University of Southampton

Faculty of Medicine Health & Life Sciences

School of Medicine

IMPROVING THE APPLICATION OF IMPLANTABLE CARDIOVERTER DEFIBRILLATOR THERAPY: AN EVALUATION OF NEW APPROACHES TO PATIENT SELECTION

By

Paul A Scott

Thesis For The Degree Of Doctor Of Medicine

May 2011

Supervisors: Prof John Morgan and Prof Mark Hanson

UNIVERSITY OF SOUTHAMPTON

ABSTRACT

FACULTY OF MEDICINE, HEALTH AND LIFE SCIENCES; SCHOOL OF MEDICINE

DM thesis

IMPROVING THE APPLICATION OF IMPLANTABLE CARDIOVERTER DEFIBRILLATOR THERAPY: AN EVALUATION OF NEW APPROACHES TO PATIENT SELECTION

By Paul A Scott

ICD therapy effectively prevents SCD in high risk patients. However there are problems with its current application. Key amongst these relate to the epidemiology of ICD utilisation and selection of patients for ICD therapy. The aims of this thesis were to examine the requirements for ICD therapy in England and Wales, as well as the impact of clinical setting on ICD prescription rates, and evaluate the potential use of serum/plasma biomarkers (including proteomic markers) and scar quantification by LGE-CMR, to select patients for ICD therapy.

Several methodologies were employed, each tailored to address specific aims: (i) single centre retrospective analyses were performed to estimate the requirement for prophylactic post-MI ICD therapy and the effect of clinical setting on ICD prescription rates; (ii) meta-analytical techniques were used to assess the value of an individual biomarker (BNP) to predict SCD or appropriate ICD therapy in published studies; (iii) a prospective study evaluating the ability of serum proteomic biomarkers, as well as 5 individual cardiovascular biomarkers, to predict prognosis in ICD recipients was performed; (iv) the association between the extent of LV scar and appropriate ICD therapy was retrospectively evaluated in a single centre study of patients who had undergone LGE-CMR prior to ICD implantation.

My results suggest that ICD therapy is significantly underused in England and Wales, and this underuse may be greatest in areas served by a DGH. The meta-analysis and prospective study demonstrated that biomarkers can predict the occurrence of SCD, appropriate ICD therapy, and identify patients with little potential to gain significant benefit from ICD therapy. I also found that the extent of myocardial scar, quantified by LGE-CMR, is strongly associated with the occurrence of spontaneous ventricular arrhythmias.

Both serum/plasma biomarkers and scar quantification by LGE-CMR may be valuable risk stratification tools to guide ICD use. However, their incremental benefit in addition to currently available risk prediction models remains unclear, and further work is needed.

Contents

List Of Figures

List Of Tables

DECLARATION OF AUTHORSHIP

I, **Paul A Scott,** declare that the thesis entitled:

Improving The Application Of Implantable Cardioverter Defibrillator Therapy: An Evaluation Of New Approaches To Patient Selection

and the work presented in the thesis are both my own, and have been generated by me as the result of my own original research. I confirm that:

- this work was done mainly while in candidature for a research degree at this University;
- 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;
- where I have consulted the published work of others, this is always clearly attributed;
- 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;
- I have acknowledged all main sources of help;
- 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;
- parts of this work have been published as:

Scott PA, Gorman S, Andrews NP, Roberts PR, Kalra PR. Estimation of the requirement for implantable cardioverter defibrillators for the primary prevention of sudden cardiac death post-myocardial infarction based on UK national guidelines (2006). *Europace* 2008;**10**:453-7.

Scott PA, Barry J, Roberts PR, Morgan JM. Brain natriuretic peptide for the prediction of sudden cardiac death and ventricular arrhythmias: a meta-analysis. *European Journal of Heart Failure* 2009;**11**:958-66.

Scott PA, Turner NG, Chungh A, Morgan JM, Roberts PR. Varying implantable cardioverter defibrillator referral patterns from implanting and non-implanting hospitals. *Europace* 2009;**11**:1048-51.

Scott PA, Townsend PA, Ng LL, Zeb M, Harris S, Roderick PJ, Curzen NP, Morgan JM. Defining Potential To Benefit From Implantable Cardioverter Defibrillator Therapy – The Role of Biomarkers. *Europace* 2011;**13**:1419-27.

Scott PA, Morgan JM, Carroll N, Murday DC, Roberts PR, Peebles CR, Harden SP, Curzen NP. The Extent of Left Ventricular Scar Quantified By Late Gadolinium Enhancement MRI Is Associated With Spontaneous Ventricular Arrhythmias In Patients With Coronary Artery

Disease And Implantable Cardioverter Defibrillators. *Circulation: Arrhythmia and Electrophysiology* 2011;**4**:324-30.

- parts of this work have been presented as:

Scott PA, Gorman S, Kalra PR. Estimation of the requirement for implantable cardiac defibrillator post myocardial infarction based on nice primary prevention guidelines (2006). Heart Rhythm Congress, Oct 2008.

Scott PA, Turner NG, Chungh A, Barry J, Yue AM, Roberts PR, Morgan JM. The Effect of Hospital Type on ICD Implantation Rates. Heart Rhythm Society, May 2009.

Scott PA, Barry J, Roberts PR, Morgan JM. A meta-analysis of Brain Natriuretic Peptide for the prediction of sudden cardiac death and ventricular arrhythmias. Heart Rhythm Society, May 2009.

Scott PA, Barry J, Roberts PR, Morgan JM. A meta-analysis of Brain Natriuretic Peptide for the prediction of sudden cardiac death and ventricular arrhythmias. Europace, June 2009.

Scott PA, Zeb M, Yue AM, Curzen NP, Townsend PA, Ng LL, Roberts PR, Morgan JM. The Use of Biomarkers to Refine Patient Selection For ICD therapy. Heart Rhythm Congress, Oct 2010.

Scott PA, Carroll N, Murday D, Zeb M, Yue AM, Roberts PR, Peebles C, Harden S, Morgan JM, Curzen NP. Scar Quantification Assessed by Contrast-Enhanced MRI Predicts Spontaneous Ventricular Arrhythmias In patients with Coronary Artery Disease and Implantable Cardioverter Defibrillators. Heart Rhythm Congress, Oct 2010.

Scott PA, Carroll N, Murday D, Zeb M, Yue AM, Roberts PR, Peebles C, Harden S, Morgan JM, Curzen NP. Scar Quantification Assessed by Contrast-Enhanced MRI Predicts Spontaneous Ventricular Arrhythmias In patients with Coronary Artery Disease and Implantable Cardioverter Defibrillators. Heart Rhythm Society, May 2011.

Signed: ……..

Date:…….

Acknowledgements

I would especially like to thank Professor John Morgan for his excellent guidance and constant encouragement. I would also like to acknowledge the invaluable contributions made by Dr Nick Curzen, Dr Paul Roberts, Professor Paul Townsend and Professor Mark Hanson.

I am grateful to Dr Sarah Gorman for helping with data collection for the study estimating the requirement for ICD therapy for the primary prevention of sudden cardiac death post-myocardial infarction (Chapter 3).

I am grateful to Dr Nick Turner and Aman Chungh for their assistance with data collection for the study assessing the effect of clinical setting on ICD prescription rates (Chapter 4).

I am grateful to Dr James Barry for repeating data extraction for the BNP meta-analysis (Chapter 5).

I would like to thank Professor Leong Ng and his team for performing the NT-proBNP, sST2, GDF-15, CRP and IL-6 assays (Chapters 6 and 7).

I would like to thank Dr Bashar Zeidan for his help and advice regarding the proteomic analysis (Chapter 7).

I would like to thank Nicola Carroll for performing CMR scar quantification in 15 patients to allow assessment of the reproducibility of the scar analysis methodology (Chapter 8).

I would like to thank Scott Harris for his advice concerning statistics.

I would like to thank the cardiac rhythm management physiologists at Southampton University Hospital, for their constant support with patient recruitment.

I would like to thank Medtronic for providing the financial support that made this work possible.

Definitions And Abbreviations

ACE-I	Angiotensin converting enzyme inhibitor
AF	Atrial fibrillation
ARB	Angiotensin receptor blocker
BNP	Brain natriuretic peptide
BPM	Beats per minute
CAD	Coronary artery disease
CMR	Cardiac magnetic resonance
CRP	C-reactive protein
CRT-D	Cardiac resynchronisation defibrillator
DGH	District general hospital
EPS	Electrophysiological study
GDF-15	Growth differentiation factor-15
ICD	Implantable cardioverter defibrillator
IL-6	Interleukin-6
LV	Left ventricle
LVEF	Left ventricular ejection fraction
LVSD	Left ventricular systolic dysfunction
MI	Myocardial infarction
NICE	National Institute for Health and Clinical Excellence
NICM	Non-ischaemic cardiomyopathy
NSVT	Non-sustained ventricular tachycardia
NT-proBNP	N-terminal pro-brain natriuretic peptide
NYHA	New York Heart Association functional class
PPM	Permanent pacemaker
RCT	Randomised controlled trial
RR	Relative risk
SCD	Sudden cardiac death

SELDI-TOF MS	Surface-enhanced laser desorption/ionisation time-of-flight mass spectrometry
SHF	Systolic heart failure
sST2	Soluble ST2
TTE	Transthoracic echocardiogram
VA	Ventricular arrhythmias
VF	Ventricular fibrillation
VT	Ventricular tachycardia

1. INTRODUCTION

1.1. Heart Failure And Asymptomatic Left Ventricular Dysfunction

1.1.1. Definition, Epidemiology and Aetiology

Heart failure is a complex clinical syndrome that can result from any structural or functional cardiac disorder that impairs the ability of the ventricle to fill with or eject blood (1). The principal symptoms of heart failure are dyspnoea and fatigue, which may limit exercise tolerance, and fluid retention, which may lead to pulmonary congestion and peripheral oedema.

Though diagnostic definitions vary, the epidemiology of heart failure in developed countries is relatively well established. Overall between 2% and 3% of the adult population have heart failure, though prevalence rises steeply with age, with 10-20% of 70 to 80-year-olds affected (2). In younger age groups heart failure is more common in men, though the prevalence is relatively equal between the sexes in the elderly (2). In addition, the prevalence of heart failure is increasing (2, 3). This is due to both the ageing population and improvement in the management of patients with structural heart disease and those suffering coronary events (2, 3). The resulting economic burden of heart failure is significant, with an estimated direct and indirect cost in the United States alone for 2007, of $33.2 billion (4). About two-thirds of these costs relate to hospital admissions (5).

Though the clinical syndrome of heart failure may result from diseases of the myocardium, pericardium, endocardium or great vessels, the majority of cases are secondary to impairment of left ventricular (LV) function (1). Heart failure may be associated with a spectrum of abnormalities of LV function, ranging from patients with a preserved left ventricular ejection fraction (LVEF), to those with significant left ventricular systolic dysfunction (LVSD), termed systolic heart failure (SHF) (1). Although approximately half of patients with symptomatic heart failure in the community have preserved LVEF, the vast majority of clinical trials have included only patients with SHF, and there is little research to guide the management of patients with heart failure and preserved LVEF (6). Although LVSD often causes symptoms, community echocardiographic studies have demonstrated that approximately half of patients with significant LVSD are asymptomatic (7). However, it is well recognised that despite the lack of functional limitation, asymptomatic LVSD is a precursor to SHF and its presence has a significant adverse effect on prognosis (1).

Though the potential causes of SHF and asymptomatic LVSD are numerous, the most frequent aetiological factor in developed countries is coronary artery disease (CAD), which is thought to be the initiating cause in approximately 70% of cases (2). Additional common causes include

non-ischaemic dilated cardiomyopathy (NICM), valve disease and systemic hypertension (2). Many patients however, have multiple potential causes (e.g. CAD, hypertension, diabetes mellitus, atrial fibrillation) and establishing the primary aetiology of heart failure in such cases can be difficult.

1.1.2. Prognosis

Heart failure, irrespective of the aetiology, is a lethal condition. Early data from the Framingham Heart Study demonstrated that in 652 patients who developed heart failure between 1948 and 1988 (51% men, mean age 70 years), 1-year and 5-year survival rates after the onset of symptoms were 57% and 25% in men, and 64% and 38% in women (8). Despite significant advances in the therapeutic management of heart failure prognosis remains poor. In a contemporary cohort study of 2445 patients (43% men, mean age 76 years) discharged from hospital after an admission with heart failure in one metropolitan area in the United States in 2000, 1-year and 5-year survival rates after discharge were 63% and 22% respectively (9).

Although the natural history of asymptomatic LVSD is not as well described as that of heart failure, there are data from both community-based studies and randomised controlled trials (RCTs) demonstrating an adverse prognosis (7). In community-based studies asymptomatic LVSD has been associated with an increased risk of both cardiovascular and all-cause mortality (10, 11). In the placebo groups of RCTs, which have included over 3500 patients, the annual mortality rates of patients with asymptomatic LVSD have ranged from 5.1% to 10.5% (12-16).

1.1.3. Mode of Death

In heart failure there is significant variation in mode of death between patients. While some patients die suddenly within a short period from the onset of symptoms (sudden cardiac death - SCD), others die of progressive heart failure (pump failure death) (17). The relative contribution of each mode of death to overall mortality in heart failure has been thoroughly evaluated in RCTs, though epidemiological data are also available.

Epidemiological studies provided early data concerning the importance of SCD in heart failure. Among 461 members of the Framingham Heart Study who developed congestive heart failure, the 4-year mortality rate was 55% in men and 24% in women, and 40-50% of these deaths were sudden (18). These findings have persisted despite modern heart failure management. Mehta *et al*. performed a contemporary observational study of 396 patients with a new diagnosis of heart failure, at two UK hospitals over a two year period (2004-2005). During a median follow-up of 10 months there were 59 deaths, of which 52% were classified as due to progressive heart failure and 22% SCD (19).

In contrast to the results of the cohort study by Mehta *et al*., data from RCTs have consistently demonstrated that SCD is the most frequent cardiovascular mode of death in heart failure patients, accounting for around a half of all deaths (17, 20). Poole-Wilson *et al*. analysed the mode of death in 3164 heart failure patients (83% NYHA III/IV) enrolled in the ATLAS trial, a study of the ACE-inhibitor Lisinopril (20). Over a median follow-up of 46 months there were 1383 deaths, of which 589 were classified as sudden and 445 due to pump failure. These findings were confirmed by an analysis of 10,538 heart failure patients enrolled in 6 RCTs or registries by Mozaffarian *et al.* (17). During 16,735 person-years of follow-up 2014 deaths occurred, of which 1014 were classified as SCD and 684 as due to pump-failure.

However, RCTs have some important limitations with respect to assessment of mode of death. Firstly, they do not accurately reflect the population of patients with heart failure in the community. The participants in RCTs tend to be in their late 50s and early 60s and approximately 80% are male (15, 17, 21, 22). In contrast, in the community nearly 90% of patients are ≥65 years, 50% ≥80 years, and the distribution of sexes more equal (23, 24). In addition, other factors, such as survival bias, where patients included in RCTs are 'natural survivors' of the early high risk period of heart failure may also be important (19). It is likely that some of these factors partly explain the differences in findings between the epidemiological and trial data.

An additional factor that complicates the assessment of mode of death is the difficulty in classification, with a significant variation in the definition of SCD used in studies. Narang *et al*. performed a systematic review of 27 studies that reported mode of death in at least 50 heart failure patients (25). Though most studies defined SCD by a specified time interval between death and antecedent change in clinical status, no standard time interval was used, with definitions ranging from 15 mins to 24 hours. Furthermore, in some cases death occurs suddenly, but after a recent worsening in cardiac symptoms (26).

Though less data are available, evidence from the placebo-arms of RCTs suggest that both modes of death are also important in patients with asymptomatic LVSD. In the 2117 patients in the placebo arm of the SOLVD study, a randomised trial of Enalapril in patients with asymptomatic LVSD, there were 334 deaths (16%) over a mean follow-up of 37 months (15). Of these deaths 105 (31%) were classified as due to sudden death and 106 due to progressive pump failure (32%). In the 1116 patients in the placebo arm of the SAVE study, a randomised trial of the ACE-I Captopril in patients with asymptomatic LVSD, there were 275 deaths (25%) over a mean follow-up of 42 months (14). Of these deaths 75 (27%) were classified as sudden

unexpected deaths, 50 (18%) sudden deaths with preceding symptoms, and 58 (21%) deaths due to progressive pump failure.

1.2. Sudden Cardiac Death

1.2.1. Definition and Incidence

SCD is a major public health problem, causing an estimated 100,000 adult deaths per year in the United Kingdom and four times that in the United States (27, 28). Although most documents now define SCD as death that occurs within 1 hour or less from the onset of symptoms, or that which occurs during sleep, there is variation between studies in the definition used, and this strongly influences its estimated incidence (29, 30). Using the definition of death within an hour of symptom onset, the proportion of all natural deaths due to SCD is 13% (31). When all deaths occurring within a 24-hour period of symptom onset are included this rises to 18.5%, though the proportion of these deaths that are due to a cardiac cause is consequently reduced (31, 32).

1.2.2. Population Subgroups at Risk of SCD

In the vast majority of cases, SCD occurs in patients with structural heart abnormalities, though this may not have been previously recognised. The most common condition underlying SCD risk is CAD and its consequences, which accounts for up to 80% of cases (33). NICM is probably the second most common predisposing condition, accounting for up to 10% of cases (33). In a small proportion of cases (5-10%) SCD occurs in the context of a structurally normal heart. It is likely that a number of these patients will have one of a group of inherited conditions that affect the electrical properties of the heart, termed ‘channelopathies’. These include the long QT syndrome, short QT syndrome, Brugada syndrome, and catecholaminergic polymorphic ventricular tachycardia, all of which can precipitate SCD without overt structural changes in the heart (34).

The highest risk population subgroups for SCD are patients with LVSD, with or without symptoms of heart failure, and survivors of out-of-hospital cardiac arrest (35). It is important to note though, that despite their high incidence of SCD, because of their small population size, these high-risk subgroups do not contribute most SCD cases (35). In fact, the larger, lower risk populations, because of their size, generate the largest absolute number of SCD events. However, because of their high absolute risk, observational studies of risk and interventional trials of therapy have been carried out primarily in the highest risk subgroups.

1.2.3. Mechanisms of SCD

The rhythm most often recorded at the time of cardiac arrest is ventricular fibrillation (VF), and this is likely to hold true for the subset of patients with LVSD (36, 37). It is thought that in most cases VF will have degenerated from ventricular tachycardia (VT) (38, 39). Though bradyarrhythmias and pulseless electrical activity may occur they are recorded less frequently. However, the true incidence of bradyarrhythmias is difficult to assess, as a rhythm beginning as VF may appear to be asystole once the first ECG is recorded. This understanding is informed by data from patients who have suffered SCD or resuscitated cardiac arrest whilst undergoing ambulatory monitoring (37, 40).

In 1989 Bayés de Luna *et al*. combined data from seven studies of ambulatory patients dying suddenly while undergoing Holter recording (37). In 157 episodes of SCD, 131 (83.4%) were due to ventricular arrhythmias and 26 (16.6%) bradycardia. Of the 131 cases of SCD due to ventricular arrhythmias, in 98 cases VT degenerated into VF, in 13 cases VF appeared without any significant preceding arrhythmia, and in 20 cases the rhythm was polymorphic VT. Huikuri *et al*. used implantable ECG loop-recorders to document the cardiac rhythm at the time of cardiac arrest or peri-arrest, in 312 post-MI patients with significant LVSD (mean LVEF 31±6%) (40). During a follow-up of 2 years, 25 (8%) patients experienced the primary end-point of VF or sustained symptomatic VT. There were 8 SCDs, 6 due to VF and 2 to fast VT (ventricular rate 194-220 bpm), 3 resuscitated cardiac arrests, 2 due to VF and 1 to fast VT, 2 episodes of syncope secondary to VF, and 12 episodes of sustained symptomatic VT (ventricular rate <194 bpm).

While the arrhythmias that lead to SCD are well described, the pathophysiology underlying the genesis of these rhythm disturbances is complex and less well understood. However, it is thought to involve the interaction of a triggering event and an abnormal substrate, that induces electrical stability and a subsequent malignant ventricular arrhythmia (39, 41).

The substrate for SCD varies depending on the underlying heart disease, as well as the initiating arrhythmia (38, 42). It has been well studied in monomorphic VT, the most frequent lethal arrhythmia in patients with LVSD, partly due to the relative reproducibility of the arrhythmia (43, 44). Monomorphic VT is generally associated with a fixed arrhythmic substrate, which in patients with structural heart disease is usually a region of ventricular scar tissue (44). The most common cause of scar is myocardial infarction, however in patients with other forms of structural heart disease scar tissue has different origins. These include myocardial fibrosis, in patients with NICM and hypertrophic cardiomyopathy, and scars created during cardiac surgery, such as surgical correction of Tetralogy of Fallot (45). Ventricular scars consist of regions of

dense fibrosis, with collagen and fibrocytes, interspersed with regions of surviving myocyte bundles (46). The dense fibrosis creates areas of conduction block, that can define borders for re-entry circuits, as well as areas of slow conduction (46). It is the slow conduction and fibrous anatomical barriers that set the stage for re-entry.

In patients with CAD, both acute myocardial infarction and myocardial ischaemia can also provide the substrate for malignant ventricular arrhythmia that lead to cardiac arrest (42). However, the degree to which these factors play a role in the majority of cases of SCD is difficult to establish, as many of the hallmarks of acute MI may not be evident in the acute phase of infarction, and ischaemia may only be transient and, without ST-segment monitoring, difficult to identify. Despite these difficulties, is has been estimated that acute MI accounts for only a minority of cases of SCD (39).

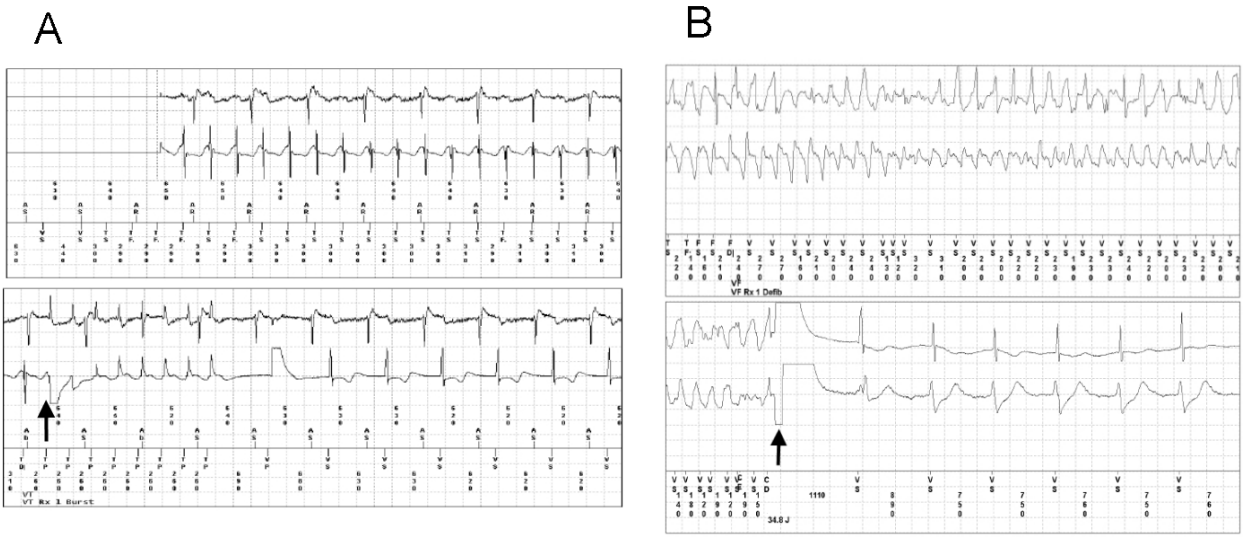
In heart failure, a number of structural and functional changes take place that are likely to be important in the pathophysiology of SCD (41). These include, but are not limited to, action potential prolongation, alterations in calcium homeostasis, abnormal electrical conduction, and altered neurohormonal signalling (41). In addition, genetic predisposition may play a role in some patients (47).

1.3. Implantable Cardioverter Defibrillators

1.3.1. The Technology

Since their introduction in 1980, implantable cardioverter defibrillators (ICDs) have revolutionised the management of patients at high SCD risk (48). ICDs work by accurately recognising the occurrence of the potentially life-threatening ventricular arrhythmias that cause SCD, and effectively terminating them, either by anti-tachycardic pacing (ATP) or delivery of shock therapy (Figure 1-1) (49).

Figure 1-1 Normal Functioning Of An ICD
(A) Stored data are shown from an episode of ventricular tachycardia, successfully treated with anti-tachycardia pacing by a dual-chamber ICD. From the top the tracings represent electrograms (egms) from the atrial and ventricular leads, and from an annotated marker channel. At the start of the recording the ventricular egm shows a repetitive rhythm, with an identical morphology, at a rate of approximately 200 bpm. The tachycardia is successfully terminated back to sinus rhythm by 8 paced beats, at a slightly faster rate than the arrhythmia (arrow). (B) Stored data are shown from an episode of ventricular fibrillation, successfully treated with shock therapy, in a patient with a single-chamber ICD. From the top the tracings represent egms from the ventricular lead, shock coil, and from an annotated marker channel. At the start of the recording both egms show a rapid, irregular, chaotic rhythm. The tachycardia is successfully terminated back to sinus rhythm by a 34.8 J shock (arrow).



1.3.2. Randomised Trials of ICD Therapy

ICD therapy has been evaluated by multiple large RCTs. These trials predominantly included patients with significant LVSD, a large proportion of whom had symptoms of heart failure, reflecting the population at highest SCD risk. The underlying aetiology of the LVSD was CAD in most cases, though a small proportion of patients had NICM. There have been no randomised trials of ICD therapy in patients with heart disease of other aetiologies, principally due to the relative rarity of these conditions.

The trials evaluated ICD therapy in two different clinical scenarios – patients who have survived life-threatening ventricular arrhythmias (secondary prevention), and patients who are thought to be at high risk of developing a life-threatening arrhythmia, but who have not yet done so (secondary prevention). In both settings ICDs are highly efficacious (21, 22, 50).

