eGFR post discharge were not available. This in part was since data were recorded in different formats at each centre and our objective was to get a large number of subjects from several centres to give a better representative overview. Further prospective evaluation is required to look at these factors as well as longer term effects in more detail.

CONCLUSIONS

Taken in entirety, the overall utilisation of secondary prevention was very good in this primary angioplasty population with commendable use of beta blockers, ACE inhibitors and statins in the elderly and in those with CKD. There may however be room to improve even with the use of ACE inhibitors in those with worst CKD stage. These decisions should be made carefully on an individual basis, with close monitoring of renal function once initiated. Where difficulties are encountered, it may be that a lower dose will have to be accepted or titration may have to be slower. This is particularly important for those at greatest risk, such as patients with impaired LV function and/or CKD.

CHAPTER 7- GENERAL DISCUSSION

Cardio renal disease by virtue of its complexity, exaggerated CV risks and paucity of evidence remains one of the most challenging conditions to treat. This thesis has focused on the different stages of this relationship ranging from the concomitant presence of shared risk factors to death in patients with ESKD.

The hypotheses generating this thesis were based on assumptions that one would deduce from clinical experience for example that iron deficiency would be more common in acute heart failure and that it would be driven by hepcidin up regulation secondary to the inflammatory processes that accompany decompensation. The prevalence of iron deficiency in acute heart failure patients was indeed significantly greater than in chronic stable heart failure patients, however it was absolute iron deficiency that was significantly more common than functional iron deficiency. This was reflected in the lower serum hepcidin levels that were seen in the decompensated heart failure patients compared to the stable CHF patients. Even when corrected for baseline inflammation (CRP levels), the relationship between hepcidin and ferritin remained unchanged. Hence hepcidin appeared to be driven by low iron levels rather than the accompanying inflammation. Another key finding was the significant improvement in iron status during the weeks following decompensation and a suggestion that transferrin saturation and serum iron levels at 1 month post decompensation was prognostically important from a CV outcome point of view. Despite the prevalence of renal dysfunction in ADHF, there did not appear to be a relationship

with iron status. The study was not specifically powered to answer this question and a larger cohort analysis may shed more light on the link between serum hepcidin, CKD and iron status. The findings from this study are nevertheless of great clinical relevance as it would suggest that there is no indication to treat iron deficiency in the acute decompensated state however it would be important to exclude this at 4 week follow up. Studies have already proven that iron replacement therapy is associated with improved QoL and fewer heart failure hospitalisations. The presence of iron deficiency has even been implicated as a reason for poor clinical response and poor cardiac remodelling after cardiac resynchronisation therapy. (224) The results of IRON STATS DHF would be validated further by a study that prospectively evaluates the change in iron status in a stable heart failure cohort who during follow-up have an episode of decompensation. This would be key in understanding the mechanism driving the onset of absolute iron deficiency in acute heart failure.

Patients who have concomitant CKD and heart failure and who otherwise fulfil current criteria for implantation of complex devices (CRT) were demonstrated to have comparable symptomatic benefit with no added risks from device implant. Despite clinical concerns regarding higher infection rates and post procedural complications in the presence of CKD, this thesis demonstrates that there was no significant difference in this between those with and without significant CKD. As expected, poor renal function at baseline is associated with worse mortality. Mortality was also worse in those with labile renal function prior to device implant. Changes in renal function in the months leading up to device implant were not associated with increased procedural risk. To comprehensively ascertain

the impact of CRT on progression of renal dysfunction, a meta analysis of large randomised controlled trials with CRT devices where renal function has been reported is required. Nevertheless, the results of this CRT-CKD study offer reassurance to the implanting clinician that devices must be implanted in those with CKD who otherwise fulfil the criteria for complex device implantation.