Four RCTs, enrolling 2023 patients in total, have investigated the benefit of ICD therapy in survivors of potentially life-threatening ventricular arrhythmias with LVSD (secondary prevention) (51-54). Lee *et al*. performed a meta-analysis of these studies (55). In a fixed effects model defibrillator therapy was associated with a 25% relative risk reduction in all-cause mortality (p=0.0002), with an absolute risk reduction of 7%.

The benefit of primary prevention ICD therapy has been demonstrated in a number of meta-analyses (21, 55, 56). Nanthakumar *et al*. performed a meta-analysis of 10 RCTs that enrolled 7253 patients (56). Using the random effects model there was a 25% relative risk reduction in all-cause mortality in the ICD group (p=0.003), with an absolute mortality risk reduction of 7.9%.

Though the number of patients with NICM included in the RCTs was small, the benefit of ICD therapy in these patients has been established using meta-analytical techniques. Desai *et al*. found that in both primary and secondary prevention settings patients with NICM benefitted as much as those with CAD, though the small number of patients in the secondary prevention analysis meant that this result was not statistically significant (57).

1.3.3. The Epidemiology of ICD Utilisation

Despite the large body of evidence supporting their use and their widespread endorsement by national and international guidelines, surveys have consistently demonstrated significant geographical variation in ICD implantation rates (2, 32, 50, 58-61). Early surveys in 1997 found that implant rates, including new and replacement devices, varied from 7/million/year in the UK to 49/million/year in Germany, the highest implanter in Europe, to 133/million/year in the USA

(61, 62). Although implant rates have risen significantly in the last decade worldwide, these geographical differences have persisted. In 2006, total ICD implant rates were ~65/million in the UK, compared to ~190/million in Germany and ~370/million in the USA (Figure 1-2) (63). There are no major differences in cardiovascular mortality rates between the European countries and the USA, and the European and US guidelines are broadly similar with respect to the indications for ICD therapy (2, 50, 64, 65). It is therefore likely that these geographical differences represent variation in clinical practice rather than the need for ICD therapy. Furthermore, it remains unclear to what degree this geographical variation in ICD utilisation represents an underuse in low implanting areas or an overuse in high implanting areas.

There are number of factors that may contribute to this observed disparity. However three issues that are likely to be of paramount importance are the number of ICD implanting centres and electrophysiologists per population, the development of local referral strategies for potential ICD recipients and the financial circumstances of the healthcare system (64).

The first issue - the number of ICD implanting centres and electrophysiologists in a country – is likely to be of key importance. In four of the larger European countries, France, Germany, Italy and the UK, there is a degree of correlation between the number of implanting centres and ICD implant rates (64). In Italy and Germany, where there are 4.4 and 6.8 implant centres per million population respectively, implants have been consistently higher than in France and the UK, where there are 1.4 and 0.7 implant centres per million, respectively (64). The second issue is the presence of poorly developed local referral strategies and care pathways for ICD patients. American and European studies have consistently demonstrated that many patients with heart failure who potentially meet criteria for prophylactic ICD implantation are not referred to their local implanting centre for consideration of device therapy, and furthermore only a minority of those referred are actually implanted with a device (64, 66). Thirdly, the financial circumstances of the healthcare system may be important. ICDs are an expensive technology and although RCTs have demonstrated clear clinical effectiveness when used in specific populations, their cost-effectiveness is less clear cut. The cost-effectiveness of ICD therapy will depend on a number of factors including the associated healthcare costs in the individual country as well as the assumptions made concerning clinical effectiveness, battery longevity and the SCD risk of the patient population they are used in (67, 68).

In England and Wales new or costly therapies are evaluated by the National Institute for Health and Clinical Excellence (NICE), an independent organisation responsible for providing guidance to clinicians and healthcare purchasers. NICE initially published its guidance on ICD use in 2000. Although NICE anticipated that its guidance would require the implantation of

approximately 50 first and replacement devices per million of the population per year, the exact number of devices that should be implanted based on the guidance was not known. In 2005 Plummer *et al*. performed a one month audit of 336 patients admitted to a coronary care unit in the hospitals serving one district, to try to assess this more accurately (69). Applying NICE criteria, they found that the incidence of ICD indications based on their data was 98.4/million/year, far in excess of the national implant rate at the time of the study. Additional studies by the same group have given consistent results, supporting the conclusion that despite national guidance ICDs were significantly underused in the UK (70, 71).

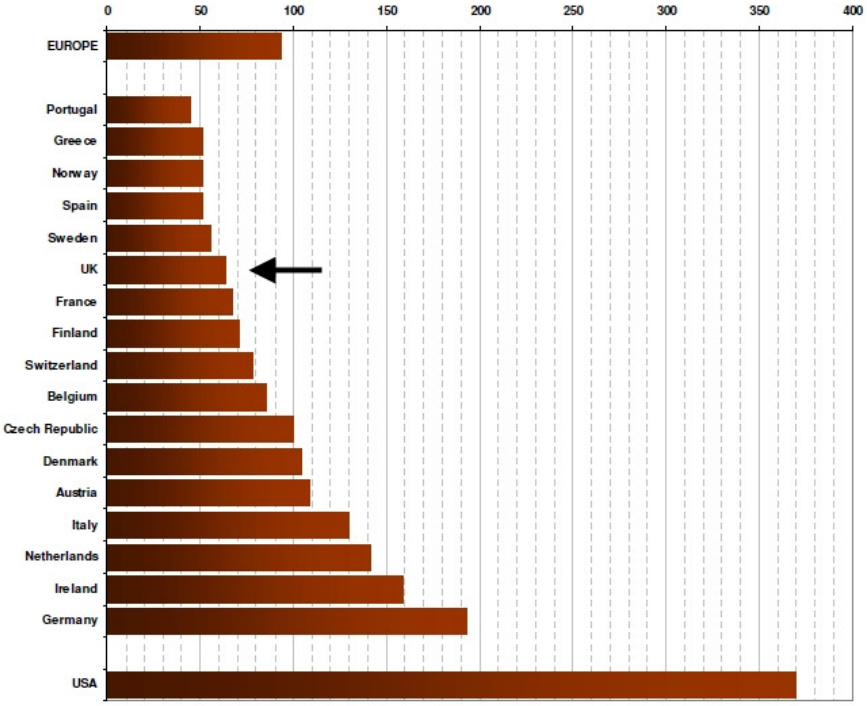
Since the initial NICE guidance was published, the evidence supporting ICD therapy has increased with the publication of several landmark primary prevention trials, and in 2006 NICE updated its guidance to take account of these new data (58, 59, 72). The revised guidelines, which broadened the indications for prophylactic device use, are likely to significantly increase the required UK implant rate, though the true need for prophylactic ICD implantation in the UK is not known.

Despite national guidance from NICE, there is great geographical variability in ICD implant rates within England and Wales (Figure 1-3). The first national survey of ICD use, published in 2005, assessed regional implant rates from 1998-2002 based on postcode (73). The survey found that the implant rate of new devices gradually increased over the 5 year study period – from 12/million/year in 1998 to 31.5/million/year in 2002. However, over the 5-year study period, the difference between the highest (39 total implants/million/year) and lowest (9/million/year) implanting areas was over 4-fold. These findings have persisted in more recent analyses. The 2006 national survey, which assessed implant rates from 2004-2006, demonstrated a nearly three-fold difference in new implantation rates between the lowest (26/million/year) and highest (73/million/year) implanting regions (63).

The number of patients with indications for ICD therapy in the population will depend on a number of factors, including the incidences of CAD and heart failure, and survival from aborted cardiac arrest. These factors are known to vary within the UK. However they are unable to explain the magnitude of the differences in implantation rates observed between different areas. The reason for this regional disparity in ICD implantation rates in the UK is unknown, but is likely to be multifactorial. Some of the factors underlying the variation in implant rates between different countries outlined above may also be important in explaining the regional disparity within the UK. The provision of ICD services is not uniform across the UK and this may have an impact (73). Furthermore, an effective ICD implantation service needs well developed local pathways for identifying and referring patients for consideration of ICD therapy, and this

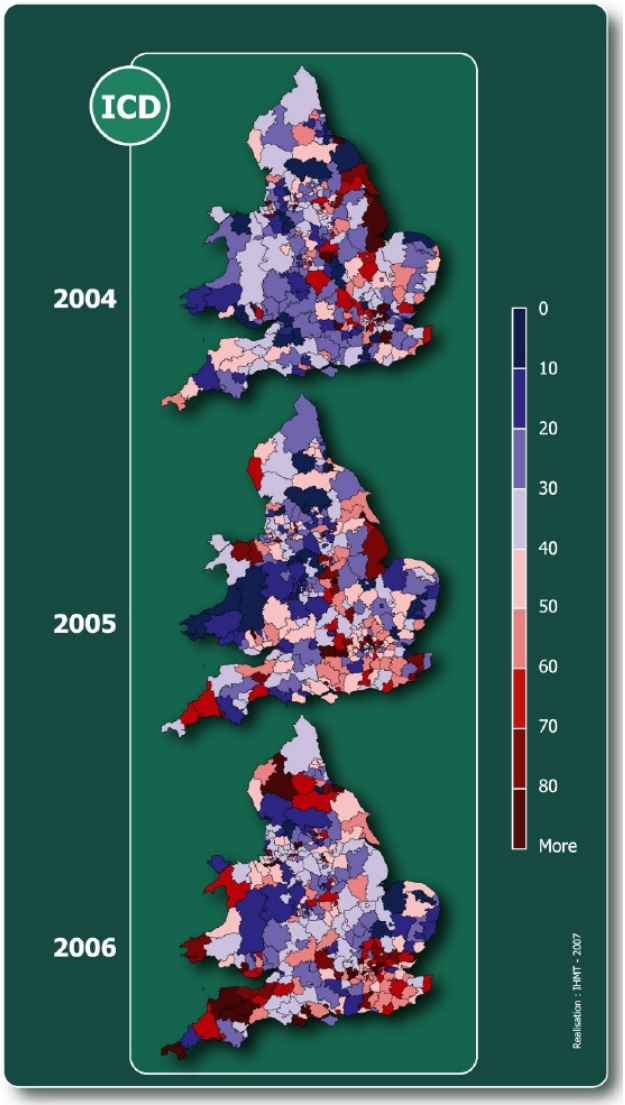
requires network-wide planning and significant long term investment (73). Again this is likely to be subject to regional variation and may affect implant rates.

Figure 1-2 ICD Implant Rates In Europe
Total ICD implant rates for 2006, per million population, for Europe as a whole, various European countries, and the USA. The UK implant rate (arrow), at approximately 65/million, is significantly below the European average (over 90/million), and over five times lower than in the USA (~370/million) (63). Copyright © 2010, Re-used with the permission of The Health and Social Care Information Centre. All rights reserved.



Data supplied by NHS The Information Centre for health and social care

Figure 1-3 Map Of ICD Implants In England And Wales
Map of new ICD implant rates in England and Wales (2004-2006). The colour scale on the right of the figure refers to yearly implant rates per million population. The map demonstrates significant regional variation in implant rates, with an approximate three-fold difference between the highest and lowest implanting regions in 2006 (63). Copyright © 2010, Re-used with the permission of The Health and Social Care Information Centre. All rights reserved.



Data supplied by NHS The Information Centre for health and social care

1.4. Selection Of Patients For ICD Therapy

1.4.1. SCD Risk Stratification

Despite recent advances in the management of cardiovascular disease, survival following out-of-hospital cardiac arrest remains poor. In a recent prospective study of 20,520 out-of-hospital cardiac arrests in 10 different sites in North America from 2006-2007, average survival to hospital discharge was 4.4% (74). Therefore, although ICD therapy effectively reduces mortality in the secondary prevention setting, the vast majority of patients that suffer a life-threatening arrhythmia do not survive to be considered for a device. As a consequence, the use of primary prevention ICD therapy in patients who are at highest risk of SCD but have not yet suffered a life-threatening arrhythmia, is of paramount importance in reducing overall SCD rates. Central to this concept is the ability to identify which patients are at highest risk of SCD, termed SCD risk stratification.

1.4.2. Current SCD Risk Stratification Tools

SCD risk stratification has been studied primarily in patients with LVSD and those who have suffered a previous MI, as these groups are well known to be at increased SCD risk. A large number of tests have been evaluated. These include tests of LV function, autonomic function, ventricular repolarisation, and the presence or absence of spontaneous or inducible ventricular arrhythmias. The diverse nature of these tests reflects the complex pathophysiology underlying ventricular arrhythmogenesis. The clinically relevant risk stratification tests are:

1.4.2.1. Left Ventricular Ejection Fraction

Depressed LVEF, as measured by echocardiography, contrast and radionuclide ventriculography, or magnetic resonance imaging, has long been recognised to be the most important determinant of all-cause mortality in patients with CAD (75, 76). Contemporary studies have confirmed these early findings and demonstrated that in both ischaemic and non-ischaemic cardiomyopathy, a reduced LVEF is consistently the strongest predictor of SCD, as well as all-cause mortality (77, 78). In 14,609 post-MI patients enrolled in the VALIANT trial, depressed LVEF was the most powerful predictor of SCD (77). In the first 30 days following MI, each decrease in 5 percentage points in LVEF was associated with a 21 percent increase in the risk of SCD or cardiac arrest with resuscitation. In another prospective study of 343 patients with NICM, LVEF was the only significant predictor of arrhythmic events in multivariable analysis, with a relative risk of 2.3 per 10% decrease in LVEF (78). As a result of these robust data, a depressed LVEF has been the main entry criterion used in RCTs of primary prevention ICD therapy.

1.4.2.2. Ambulatory Monitoring

A number of studies have found an association between the presence of non-sustained ventricular tachycardia (NSVT) on ambulatory monitoring and SCD, in both ischaemic and non-ischaemic cardiomyopathy (79, 80). However, although it is used as an important determinant in the latest NICE guidance on ICD use, it plays only a minor role in selection of patients for ICD therapy in the North American guidelines, and its use is not advocated in the most recent European guidelines (2, 50, 72). Furthermore, more recent evidence has cast doubt on its predictive accuracy in the modern era (81).

1.4.2.3. Electrophysiological Studies (EPS)

Following the finding that post-MI patients with inducible ventricular arrhythmias had a significantly increased risk of SCD, EPS was for a long time considered the "gold standard" SCD risk stratification test in CAD patients (82-84). However more recent studies have suggested that non-inducible patients are still at high risk of SCD, casting doubt on the prognostic value of EPS in CAD (85, 86). EPS has no significant prognostic role in NICM (87, 88).

1.4.2.4. QRS Width

Early observational studies suggested that QRS prolongation was a significant marker of poor outcomes in patients with depressed LVEF, especially those with CAD (89). However, data from RCTs have provided conflicting results, and again, although QRS width is employed in the current NICE guidelines, its use to guide ICD therapy is not advocated in North American or European guidelines (2, 50, 72).

1.4.2.5. Microvolt T-wave Alternans (MTWA)

Multiple trials have demonstrated that MTWA testing is predictive of malignant arrhythmias. A meta-analysis of 19 studies, evaluating MTWA in 2608 patients over an average of 21 months follow-up, found a positive predictive value of 19.3% and negative predictive value of 97.2% (90). However, patients with an indeterminate result were excluded from the analysis, and the high proportion of such patients (20-40%) is a significant limitation of MTWA. Furthermore, more recent large prospective studies have not confirmed these earlier findings, and the role of MTWA in SCD risk stratification is currently unclear (91).

1.4.2.6. Other Tests

In addition, a number of other risk stratification tests have some predictive ability though they are not in widespread clinical use. These include tests of autonomic function, the signal-averaged ECG, and changes in the ECG QT segment (92).

1.4.3. Guidelines for the Selection of Patients for ICD therapy

Despite the variety of risk stratification tests available contemporary guidelines for the use of prophylactic ICD therapy in patients with CAD or NICM are based primarily on the presence of a reduced LVEF (Table 1-1) (2, 36, 50). Although consensus US and European guidelines for the prevention of SCD, published in 2006, recommend the use of MTWA and EPS for the risk stratification of SCD, only the use of EPS is advocated in the selection of patients for prophylactic ICD therapy (36). Both contemporary US and European guidelines suggest that patients with a significantly depressed LVEF (≤35%) should be considered for an ICD without the need for additional testing (2, 50), while the most recent US guidelines, published in 2008, suggest that patients with a higher LVEF (<40%) may benefit from further evaluation in the form of EPS and Holter monitoring prior to ICD implantation (50). The central role of LVEF in SCD risk stratification primarily reflects the fact that reduced LVEF has been the primary criterion used for inclusion in many of the RCTs of ICD therapy.

The most recent NICE guidance also uses LVEF as the primary determinant for prophylactic ICD implantation, though patients also require additional testing in the form of ambulatory monitoring, EP testing, and a broad QRS (Table 1-2) (72). Although broadly similar to the US and European guidelines there are important differences between NICE guidance and the other contemporary guidelines. First, NICE guidance does not cover the use of prophylactic ICD therapy in patients with NICM. Second, NICE uses QRS width as a risk stratifier. Part of the explanation for these differences in guidelines relates to the focus of NICE on cost effectiveness. The NICE guidance is significantly more exclusive than the US and European guidelines, and attempts to focus the use of ICD therapy in patient groups where it is likely to be most cost effective as well as clinically efficacious (72).

Table 1-1 International recommendations concerning the use of ICD therapy for the primary prevention of SCD in patients with coronary artery disease and NICM (2, 50)

ACC/AHA/ESC 2006 Guidelines (36)	
Class I	Patients who have an LVEF ≤30-40% due to prior MI, are at least 40 days post-MI, are in NYHA functional Class II or III and are receiving optimal medical therapy with a reasonable expectation of survival with good functional status for >1 year Patients with NICM who have an LVEF ≤30-35% , are in NYHA functional Class II or III, and are receiving optimal medical therapy with a reasonable expectation of survival with good functional status for >1 year
Class IIa	Patients who have an LVEF ≤30-35% due to prior MI, are at least 40 days post-MI, are in NYHA functional Class I and are receiving optimal medical therapy with a reasonable expectation of survival with good functional status for >1 year
ACC/AHA/HRS 2008 Guidelines (50)	
Class I	Patients who have an LVEF ≤35% due to prior MI, are at least 40 days post-MI and are in NYHA functional Class II or III Patients with NICM who have an LVEF ≤35% and are in NYHA functional Class II or III Patients who have an LVEF <30% due to prior MI, are at least 40 days post-MI and are in NYHA functional Class I Patients who have an LVEF <40% due to prior MI, NSVT on ambulatory monitoring and inducible VF or sustained VT at electrophysiological study
ESC 2008 Guidelines (2)	
Class I	Patients who have an LVEF ≤35% due to a prior MI, are at least 40 days post-MI, are in NYHA functional Class II or III, and are receiving optimal medical therapy with a reasonable expectation of survival with good functional status for >1 year Patients with NICM who have an LVEF ≤35% and who are in NYHA functional Class II or III, receiving optimal medical therapy, with a reasonable expectation of survival with good functional status for >1 year

ACC, American College of Cardiology; AHA, American Heart Association; ESC, European Society of Cardiology; HRS, Heart Rhythm Society.

Table 1-2 NICE guidance on the use of ICD therapy for primary prevention following myocardial infarction (72)

A history of previous (more than 4 weeks) myocardial infarction (MI) and:

Either

left ventricular dysfunction with an LVEF of less than 35% (no worse than class III of the New York Heart Association functional classification of heart failure),

and

non-sustained VT on Holter (24-hour electrocardiogram [ECG]) monitoring,

and

inducible VT on electrophysiological testing

or

left ventricular dysfunction with an LVEF of less than 30% (no worse than class III of the New York Heart Association functional classification of heart failure)

and

QRS duration of equal to or more than 120 milliseconds

1.4.4. Limitations of LVEF-based Strategies

Although LVEF plays a key part in contemporary ICD guidelines, there are a number of limitations in its use as the primary determinant for ICD implantation (93, 94). These limitations become clear when the sensitivity and specificity of LVEF to predict SCD are examined.

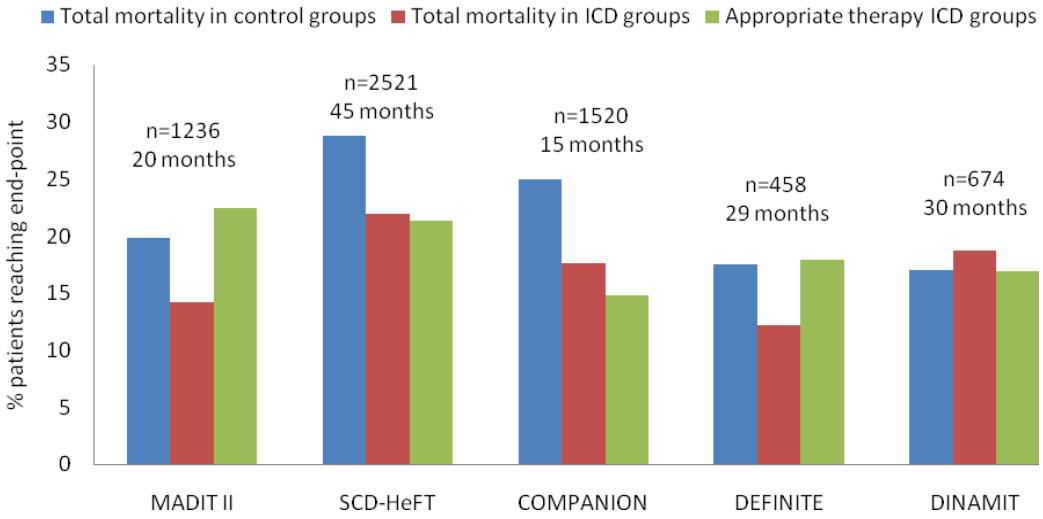
First, the sensitivity of reduced LVEF to predict SCD is relatively low. In a review of 8 studies that used LVEF, with cut-off values ranging from 30-40%, to predict SCD after MI, Buxton *et al*. found that the mean sensitivity ranged from 22-59% (94). These findings also hold true when LVEF is applied as a risk stratifier in the general population, rather than specific high-risk subgroups. In the Oregon Sudden Unexpected Death Study, a prospective study of all cases of SCD in Multnomah County, Oregon, LVEF was above 35% in 70% of people who had had LVEF estimation prior to cardiac arrest (95). The consequence of this low sensitivity is that the vast majority of patients who suffer SCD, or are resuscitated from cardiac arrest, will never have been considered for a prophylactic ICD under current LVEF-based guidelines.

Second, the specificity of reduced LVEF to predict SCD is poor. Germano *et al*. investigated the rate of appropriate ICD therapy and SCD, in a review of 7 large RCTs of ICD therapy (96). In each of 5 recent trials, with a mean follow-up ranging from 15-45 months, less than a third of patients experienced appropriate ICD therapy, while the rate of overall mortality in the control arm was also less than 30% (Figure 1-4). These findings are consistent with registry data, where approximately half of implanted patients do not receive appropriate ICD therapy during long-term follow-up (97, 98). In 463 patients with NYHA class II/III heart failure who received a CRT-D device (75% primary prevention) included in two prospective ICD registries, the cumulative rate of appropriate ICD therapy at 7 years was 50.4% (97). In another single-centre prospective registry, including 442 patients implanted with an ICD (16% CRT-D, 59% primary prevention), the cumulative incidence of appropriate ICD therapy was 52% at 7 years (98). The consequence of this low specificity is that the majority of patients implanted with ICDs based on current guidelines will not receive life-saving therapy from their device.

Third, the specificity of reduced LVEF for SCD, rather than non-sudden death, which is predominantly due to pump failure, is poor. Although patients with a low LVEF, compared to those with a higher LVEF, have an increased risk of SCD, they have a similarly increased risk of non-sudden death. Buxton *et al*. reviewed 12 studies that compared the specificity of reduced LVEF, using cut-off values of 30% to 40%, to predict SCD versus non-sudden death (94). Overall, the relative risk of SCD associated with a reduced LVEF was similar to that of non-sudden death. Therefore a reduced LVEF is a marker of total cardiovascular mortality risk and is not specific to mode of death. The importance of this concept lies in the fact that ICD therapy

reduces mortality due to arrhythmic SCD, but has no effect on mortality relating to other causes of death, specifically pump failure. Using reduced LVEF to guide ICD use will mean that ICDs are implanted in a number of patients who are likely to suffer from SCD, but also a similar number of patients who are likely to suffer a non-sudden mode of death, such as pump failure, and therefore not benefit from the device.

Figure 1-4 Outcomes In RCTs Of ICD Therapy
Rates of mortality and appropriate ICD therapy in 5 contemporary RCTs of ICD therapy. Rates are given for total mortality in the control groups, and total mortality and appropriate ICD therapy in the ICD groups. Number of patients included (n) and mean/median follow-up times are given for each trial. The trials included are the Multicenter Automatic Defibrillator Implantation Trial II (MADIT II) (58), Sudden Cardiac Death in Heart Failure Trial (SCD-HeFT) (59), Comparison of Medical Therapy, Pacing, and Defibrillation in Heart Failure (COMPANION) (99), Defibrillators in Non-Ischemic Cardiomyopathy Treatment Evaluation (DEFINITE) (100), and the Defibrillator in Acute Myocardial Infarction Trial (DINAMIT) (101). For the COMPANION trial data are shown for the control and CRT-D groups only.



1.4.5. Which Low LVEF Patients Do Not Benefit from ICD Therapy?

Overall, prophylactic ICD therapy improves survival in patients selected on the basis of a reduced LVEF (22). However, as described above, an LVEF-based risk stratification model has important limitations and not all implanted patients derive benefit from their device (50, 102). Two specific identifiable subpopulations of implanted patients do not significantly benefit from ICD therapy despite fulfilling international implant criteria: some patients never receive appropriate ICD therapy during long-term follow-up, reflecting the poor specificity of LVEF to predict SCD, while others have a high mortality despite an ICD, reflecting the lack of specificity of LVEF for SCD, rather than non-sudden death.

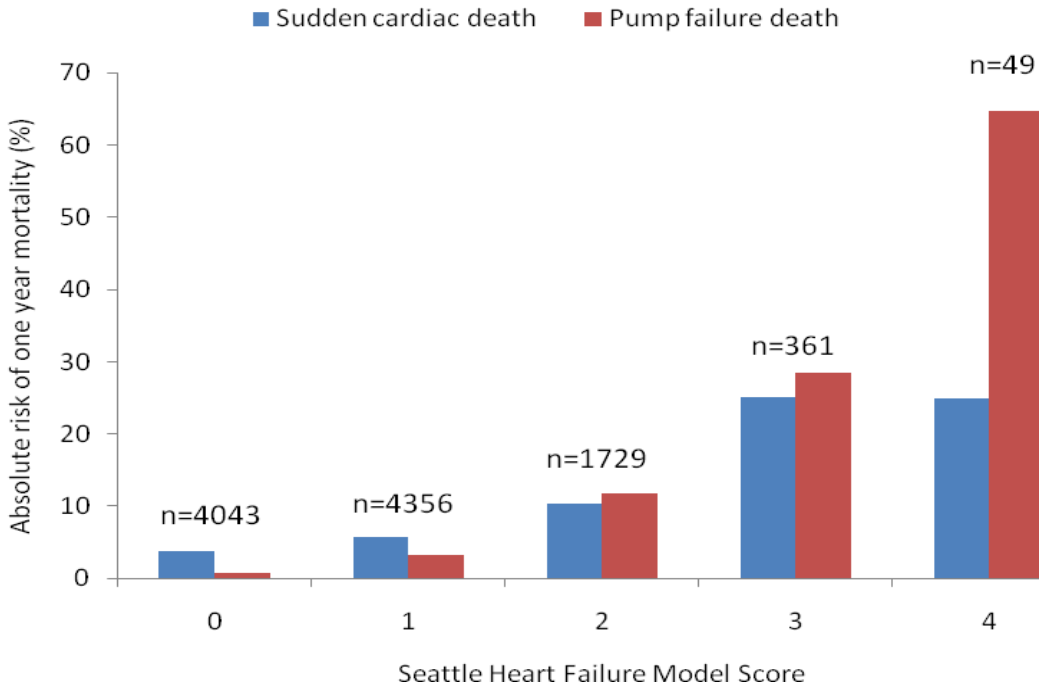
Due to the low specificity of reduced LVEF to predict SCD, up to a half of implanted patients never receive appropriate therapy during long-term follow-up (96-98). Furthermore, a significant proportion of these appropriate therapies are likely to have been for arrhythmias that would not have been life-threatening. Therefore, despite a reduced LVEF, many patients implanted with a prophylactic ICD are actually at relatively low SCD risk.

In contrast, other patients, who have either advanced heart failure or other co-morbidities (such as advanced chronic kidney disease), will have high mortality despite ICD therapy as modification of their SCD risk does not offer significant survival benefit (101, 103). The benefits of ICD therapy on mortality are not seen until after the first year and therefore patients whose predicted life expectancy, for any reason, does not significantly exceed this time-frame should not be considered for a device (50). Although this includes patients with significant non-cardiac life-limiting co-morbidities, it is most relevant with respect to patients with advanced cardiac failure, whose risk of non-sudden cardiac death is significantly elevated. This is especially important as reduced LVEF is as strong a risk factor for sudden as non-sudden death (94). Although overall SCD is the commonest mode of death in patients with LVSD, in patients with advanced heart failure progressive pump failure predominates (17). Mozaffarian *et al*. used the Seattle Heart Failure Model, a prognostic model that incorporates 24 clinical variables, to investigate mode of death in 10,538 ambulatory patients with heart failure enrolled in 6 RCTs or registries (17). Overall there were 2014 deaths, of which 1014 were classified as SCD and 684 as due to pump failure. However, in the 410 patients with the two highest model scores, reflecting more advanced heart failure, pump failure was most the frequent mode of death, accounting for 128 deaths, compared to 100 due to SCD (Figure 1-5).

A further issue with ICD therapy in patients with advanced heart failure is that although SCD may be prevented by appropriate device therapy, in many patients this does not lead to a significant increase in life expectancy, as patients suffer death from pump failure soon

afterwards. In this situation ICD therapy serves only to change the mode of death (102). This possibility is supported by the results of RCTs. In the DINAMIT study, an RCT of early ICD therapy following MI, 55 patients who suffered appropriate ICD therapy were examined for their long-term outcomes (104). Of these patients 40% had died within a year of the ICD therapy, and this was independent of age, LVEF and low heart rate variability. This is consistent with the MADIT-II trial, where patients that received appropriate ICD therapy had a significantly increased risk of a first heart failure event (HR 1.90; $p=0.01$) and recurrent heart failure (HR1.74; $p<0.001$) (105).

Figure 1-5 Mode Of Death In Heart Failure By SHFM Score
Absolute risk of 1-year mortality from SCD and pump failure death according to the modified Seattle Heart Failure Model Score (SHFM), in 10,538 ambulatory patients with predominantly systolic heart failure (NYHA II-IV) enrolled in 6 RCTs or registries. The SHFM is a validated multivariable risk model that predicts both all-cause and cause-specific mortality in heart failure patients. The modified SHFM evaluated in this study used the following clinical variables: age, gender, systolic blood pressure, ischaemic origin of heart disease, NYHA class, LVEF, angiotensin-converting enzyme inhibitor use, angiotensin receptor blocker use, β-blocker use, statin use, frusemide equivalent daily dose in milligrams per kilogram, serum sodium, digoxin use, carvedilol use, and serum creatinine. The number (n) of patients in each group are given (17).



1.4.6. Identifying Patients with Little Potential to Benefit

Although it is apparent that some patients meeting current ICD implant criteria do not gain significant benefit from their device, it is not clear how best to identify such patients prior to device implantation. Most work in this area has focused on identifying patients with an increased risk of non-SCD, whose mortality despite an ICD is high. Current international guidelines use NYHA functional class to define a patient group with advanced heart failure, with ICD therapy contraindicated for patients in class IV (2, 50). However, NYHA class is a relatively inaccurate prognostic variable and there is significant variation between rates of SCD and pump failure death within NYHA classes (17). Many patients in NYHA class II/III are at higher risk of pump failure than some patients in NYHA IV (17). A variety of alternative strategies to identify these high risk patients have been evaluated, including the use of individual clinical characteristics, the presence of cardiac and non-cardiac co-morbidities, and more complex risk scores.

Individual clinical characteristics that have been evaluated include renal dysfunction and advanced age. In a retrospective analysis of 229 patients with new ICD implants, Cuculich *et al*. found that the 15 month mortality in the 35 patients with chronic kidney disease (CKD) (serum creatinine ≥2 mg/dL or dialysis use) was significantly higher than those without CKD (48.6% vs. 8.2%, $P < 0.00001$) (103). In a retrospective cohort study of 502 consecutive patients with predominantly new ICD implants, Pellegrini demonstrated the importance of age in patient selection (106). Compared with younger patients (<65 years at implant), older patients (>75 years) were at significantly increased risk of death (HR 4.7, $p<0.001$).

Studies evaluating the effect of co-morbidity on benefit from ICD therapy have given conflicting results. Lee *et al*. examined the effect of age, gender and co-morbidity on survival, in a community-based study of 2467 ICD recipients in Ontario, Canada (107). Overall the 2-year mortality rate was 7.8%, and older age at implant, prior heart failure and the presence of non-cardiac co-morbidities, were all associated with an increased the risk of death. From their data, the authors suggested that greater attention to the presence of heart failure status and non-cardiac co-morbidities may improve outcomes in ICD recipients. Chan *et al*. also evaluated the effect of age and co-morbidities on benefit from ICD therapy, in a prospective study of 965 patients with LVEF ≤35% and no previous ventricular arrhythmias, of which 494 received an ICD (108). In contrast to the study by Lee *et al*. the authors found that, over a mean follow-up of 34 months, ICD therapy was associated with lower all-cause mortality, even among older patients and those with major co-morbidities.

Two studies have evaluated the ability of more complex risk scores, reflecting baseline pre-implantation predicted mortality risk, to identify patients less likely to benefit from ICD therapy. Goldenberg *et al*. evaluated the relationship between a derived risk score, made up of 5 clinical variables (NYHA functional Class >II, age >70 years, blood urea nitrogen >26 mg/dl but <50mg/dl, QRS duration >0.12 s, and AF at baseline), and benefit from ICD therapy, in the 1232 patients enrolled in the MADIT-II trial (109). The investigators also described a group of 60 'very high risk' (VHR) patients, with advanced renal dysfunction (BUN >50 mg/dl and/or serum creatinine >2.5 mg/dl), that were analysed separately. Patients in the VHR group, whose overall 2-year mortality was 50%, did not benefit from ICD therapy (HR 1.0, p>0.99). Furthermore, the 345 patients with none of the five risk markers, whose overall 2-year mortality was 9%, also derived no benefit from an ICD (HR 0.96; p >0.91). Based on their results, Goldenberg *et al*. proposed a U-shaped curve for the relationship between baseline predicted mortality risk, and benefit from ICD therapy, with patients at highest and lowest risk not gaining significant benefit from their device (Figure 1-6).

Levy *et al*. used a modified version of the Seattle Heart Failure Model (SHFM) to examine the baseline predicted mortality risk and the relative and absolute benefit from ICD therapy, in 2487 patients enrolled in the SCD-HeFT trial (110). The investigators used the SHFM to divide the patients into 5 equally sized groups of increasing predicted baseline risk. Patients in the group with the highest baseline predicted risk of death (n=497), whose observed annual mortality in the placebo arm was 17.6%, gained no significant benefit from ICD therapy (RR 0.98, p=0.89), despite the highest rate of appropriate ICD therapy (33% over 3.8 years) (Figure 1-7). By extrapolating their data, the authors suggested that the benefit of ICD therapy may approach null when annual predicted mortality reaches 20-25%. In contrast to the study by Goldenberg *et al*, the study by Levy *et al*. did not find a group whose risk of SCD was too low to benefit from their device.

All of the proposed strategies to identify patients that are unlikely to gain significant benefit from ICD therapy have important limitations. The use of individual risk factors, such as age or the presence of co-morbidities, may be too simplistic, and furthermore, data on the value of such an approach are conflicting (107, 108). In contrast, risk scores, such as the SHFM, can be relatively complex, requiring the availability of multiple clinical and laboratory variables, and their utility has only been evaluated in the context of RCTs, where the enrolled patients may not be representative of those in 'real world' practice (109, 110).

Figure 1-6 Effect Of Pre-Implantation Mortality Risk In MADIT II
Observed Kaplan-Meier mortality at 2 years for the control and ICD arms of the MADIT II trial (109). Patients are grouped by number of clinical risk factors (NYHA functional Class >II, age >70 years, blood urea nitrogen >26 mg/dl but <50mg/dl, QRS duration >0.12s, and AF at baseline), and by the presence of significant renal dysfunction (BUN >50 mg/dl and/or serum creatinine >2.5 mg/dl), who are in the very high risk group (VHR). The number of patients (n) in each group are given. Although mortality in patients at moderate predicted overall mortality risk (groups with 1 or more clinical risk factors) was reduced by ICD therapy, mortality in the lowest (no risk factors) and highest (VHR group) risk groups was not reduced by ICD therapy.

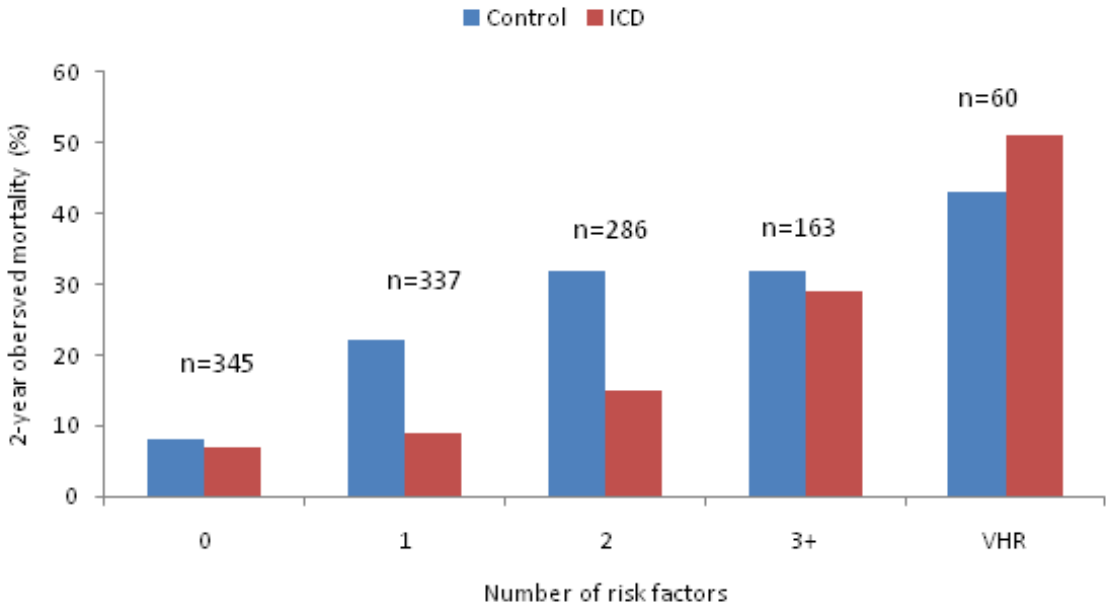
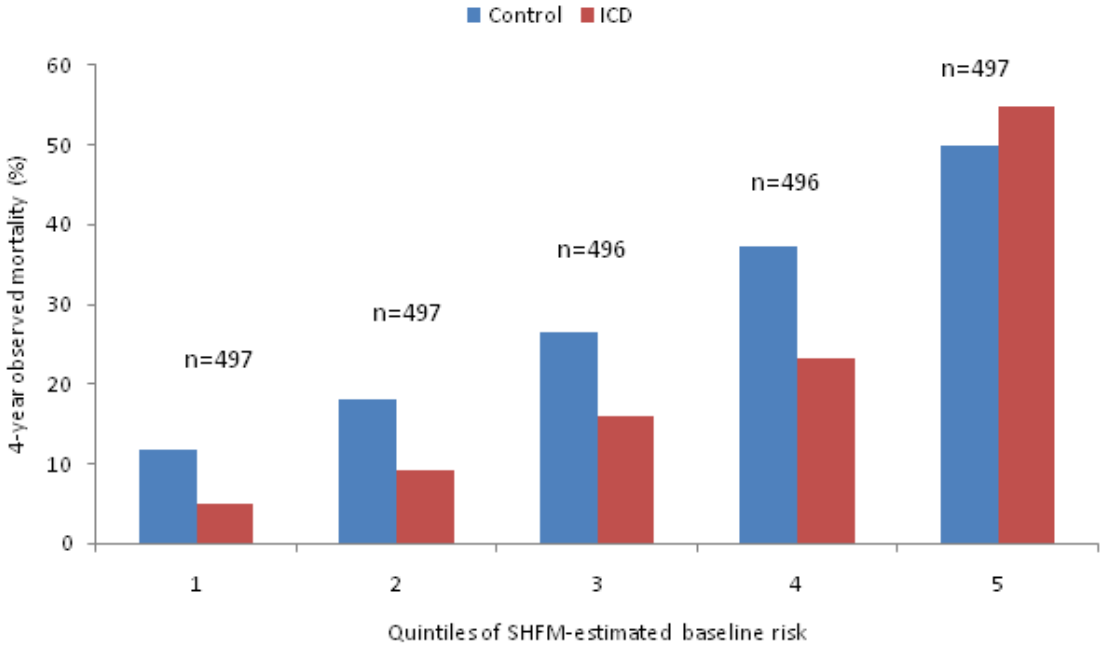


Figure 1-7 Effect Of Pre-Implantation Mortality Risk In SCD-HeFT
Observed Kaplan-Meier mortality at 4 years for the control and ICD arms of the SCD-HeFT trial (110). Patients are grouped by Seattle Heart Failure Model (SHFM) estimated quintiles of baseline predicted mortality risk. The number of patients (n) in each group are given. Although mortality in patients in the lowest 4 quintiles of risk (quintiles 1-4) was reduced by ICD therapy, in patients in the highest risk group (quintile 5) mortality was not reduced by ICD therapy.



1.5. Biomarkers And Prognosis In Patients With Low LVEF

A number of individual serum and plasma biomarkers have been shown to be powerful predictors of adverse outcomes in patients with reduced LVEF in a variety of clinical settings. These include biomarkers that predict all-cause mortality, biomarkers that predict SCD, and biomarkers that predict the occurrence of ventricular arrhythmias in ICD recipients.

1.5.1. Biomarkers and All-Cause Mortality in Low LVEF Patients

A range of individual plasma/serum biomarkers have demonstrated associations with all-cause mortality in patients with heart failure or asymptomatic LVSD. Although no specific classes for biomarkers are accepted, they may be grouped into categories based on pathophysiology: markers of inflammation, oxidative stress, extracellular matrix remodelling, neurohormones, myocyte injury, myocyte stress, and novel biomarkers that do not clearly fit into any of the previous categories (111).

1.5.1.1. Inflammation

Inflammation is central to the pathogenesis and progression of heart failure, and biomarkers of inflammation and inflammatory pathway activation are highly predictive of prognosis in patients with reduced LVEF (112). Rauchaus *et al*. prospectively evaluated multiple inflammatory cytokine levels in 152 patients with heart failure (121 patients in NYHA class II-III) (113). During a mean 34 months follow-up there were 62 deaths. In univariate analyses tumour necrosis factor-alpha (TNF-α) ($p<0.0001$), soluble TNF-receptors 1 and 2 (sTNF-R1/sTNF-R2) ($p<0.0001$), interleukin-6 (IL-6) ($p=0.005$), and soluble CD14 receptor ($p=0.0007$), were all predictive of death. In multivariable analysis the strongest predictor was sTNF-R2 ($p<0.001$), which proved better than LVEF.

Deswal *et al*. analysed circulating levels of two inflammatory cytokines, tumour necrosis factor (TNF) and IL-6, and their cognate receptors, in 1200 patients with advanced heart failure enrolled in a multicentre placebo-controlled trial of Vesnarinone, an inotropic drug (114). All patients were NYHA class III-IV, and the aetiology of heart failure in the majority (58%) was ischaemic. In the placebo group, which contained 384 patients, there were 65 deaths, 31 each due to SCD and pump failure, and TNF ($p=0.02$), IL-6 ($p=0.002$), sTNF-R1 ($p=0.0001$), and sTNF-R2 ($p=0.0001$), were all independent predictors of all-cause mortality in multivariable analysis. Although the predictive relationship of biomarkers to SCD was not specifically evaluated, levels of TNF and IL-6 were not significantly different between patients that died of SCD and those that died of pump failure.

1.5.1.2. Oxidative Stress

Oxidative stress is a result of an imbalance between reactive oxygen species and endogenous antioxidant defence mechanisms. This imbalance is important in the pathogenesis and progression of heart failure (115). An elevated level of uric acid, which is associated with the production of oxidants, is independently associated with an adverse prognosis in heart failure (116). In addition, plasma myeloperoxidase concentration, a marker of oxidative stress, correlates with heart failure severity and is an independent marker of all-cause mortality (117-119).

1.5.1.3. Extracellular-Matrix Remodelling

Myocardial remodelling is an integral process in the progression of heart failure, and markers of extracellular-matrix remodelling, such as aminoterminal propeptide type III procollagen, predict all-cause mortality in chronic heart failure (120).

1.5.1.4. Neurohormones

Activation of neurohormonal systems, especially the sympathetic and renin-angiotensin-aldosterone systems (RAAS), play a central role in the progression of heart failure, and a number of different neurohormones have shown prognostic value in heart failure (121). Latini *et al*. measured multiple different plasma neurohormones in 4300 patients with moderate to severe heart failure enrolled in the Val-HeFT study (122). In univariate analyses big endothelin, plasma renin and norepinephrine, were all predictive of all-cause mortality during follow-up.

1.5.1.5. Myocyte Injury

In addition to ischaemic damage, myocyte injury may occur as a result of oxidative stress, neurohormonal activation, or inflammation. Cardiac troponin (cTn) is a highly specific marker of myocardial injury, and an elevated troponin level is associated with total mortality in both acute and chronic heart failure, irrespective of the aetiology of the heart disease (123, 124). Horwich *et al*. evaluated the cTn level in 238 patients with advanced heart failure referred for transplantation (123). cTn was elevated in 117 patients (49%) and associated with a higher pulmonary wedge pressure ($p=0.002$), lower cardiac index ($p=0.0001$), and increased mortality during follow-up ($p<0.0001$). Peacock *et al*. evaluated admission cTn levels in 84,872 patients hospitalised with acute decompensated heart failure (124). An elevated cTn level was found in 4240 patients (6.2%) and was associated with a lower admission systolic blood pressure ($p<0.001$), a lower LVEF ($p<0.001$), and higher in-hospital mortality ($p<0.001$).

1.5.1.6.Myocyte Stress

A handful of biomarkers that reflect myocyte stress have been studied in patients with LVSD. These include the natriuretic peptides and soluble ST2.

There are three major natriuretic peptides (NPs), atrial natriuretic peptide (ANP), B-type natriuretic peptide (BNP), and C-type natriuretic peptide, all of which protect the cardiovascular system from the effects of volume overload found in the context of cardiac failure (125). ANP and BNP are released primarily from the heart, but circulate as hormones to act in various tissues in the body, inducing vasodilation, natriuresis, and diuresis. Though ANP is preferentially synthesized and secreted from the atria and BNP from the ventricles, under pathological conditions both can be synthesised in either chamber (126).

In the setting of volume expansion or pressure overload, the resulting myocardial wall stress initiates synthesis of pre–proBNP in the ventricular myocardium (125). The peptide is then cleaved first to $proBNP_{1-108}$, then to the biologically active BNP_{1-32} and the inactive amino-terminal fragment (NT-proBNP) (125). Once released, BNP results in improved myocardial relaxation and serves an important regulatory role, counteracting acute increases in ventricular volume by opposing the vasoconstriction, sodium retention, and antidiuretic effects of the RAAS (127).

As well as becoming established tests in the diagnosis of heart failure, there is increasing evidence that NP levels also give valuable prognostic information. Doust *et al*. performed a systematic review of 19 studies that assessed the accuracy of BNP (or NT-proBNP) to predict total mortality in patients with heart failure or LVSD (128). In patients with symptomatic heart failure, each 100 pg/ml increase in BNP was associated with a 35% increase in the relative risk of death. A raised BNP was also associated with an increased risk of death in patients with asymptomatic LVSD.

The ST2 gene is a member of the interleukin-1 receptor family, and consists of both a transmembrane receptor form, and a soluble receptor form (sST2) that can be detected in serum (129). The ST2 gene is up-regulated on the application of mechanical stress to cardiac myocytes and is thought to play an important role in regulating the myocardial response to mechanical overload in stretched cardiomyocytes, in a similar manner to BNP (129, 130). sST2 predicts all-cause mortality in the post-MI setting, as well as in patients with heart failure of ischaemic and non-ischaemic aetiology (129, 131).

Sabatine *et al*. measured sST2 at baseline in 1239 patients with ST-elevation MI, enrolled in the CLARITY-TIMI 28 trial, a trial of adjunctive Clopidogrel in the treatment of MI (131). sST2 was a powerful predictor of cardiovascular death or development of heart failure over 30-days follow-up, independent of both NT-proBNP and LVEF. Rehman *et al*. measured sST2 in 346 patients with acute heart failure (129). In an analysis including LVEF and NT-proBNP, sST2 was a powerful predictor of 1-year mortality. Taken together, these data suggest that NT-proBNP and sST2 may reflect complementary pathophysiological pathways involved in myocardial stress, and as such give prognostic information in patients with LVSD, that is both independent of one another and LVEF (131).

1.5.1.7. New Biomarkers

A novel biomarker that does not fit clearly into the above categories is growth differentiation factor-15 (GDF-15), a protein belonging to the transforming growth factor-β superfamily (132). GDF-15 expression is upregulated in cardiac myocytes by a range of stressors, including reactive oxygen species and inflammatory cytokines (133). GDF-15 is not normally expressed in the heart, but its expression is induced by experimental pressure overload and in mouse models of NICM, and it is found at increased circulating levels in patients with heart failure (134, 135).

GDF-15 has also been shown to predict mortality in patients with heart failure, independent of LVEF and NT-proBNP. Kempf *et al*. explored the prognostic utility of GDF-15 in 455 patients with LVSD (median LVEF 32%) (136). Over a median follow-up of 40 months 117 patients died. Ln GDF-15 was a significant predictor of mortality ($p<0.001$), independent of both LVEF and NT-proBNP. GDF-15 is also an independent predictor of mortality in patients undergoing CRT implantation (137). Foley *et al*. evaluated the prognostic value of GDF-15 in 158 patients with heart failure undergoing CRT (137). Over a median follow-up of 950 days 40 patients died of cardiovascular causes. GDF-15 was a predictor of cardiovascular mortality ($p=0.005$), independent of LVEF and NT-proBNP.

1.5.2. Biomarkers and SCD

The ability of individual serum/plasma biomarkers to predict SCD has been evaluated in three broad groups of patients – patients with heart failure or asymptomatic LVSD, post-MI patients, and apparently healthy people. In each context studies have demonstrated a significant association between biomarker levels and the occurrence of SCD (Table 1-3).

1.5.2.1. Patients with Heart Failure or Asymptomatic LVSD.

Berger *et al*. examined the association of 4 markers of neurohormonal activation and myocyte stress - BNP, NT-proBNP, N-terminal ANP (N-ANP), and big endothelin - with SCD, in 452 ambulatory patients with heart failure and LVEF <35% (138). The aetiology of heart failure in the majority of these patients (65%) was NICM. During a mean follow-up of 592±387 days there were 89 deaths, of which 44 were due to SCD. In univariate analyses, log BNP (p=0.0006), log NT-proBNP (p=0.0057), log N-ANP (p=0.0028), and big endothelin (p=0.0326), were all predictive of SCD. However in multivariable analysis only log BNP (p=0.0006) was still significant, while other traditional clinical predictors, including LVEF and NYHA class, were not. The association between BNP/NT-proBNP levels and SCD in patients with heart failure or asymptomatic LVSD, has been reproduced by other investigators (139-142).