The benefits of implanting defibrillators in an advanced CKD population are however less well understood. The findings of my CRASH-ILR study suggest that in haemodialysis patients, SCD due to VT/VF is uncommon and tachyarrhythmias are more likely to reflect the final mode of death in an otherwise complex downward spiral. Asymptomatic bradyarrhythmia and subsequent implantation of devices occurred in 10% of our cohort. This could be a reflection of the haematological and metabolic derangements that accompany cardio renal disease. The hypothesis around the prevalence of brady arrhythmias needs to be tested in much larger dialysis patient cohorts. A study utilising the much smaller and cosmetically more appealing Reveal linq devices or St Jude's Confirm devices, both with remote download capabilities will ensure better availability of ECG data. If proven, phenotypes can be described and potential risk factors can be identified and pacemaker implantation could offer a simple cost effective therapy for specific patients with cardiorenal disease; this may or may not translate to improvements in SCD.

With new therapies and better emergency care, such as PPCI, even those with CKD receive better treatment. Current practices as assessed by my secondary prevention following PPCI study, demonstrated that the utilisation of secondary

prevention medication in the presence of CKD is much better than historical data would suggest. Secondary prevention medication such as ACE inhibitors, beta-blockers and mineralocorticoid antagonists (MRA) are tolerated well post PPCI and would benefit from aggressive uptake and up titration where possible.

In summary, this thesis answered the questions raised at the start but in the process, also generated new questions and ideas for further projects. A prospective study of iron status in heart failure patients with long periods of follow up is warranted as is a larger study of loop recorder ECG monitoring in haemodialysis patients. Meta analytical studies based on individual patient data from large CRT trials would help establish the impact of CRT on renal function.

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INFORMED CONSENT

TITLE: IRON STATUS IN DECOMPENSATED HEART FAILURE (IRON STATS-DHF)

Protocol Version 1.0 (21/06/2012)

NAME OF LOCAL LEAD RESEARCHER: Dr. PAUL KALRA

SUBJECT ID or HOSPITAL NO: _____

Please initial box

1 I confirm that I have read and understand the information sheet dated …….. (version …….) for the above study and have had the opportunity to consider the information and ask questions. ☐

2 I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected. ☐

3 I understand that sections of any of my medical notes relating to my taking part in the study may be looked at by responsible individuals from Portsmouth Hospitals NHS Trust or from the appropriate regulatory authority. where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records. ☐

4 I understand that my general practitioner will be informed of my participation in this study. ☐

5 I agree to have blood samples (15mls on each occasion) taken during research visits for study related analyses. ☐

6 I understand that the blood samples drawn from me may go to other centres for further analysis but will only be labelled with a study number. None of these centres will have any access to my personal details. ☐

7 I understand that the samples of my blood may be stored for future tests (such as biomarkers, but NOT genetic tests). This is on the understanding that these investigations will be for medical research only and my results will be kept confidential. ☐

8 I understand that data collected during the study will be stored in an NHS computer database in an anonymised form and the information will be kept ☐

securely and confidentially.

9 I agree to take part in the above study. ☐

Name of Subject (BLOCK CAPITALS)	Date	Signature
Name of Person taking consent	Date	Signature
Researcher/witness	Date	Signature

1 copy for subject; 1 for researcher; 1 to be kept with hospital notes.

Parameter	Parameter exhibiting correlation	r and p values
3 month ferritin	Baseline ferritin	r = 0.807, p = 0.001
	Baseline serum iron	r = 0.643, p = 0.003
	Baseline serum urate	r = 0.457, p = 0.049
	3 month serum iron	r = 0.456, p = 0.43
	3 month TSAT	r = 0.525, p = 0.017
3 month TSAT	Baseline ferritin	r = 0.548 and p =
	Baseline serum urea	0.011
	Baseline serum urate	r = 0.795, p = 0.001
	Baseline 6MWT distance	r = 0.620, p = 0.006
		r = -0.496, p = 0.031
3 month serum iron	Baseline ferritin	r = 0.508, p = 0.019

Table 1 (Appendix)- Significant correlations of 3 month iron biomarkers in the stable heart failure cohort of the comparator arm (IRON STATS- DHF study).

TSAT- transferrin saturation; 6MWT- 6 minute walk test