Pascual-Figal *et al*. evaluated the relationship between sST2 and SCD, in a nested case control study of ambulatory heart failure patients (NYHA II/III, LVEF ≤45%) enrolled in a registry (143). The investigators identified 36 cases of SCD and matched 63 control patients, based on age, sex and LVEF. sST2 concentrations were significantly higher in SCD patients than controls (0.23 ng/ml [lower quartile to upper quartile 0.16-0.43 ng/ml] vs. 0.12 ng/ml [lower quartile to upper quartile 0.06-0.23 ng/ml]; p=0.001). Furthermore, the association of sST2 with SCD was independent of NT-proBNP levels.

1.5.2.2. Post-Myocardial Infarction Patients

Tapanainen *et al*. prospectively evaluated the accuracy of ANP, N-ANP, BNP and depressed LVEF, to predict SCD in 521 survivors of acute MI (144). During a mean follow-up of 43±13 months there were 33 deaths, of which 16 were due to SCD. In univariate analyses, BNP (relative risk [RR] 4.4, p=0.011), ANP (RR 4.1, p=0.014) and N-ANP (RR 3.4, p=0.018), had a similar accuracy to LVEF (RR 4.9, p=0.013), in predicting SCD. After adjusting for clinical variables only elevated BNP (p = 0.02) and low LVEF (<40%) (p = 0.03) remained as significant predictors of SCD. It should be noted that there was a high use of contemporary post-MI medical therapy in the cohort, including 97% beta-blockade.

1.5.2.3. Apparently Healthy People

The association between biomarkers and SCD has been assessed in apparently healthy men and women. Albert *et al*. assessed the relationship between CRP, homocysteine, plasma lipids levels and SCD, in a nested case-control study of men enrolled in the Physicians' Health Study (145). Ninety-seven cases of SCD and one hundred and ninety-two matched controls were included. Baseline CRP level was significantly associated with SCD – men in the highest quartile of CRP

were at a 2.78-fold (95% CI 1.35 to 5.72) increased risk of SCD, compared with men in the lowest quartile, which persisted after adjustment for known risk factors. Neither homocysteine nor lipid levels were predictive.

Korngold *et al*. investigated the relationship between two biomarkers, NT-proBNP and CRP, and SCD in apparently healthy women (146). They performed a nested case-control study of 99 cases of SCD and 294 matched controls, taken from 121,700 patients enrolled in the Nurses' Health Study. When examined continuously both NT-proBNP and CRP were associated with SCD risk (p value for trend 0.04 and 0.03 respectively). After adjustment for known CAD risk factors, the relationship with NT-proBNP was strengthened, but the association with CRP was no longer significant. Women with NT-proBNP levels above a pre-specified cut-point of 389 pg/mL were at increased risk of SCD (RR 5.68, p=0.003).

Table 1-3 Biomarkers Associated With The Occurrence Of SCD In Different Settings

Biomarker	Role of biomarker
Patients with LVSD	
Brain Natriuretic Peptide (138, 141)	Natriuretic peptide largely released from the ventricles
N-terminal pro Brain Natriuretic Peptide (138-140)	N-terminal fragment co-secreted with BNP
N-terminal Atrial Natriuretic Peptide (138)	N-terminal fragment co-secreted with ANP
Big endothelin (138)	Precursor to endothelin, a vasoactive peptide involved in vascular homeostasis
Soluble ST2 (143)	Plays an important role in regulating the myocardial response to mechanical overload in stretched cardiomyocytes, in a similar manner to BNP
Post-Myocardial Infarction	
Brain Natriuretic Peptide (144)	As above
N-terminal Atrial Natriuretic Peptide (144)	As above
Atrial Natriuretic Peptide (144)	Natriuretic peptide largely released from the atria
Apparently Health People	
N-terminal pro Brain Natriuretic Peptide (146)	As above
C-reactive protein (145)	Acute phase reactant marker of systemic inflammation

1.5.3. Biomarkers and Ventricular Arrhythmias in ICD Recipients

The ability of biomarkers to predict malignant arrhythmias in ICD recipients has been assessed in 9 studies, enrolling over 800 patients (147-155). These studies, which included patients with LVSD of an ischaemic and non-ischaemic aetiology, demonstrated that biomarkers are able to predict the occurrence of malignant ventricular arrhythmias with appropriate ICD therapy in ICD recipients (Table 1-4).

A number of studies have demonstrated that BNP (or NT-proBNP) independently predicts the occurrence of malignant arrhythmias in patients with ICDs (147-149, 151-153, 155). However, many of these were limited by a small sample size, enrolling less than 100 patients each (148, 150-152). Three of the larger studies reported that patients with BNP levels above the study median had significantly more malignant arrhythmias than those below this cut-off (risk ratios between 2.2 and 3.8) (147, 153, 155). Multivariable regression analyses in these studies examining traditional clinical and echocardiographic risk factors for SCD, found BNP most strongly predicted malignant arrhythmias and performed better than reduced LVEF. One study, by Yu *et al*., compared the predictive accuracy of NT-proBNP to EPS, in 99 patients who had undergone prophylactic ICD placement following MI (153). During a mean follow-up of 556±122 days, 23 patients received appropriate device therapy for VF/VT. On multivariable Cox regression analysis, only NT–proBNP level at or greater than median (497 ng/L) was a significant predictor for VT/VF occurrence (p=0.047).

Two studies have investigated a broader range of serum biomarkers to predict the occurrence of appropriate ICD therapy (147, 154).

Blangy *et al*. prospectively evaluated the ability of markers of cardiac fibrosis [procollagen type I aminoterminal peptide (PINP), procollagen type III aminoterminal peptide (PIIINP), membrane metalloproteinase I (MMP-1)], BNP and CRP, to predict appropriate ICD therapy (147). They followed 121 patients with ICDs and CAD over 12 months. During this time 38 patients had appropriate device therapy for VT. In a multivariable analysis, LVEF <35% (Odds Ratio [OR] 2.19, p=0.049), BNP (OR 3.75, p=0.014), CRP (OR 3.2, p=0.006), PINP (OR 3.71, p=0.009), and PIIINP (OR 0.21, p=0.003), were all significant predictors of device therapy for VT.

Kanoupakis *et al*. prospectively evaluated the association of markers of collagen turnover and the occurrence of malignant ventricular arrhythmias (154). Serum C-terminal propeptide of collagen type-I (CICP), C-terminal telopeptide of collagen type-I (CITP), MMP-1, and tissue inhibitor of MMP-1 (TIMP-1), were measured in 70 patients with NICM undergoing

prophylactic ICD implantation. During 12 months follow-up 14 patients received appropriate ICD therapy. Compared to patients that did not receive appropriate ICD therapy, patients that did had significantly higher levels of CITP (0.46±0.19 ng/ml vs. 0.19±0.07 ng/ml, $p<0.001$), MMP-1 (27.7±1.6 ng/ml vs. 24.1±2.5 ng/ml, $p<0.001$), and TIMP-1 (89±14 ng/ml vs. 58±18 ng/ml, $p=0.008$).

Table 1-4 Biomarkers Associated With The Occurrence Of Appropriate ICD Therapy

Biomarker	Role of biomarker
Natriuretic peptides	
Brain Natriuretic Peptide (147, 155)	Natriuretic peptide largely released from the ventricles
N-terminal pro Brain Natriuretic Peptide (149, 153)	N-terminal fragment co-secreted with BNP
Inflammatory markers	
C-reactive peptide (147)	Acute phase reactant marker of systemic inflammation
Markers of collagen turnover	
Procollagen type I aminoterminal peptide (PINP) (147)	Marker of collagen turnover
Procollagen type III aminoterminal peptide (PIIINP) (147)	Marker of collagen turnover
Membrane metalloproteinase I (MMP-1) (154)	Extracellular matrix degradation enzyme
C-terminal telopeptide of collagen type-I (CITP) (154)	Marker of tissue degradation of type-I collagen
Tissue inhibitor of MMP-1 (TIMP-1) (154)	Inhibitor of extracellular matrix degradation enzyme MMP-I

1.6. Biomarker Discovery Using A Proteomic Approach

1.6.1. Overview

The standard approach to the assessment of proteins in a biological sample, such as serum or plasma, involves techniques such as enzyme-linked immunosorbent assay (ELISA), which determine the level of individual proteins. While such an approach gives accurate information about the quantity of specific proteins, it is, by its nature, limited by its lack of breadth. In contrast, proteomic techniques allow hundreds of proteins in a given sample tissue to be analysed simultaneously (156). Though proteomic techniques have many applications, one of the most clinically relevant is as a tool for the discovery of potentially novel biomarkers (156, 157).

The basic principles of proteomic biomarker discovery are similar irrespective of the specific technological platform used or biological samples studied (158). First, biological samples are analysed using a quantitative platform, to make an assessment of the abundance of the different proteins present, or protein profile, of the sample. The protein profiles are then compared between a group of samples taken from patients with a specific condition, or cases, and suitably chosen controls, looking for differentially expressed proteins that distinguish cases from controls. If desired, the identity of these differentially expressed proteins can then be established.

A variety of different technological platforms have been used to separate proteins in proteomic biomarker studies (156). These platforms can be broadly classified into two different groups: gel-based approaches, where proteins are separated using electrophoresis, and non-gel based approaches, where proteins are separated by other techniques such as liquid chromatography (156, 158).

1.6.2. Biomarker Discovery Using SELDI-TOF MS

Surface-enhanced laser desorption ionisation time-of-flight mass spectrometry (SELDI-TOF MS) is a proteomic technique that uses on-chip retentate chromatography to separate complex protein mixtures (159). The SELDI-TOF MS technology employs the use of ProteinChip arrays, which contain 8 or 16 chemically treated spots. Biological samples of interest are loaded onto the array spots, with or without prior pre-fractionation. Each spot contains a solid-phase chromatographic surface, designed to retain proteins according to a general or specific physicochemical property of the protein, yielding an on-surface chromatographic protein separation for binding proteins at a particular binding condition. The spots are then washed to remove any unbound protein, and ionised by the addition of a matrix chemical – or energy

absorbing molecule. A laser then vaporises the ionised peptides, which are accelerated in an electric field and sent to a flight tube, at the end of which the detector is located. For a given electrical field voltage, the time of flight (TOF) to the detector is proportional to the ratio of the mass to charge (m/z), with smaller molecules flying faster than larger ones. For each biological sample applied to an individual spot, mass spectra are produced that reflect the presence and quantity of the individual peptides present in the sample, that have been captured and bound by the specific chromatographic surface of the ProteinChip array spot (Figure 1-8).

The technique of SELDI-TOF MS has been widely applied in the search for diagnostic serum/plasma biomarkers in a variety of different non-cardiac conditions.

SELDI-TOF MS first came to prominence as a technique for biomarker discovery with a publication by Petricoin *et al*., describing its use in the diagnosis of early-stage ovarian cancer (160). Proteomic spectra were generated from the serum of 100 patients with ovarian cancer and 116 controls. Patients were separated into a "training" set of 50 cases and 50 controls, and a separate "test" set of 50 cases and 66 controls. Using the spectra from the training set, the investigators employed a bioinformatic approach to develop an algorithm to discriminate cancer patients from controls, that used the peak amplitudes (or signal to noise ratio, S/N) of 5 separate peaks. In the test set, the algorithm identified all 50 cancer cases correctly and 63 of the 66 controls correctly, yielding a sensitivity of 100%, specificity of 95% and positive predictive value of 94%.

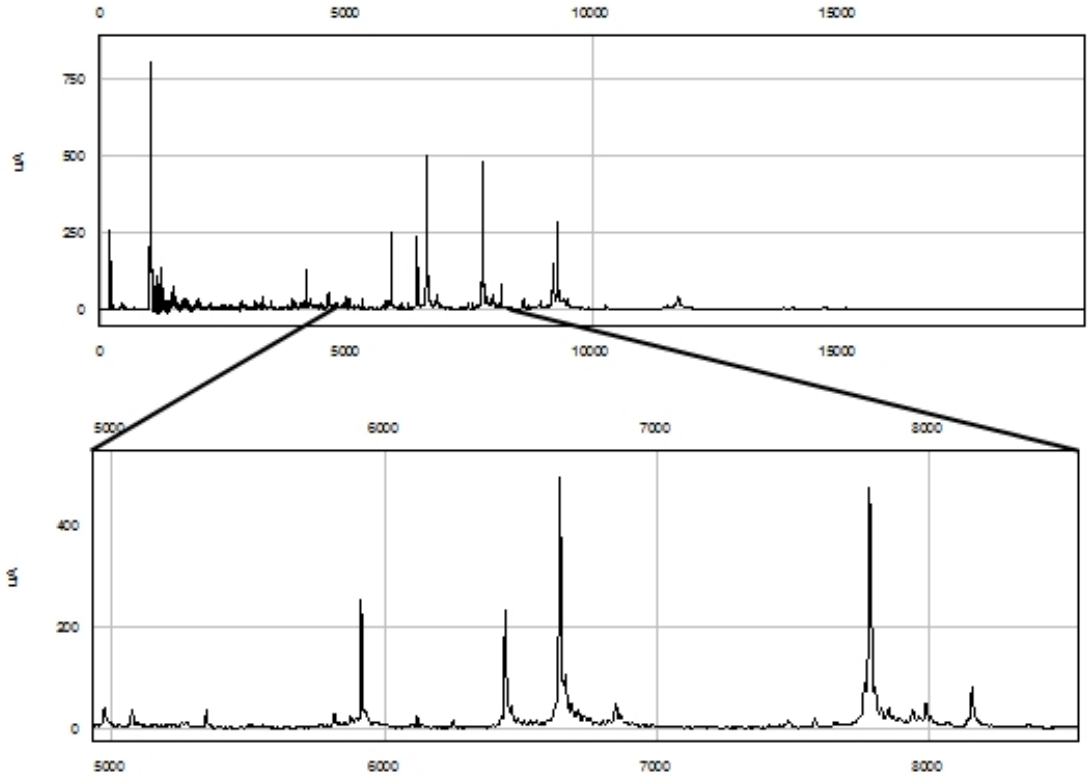
This approach has been successfully employed by other investigators to search for diagnostic protein patterns, or proteomic 'fingerprints', associated with specific conditions (161-164). Papadopoulos *et al*. used SELDI-TOF MS in the diagnosis of human African trypanosomiasis (sleeping sickness) (161). Spectra were generated from serum samples from 85 patients with sleeping sickness and 146 controls. Again, spectra were grouped into training (n=122) and test (n=109) sets. The training set was used to identify a distinct protein pattern, characteristic of sleeping sickness, made up of multiple differentially expressed protein peaks. When evaluated in the test set this proteomic fingerprint identified cases with a sensitivity of 100% and specificity of 98.6%. Adam *et al*. evaluated the use of SELDI-TOF MS in the diagnosis of prostate cancer, in 167 patients with cancer, 77 patients with benign prostatic hyperplasia and 82 matched controls (162). In a training set (n=326) the authors developed a classification algorithm made up of 9 differentiating protein peaks. In the test set (n=60) the algorithm demonstrated a sensitivity of 83% and specificity of 97% for the diagnosis of prostate cancer.

Since the publication of these early SELDI-TOF MS biomarker discovery studies some of the potential limitations of this protein pattern approach have been highlighted (165-167). One potential problem is that of data overfitting (168). It has been suggested that the apparent discrimination between cases and controls demonstrated by these studies, may be explained simply by chance, due to the overfitting that may occur when a multivariable model is used to fit a very large number of possible predictors, as found with MS peaks, to discriminate between patients with and without disease (168, 169). An additional problem is the introduction of potential procedural bias, for example by systematic differences in the collection and pre-analytical processing of samples e.g. case and control patients being collected at different geographical sites, using different equipment, at a different time of day (168).

The use of a proteomic fingerprint approach to develop diagnostic algorithms, based on multiple SELDI-TOF MS peaks, makes no assumptions about the identities of the proteins constituting the fingerprint. In contrast, some more recent studies have used an alternative approach, focussing on a small number of differentiating protein peaks, the identities of which are established, and in some cases subsequently measured in the same or an additional dataset using more accurate tests such as an ELISA assay (170-174). Such an approach may avoid some of the potential limitations of applying SELDI-TOF MS to biomarker discovery.

Agranoff *et al*. evaluated such an approach in the diagnosis of tuberculosis (TB) (170). Proteomic profiles were generated from serum using SELDI-TOF MS in 179 patients with TB and 170 controls, which were randomly allocated to training (n=193) and test sets (n=156). An algorithm was developed in the training set, which had a sensitivity of 94% and specificity of 95% to distinguish cases from controls in the test set. The authors then established the identities of two of the most discriminative proteomic peaks as serum amyloid A1 and transthyretin. These proteins, as well as another two related proteins, were then measured in serum using ELISA assays in a second dataset, containing 18 patients with TB and 23 controls. In this second dataset a multivariable model, using the results of the 4 ELISA assays, gave a sensitivity of 88% and specificity of 74% for the diagnosis of TB. This approach has been successfully used be other investigators (171-174).

Figure 1-8 Example Of A SELDI-TOF Mass Spectrum
The x-axis is the ratio of mass-to-charge (*m/z*), which corresponds to the molecular weight of each peak, and the y-axis represents the peak intensity. The upper spectrum shows a m/z range of 0 to 18,000. In the lower spectrum the 5,000 to 8,000 section has been enlarged. Multiple peaks can be seen in the spectra, which correspond to individual proteins or protein fragments. The peak height, or intensity, corresponds to the relative abundance of the individual protein in the sample being tested.



1.6.3. Proteomic Biomarker Discovery In Cardiovascular Disease

Although proteomic techniques have been employed widely in the search for biomarkers in patients with cancer and infectious diseases, they have also been used for plasma/serum biomarker discovery in cardiovascular disease (156, 175). Non-SELDI proteomic techniques have been used to identify plasma/serum biomarkers in patients with acute coronary syndromes and ischaemic stroke, and SELDI-TOF MS has been used to identify plasma biomarkers associated with peripheral arterial disease (PAD) and idiopathic pulmonary arterial hypertension (IPAH) (176-179).

Wilson *et al*. compared SELDI-TOF mass spectra between patients with PAD (n=45) and matched controls (n=43), and found 5 differentially expressed protein peaks (178). Using western blot analyses and immunoaffinity studies the investigators identified three of these peaks as representing β2-microglobulin, cystatin C and lysozyme C. The authors subsequently validated their findings by measuring β2-microglobulin using an ELISA assay in a further 20 PAD patients and 20 controls. Abdul-Salam *et al*. employed SELDI-TOF MS to screen for plasma biomarkers associated with IPAH, in 27 patients with IPAH and 26 controls (180). The expression of one protein peak was found to be significantly different between IPAH and control patients, and the identity of this peak was established as a truncated form of complement C4a.

Proteomic techniques have also been employed to search for serum/plasma biomarkers in patients with heart failure or asymptomatic LVSD.

Willingale *et al*. used matrix-assisted laser desorption ionisation time of flight mass spectrometry (MALDI-TOF MS), a related proteomic technique to SELDI-TOF MS, to look for diagnostic markers of systolic heart failure (SHF) (181). Plasma proteomic profiles were generated in a training set of 100 patients with SHF (LVEF <35%) and 100 healthy controls. An algorithm using 18 differentiating protein peaks diagnosed SHF with a receiver operator characteristic (ROC) score of 0.997 in the training set and 0.998 in a separate test set. The same group used similar techniques to examine the benefit of MALDI-TOF MS proteomic analysis in addition to NT-proBNP measurement in the diagnosis of SHF (182). In a training set of 100 patients with heart failure and 100 controls, 6 proteomic peaks were identified with diagnostic power independent of NT-proBNP. In the training set ROC scores for the diagnosis of SHF were 0.91 for NT-proBNP alone, improving to 0.99 with the addition of a model including the 6 proteomic peaks. These findings were consistent in the test set (ROC area 0.995).

Pinet *et al*. used SELDI-TOF MS to search for plasma markers of post-MI left ventricular remodelling (LVR) in 93 survivors of anterior q-wave MI enrolled in a multicentre study (183). Proteomic profiling was performed on blood taken on day 5 of hospitalisation. Patients were followed-up at 3 months and 1 year following MI with a transthoracic echocardiogram. Patients were divided into 3 groups (no, low or high remodelling) based on the degree of post-MI LVR, and mass spectra were compared between groups. Four protein peaks were differentially expressed between patients with no LVR and patients with high LVR, and were subsequently found to represent a post-translational variant of the α1-chain of haptoglobin (Hpa1) and haemoglobin.

To date, there have been no studies evaluating a proteomic approach to identify plasma or serum biomarkers associated with overall mortality, SCD, or the occurrence of ventricular arrhythmias, in patients with LVSD.

1.7. Scar Assessment By LGE-CMR

1.7.1. Overview

Gadolinium-DTPA (Gd-DTPA) is a contrast agent used in cardiac magnetic resonance (CMR) imaging, that rapidly diffuses outside the capillaries but is unable to cross intact cellular membranes (184). In the normal myocardium, tissue volume is predominantly intracellular, and therefore the uptake of Gd-DTPA is low (185). However, in areas of diseased myocardium the uptake of Gd-DTPA is increased, and the tissue appears hyperenhanced on CMR images (185). In the setting of myocardial necrosis, found in the context of MI, Gd-DTPA passively diffuses across the ruptured myocyte membranes into the intracellular space (Figure 1-9) (184). In the presence of collagenous scar tissue, the extracellular space is expanded in comparison to normal myocardium, leading to accumulation of Gd-DTPA and consequent hyperenhancement.

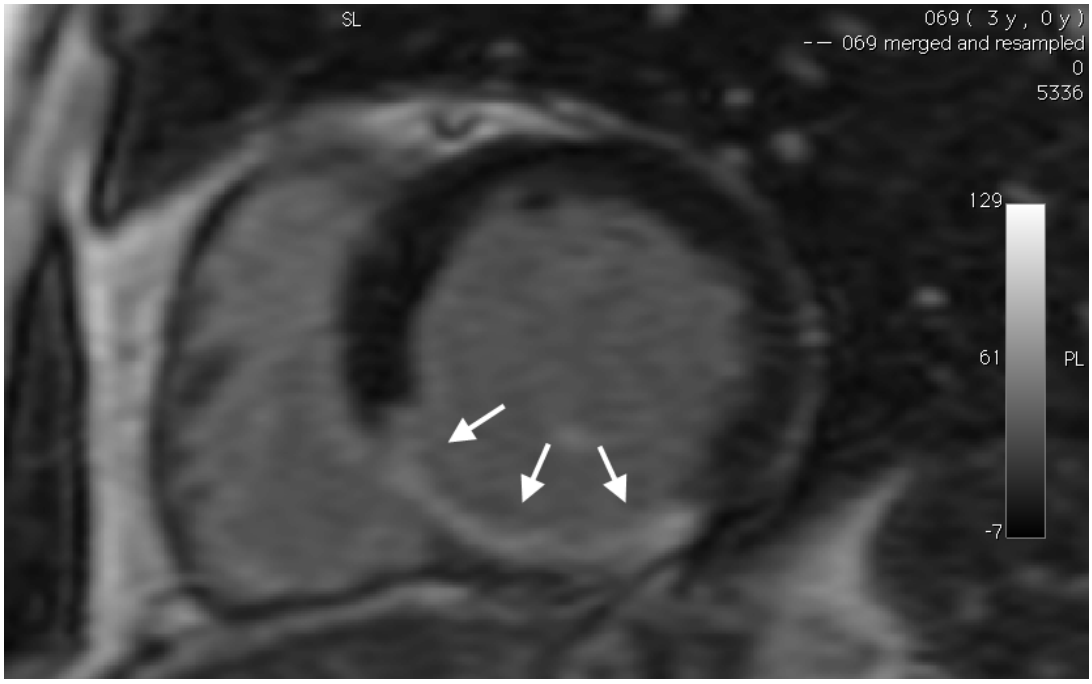
1.7.2. LGE-CMR to Predict Outcomes in Patients with Low LVEF

Late gadolinium enhancement cardiac magnetic resonance imaging (LGE-CMR) can accurately and reproducibly identify areas of myocardial scar tissue (186, 187). Furthermore, its high spatial resolution enables discrimination between subendocardial and transmural scar (186). The amount, as well as the transmural extent, of myocardial scar tissue on LGE-CMR has been shown to predict overall mortality in patients with CAD independently of reduced LVEF in a range of settings, including patients with an ischaemic cardiomyopathy, patients with a previous MI, and patients without previously diagnosed CAD (188-190).

Kwon *et al*. evaluated the association between the extent of LV scar, measured by LGE-CMR, and a composite end-point of all-cause mortality or cardiac transplantation, in an observational study of 349 patients with CAD and severely reduced LVEF (mean LVEF 24%) (188). Over a mean follow-up of 2.6±1.2 years there were 56 events (51 deaths and 5 transplants). In multivariable analysis the strongest predictor of the composite end-point was the amount of LV scar ($p=0.005$), quantified as a percent of the total myocardium, whilst LVEF was not a significant predictor. Roes *et al*. evaluated the prognostic significance of LV scar in 231 patients with previous MI (189). During a median follow-up of 1.7 years 19 patients died. The degree of LV scar on LGE-CMR, quantified both as the spatial extent of scar ($p<0.001$) and the amount of transmural scar ($p=0.003$), predicted mortality. Cheong *et al*. studied the association between the extent of scar and a composite end-point of death or cardiac transplantation, in 857 consecutive patients who underwent LGE-CMR at a single centre (190). During a median follow-up of 4.4 years 252 patients reached the composite end-point. In multivariable analysis, the amount of LV scar was an independent predictor of the composite end-point both in the 642 patients with CAD ($p=0.018$) and the 215 patients without CAD ($p=0.004$).

However, although the association between the extent of LV scar, quantified by LGE-CMR, and overall mortality is well established, since both SCD and non-sudden death contribute to overall mortality in patients with CAD it is unclear how accurately it predicts the occurrence of SCD, and therefore its use in selecting patients for ICD therapy.

Figure 1-9 LGE-CMR Image Of Left Ventricular Scar
Short axis LGE-CMR image through the mid-LV cavity in a patient with a full thickness inferoseptal MI (arrows). The normal myocardium is black while the infarcted area has taken up gadolinium and appears brighter.



1.8. Key Areas Of Uncertainty

The preceding literature review has demonstrated a number of areas of uncertainty regarding the current use of ICD therapy. Key amongst these are issues regarding the epidemiology of ICD utilisation in England and Wales, as well as SCD risk stratification and patient selection for ICD therapy.

1.8.1. The Epidemiology of ICD Use

The true need for prophylactic ICD implantation in England and Wales is not known. NICE initially published national guidance on ICD implantation in 2000. Early studies suggested that using the initial NICE criteria the incidence of ICD indications was far in excess of contemporary implant rates (69). In 2006 NICE revised their guidelines broadening the indications for prophylactic device use (72). The updated guidelines are likely to significantly increase the required implant rate and the current need for prophylactic ICD therapy for patients with CAD is unknown.

Despite national guidance concerning the use of ICD therapy there is significant regional variation in ICD implant rates within England and Wales (63). It is not possible to explain these geographical differences on variations in the prevalence of CAD, the primary condition underlying SCD risk, and the reason for this regional disparity is unknown. One possible factor that has not been investigated in a UK setting is the impact of clinical setting (regional centre vs. district general hospital) on implantation rates.

1.8.2. SCD risk Stratification and Patient Selection for ICD Therapy

Although ICD therapy effectively reduces mortality in patients at high SCD risk identifying patients for a prophylactic device remains a challenge. LVEF assessment plays a key role in contemporary ICD guidelines, including NICE guidance. However its predictive accuracy is poor with low sensitivity and specificity for predicting SCD (93, 94). There is a significant need for more accurate SCD risk stratification tools to improve the clinical and cost-effectiveness of ICD therapy. Three specific areas that warrant further evaluation in the risk stratification of SCD are:

(i) The potential role of BNP (or NT-proBNP) in predicting SCD. A number of studies have evaluated the association between BNP and the occurrence of SCD and ventricular arrhythmias. However, many of these have been limited by small sample size and the accuracy of BNP in predicting SCD and ventricular arrhythmias is unclear.

(ii) The potential role of left ventricular scar quantification by LGE-CMR in predicting SCD. The association of the extent of left ventricular scar, quantified by LGE-CMR, and overall mortality in patients with CAD is well established. However, it is unclear how strongly it is associated with the occurrence of SCD, and therefore its potential utility in selecting patients for ICD therapy.

(iii) The potential use of proteomic techniques in identifying prognostic biomarkers in patients with LVSD. Proteomic techniques have been successfully used to identify biomarkers in a range of cardiac and non-cardiac conditions. However their potential utility in identifying prognostic biomarkers in patients with LVSD has not been evaluated.

Prophylactic ICD therapy improves overall survival in patients selected using an LVEF-based risk stratification model (22). However, there are subpopulations of implanted patients that do not derive significant benefit from their device (50, 102). Some implanted patients never receive device therapy during long term follow-up, while others, who have either advanced heart failure or other co-morbidities, have high mortality which is not significantly reduced by ICD therapy (96, 98, 101, 103). Identifying these patient groups is an important component of refining the application of ICD therapy, however there is currently no consensus on how best to do this. Biomarkers are available that reflect multiple complementary pathophysiological pathways in heart failure, and as such are powerful predictors of prognosis in patients with LVSD (111). However, the value of biomarkers in identifying patients with greatest potential to gain benefit from ICD therapy is not known.

There is a significant clinical need to identify those patients who have the greatest potential to benefit from ICD therapy.

1.9. Aims

Given the need to improve the application and uptake of ICD therapy the specific aims of this thesis were:

(i) To estimate the requirement for ICD therapy for the primary prevention of SCD post-MI, based on the revised NICE guidelines (2006) (Chapter 3).

(ii) To assess the effect of clinical setting (regional cardiothoracic centre vs. district general hospital) on ICD prescription rates (Chapter 4).

(iii) To use meta-analytical techniques to evaluate the accuracy of BNP/NT-proBNP to predict SCD and the occurrence of ventricular arrhythmias in published studies (Chapter 5).

(iv) To assess the value of biomarkers in identifying patients' potential for survival benefit from ICD therapy (Chapter 6).

(v) To use proteomic techniques to identify serum biomarkers associated with LVSD, and prospectively explore the association of these proteomic biomarkers with mortality and the occurrence of ventricular arrhythmias in a cohort of patients with ICDs on the background of LVSD (Chapter 7).

(vi) To assess whether the extent of left ventricular scar, quantified by LGE-CMR, is associated with the occurrence of appropriate ICD therapy in patients with ICDs and CAD (Chapter 8).

1.10.Hypotheses

With reference to the specific aims described above this thesis tested the following hypotheses:

(i) There is significant underuse of ICD therapy for the primary prevention of SCD in patients following MI in England and Wales.

(ii) The prescription of ICD therapy is influenced by clinical setting, and is significantly higher in areas served by a regional cardiothoracic centre than a district general hospital.

(iii) The biomarkers BNP/NT-proBNP predict the occurrence of SCD and appropriate ICD therapy with comparable accuracy to currently available risk stratification tests such as LVEF.

(iv) That individual serum/plasma biomarkers can identify patients who are unlikely to gain significant benefit from ICD therapy.

(v) That a high-throughput proteomic approach, using SELDI-TOF MS, can identify serum biomarkers associated with LVSD and that these proteomic biomarkers are associated with prognosis in ICD recipients with LVSD.

(vi) That in patients with CAD, LV scar burden, quantified by LGE-CMR, may be more strongly associated with the occurrence of appropriate ICD therapy, as a surrogate for arrhythmic SCD, than LVEF.

2. GENERAL METHODS

2.1. Approach

This thesis contains 6 separate data chapters, each corresponding to a separate study or analysis, and designed to test hypotheses (i) through to (vi) described previously. Chapters 3, 4 and 8 detail separate retrospective observational studies designed to address hypotheses (i), (ii) and (vi) respectively. Chapter 5 is a meta-analysis of published data addressing hypothesis (iii). Chapters 6 and 7 describe two separate analyses of data from one prospective study, evaluating the role of serum/plasma biomarkers in patient selection for ICD therapy, and address hypotheses (iv) and (v) respectively.

2.2. Ethical Considerations

The prospective study, evaluating the role of serum/plasma biomarkers in patient selection for ICD therapy (Chapters 6 and 7), was undertaken in accordance with the Declaration of Helsinki of the World Medical Association. It was also performed with the approval of the Southampton and South West Hampshire Research Ethics Committee (local ethics number 08/H0502/54) and the University Hospital Southampton NHS Foundation Trust Research and Development department (study number CAR0357).

All patients participated in the prospective study voluntarily. They were all deemed competent to consent and able to consider fully and rationally the implications of taking part. They were provided with adequate information in the form of a detailed patient information sheet and also given the opportunity to discuss further with the principal investigator. The written informed consent of each patient was obtained before entry into the study. Participants were able to withdraw consent at any time without hindrance or detriment to their future treatment. Measures were taken to safeguard patient confidentiality. The use of identifiable information was minimised and data anonymised wherever possible. Research records, both paper and electronic, and anonymously labelled specimens were stored in a secure manner only accessible to authorised personnel. Confidential information was used only for the purpose it was obtained and no individual was identifiable from published results.

The studies assessing the requirement for ICD therapy for the primary prevention of SCD post-MI (Chapter 3), the effect of clinical setting on ICD prescription rates (Chapter 4), and the association of LGE-CMR scar quantification with the occurrence of appropriate ICD therapy (Chapter 8) were all retrospective observational studies and therefore did not require Research Ethics Committee approval.

2.3. Subject Recruitment

Patients in the prospective study, evaluating the role of serum/plasma biomarkers in patient selection for ICD therapy, were recruited from patients attending the Wessex Cardiothoracic Unit (Southampton University Hospital) device service. Two different patient populations were enrolled:

(i) *Control Patients.* This group comprised patients with a permanent pacemaker (PPM) and preserved LVEF. Patients with a high percentage of right ventricular pacing (>30%), or history, signs or symptoms of heart failure, were excluded.

(ii) *Left Ventricular Systolic Dysfunction Patients*. This group comprised patients with LVSD, and an ICD or cardiac resynchronisation defibrillator (CRT-D). All patients were on stable optimal medical therapy. None had heart failure admissions or therapy changes in the six weeks prior to enrolment.

Additional exclusion criteria for both groups were pregnancy, an acute coronary syndrome or surgery of any type within the preceding 6 weeks.

One hundred and thirty-seven patients enrolled in the prospective study were included in both analyses described in Chapters 6 and 7.

2.4. Setting

The first study, assessing the incidence of ICD indications for the primary prevention of SCD following MI (Chapter 3) was performed at Portsmouth Hospitals NHS Trust. The other studies (Chapters 4 and 6-8) were performed at Southampton University Hospitals NHS Trust.

Portsmouth Hospitals NHS Trust is a large District General Hospital Trust, providing local hospital services to a population of approximately 560,000 people. Southampton University Hospitals NHS Trust provides local hospital services to around half a million people that live in Southampton and south Hampshire. It also provides a regional cardiothoracic service, including complex coronary intervention, interventional electrophysiology and devices, and cardiothoracic surgery, to approximately 2.8 million people in central southern England.

Both local areas served by the two hospitals have higher rates of heart disease than the national average, especially in younger patients. The directly standardised rates (per 100,000) of death due to CAD for all ages (2003-2007), were 117 (95% confidence intervals [CI] 111-123) for

Portsmouth City Primary Care Trust (PCT), 106 (95% CI 101-112) for Southampton City PCT, and 95 (95% CI 95-95) for England as a whole (191). The equivalent values for people under 75 years of age were 104 (95% CI 97-110) for Portsmouth City PCT, 99 (95% CI 93-105) for Southampton City PCT, and 45 (95% CI 45-45) for England as a whole.

2.5. General Statistics

Data were collected and stored in accordance with the Data Protection Act. Statistical analyses were performed using Statistical Package for Social Sciences version 17 (SPSS Inc., Chicago, IL, USA), Review Manager 4.2.8 (Cochrane Collaboration, Oxford, United Kingdom) and Meta-Disc 1.4 analysis software (192).

Categorical variables are expressed as percentages (numbers). Continuous variables are expressed as mean ± standard deviation if normally distributed, and as median (lower quartile to upper quartile) if not normally distributed. Group comparisons of categorical data were evaluated using the x^2 test. For normally distributed continuous variables, group comparisons were made using a two-tailed, unpaired t-test. For non-normally distributed continuous variables, group comparisons were made using the Mann-Whitney *U* test for two groups. The Jonckheere-Terpstra test was used to assess the trend across more than two groups.

In prospective studies, predictors of outcomes were investigated in Cox proportional hazards models. In all analyses the proportional hazards assumption was checked using Schoenfeld residuals (193).

In all analyses $p < 0.05$ was considered significant, except for inclusion in multivariable models.

3. NEED FOR ICD THERAPY

3.1. Introduction

In 2000, NICE published its initial guidance on the indications for ICD therapy in England and Wales. Since then the evidence base for the use of ICD therapy for the primary prevention of SCD following MI has extended, and in 2006 NICE guidance was updated to take account of these expanded indications (Table 1-2) (58, 59, 72). It remains uncertain what impact the revised NICE guidance will have on absolute ICD implantation rates and the associated health resources. Previous studies assessed the incidence of ICD requirements based on the initial guidelines (69-71). However, the revised indications are likely to increase this rate and the true need for ICD implantation for primary prevention post-MI is not known.

The aim of this study was to estimate the requirement for ICD therapy for the primary prevention of SCD post-MI, based on the revised NICE guidelines (2006). The study tested the hypothesis that there is significant underuse of ICD therapy for the primary prevention of SCD in patients following MI in England and Wales.

3.2. Methods

Portsmouth Hospitals NHS Trust is a large DGH Trust serving a population of approximately 560,000 people. All patients with acute MI are admitted via a single site. Using ICD-10 clinical coding all patients during a 6 month period from February to July 2006 with any discharge diagnosis of MI were identified. All transthoracic echocardiograms (TTE) reports are stored on a database (Tomcat Systems Ltd, Belfast, NI), which was searched to obtain TTE reports during or early after hospital admission. If there were multiple reports the most recent was used. Hospital notes were reviewed for patients whose LV systolic function was reported as "severely impaired" (or "severe dysfunction") or "moderate-severely impaired" (or "moderate-severe dysfunction"). Age, sex and type of infarct (ST-elevation or non ST-elevation) were recorded. The most recent electrocardiogram (ECG) prior to discharge (or death) was evaluated and QRS width noted. Documented contraindications to an ICD and evidence of ventricular arrhythmias which might meet secondary prevention guidelines for an ICD were also noted.

NICE criteria (Table 1-2) were used to estimate the incidence of potential post-MI primary prevention ICD indications in the cohort. With reference to TTE assessment of LV systolic function, based on the departmental reporting criteria, a pragmatic assumption that "severely impaired" reflected LV ejection fraction (LVEF) <30% and "moderate-severely impaired" 30-35% was made. As there was no systematic screening for post-MI primary prevention ICD indications in the hospital at the time of the study, anticipated results from Holter monitoring

and EPS were extrapolated from published data (Table 3-1) (85, 86, 194-197). Patients who died within 4 weeks of their MI (as they would not meet NICE guidance), had a "secondary prevention" indication for an ICD, or had a documented contraindication to an ICD were excluded.

Table 3-1 Assumptions Used To Calculate The Incidence Of Primary Prevention ICD Indications In Post-MI Patients

- 35-36% of patients surviving an MI with EF <35% and NSVT on Holter monitoring will have a positive EPS (85, 86).
- 12-41% of patients surviving an MI with EF <35% will have NSVT on Holter monitoring (194-197).

3.3. Results

Five hundred and forty-six patients admitted to hospital during the 6 month study period with an ICD-10 diagnosis of MI were identified. Four hundred and four (74%) had reported TTEs, of which 50 had estimated LVEF <35%. Of these 50 patients, 1 set of notes was unavailable and therefore 49 were evaluated in more detail - 27 with "severely impaired" and 22 with "moderate-severely impaired" LV systolic function (Table 3-2). Under NICE guidance all post-MI patients with LVEF <35% should be considered for an ICD. However the need for further testing prior to device implantation depends on the severity of LV impairment. Patients with LVEF <30% require only a QRS width of ≥120msec, while patients with LVEF 30-35% require both NSVT on Holter monitoring and inducible VT at EPS. Consistent with NICE guidelines, these two patient groups were considered separately (Figure 3-1).

Of the 27 patients with "severely impaired" LV systolic function (assumed equivalent to LVEF <30%), 6 died within 4 week of their MI, 4 had a "secondary prevention" indication for an ICD and 1 had limited life expectancy due to non-cardiac co-morbidity. Of the remaining 16, 7 patients had a QRS width greater than or equal to 120msec and would have met the guidance for consideration of ICD implantation without need for further testing assuming LVEF remained <30% after 4 weeks. The remaining 9 with a narrow QRS would need to meet the same requirements as those with LVEF 30-35% (NSVT on Holter monitoring and inducible VT at EPS) and are discussed below (Figure 3-1).

Of the 22 patients with “moderate-severely impaired” LV systolic function (assumed equivalent to LVEF 30-35%), 2 died before 4 weeks and 2 had a “secondary prevention” indication for an ICD (Figure 3-1). To meet NICE criteria the remaining 18, in addition to the 9 with LVEF <30% but narrow QRS, would need to demonstrate NSVT on Holter monitoring and inducible VT at EPS. Making the assumptions outlined in Table 3-1 gives an estimate of between 1 and 4 patients over the six-month study period requiring an ICD for this indication.

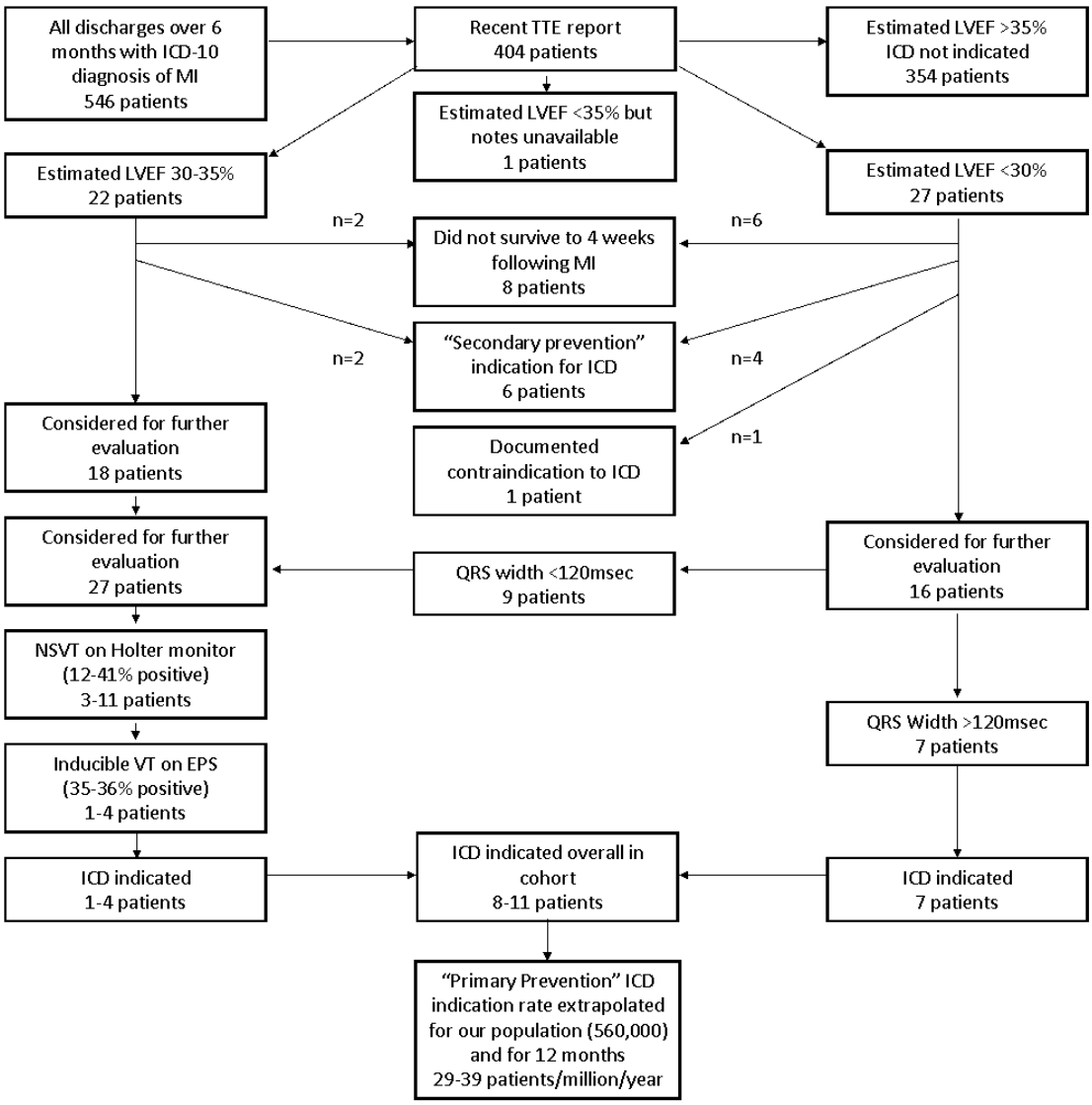
Therefore, out of 546 post-MI patients identified over a six month period, between 8 and 11 met the NICE post-MI primary prevention criteria for potential ICD indication. Extrapolating this for the hospital catchment population (560,000 people), for the whole year, gives an incidence of 29-39 patients/million/year.

Table 3-2 Details Of 49 Patients With "Moderate-Severe" And "Severe" LV Dysfunction In The Cohort

	Left ventricular systolic function as assessed on TTE	
	Moderate-Severe dysfunction (n=22)	Severe dysfunction (n=27)
Age (years)	70	69.5
Male Sex % (no.)	73 (16)	81 (22)
Infarct Type % (no.)		
STEMI	45 (10)	30 (8)
Non-STEMI	55 (12)	70 (19)
QRS width % (no.)		
<120 ms	73 (16)	63 (17)
120-149 ms	23 (5)	30 (8)
>150 ms	0 (0)	7 (2)
Paced	5 (1)	0 (0)
Timing of TTE following MI % (no.)		
1-7 days	77 (17)	78 (21)
8-14 days	14 (3)	11 (3)
2-4 weeks	5 (1)	0 (0)
4 weeks	5 (1)	11 (3)

STEMI, ST-elevation myocardial infarction; Non-STEMI, non ST-elevation myocardial infarction.

Figure 3-1 Algorithm For Calculating ICD Indications



ICD, implantable cardioverter defibrillator; TTE, transthoracic echocardiogram; LVEF, left ventricular ejection fraction; NSVT, non-sustained ventricular tachycardia; VT, ventricular tachycardia; EPS, electrophysiological study.

3.4. Discussion

The published UK ICD data corresponding best to the study period give a new implantation rate of just over 40/million/year (2005) (198). My estimate of 29-39/million/year relates only to post-MI primary prevention. A recent estimate of the incidence of ICD secondary prevention indications based on current NICE guidance was 76/million/year (69). Taken together these imply a combined ICD indication incidence of approximately 105-115/million/year. This suggests there is currently considerable under-provision of ICD therapy in the UK, though requirements may not be as high as some studies have proposed (69, 70).

An increase in device use to achieve the estimated implantation rate based on my findings would have significant cost implications. Using the published NICE costing of £20102 per device, which includes follow-up appointments and replacement/repair costs (though based on 2004 costs), but not the potential cost saving of a reduction in cardiac events, would mean an extra cost of approximately £74-86 million ($152-177 million, 103-120 million Euro) per year to the NHS (199).

The only other studies to assess the incidence of ICD indications based on NICE guidance used different methodology, and as they were published prior to the new guidance used the MADIT II study criteria for reference (58, 69, 70). Both studies produced significantly higher estimates of the overall incidence of patients requiring consideration for ICD implantation (453/million/year and 504/million/year), and there are a number of potential reasons for this disparity. The more inclusive MADIT II criteria did not use QRS width as a determinant – in my study only 37% of patients with LVEF <30% had a broad QRS. The new NICE guidance also states that patients must be at least 4 weeks after their infarct to be considered for device therapy – in my study only 78% of patients with LVEF <30% survived to this point. Furthermore in 2005, two years after the publication of the MADIT II trial, the highest total (not new) ICD implantation rate in Europe in 2005 was approximately 170/million/year (Germany), which is more in keeping with my estimate (198).

In my cohort 9% of post-MI patients had an LVEF <35%, which is lower than in other studies (11-16%) (81, 194). The diagnosis of MI in my study was based on the modern, more inclusive diagnostic criteria using troponin. In contrast other published studies primarily enrolled patients with ST-elevation MIs, who are likely to have suffered larger infarcts with more myocardial damage. Contemporary treatment of acute MI may also contribute to better preservation of LV function. The number of patients with potential “secondary prevention” ICD indications in my cohort was 6, giving an incidence of 21/million/year, which is significantly lower than other

studies (69). This is likely to be related to my study methodology where only those patients with LVEF <35% were included; a "secondary prevention" indication but LVEF ≥35% will have been missed. In addition patients who presented with VF/VT without a significant troponin rise would also have been excluded.

In this study I estimated the incidence rather than prevalence of ICD requirements. In order to screen for the prevalence in the study population one would need to assess the records of all patients with heart failure and CAD in both outpatient and inpatient settings. In view of the potential logistical difficulties, I felt that estimation of incidence would still provide meaningful data. However the estimate will be considerably lower than the equivalent prevalence.

To improve the uptake of primary prevention device therapy the implementation of clinical pathways enabling the systematic identification and early assessment of potential candidates are needed. The 2006 NICE guidance advocates further testing for patients with LVEF 30-35% (Holter monitoring and EPS). It has been suggested that this guidance has not been widely followed because EPS, which is only available in larger cardiac departments, is perceived as a block (27). In my cohort the vast majority of patients requiring consideration for ICD implantation (64-88%) were identified purely by ECG criteria (QRS >120msec). Therefore the incremental benefit of further testing in addition to TTE and ECG in identifying high risk patients is relatively small. These data suggest that merely evaluating LVEF and QRS width should be an initial priority when setting up a robust assessment programme.

3.4.1. Limitations

Some of the data were incomplete. Approximately three-quarters of patients with a discharge diagnosis of MI had a TTE. Although this may have led to an underestimation of the ICD indication incidence it is highly likely that patients who did not have a TTE were less likely to have had an ICD indication. For example, in some instances the primary reason for admission will not have been an MI and these patients may be less likely to have an inpatient TTE. Patients with a clinically small infarct and no evidence of heart failure may also be less likely to receive an echo. However even if it is assumed that the group of patients for whom full data were unavailable were identical in terms of spread of clinical features to those for whom data were complete, the adjusted rate would be approximately 39-52/million/year and as such unlikely to dramatically alter my conclusions.

At the time of the study there was no screening program for post-MI primary prevention ICD implantation at Portsmouth Hospitals NHS Trust, and most TTEs were performed in the week after admission. Following MI early LV remodelling may occur and according to NICE

guidance assessment of ICD need should be made with evaluation of LVEF at least 4 weeks post-MI. As such this study truly estimates the number of patients requiring further assessment for contemplation of ICD implantation as opposed to an absolute indication for device therapy. Both beneficial and adverse LV remodelling can occur and I consider that my estimate of ICD requirement represents a realistic approximation. Furthermore, I believe it provides valuable insights into the services that need to be introduced to make a timely assessment of such patients.

TTE, though the most widely used tool to assess LVEF, suffers from intra- and inter-observer variability and some patients have limited echo windows. However TTE remains the investigation of choice when assessing cardiac structure and function in clinical practice and was the method used in the majority of ICD trials (58, 59, 200). In Portsmouth Hospitals NHS Trust's Cardiac department, LV systolic function is described qualitatively, and in converting this into an approximate ejection fraction I have had to make some assumptions. However, this study reflects 'real world' practice in a busy DGH where the majority of acute infarcts will present. It is likely that by taking this approach I have identified the vast majority of cases that would require further detailed assessment of LVEF in early follow-up.

In common with other National and International guidelines, current NICE guidance relies heavily on LVEF as the primary determinant for ICD need (36). The use of qualitative assessments of LV function, as in this study, and the inherent problems in converting these into an LVEF, highlight the potential difficulties of the assessment of LVEF. Accurate and reproducible estimation of LVEF is an essential pre-requisite for any potential ICD screening program, and it may be that the more widespread use of other imaging modalities needs to be considered.

3.5. Conclusions

This study highlights the current significant under-provision of ICD therapy for the primary prevention of SCD post-MI in the UK. The latest published UK ICD data give a new implantation rate of ~40/million/year. Combining my results with published data for NICE secondary prevention indications, gives a combined ICD indication incidence of approximately 105-115/million/year. In order to improve the uptake of primary prevention device therapy the implementation of clinical pathways that enable the systematic identification and early assessment of potential candidates are needed. Implantation rates for primary prevention indications are unlikely to significantly improve until there is widespread introduction of such programs.

4. CLINICAL SETTING

4.1. Introduction

Despite national guidance surveys have consistently found considerable regional variation in ICD utilisation in England and Wales (72, 73). The most recent survey, in 2006, demonstrated a nearly three-fold difference in new ICD implantation rates between the lowest (26/million) and highest (73/million) implanting regions (63). Although the appropriate rate of ICD use in the UK is not known, my research, the results of which are detailed in Chapter 3, as well as studies by other investigators, have estimated that the implantation rate based on published guidelines should be between 105-504/million/year (69, 70). It is therefore likely that these differences reflect a significant underuse in regions with low implant rates. In order to improve ICD uptake in the UK it is important to understand the reasons underlying this regional variation.

The aim of this study was to assess the effect of clinical setting (regional centre vs. DGH) on ICD prescription rates in a UK setting. The study tested the hypothesis that the prescription of ICD therapy is influenced by clinical setting, and is significantly higher in areas served by a regional cardiothoracic centre than a DGH.

4.2. Methods

The Wessex Cardiothoracic Unit is the regional ICD implanting centre for the Central South Coast Cardiac Network. It serves 8 DGHs, covering a population of approximately 2.8 million people. All new ICD implants performed at the Wessex Cardiothoracic Unit over a 4 year period (2005 –2008) were retrospectively audited. To prevent underestimating implant rates only patients from hospitals for which the Wessex Cardiac Unit was the sole ICD implanting centre for the duration of the study period were included. Patients from DGHs with an implanting service, or that were known to refer patients to another implanting centre, were excluded. The lead Consultant Cardiologist or Cardiologist with an interest in devices from the remaining DGHs was contacted to determine their ICD referral pathways, and again hospitals that referred patients to another centre were excluded. From the original 8 DGHs, 5 were excluded – 2 that had an implanting service and 3 that referred patients to another implanting centre. This left patients from 4 different hospitals in the analysis – 1 regional centre and 3 DGHs.

Patients' hospital notes were reviewed and the referring hospital determined. For patients admitted to hospital as an emergency their initial admission hospital was taken as the referring hospital. For patients admitted electively the hospital where they were initially seen by a cardiologist was taken as the referring hospital. Patients from out of region (e.g. visiting or on

holiday) were excluded from the analysis. To assess for evidence of referral bias, resulting from patients being referred straight from primary care to the Regional centre and bypassing their local DGH, the postal addresses of all patients in the Regional Centre group were checked to determine whether they were within the regional centre catchment area.

Patients were categorised into 3 different groups depending on their referring hospital type – regional centre (1 hospital), DGH with a device specialist but without an implanting service (1 hospital), DGH without a device specialist and no implanting service (2 hospitals). For patients referred from each hospital type, the overall yearly ICD implant rates based on the local population were assessed. Patients with NICM are specifically excluded from the latest NICE guidance on primary prevention ICD use. Therefore to assess the relationship between hospital type and primary prevention ICD use, implant rates for the primary and secondary prevention of CAD were calculated. Figures for local hospital populations were taken from data published by each hospital. For the group composed of 2 different DGHs an overall implant rate was calculated based on the combined population. Both ICDs and CRT-Ds were included. For the purposes of the study, patients who presented with syncope whose subsequent investigation led to device implantation were classified as secondary prevention. Group comparisons of categorical data were evaluated using Yates' x^2 test.

4.3. Results

During the study period there were 459 new ICD implants. After exclusion of patients from the five DGHs implanting devices or referring patients to other centres, 381 patients from 4 different hospitals were included in the analysis. Patient characteristics are shown in Table 4-1. The majority of patients were male (80%) and had CAD (70%). Most patients received a dual chamber ICD (48%) or a CRT-D device (31%). The use of EPS decreased during the study period from 61% of implants in 2005, to 28% in 2006, 18% in 2007, and 25% in 2008. Overall only 29% of patients who had a CAD primary prevention ICD implanted had EPS to guide device use.

The overall ICD implant rate was 68/million/year, which remained static over the 4 year study period (Table 4-2). The overall implant rate for CAD was 47/million/year, which again was relatively constant. At the start of the study period in 2005 only a minority of the CAD implants were for primary prevention indications (29%). However the proportion of prophylactic devices implanted increased such that in 2008 nearly half of devices (45%) were implanted for this indication.

Overall implant rates were significantly higher in areas where the local hospital was a regional cardiothoracic centre (103/million/year), compared to DGH with a device specialist (49/million/year) and DGH without a device specialist (48/million/year). These findings were consistent throughout the study period (Table 4-2). The disparity in implant rates between different hospital types was greatest with respect to CAD primary prevention indications – regional centre (29/million/year), DGH with a device specialist (14/million/year), and DGHs without a device specialist (9/million/year). The proportion of devices implanted for CAD primary compared to secondary prevention indications was significantly higher in patients from an area served by a regional centre than a DGH without a device specialist ($p=0.04$). During the study period the CAD primary prevention implant rate for patients served by a regional centre did not greatly alter (range 26-34/million/year). However, the primary prevention implant rate progressively increased in the areas served by a DGH with (7 to 27/million/year) or without (6 to 14/million/year) a device specialist.

Of the 205 patients in the regional centre group, 8 had been referred from primary care directly to the regional centre, bypassing their local DGH. Of these 8, 4 were from the catchment areas of local DGHs that had been excluded from the analysis, 2 were from the catchment area of the DGH with a device specialist and 2 from the DGHs without a device specialist.

Table 4-1 Characteristics Of 381 Patients Included In The Analysis

	Regional Cardiothoracic Centre (n=205)	DGH with device specialist but not implanting (n=109)	DGH without device specialist or implanting (n=67)	Overall (n=381)
Age (years)	65	65	65	65
Male Sex % (no.)	80 (165)	76 (83)	82 (55)	80 (303)
Type of heart disease % (no.)				
CAD	65 (134)	74 (81)	75 (50)	70 (265)
NICM	18 (37)	12 (13)	13 (9)	15 (59)
Other	17 (34)	14 (15)	12 (8)	15 (57)
Admission type % (no.)				
Elective	52 (106)	50 (54)	36 (24)	48 (184)
Emergency	48 (99)	50 (55)	64 (43)	52 (197)
Device Type % (no.)				
ICD single chamber	18 (36)	25 (27)	25 (17)	21 (80)
ICD dual chamber	46 (95)	50 (54)	48 (32)	48 (181)
CRT-D	36 (74)	26 (28)	27 (18)	31 (120)
CAD ICD indication % (no.)				
Primary prevention	43 (58)	33 (30)	26 (13)	38 (101)
Secondary prevention	57 (76)	63 (51)	74 (37)	62 (164)

Table 4-2 ICD Yearly Implant Rates By Hospital Type

	ICD implant rate (per million population)				
	2005	2006	2007	2008	Overall
All hospitals					
Total	74	70	62	65	68
CAD Primary prevention	16	17	16	23	18
CAD Secondary prevention	39	27	23	28	29
Regional Cardiothoracic Centre					
Total	106	130	96	78	103
CAD Primary prevention	34	30	26	26	29
CAD Secondary prevention	44	42	30	36	38
DGH with device specialist but not implanting					
Total	48	41	43	62	49
CAD Primary prevention	7	13	9	27	14
CAD Secondary prevention	32	18	18	23	23
DGH without device specialist and not implanting					
Total	69	32	43	49	48
CAD Primary prevention	6	6	11	14	9
CAD Secondary prevention	43	20	23	23	27

4.4. Discussion

These results show a significant variation in ICD implantation rates between areas served by different hospital types. There was approximately a two-fold difference in implant rates between areas whose local hospital is a regional cardiothoracic centre and those whose local hospital is a DGH. The difference in implant rates was most significant with respect to CAD primary prevention indications, where overall implant rates were 2-3 times higher in an area served by a regional centre. Although the appropriate rate of ICD use in the UK is not known, studies have estimated the likely implantation rate based on published guidelines to be between 105-504/million/year. It is therefore likely that these differences reflect an underuse in DGHs rather than an overuse in the regional centre, and in fact may actually represent an underuse in regional centres as well, though not as great as in DGHs. This is supported by the significantly higher implant rates in other European countries and North America (63).

The findings are consistent with the most recently published UK Pacemaker and ICD National Survey covering 2004-2006 (63). Over the 3-year survey period the Southampton City Primary Care Trust (PCT), whose local hospital is a regional cardiothoracic centre, had the highest regional annual ICD implantation rate with an average 66/million. In comparison two PCTs served by local DGHs without device specialists, had average yearly implant rates of 32-34/million. Another PCT, served by a DGH with a device specialist, had an average annual implant rate of just over 50/million. In the national survey CRT devices, which included both CRT pacemaker and CRT-D devices, were counted separately and data concerning the proportion of CRT devices that had defibrillation capacity were not given. There is likely to be significant variation in practice with regard to the use of resynchronisation therapy in patients undergoing ICD implantation (201). Furthermore PCTs do not directly correspond to hospital catchment areas. For these reasons it is difficult to directly compare my data with those from the survey, or make meaningful comment on the pattern of device use in areas served by other regional centres, without further information concerning local practice.

Although most patients in England and Wales are seen in their local hospital, General Practitioners can choose to refer patients to other secondary care providers, which could potentially affect the results. There was some evidence of referral bias, with respect to patients being referred from primary care directly to the regional centre, bypassing their local DGH. In most cases the reason for this was that patients had been seen at the regional centre previously for a cardiac problem. The number of patients involved (8 patients) was small and therefore would not affect the nature of my conclusions.

The effect of hospital type and size on ICD implant rates has been assessed in other healthcare systems. Birnie *et al*. evaluated the use of ICDs in 82,331 Canadian and US survivors of out-of-hospital cardiac arrest (202). They found that patients initially admitted to a teaching hospital were more than 3 times as likely to receive an ICD as those admitted to a non-teaching hospital, and those admitted to a larger hospital (defined as >100 beds) were 2.9 times more likely to receive a device than those admitted to a smaller one. Shah *et al*. analysed the use of ICDs in 10,148 heart failure patients discharged from hospital who met criteria for prophylactic device therapy as part of the GWTG-HF (Get With The Guidelines–Heart Failure) US registry (66). They found wide (35-fold) variation in ICD use in eligible patients between hospitals. They also found that use of ICD therapy was higher in hospitals that were larger, had an academic affiliation, and provided cardiac procedures (e.g. percutaneous coronary intervention and coronary artery by-pass grafting).

The reasons underlying my findings are likely to be multifactorial. The diffusion of new evidence-based innovations in healthcare is often slow, and the translation of knowledge into practice and penetration of guidelines are likely to be important factors in explaining the lower ICD use in DGHs (203). Countries with a greater number of device specialists, such as the USA and Germany, tend to implant a larger number of devices than those with fewer, such as the UK (60, 61, 63). Therefore the availability of device specialists and implanting centres, or the perceived length of wait to see a device specialist, may be important issues, especially in smaller DGHs where patients admitted with heart failure or a myocardial infarction are less likely to be managed by a cardiologist. ICDs are an expensive technology and are associated with important morbidities, such as inappropriate device therapy and infection. Both these factors may concern referring physicians. The initial NICE guidance, published in 2000, required patients to be inducible at EPS to meet criteria for a prophylactic ICD. It has been suggested that EPS, only available in larger cardiac departments, was perceived as a block to patients receiving a prophylactic device (27). Although in the updated NICE guidance, published in 2006, EPS is not as central in determining device eligibility, this may still be an important issue.

4.4.1. Limitations

A small number of patients from the hospitals included in the analysis may have been referred to other implanting centres. However the number of patients concerned is likely to be small and as such unlikely to significantly influence my conclusions. This is supported by the results of the National ICD Survey, which suggests that at least for 2005-6, implant rates for areas served by DGHs in the Central South Coast Cardiac network are significantly lower than those served by the regional centre (63). As a result of problems with capacity, for a 3 month period at the

start of 2007 the Wessex Cardiothoracic Unit was closed to new referrals for device implantation. Prospective device patients will therefore have been referred to other implanting centres. Although again this may have led to an underestimation of the DGH implantation rates, the numbers are likely to be small and discounting the data for 2007 makes little difference to the results. Lastly, implant rates were not corrected for local demographics that may influence the incidence of device indications. However, this is unlikely to have made a significant difference to implant rates and therefore my conclusions.

4.5. Conclusions

These results suggest that ICD implant rates are affected by clinical setting and are significantly higher in areas served by a regional cardiothoracic centre compared to a DGH. In order to increase UK ICD implant rates both an increase in device specialists and the widespread implementation of clinical pathways to identify prospective primary prevention patients may be needed.

5. BRAIN NATRIURETIC PEPTIDE

5.1. Introduction

BNP is released by the heart in response to myocardial stretch and increased intravascular volume (125). BNP is a powerful predictor of mortality, independent of LVEF, in patients with chronic heart failure, asymptomatic LVSD, acute coronary syndromes and stable coronary disease (125). The role of BNP in predicting SCD and ventricular arrhythmias (VA) has also been investigated in a number of studies. However, many of these have been limited by small sample size, and the predictive accuracy of BNP is unclear.

The aim of this study was to use meta-analytical techniques to evaluate the accuracy of BNP/NT-proBNP to predict SCD and the occurrence of VA in published studies. The study tested the hypothesis that BNP/NT-proBNP predict the occurrence of SCD and appropriate ICD therapy with comparable accuracy to currently available risk stratification tests.

5.2. Methods

5.2.1. Search Strategies

MEDLINE, EMBASE, and CINAHL (January 1984–October 2008) were searched to find primary references and reviews together with published bibliographies and the Cochrane library. Studies were included that evaluated the relationship of BNP or NT-proBNP to SCD or VA. The following medical subject heading search terms were used: brain natriuretic peptide, natriuretic peptides, cardiac arrhythmias, sudden death, sudden cardiac death, ventricular tachycardia, ventricular fibrillation, predictive value of tests and implantable defibrillators. The search was restricted to English language literature and human subjects. The date limits were chosen because natriuretic peptides were not in research use prior to 1984. Studies where the results were reported so that a 2x2 table of results could not be constructed and those involving overlapping or duplicate cohorts of patients were excluded.

5.2.2. Types Of Study

Two categories of peer-reviewed papers were identified:

(i) Studies examining the accuracy of BNP/NT-proBNP in predicting SCD in patients without ICDs.

(ii) Studies examining the accuracy of BNP/NT-proBNP in predicting the occurrence of VA in patients with ICDs.

5.2.3. Data Extraction

Studies were assessed for eligibility and data extracted by two independent investigators (myself and Dr James Barry). When there were differences between observers they reviewed the papers together to reach joint conclusions. Each reviewer extracted the data to construct a 2x2 table for each study.

5.2.4. Statistics

Results were analysed using Review Manager 4.2.8 (Cochrane Collaboration, Oxford, United Kingdom) and Meta-Disc 1.4 analysis software (192). Summary estimates of the univariate relative risk (RR) and likelihood ratios were calculated using the random effects model based on DerSimonian and Laird's meta-analytic statistical method (204). This gives more equal weighting to studies of different precision in comparison to a simple inverse variance weighted approach, so accommodating between study heterogeneity (205). In the case of papers that gave multiple cut-off points for BNP analysis, the score that gave the maximum overall accuracy was chosen. In the case of analyses with empty cells 0.5 was added to all cells to avoid computational errors.

As studies used different cut-off points for defining raised BNP Spearman's correlation coefficient between sensitivity and specificity was calculated (206). This looks for the presence of a threshold effect, where variations in sensitivity and specificity are related to differences in the cut-off point used to define an abnormal result. If there is no evidence of a threshold effect then likelihood ratios can be pooled.

Subgroup analyses were performed based on patient populations. In the analysis of studies examining BNP to predict SCD in patients without ICDs, subgroups of studies that only enrolled patients with known structural heart disease or symptomatic heart failure, and those that only included patients with LVEF <40%, were evaluated. In the analysis of studies examining BNP to predict appropriate ICD therapy the subgroup of studies that only included patients with CAD was examined.

Meta-regression analysis was performed using the linear weighted inverse variance method to evaluate the relationship between mean LVEF in each study and the log odds ratio of BNP as a predictor of SCD or appropriate ICD therapy (207).

For all meta-analyses Cochran's χ^2 test was performed to assess between study heterogeneity and the I^2 statistic was quantified (208). In all analyses a P value less than 0.05 was considered

as significant. Publication bias was assessed using a funnel plot and the correlation coefficient, Kendall's tau, comparing sample size to RR (209).

5.3. Results

From the initial 1006 citations generated by the search strategy, 20 studies met the inclusion criteria. Six of these studies were excluded - 4 because 2x2 tables could not be constructed (210-213), one because there were no episodes of SCD during follow-up (214), and one was a duplicate cohort (215). This left 14 studies for analysis - 6 examining the accuracy of BNP in predicting SCD in patients without ICDs (Table 5-1) and 8 examining the role of BNP in predicting the occurrence of VA in patients with ICDs (Table 5-2). These two types of study were analysed separately.

5.3.1. BNP to Predict SCD in Patients without ICDs

The six studies included 3543 patients (138-142, 144). The mean age of the participants ranged from 40 to 66 years. The mean percentage of men ranged from 54 to 87. The mean LVEF ranged from 20 to 46%, while one study did not report LVEF (142). There was a wide variation of subject populations, including patients with congestive heart failure, patients with reduced LVEF, post-MI patients, and type 2 diabetics on haemodialysis. Four studies measured NT-proBNP and two BNP. All studies expressed BNP as a dichotomous variable using either the median (1 study) or a "best cut-off" value (5 studies).

During a mean follow-up of 19 months to 4 years there were 310 cases of SCD. SCD was more frequent in patients with raised BNP (RR 3.68; 95% CI 1.90, 7.14) (Table 5-3 and Figure 5-1). There was statistical heterogeneity between studies (P=0.003). Spearman's correlation between sensitivity and specificity was 0.257 (p=0.623) suggesting no evidence of a threshold effect. Therefore pooled positive and negative likelihood ratios were calculated (Table 5-3 and Figure 5-2). The summary positive likelihood ratio (PLR) was 1.70 (95% CI 1.44 to 2.01) and negative likelihood ratio (NLR) 0.45 (95% CI 0.28 to 0.73). There was statistical heterogeneity for the PLR (P=0.0004) and NLR (P=0.0049). In the subgroup of five studies that included only patients with known heart disease (n=2104) the relative risk of SCD in patients with a raised BNP was 4.59 (95% CI 2.41, 8.74) (Table 5-3) (138-141, 144). In the subgroup of two studies that included only patients with LVEF <40% (n=530) the RR was 20.13 (95% CI 3.96 to 102.37) (138, 140).

A sensitivity analysis suggested the heterogeneity was accounted for by two studies that included patient populations at the extremes of heart disease severity (138, 142). In the study by

Berger *et al*. the mean LVEF was 20% (138). In the study by Winkler *et al*. evidence of heart disease was not an inclusion criteria, and although LVEF was not reported it is likely to have been significantly higher than the other studies (142). Excluding these studies, the pooled RR was not greatly altered (3.72, 95% CI 2.31, 6.0) with no heterogeneity (P=0.34, I^2 = 11%). Although the risk of SCD associated with a raised BNP tended to be higher in patients with a lower LVEF, meta-regression analysis showed no statistically significant influence of LVEF on the diagnostic odds ratio of BNP as a predictor of SCD (p=0.96). Though as only 5 studies gave detailed information concerning LVEF the power of this analysis is limited. There was no significant evidence of publication bias (Kendall's tau = 0. 47, P = 0.27).

Table 5-1 Studies Evaluating BNP To Predict SCD In Patients Without ICDs

Study	Year	Cases	Patients	Heart disease	Age	Male (%)	LVEF (%)	Beta-blocker use	ACE-I use	Hormone	Cut-off (median or best)	End-point	Months follow-up	No at end-point
Bayes-Genis (135)	2007	494	Symptomatic heart failure	Any	63	78	31	67	76	NT-proBNP	>908 ng/L (best cut-off)	Witnessed death within 1 hour, unwitnessed death without worsening heart failure or death during attempted resuscitation	31	50
Berger (134)	2002	452	LVEF <35%	CAD/ NICM	54	87	20	30	89	NT-proBNP	>130 pg/mL (best cut-off)	Witnessed death within 1 hour of symptoms or unexpected, unwitnessed death in a patient known to have been well or	19	44
Tapanainen (140)	2004	521	Post-MI	CAD	61	77	46	97	44	BNP	>23.0 pmol/l (best cut-off)	Witnessed death within 1 hour of symptoms, unwitnessed death without worsening heart failure or death during attempted resuscitation	43	16
Tigen (136)	2007	78	LVEF <40% and sinus rhythm	NICM	40	69	25	81	98	NT-proBNP	>4500 pg/ml (best cut-off)	Abrupt loss of consciousness within 1 hour of the symptoms onset	22	4
Watanabe (137)	2006	680	Patients with clinics findings of heart failure	Any	66	69	42	39	71	BNP	>200 pg/ml (best cut-off)	Sudden, unexpected death without worsening heart failure	26	36
Winkler (138)	2008	1255	Type 2 diabetics on haemodialysis	NR	66	54	NR	NR	NR	NT-proBNP	>3361pg/ml (median)	Not stated	48	160

NR, not reported.

Table 5-2 Studies Evaluating BNP To Predict Ventricular Arrhythmias In Patients With ICDs

Study	Year	Cases	Patients	Heart disease	Age	Male (%)	LVEF (%)	Beta-blocker use	ACE-I use	Hormone	Cut-off (median or best)	End-point	Months follow-up	No at end-point
Blangy (143)	2006	121	Prior MI, sustained VT	CAD	68	90	36	75	83	BNP	>64 ng/L (median)	Occurrence of VT	12	38
Budeus (144)	2008	93	LVEF <30%	CAD	68	89	25	81	94	BNP	>265 pg/ml (best cut-off)	Appropriate ICD therapy for VT or VF	33	43
Klein (145)	2006	250	Meeting criteria for ICD implantation	Any	63	77	40	70	72	NT-proBNP	>405 ng/L (median)	Appropriate ICD therapy for VT or VF	18	46
Klingenberg (146)	2006	50	LVEF <30%	CAD	59	84	20	80	88	NT-proBNP	>2536 pg/ml (best cut-off)	Appropriate ICD therapy for VT or VF	12	16
Manios (147)	2005	35	Prior MI, LVEF <35% and primary prevention ICD	CAD	64	97	28	77	88	NT-proBNP	880 pmol/L (best cut-off)	Appropriate ICD therapy for VT or VF	12	11
Nagahar (148)	2008	54	Meeting criteria for ICD implantation	Any	53	74	48	56	46	BNP	187 pg/mL (best cut-off)	Appropriate ICD shock or SCD	15	21
Verma (151)	2006	345	Meeting criteria for ICD implantation	Any	63	84	29	71	64	BNP	>283 ng/l (median)	Appropriate ICD therapy for VT or VF	13	63
Yu (149)	2007	99	Prior MI	CAD	68	86	36	74	80	NT-proBNP	>497 ng/L (median)	Appropriate ICD therapy for VT or VF	18	23

Table 5-3 Summary Estimates Of Likelihood Ratios And Relative Risks In 14 Studies Of BNP To Predict SCD Or VA

Summary estimates	Relative Risk (95% CI)	Positive Likelihood Ratio (95% CI)	Negative Likelihood Ratio (95% CI)	No of studies
Studies to predict SCD				
All	3.68 (1.90, 7.14)	1.70 (1.44, 2.01)	0.45 (0.28, 0.73)	6
Subgroups:				
Known heart disease	4.59 (2.41, 8.74)	1.81 (1.53, 2.14)	0.36 (0.19, 0.67)	5
LVEF ≤40%	20.13 (3.96, 102.37)	2.13 (1.1, 4.3)	0.082 (0.02, 0.39)	2
Studies to predict appropriate ICD therapy				
All	2.54 (1.87, 3.44)	1.92 (1.46, 2.52)	0.54 (0.45, 0.65)	8
Subgroups:				
CAD	2.76 (1.57, 4.84)	2.26 (1.41, 3.61)	0.50 (0.34, 0.73)	5

Figure 5-1 Summary Of The Relative Risk Of SCD In Patients With An Elevated BNP

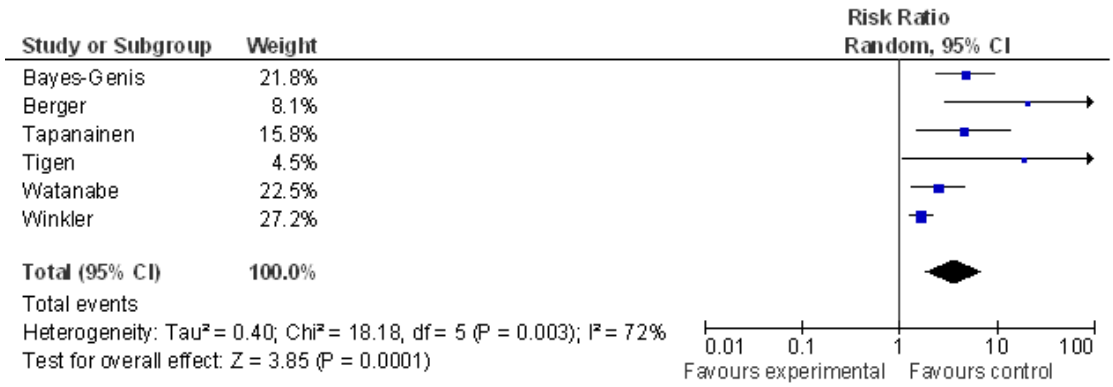
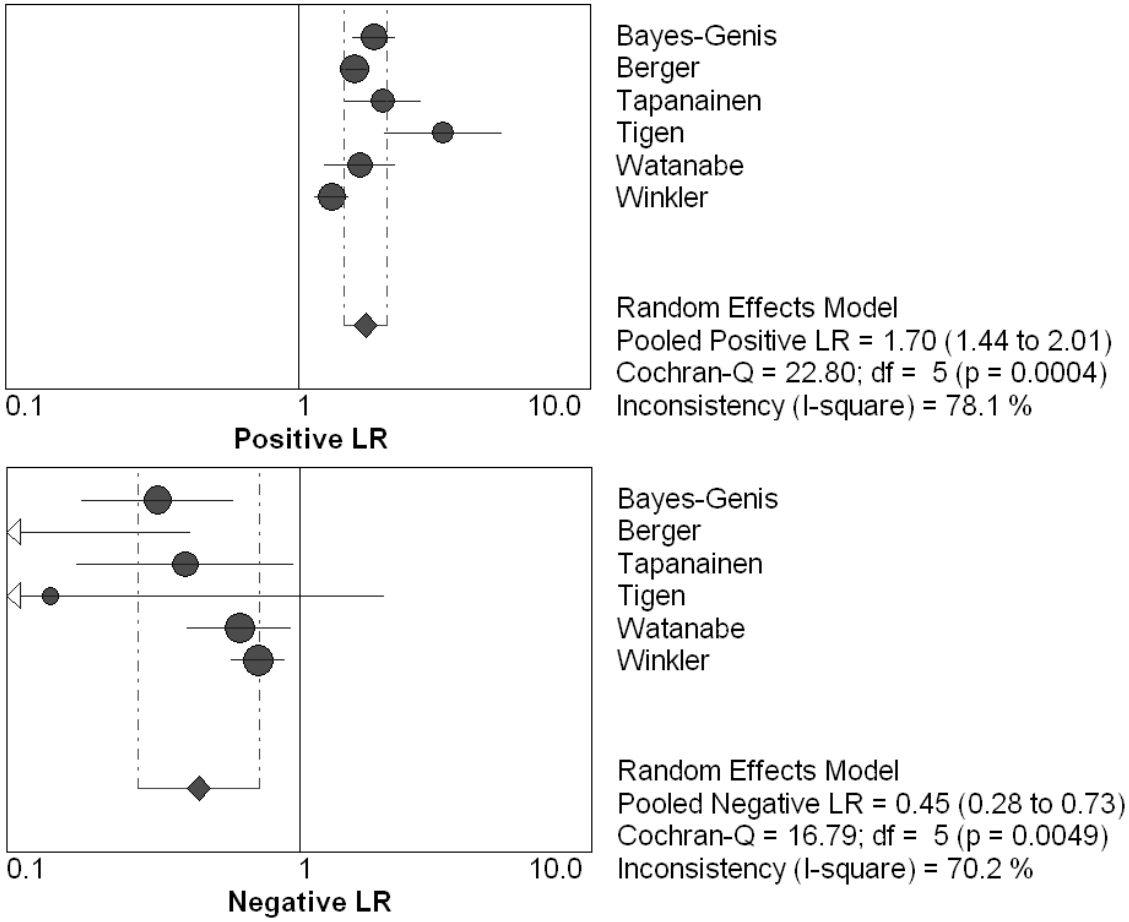


Figure 5-2 Summary Of Likelihood Ratios Of An Elevated BNP To Predict SCD



5.3.2. BNP to Predict VA in Patients with ICDs

The eight studies enrolled 1047 patients (147-153, 155). The mean age of study participants ranged from 53 to 68 years, and percentage of patients that were male ranged from 77 to 97. There was some heterogeneity between studies with respect to design. Five studies only enrolled patients with CAD, while three included patients with other types of heart disease. Four studies measured BNP and four NT-proBNP. All studies expressed BNP as a dichotomous variable using either the median (4 studies) or a "best cut-off" value (4 studies). The majority of the studies (6) used the occurrence of appropriate ICD therapy for VT or VF as the primary end-point, while one study used the occurrence of VT, and another SCD or appropriate ICD shock therapy.

During a mean follow-up of 12 to 33 months 261 met the primary end-point. The primary end point was more common in patients with a high BNP (RR 2.54; 95% CI 1.87, 3.44) without heterogeneity (Table 5-3 and Figure 5-3). Spearman's correlation between sensitivity and specificity was 0.167 (p=0.693) suggesting no evidence of a threshold effect, and therefore pooled likelihood ratios were calculated (Table 5-3 and Figure 5-4). The PLR was 1.92 (95% CI 1.46, 2.52) with significant heterogeneity (P=0.0019). The NLR was 0.54 (95% CI 0.45, 0.65) with no significant heterogeneity. In the subgroup of 5 studies that included only patients with CAD (n=398) the RR was 2.76 (95% CI 1.57, 4.84) (Table 5-3) (147, 148, 150, 151, 153).

A sensitivity analysis showed that the heterogeneity was sensitive to one small study by Klingenberg *et al*. that included only 50 patients (150). Removing this study did not significantly alter the result, with a pooled RR 2.30 (95% CI 1.83, 2.90) and no heterogeneity (P=0.38, I^2=7%). Meta-regression analysis showed no statistically significant influence of LVEF on the diagnostic odds ratio of BNP as a predictor of ICD therapy (p=0.14). The funnel plot was asymmetrical, suggesting some evidence of publication bias, with smaller studies showing a more positive result (Kendall's tau = 0.71, P = 0.014).

Figure 5-3 Summary Of The Relative Risk Of An Elevated BNP To Predict The Occurrence Of VA In Patients With ICDs

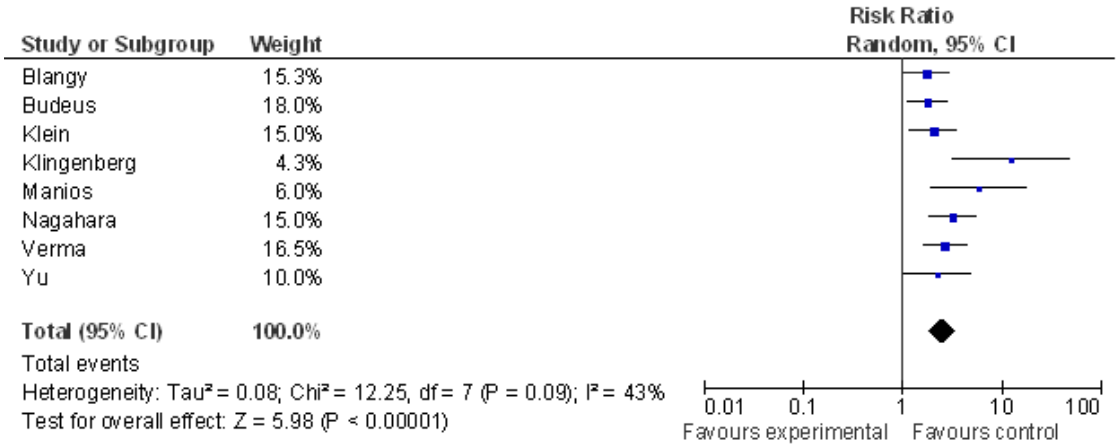
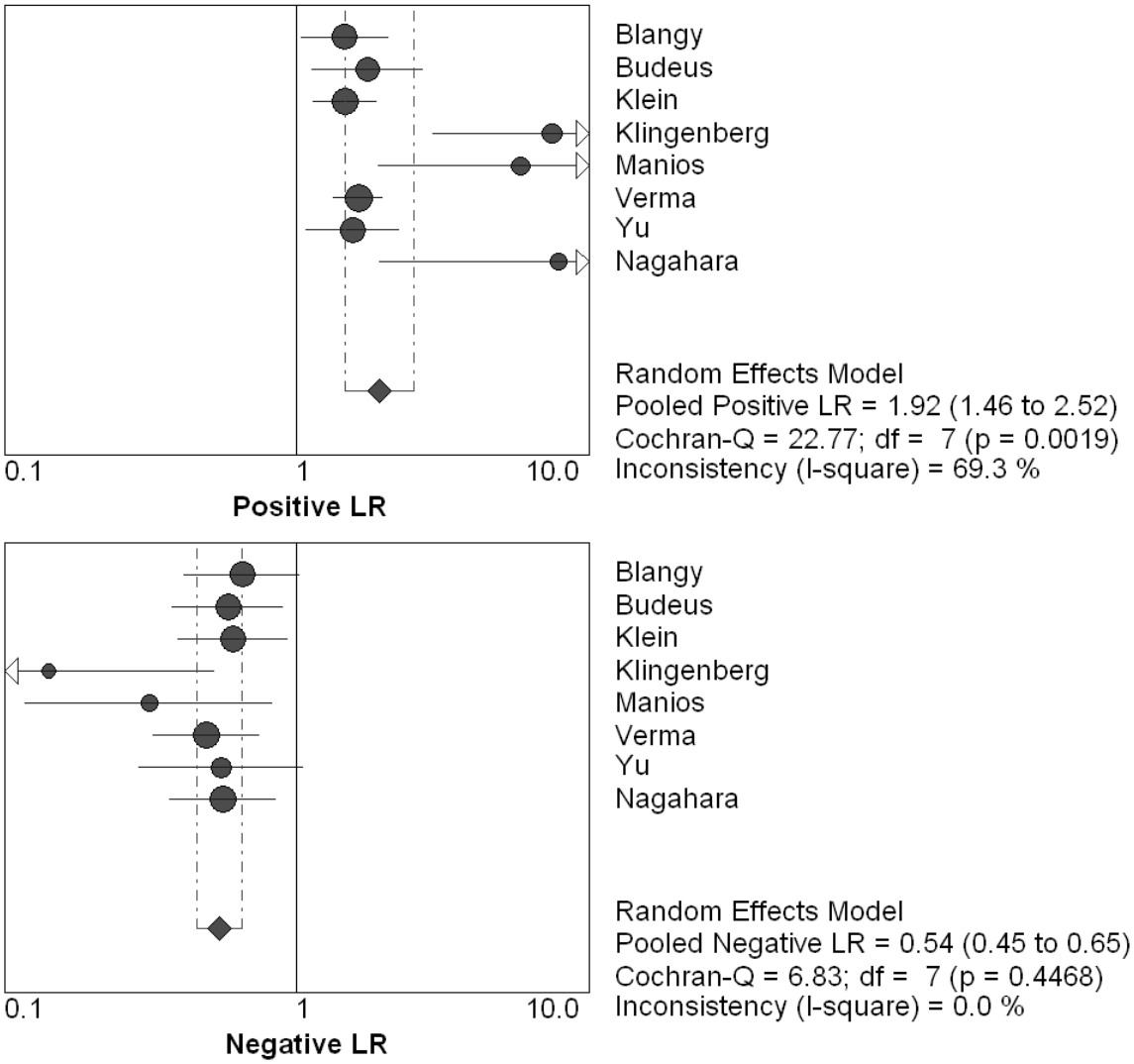


Figure 5-4 Summary Likelihood Ratios Of An Elevated BNP To Predict The Occurrence Of VA In Patients With ICDs



5.3.3. Comparison of BNP With Other Prognostic Markers

Ten out of fourteen studies performed multivariable analyses including BNP or NT-proBNP to predict SCD or the occurrence of VA. Four of the six studies with SCD as an end-point performed multivariable analysis, all included LVEF and 2 NYHA class. In all 4 analyses BNP was an independent predictor of SCD, and in 3 it had either the highest risk ratio or was the only significant predictor. Six out of the eight studies with VA as an end-point performed multivariable analysis. All included LVEF and 4 NYHA class. BNP was predictive in 4 of these, being either the only significant predictor or with the highest risk ratio. One study compared the accuracy of NT-proBNP to EPS in predicting the occurrence of VA in 99 patients with CAD (153). While NT-proBNP was predictive in univariate and multivariable analysis EPS was not.

5.4. Discussion

I have performed a meta-analysis of BNP to predict malignant arrhythmias, including data on over 4500 patients. I have found that BNP predicted both the occurrence of SCD in patients without ICDs and the occurrence of VA in patients with devices. In patients without ICDs a raised BNP predicted nearly a four-fold increase in the risk of SCD compared to patients with a lower BNP. In the subgroup of patients with known heart disease there was over a four and a half-fold increase in risk. In patients with ICDs a raised BNP predicted over a two-fold increase in the risk of VA occurrence.

These results are consistent with findings from previous clinical studies. In a recent systematic review, Doust *et al*. examined the accuracy of BNP to predict overall mortality in patients with heart failure or asymptomatic left ventricular dysfunction (128). They identified 19 prospective studies of patients with heart failure and 5 of patients with asymptomatic left ventricular dysfunction. The studies varied significantly in terms of design, with some studies assessing BNP as a continuous and others as a dichotomised measure, and primary outcome, with some studies using overall mortality and others cardiac events (variously defined). For this reason pooled estimates of effect were only given for some of the analyses, where studies could be appropriately combined. In heart failure patients, each 100 pg/ml increase in BNP was associated with a 35% increase in the relative risk of death. BNP also predicted overall mortality in the 5 studies of asymptomatic patients, though a pooled estimate was not calculated. Although the studies included in this review did not give detailed information about the mode of death (sudden vs. non-sudden), as SCD accounts for approximately half of morality in patients with heart failure, my results are in keeping with these findings (17).

My results are also consistent with findings from laboratory studies. BNP is predominantly released from the ventricles in response to myocyte stretch (125). Myocardial stretch results in several pro-arrhythmic electrophysiological changes that may lead to VA and SCD, including slowed intraventricular conduction, and the occurrence of ventricular ectopic beats and triggered after-depolarisations (216, 217).

The studies included in my analysis used different cut-off points to define high-risk, which varied significantly between studies. In the analysis with SCD as the end-point, the 'best-fit' cut-off point was as low as >130 pg/ml in one study and as high as >4500 pg/ml in another, though this latter study enrolled patients with chronic renal failure, a condition known to raise BNP (138, 140). This variation in cut-off point is at least in part related to the significant heterogeneity in patient population between the different studies, especially in the studies that used a median value as the cut-off. From the currently available data it is not possible to draw any firm conclusions regarding the most appropriate cut-off point for clinical SCD risk stratification. In addition, different cut-off values may be chosen to optimise either sensitivity or specificity, depending on the desired use of the test and the population in which it is being used – either to identify high risk patients for consideration of an ICD or to exclude lower risk patients from the need for further testing. Furthermore, BNP measurement could be used as a continuous variable in a risk prediction model combined with other risk stratification tests (109).

A consistent problem with many risk stratification tests designed to guide ICD use is a lack of specificity for SCD. Many, such as reduced LVEF, prolonged QRS, and the presence of NSVT, although indicative of a raised SCD risk are also strong predictors of non-SCD mortality (81). In the only study in my analysis to give full results concerning the ability of BNP to predict sudden and non-sudden cardiac death, it was a significant predictor of only SCD (144). However, given its proven ability to predict overall mortality in heart failure, as well as mortality in many other cardiac and non-cardiac conditions, a raised BNP is unlikely to be specific for SCD (125).

The predictive properties of other risk stratification tests, including LVEF measurement, abnormal MTWA, the presence of NSVT on ambulatory monitoring, EPS, and heart rate variability, have been evaluated in meta-analyses (90, 218, 219). The relative risk of SCD associated with a raised BNP (RR 3.68) is comparable to these tests. However, it is unclear what additional prognostic information BNP gives over and above other risk stratification tools. In 8 of the 10 studies in my analysis that performed multivariable analysis, BNP was a significant predictor of events with a lower p value than LVEF. However, with the exception of the study by Yu *et al*. that showed BNP to be superior to EPS (153), none of the multivariable models

included other tests such as EPS, MTWA, the presence of NSVT, signal-averaged ECG, or heart rate variability.

Despite being the most important SCD risk stratification test in clinical practice the accurate assessment of LVEF can be problematic. When measured by echocardiography, the most commonly used imaging modality, the accuracy of LVEF estimation is in excess of ±10% for both visual estimation and calculation by Simpson's rule (220). Furthermore measurement of LVEF does not provide an assessment of diastolic function, which also impacts on mortality. As a risk stratification tool BNP has potential benefits over LVEF measurement, as well as other currently available tests. It is simple, inexpensive, reproducible, non-invasive and widely available. These attributes make it highly suitable for serial testing to monitor VA risk over time - an approach which may have incremental benefit over a one-off measurement (142). Furthermore, rather than necessarily dichotomising risk as high or low, as many tests such as EPS or MTWA do, BNP expresses risk as a continuum and this too may be advantageous. The measurement of BNP may prove most useful in combination with other complementary risk stratification tools to improve ICD targeting, or, as some authors have suggested, to identify high-risk ICD recipients who may benefit from the use of altered device programming or prophylactic anti-arrhythmics to minimise shock therapy.

5.4.1. Limitations

My study has limitations. Delivery of ICD shock therapy is not always a surrogate for SCD (221). However, with current guidelines widening the recipient population for ICDs, the investigation of BNP as a predictor of SCD in higher risk patients is difficult as most such patients are indicated for and should already have an ICD (50). The majority of patients in the studies reviewed were men and whether the results can be generalised to women is not known. Although the use of beta-blockers and ACE-I was uniformly high in the studies enrolling patients with ICDs, their use in the studies with SCD as an end-point was variable. Indeed, in the study by Berger *et al*., only 30% of patients were taking beta-blockers (138). Also, in the studies that used SCD as an end-point the inclusion criteria were variable - one study by Winkler *et al*. (recruiting type 2 diabetics on haemodialysis) does not reflect the population that would currently be considered for an ICD (142). There was some evidence of publication bias in the analysis of patients with ICDs, with smaller studies showing a larger effect. Smaller studies are more likely to be affected by publication bias and I could have excluded these (222). However, half of the studies enrolled less than 100 patients and I wanted to be as inclusive as possible in my analysis.

5.5. Conclusions

BNP is a powerful indicator of SCD/VA risk. Its predictive power is independent of reduced LVEF. However, the risk stratification benefit of BNP measurement alone or in conjunction with other markers remains unclear and the evidence of its value does not yet mandate routine clinical use. My analysis does show that BNP measurement merits precise evaluation in studies designed to assess multiple risk stratifiers in patients at risk of SCD on the background of left ventricular myocardial damage.

6. BIOMARKERS AND ICD THERAPY

6.1. Introduction

Though ICD therapy improves survival in patients at high SCD risk, some subpopulations of implanted patients do not derive benefit (50). Specifically, in patients enrolled in RCTs who never receive device therapy, it seems that over the time horizon of the study follow-up, their SCD risk was low (96). In contrast, other patients, who have either advanced heart failure or other co-morbidities, will have high mortality despite ICD therapy, as modification of their SCD risk does not offer significant survival benefit (103, 109). Given its expense and associated morbidity, identification of patients' potential to benefit is an important component of refining the application of ICD therapy.

Biomarkers can reflect systemic vascular inflammation, myocardial stretch, neurohormonal activation and myocyte injury, and as such are powerful predictors of mortality in patients with myocardial damage, independent of the assessment of LVEF (111). Furthermore, biomarkers reflecting inflammation, myocardial stretch and collagen turnover, may also predict appropriate anti-tachycardic therapy in patients with ICDs (223-225).

The aim of this study was to assess the value of biomarkers in identifying patients' potential to gain benefit from ICD therapy. The study tested the hypothesis that individual serum/plasma biomarkers can identify patients who are unlikely to gain significant benefit from ICD therapy.

6.2. Methods

6.2.1. Study Population

Consecutive patients attending Southampton University Hospital ICD follow-up service, with LVSD and an ICD or cardiac resynchronisation defibrillator (CRT-D) were recruited. All patients were on stable optimal medical therapy. None had heart failure admissions or therapy changes in the six weeks prior to enrolment. Other exclusion criteria were pregnancy, or an acute coronary syndrome or surgery of any type within the preceding 6 weeks.

At study entry, baseline demographic data and clinical characteristics were recorded, a 12-lead resting ECG performed, and New York Heart Association (NYHA) functional class assessed. All patients had a transthoracic echocardiogram (TTE) prior to study entry. Blood was drawn from a forearm vein and collected in an EDTA tube. Samples were centrifuged at 3000 rpm for 10 minutes and plasma frozen within 1 hour of sampling at -80^{o}C pending analysis. The study complied with the Declaration of Helsinki and was approved by the local research ethics committee (Southampton and South West Hampshire Research Ethics Committee local ethics

number 08/H0502/54 and University Hospital Southampton NHS Foundation Trust Research and Development department study number CAR0357). Written informed consent was obtained from all patients.

6.2.2. Study End Points and Follow Up

Device programming was at the discretion of the treating physician. Following enrolment patients were followed up 3-6 monthly at a single centre, with a hospital visit or via a remote patient management system. Patients under remote follow-up also attended the hospital every 6 months. At each follow-up the device was interrogated. The occurrence of any ICD therapy was recorded. Appropriate ICD therapy was defined as:

(i) Antitachycardia pacing therapy (ATP) for ventricular tachycardia (VT).
(ii) Shock therapy for VT or ventricular fibrillation (VF).

Correct arrhythmia detection/discrimination was confirmed by analysis of stored electrograms by two electrophysiologists blinded to the biomarker analysis.

Three study end-points were chosen to enable exploration of the utility of biomarkers in defining patients' potential to benefit from ICD therapy. These were:

(i) All-cause mortality.
(ii) All-cause mortality or appropriate ICD therapy (reflecting event-free survival).
(iii) Survival with appropriate ICD therapy.

6.2.3. Biomarker analysis

Five plasma biomarkers that reflect a range of pathophysiological processes in LVSD were analysed:

- N-terminal pro-brain natriuretic peptide (NT-proBNP), an established marker of myocardial stretch (223).
- Growth differentiation factor-15 (GDF-15), a marker of multiple stress pathways in the heart (226).
- Serum ST2, the soluble form of ST2 (sST2), a novel marker of myocardial stretch (143).
- C-reactive protein (CRP), a marker of systemic vascular inflammation (225, 227).
- Interleukin-6 (IL-6), a marker of systemic vascular inflammation (225).

The biomarkers were chosen based on previous studies in patients with LVSD, that had demonstrated an independent association with mortality (all biomarkers) (143, 223, 225-227), sudden cardiac death (NT-proBNP and sST2) (143, 223), and the occurrence of spontaneous ventricular arrhythmias (NT-proBNP and IL-6) (223, 225).

Commercially available antibodies (R and D systems, Abindgon, Oxfordshire, UK) were used for determination of GDF-15, IL-6 and sST2 as detailed below, and NT-proBNP and CRP were assayed using in-house antibodies. The assays for NT-proBNP and CRP have been demonstrated in a previous study to detect heart failure patients in a community screening programme (228). Moreover, the in-house NT-proBNP assay shows excellent correlation with the NT-proBNP Elecsys assay (Roche diagnostics) ($n=86$, $r=0.90$, $P<0.0001$).

All assays were based on a two-site noncompetitive assay format (228). Sheep antibodies were raised to the N-terminal of human NT-proBNP, and monoclonal mouse antibodies were raised to the C-terminal. Samples or NT-proBNP standards were incubated in C-terminal IgG-coated wells with the biotinylated N-terminal antibody for 24 hours at 4°C. Detection was with methyl-acridinium ester–labelled streptavidin (MAE-streptavidin) on an MLX plate luminometer (Dynex Technologies Ltd, Worthing, UK), as described previously (226, 228). The lower limit of detection was 14.4 fmol/mL of unextracted plasma, and the within and between assay coefficients of variation were 2.3% and 4.8% respectively (229). There was no cross-reactivity of the assay with ANP, BNP or CNP (229).

The CRP monoclonal antibody used for capture of analyte was coated onto ELISA plates (100 ng per well) (228). Plates were blocked using 10% foetal calf serum (FCS). Plasma (1 µL per well) or standards were incubated in coated wells for 24 hours at 4°C. Following washes, 50 ng of a different biotinylated monoclonal CRP antibody was pipetted into wells and incubated for 2 hours at room temperature. Plates were developed with MAE-streptavidin as above.

For the GDF-15, sST2 and IL-6 assays, specific mouse monoclonal antibodies for these peptides were coated onto ELISA plates (200 ng/100 µL) (226). After incubation for 24 h, all plates were washed and blocked using 10% FCS. Plasma samples were pipetted into the wells (10, 20 and 100 µL per well for the GDF-15, sST2 and IL-6 respectively), together with appropriate standards. After another 24 hours incubation, plates were washed and biotinylated goat antibodies pipetted into the wells (5, 10 or 20 ng/100 µL for GDF-15, sST2 and IL-6 respectively). After another period of incubation of 2 hours, plates were washed and developed with MAE-streptavidin as above.

6.2.4. Statistics

Categorical variables are expressed as percentages (numbers). Normally distributed continuous variables are expressed as mean ± standard deviation and compared using the independent-

samples *t* test. Variables not normally distributed are expressed as median (lower quartile to upper quartile) and compared using the Mann-Whitney *U* test.

As NT-proBNP, sST2, GDF-15, Hs-CRP and IL-6 were not normally distributed, log transformed values were used for analysis. Univariate predictors of the three end-points were investigated in Cox proportional hazards models. For the end-points of all-cause mortality or appropriate ICD therapy, and survival with appropriate ICD therapy, multivariable analyses were also performed. Variables demonstrating a significant association ($p<0.10$) with all-cause mortality or appropriate ICD therapy (age, history of AF, Log NT-proBNP, Log, GDF-15, Log IL-6) and survival with appropriate ICD therapy (age, Log NT-proBNP) in univariate analyses were included in the multivariable Cox proportional hazards models. The multivariable models were built using a backwards stepwise approach with a significance of $p<0.05$ to remain in the model. For the end-point of all-cause mortality, in view of the small number of patients reaching the end-point (n=12), multivariable analysis was not performed. The proportional hazards assumption was checked using Schoenfeld residuals (193).

For the end-points of all-cause mortality, and all-cause mortality or appropriate ICD therapy, biomarker cut-off points were chosen to identify patients with a high risk of death (NT-proBNP and sST2) and a high chance of event-free survival (NT-proBNP). Kaplan-Meier survival analysis was used for comparison between patient groups stratified according to these biomarker cut-off points, and survival curves were compared using the log-rank test.

Statistical analyses were performed on SPSS Version 17 (SPSS Inc., Chicago, IL, USA). In all analyses $p<0.05$ was considered significant, except for inclusion in the multivariable models.

6.3. Results

6.3.1. Patient Characteristics and Clinical Outcomes

One hundred and fifty-six patients were enrolled, at a mean of 48±45 months following initial ICD implant. Demographics at study entry are shown in Table 6-1. The ICD VT treatment zone lower setting was similar in patients who did, and did not, receive appropriate ICD therapy (152±11 bpm vs. 154±24 bpm respectively; p=0.55).

Although all patients had a TTE prior to study entry the timing of TTEs with respect to study entry was variable. The majority of patients had their TTE in the 12 months prior to study entry (n=111, 71.2%), 18 patients (11.5%) had their TTE 1-2 years prior to study entry, while the remainder (n=27, 17.3%) had their TTE more than 2 years prior to study entry.

During a mean follow-up of 15±3 months from study entry 12 (8%) patients died and 47 (30%) experienced appropriate ICD therapy. Four patients who experienced appropriate ICD therapy subsequently died, leaving 43 (28%) patients who experienced appropriate ICD therapy and survived the duration of the study. The distribution of appropriate ICD therapies in these 43 patients was:

- Survival with appropriate therapy for VT (rate <182bpm) only – 17 patients
- Survival with appropriate therapy for fast VT (rate ≥182bpm) (230) – 25 patients
- Survival with appropriate therapy for VF – 1 patient

Of these 43 patients, 10 experienced 1 treated VT/VF episode, while the median number of episodes per patient was 3. Twenty-one patients experienced appropriate shock therapy, while the remainder experienced only ATP.

6.3.2. Serum Biomarkers

Baseline serum biomarker levels are shown in Table 6-2. Patients that died had significantly higher levels of NT-proBNP ($p<0.001$), sST2 ($p<0.001$), GDF-15 ($p=0.04$) and IL-6 ($p=0.04$), than patients that survived without appropriate ICD therapy (Figure 6-1). Patients that survived with appropriate ICD therapy had a significantly higher level of NT-proBNP than patients with event-free survival ($p=0.01$).

6.3.3. Predictors of All-Cause Mortality

In univariate analyses, four of the five biomarkers were significant predictors of all-cause mortality: Log sST2 (Hazard Ratio [HR] 265; 95% confidence intervals [CI] 16.47-4268; $p<0.001$), Log NT-proBNP (HR 25.30; 95% CI 3.16-202; $p=0.002$), Log IL-6 (HR 1.67; 95% CI 1.03-2.74; $p=0.04$) and Log GDF-15 (HR 3.17; 95% CI 1.03-9.76; $p=0.04$) (Table 6-3). Additional significant clinical and biochemical predictors were serum creatinine (HR per 10 µmol/l 1.12; 95% CI 1.06-1.19; $p<0.001$), haemoglobin (HR per g/dl 0.95; 95% CI 0.92-0.99; $p=0.01$) and NYHA class (HR Class III/IV vs. I 10.91; 95% CI 1.34-88.9; $p=0.03$).

6.3.4. Predictors of Event-Free Survival

Using the combined end-point of all-cause mortality or appropriate ICD therapy, predictors of event-free survival were evaluated (Table 6-4). Significant univariate predictors were Log NT-proBNP (HR 3.16; 95% CI 1.73-5.78; $p<0.001$) and advancing age (HR per 10 years 1.42; 95% CI 1.06-1.89; $p=0.02$). However, in multivariable analysis only Log NT-proBNP remained significantly predictive (HR 3.16; 95% CI 1.73-5.78; $p<0.001$).

6.3.5. Predictors of Survival with Appropriate ICD Therapy

Significant univariate predictors of survival with appropriate ICD therapy were Log NT-proBNP (HR 2.26; 95% CI 1.21-4.21; p=0.01) and advancing age (HR per 10 years 1.39; 95% CI 1.01-1.93; p=0.04) (Table 6-5). However, in multivariable analysis only Log NT-proBNP remained significantly predictive (HR 2.26; 95% CI 1.21-4.21; p=0.01).

6.3.6. Combining Biomarkers to Identify Patients Unlikely to Benefit

Using best cut-off values of sST2 and NT-proBNP (see Methods section) models that divided patients into 3 groups were developed: (i) patients with a low risk of death or appropriate ICD therapy; (ii) patients with a high risk of appropriate ICD therapy but low risk of death; (iii) patients with a high risk of death (Table 6-6).

NT-proBNP was used to identify a group of patients at low risk of death or appropriate ICD therapy. None of the 31 patients with NT-proBNP below 173 pmol/L had an event, whereas 55 of the 125 above this level did (p<0.001) (Figure 6-2). Of these 31 patients, 13 (42%) had originally received a prophylactic device, 19 (61%) had CAD, 17 (55%) had an LVEF <35% and 17 (55%) were in NYHA class II-IV.

The ability of NT-proBNP and sST2 to identify a group of patients at high risk of death were evaluated separately. For NT-proBNP, 5 of the 12 patients with a level above 2350 pmol/L died, whereas only 7 of the 145 below this level died (p<0.001) (Figure 6-2). For sST2, 7 of the 18 patients with a level above 0.43 ng/ml died, whereas only 5 of the 138 below this level died (p<0.001) (Figure 6-2).

Table 6-1 Patient Characteristics At Study Entry

	Overall (n=156)
Age (years)	71 (62-77)
Male Sex % (no.)	85 (132)
Heart Disease Type % (no.)	
Ischaemic	76 (119)
NICM	18 (28)
Other	6 (9)
Diabetes % (no.)	24 (37)
History of AF % (no.)	36 (56)
NYHA Class % (no.)	
I	35 (55)
II	42 (65)
III	22 (34)
IV	1 (2)
Device Type % (no.)	
ICD	66 (103)
CRT-D	34 (53)
ICD indication % (no.)	
Primary Prevention	37 (58)
Secondary prevention	63 (98)
QRS width (ms)	125 (100-160)
LVEF	
<30%	63 (98)
30-35%	12 (19)
35-40%	14 (22)
>40%	11 (17)
Beta-blocker % (no.)	79 (124)
ACE-I/ARB % (no.)	92 (143)
Amiodarone % (no)	28 (44)
Creatinine (µmol/l)	114 (92-141)
Haemoglobin (g/dl)	134±18

Table 6-2 Baseline Biomarker Levels In Relation To Outcome

Biomarker	Event-free survival (n=101)	Survival with appropriate ICD therapy (n=43)	All-cause mortality (n=12)	P value Death vs. Event-free survival	P Value Survival with appropriate ICD therapy vs. Event-free survival
NT-proBNP (pmol/L)	412 (135-1173)	832 (399-1167)	2025 (701-2818)	<0.001	0.01
GDF-15 (µg/L)	1.72 (0.84-3.44)	1.90 (0.87-4.56)	4.71 (1.25-9.00)	0.04	0.48
sST2 (ng/ml)	0.29 (0.22-0.37)	0.28 (0.21-0.36)	0.48 (0.30-0.54)	<0.001	0.97
CRP (mg/L)	1.58 (1.16-2.84)	1.44 (1.08-2.62)	3.41 (1.57-3.81)	0.09	0.35
IL-6 (ng/L)	0.46 (0.14-9.07)	1.35 (0.14-16.90)	10.28 (0.58-100.67)	0.04	0.32

Table 6-3 Univariate Predictors Of All-Cause Mortality

	All-cause mortality (n=12)	
	P value	Hazard Ratio (95% CI)
Age (per 10 years)	0.25	1.44 (0.77-2.69)
Diabetes	0.14	2.36 (0.75-7.46)
History of AF	0.09	2.74 (0.87-8.64)
NYHA Class (versus Class I)		
II	0.25	3.67 (0.41-32.83)
III/IV	0.03	10.91 (1.34-88.9)
QRS width (per 10ms increase)	0.16	1.12 (0.96-1.30)
LVEF (per 5% decrease)	0.25	1.28 (0.85-1.93)
Beta-blocker	0.77	1.26 (0.28-5.73)
Amiodarone	0.26	1.94 (0.62-6.13
Creatinine (per 10 µmol/l increase)	<0.001	1.12 (1.06-1.19)
Haemoglobin (per g/dl increase)	0.01	0.95 (0.92-0.99)
Log NT-proBNP	0.002	25.30 (3.16-202)
Log GDF-15	0.04	3.17 (1.03-9.76)
Log sST2	<0.001	265 (16.47-4268)
Log CRP	0.13	4.82 (0.62-37.20)
Log IL-6	0.04	1.67 (1.03-2.74)

Table 6-4 Predictors Of Event-Free Survival

	Combined Endpoint of All-cause mortality or Appropriate ICD therapy (n=55)			
	Univariate Analysis		Multivariable Analysis	
	P value	Hazard Ratio (95% CI)	P value	Hazard Ratio (95% CI)
Age (per 10 years)	0.02	1.42 (1.06-1.89)	0.34	-
Diabetes	0.75	1.10 (0.60-2.02)		
History of AF	0.08	1.62 (0.95-2.76)	0.52	-
NYHA Class (versus Class I)				
II	0.34	1.35 (0.73-2.52)		
III/IV	0.28	1.48 (0.73-3.00)		
QRS width (per 10ms increase)	0.57	1.02 (0.95-1.10)		
LVEF (per 5% decrease)	0.18	1.11 (0.95-1.30)		
Beta-blocker	0.32	1.44 (0.70-2.93)		
Amiodarone	0.70	0.89 (0.48-1.63)		
Creatinine (per 10 µmol/l increase)	0.17	1.03 (0.99-1.08)		
Haemoglobin (per g/dl increase)	1.0	1.00 (0.98-1.02)		
Log NT-proBNP	<0.001	3.16 (1.73-5.78)	<0.001	3.16 (1.73-5.78)
Log GDF-15	0.06	1.67 (0.98-2.85)	0.82	-
Log sST2	0.11	3.35 (0.76-14.83)		
Log CRP	0.54	0.80 (0.40-1.62)		
Log IL-6	0.08	1.22 (0.97-1.54)	0.22	-

Table 6-5 Predictors Of Survival With Appropriate ICD Therapy

	Survival with Appropriate ICD therapy (n=43)			
	Univariate Model		Multivariable Analysis	
	P value	Hazard Ratio (95% CI)	P value	Hazard Ratio (95% CI)
Age (per 10 years)	0.04	1.39 (1.01-1.93)	0.29	-
Diabetes	0.67	0.86 (0.41-1.78)		
History of AF	0.28	1.40 (0.76-2.57)		
NYHA Class (versus I)				
II	0.59	1.20 (0.62-2.31)		
III/IV	0.59	0.78 (0.32-1.90)		
QRS width (per 10 ms increase)	0.93	1.0 (0.91-1.01)		
LVEF (per 5% decrease)	0.39	1.08 (0.91-1.27)		
Beta-blocker	0.37	1.44 (0.64-3.24)		
Amiodarone	0.32	0.69 (0.33-1.44)		
Creatinine (per 10 µmol/l increase)	0.63	0.98 (0.92-1.05)		
Haemoglobin (per g/dl increase)	0.22	1.01 (0.99-1.03)		
Log NT-proBNP	0.01	2.26 (1.21-4.21)	0.01	2.26 (1.21-4.21)
Log GDF-15	0.27	1.41 (0.76-2.61)		
Log ST2	0.81	0.82 (0.16-4.25)		
Log CRP	0.12	0.55 (0.26-1.16)		
Log IL-6	0.38	1.13 (0.87-1.46)		

Table 6-6 Combining Biomarkers To Identify Groups Of Patients Unlikely To Gain Significant Benefit From ICD Therapy

	Group 1 Low Risk of ICD therapy and death	Group 2 High risk of ICD therapy and low risk of death	Group 3 High risk of death
Model 1	NT-proBNP <173 pmol/L	NT-proBNP ≥173 pmol/L and <2350 pmol/L	NT-proBNP ≥2350 pmol/
Total Patients in Group	31	114	11
Death	0	7	5
Survival with any appropriate ICD therapy	0	41	2
Model 2	NT-proBNP <173 pmol/L	NT-proBNP ≥173 pmol/L and sST2 <0.43 ng/ml	sST2 ≥0.43 ng/ml
Total Patients in Group	31	107	18
Death	0	5	7
Survival with any appropriate ICD therapy	0	38	5

Figure 6-1 Baseline Concentrations Of NT-ProBNP And sST2
Box plots showing the baseline concentrations of NT-proBNP (A) and sST2 (B) in patients that died and patients with event-free survival. Biomarker levels are presented as box (25th percentile, median, 75th percentile) and whisker (10th and 90th percentiles) plots.

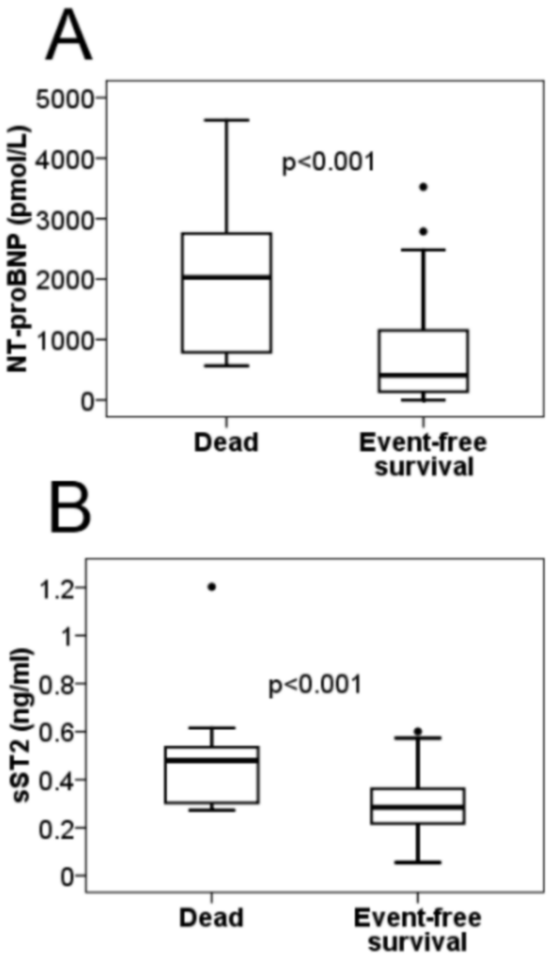
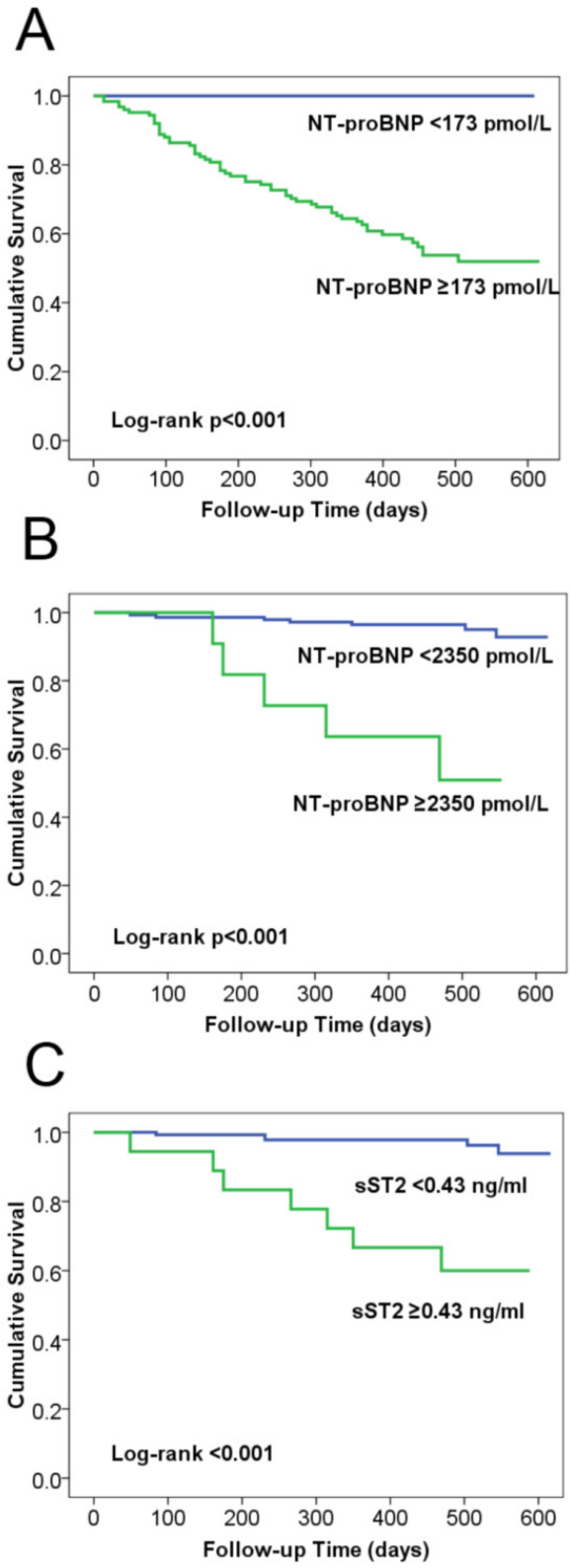


Figure 6-2 Kaplan–Meier Survival Curve Analysis
(A) Event-free survival in groups stratified by NT-proBNP level (cut-off 173 pmol/L); (B) Death in groups stratified NT-proBNP level (cut-off 2350 pmol/L); (C) Death in groups stratified by sST2 (cut-off 0.43 ng/ml).



6.4. Discussion

The main findings of this study are that the biomarkers sST2 and NT-proBNP are promising candidates for identifying patients with low potential to benefit from ICD therapy. These biomarkers identified a group of patients with advanced heart failure, whose short-term risk of death despite ICD therapy was high. NT-proBNP also identified a group of apparently low risk patients (approximately 20% of the cohort) who experienced no episodes of ICD therapy over the study's time horizon. However, the study population was patients with pre-existing rather than new ICD implants, and the follow-up was too short for this to be a clear end-point.

Although RCTs have demonstrated mortality benefit with ICD therapy, translation into clinical practice has been challenging (50, 102). Most patients implanted with an ICD based on current guidelines never receive a lifesaving device therapy (96). Furthermore, many patients with advanced heart failure will not have their life meaningfully prolonged by ICD therapy (102). This is either because they will die of another cause without receiving device therapy, or die soon after receiving appropriate ICD therapy from a non-arrhythmic (usually pump failure) death. In these patients the ICD serves only to alter the mode but not time of death. Set against these observations is the fact that ICD therapy is associated with significant morbidity, and is a high cost therapy with questionable cost-effectiveness.

To enrich ICD therapy clinical and cost-effectiveness, effort has been made to identify patients at high SCD risk. To date there is no consensus on how to do this. Importantly patients at highest SCD risk do not necessarily have the greatest potential to benefit from ICD therapy, as they are also at highest non-sudden death risk (17). An alternative approach is to identify patients meeting current guidelines who do not have the potential to gain significant survival benefit from ICD therapy.

Using their data Levy *et al*. suggested that the benefit of ICD therapy approached null when annual mortality reached 20-25% (110). Unfortunately using my data it was not possible to assess the annual mortality rate at which ICD therapy would cease to be clinically effective. However, using the data from Levy *et al*. both biomarker-defined high risk groups in my study, where the annual mortality was 36.4% in patients with NT-proBNP ≥2350 pmol/L and 33.3% in patients with sST2 ≥0.43ng/ml, may not benefit from ICD therapy.

A number of studies have evaluated the use of individual clinical characteristics to achieve this. Both serum creatinine and advancing age identify ICD recipients with a high short-term risk of death following ICD implantation (103, 106). In my study creatinine was a significant predictor

of death ($p<0.001$) and age predicted event-free survival ($p=0.02$). Both variables may prove useful combined in a model with biomarkers.

Two studies have evaluated more complex risk scores. Goldenberg *et al*. examined the relationship between a risk score, derived from 5 clinical variables, and benefit from ICD therapy, in the 1232 patients enrolled in the MADIT-II trial (109). The 345 patients with no risk markers, whose 2-year mortality was 9%, derived no benefit from an ICD (HR 0.96; $p=0.91$). The investigators also described a group of 60 patients with advanced renal dysfunction (BUN >50 mg/dl and/or serum creatinine >2.5 mg/dl), whose 2-year mortality was 50%, who also did not benefit from their device (HR 1.0; $p=0.99$). In my study, although creatinine was a predictor of death, only 2 of the 12 patients that died had a creatinine that would have merited inclusion in this group. Furthermore, of the 7 patients with an sST2 above the ROC-derived cut-off (0.43 ng/ml) that died, only 1 would have been in the group, and none of the 5 patients that died with an NT-proBNP level above the ROC-derived cut-off (2350 pmol/L) would have been in the group. Levy *et al*. used a modified version of the Seattle Heart Failure Model to examine baseline predicted mortality risk, and the relative and absolute benefit from ICD therapy, in 2487 patients in the SCD-HeFT trial (110). Patients with the highest quintile of baseline predicted risk of death gained no significant benefit from device therapy. However, neither study has yet had an impact on international guidelines (50).

A number of studies have evaluated the use of biomarkers to predict SCD and appropriate ICD therapy. The most studied of these is BNP/NT-proBNP, which predicts both SCD and ICD therapy (223). This is consistent with my results. The association of inflammatory markers with ICD therapy has been inconsistent. Although smaller studies have shown an association, other larger prospective studies have not confirmed these findings (155). In my study neither CRP nor IL-6 predicted appropriate ICD therapy.

A recent nested case-control study by Pascual-Figal *et al*. investigated the association of sST2 and SCD, in patients with heart failure in a multicentre registry (143). sST2 levels were significantly higher in 36 patients who died suddenly than 63 matched controls (0.23 ng/ml vs. 0.12 ng.ml, $p=0.001$). In contrast, I found that sST2 did not predict survival with appropriate ICD therapy, but did predict overall mortality.

A number of studies have observed that patients that receive appropriate ICD shock therapy have a high risk of subsequent death, and the possibility that shock therapy may be detrimental has been raised. In my cohort 4 patients experienced appropriate ICD therapy, of which 3 were shock therapy, and subsequently died. In comparison to the survivors, the baseline sST2 level

was significantly higher (0.42±0.09 vs. 0.30±0.12 ng/ml, p=0.04) and the NT-proBNP non-significantly higher (2009±1820 vs. 808±740 pg/ml, p=0.28) in these 4 patients. Furthermore, the mean biomarker levels were similar in patients who died with (n=4) and without (n=8) (sST2 0.53±0.30 ng/ml, NT-proBNP 1949±1056 pg/ml) experiencing appropriate device therapy prior to death. These findings are consistent with appropriate shock therapy being a marker of advanced heart failure and high mortality risk in some patients, rather than a causal factor, though no conclusions can be drawn in view of the small numbers and post-hoc nature of the analysis.

My observations that biomarkers can predict mortality in patients with advanced LVSD are consistent with those of other studies (111). The suggestion that these biomarkers may be able to accurately characterise heart failure severity, and identify patients whose disease is too advanced to gain significant benefit from ICD therapy, is novel.

The use of sST2 and NT-proBNP in a model for refining patient selection for ICD therapy could have potential benefits in comparison to other tools. Their measurement is cheap, reproducible, straightforward, and can be utilised as a continuous variable or dichotomised using a cut-off point, depending on the model of application. Furthermore, in patients with acquired heart disease, risk is a dynamic rather than static quantity, and the non-invasive nature of biomarker assessment lends itself to repeated measurement over time. However, it is unclear how the biomarkers compare to the risk scores described in the studies by Goldenberg *et al.* and Levy *et al.*, or whether they would add incremental accuracy to these proposed models or to the assessment of renal function alone (103, 106, 109, 110).

6.4.1. Limitations

The study sample is small and the number of deaths low. My study is essentially hypothesis generating and the findings need repeating in a larger cohort with validation of the optimum biomarker cut-off values.

I included patients with both primary and secondary prevention indications and therefore the study population may not fully reflect the patient population (primary prevention patients) in whom risk stratification tests are most needed. This is most relevant with respect to the low risk patient group identified by a low NT-proBNP, who experienced no episodes of appropriate ICD therapy during follow-up. However, consistent with international guidelines the identification of patients with advanced heart failure or other comorbidity, whose short-term risk of death despite ICD therapy is high, is relevant to both primary and secondary prevention patients, and for this reason secondary prevention patients were included in the study (2, 50).

All patients enrolled in my study had their devices already implanted and in many cases had had an ICD for a number of years prior to study entry. There are a number of potentially important differences between patients who already have a device in-situ and patients who are being considered for suitability for ICD therapy. Patients with significant LVSD who have survived for some years following ICD implantation may be a self-selected population of 'natural survivors' whose prognosis differs from that of patients prior to ICD implantation. Patients had to attend ICD clinic to be recruited into the study and therefore patients with a poor clinic attendance record, who may have a worse prognosis than patients who are more compliant, would be less likely to be included in the study. In addition, I included many secondary prevention patients whose risk of ventricular arrhythmias as well as potentially pump failure, may be significantly different from when their device was first implanted. This is therefore an important limitation which may reduce the generalisability of my results.

However, at study entry 75% (n=117) of patients had an LVEF ≤35% and may have had an indication for prophylactic ICD therapy based on current guidelines without the need for further testing (50). Furthermore, the death rate in my study (8%) was similar to the 1-year mortality rate in the ICD arm of MADIT-II (9%), and my rate of appropriate ICD therapy (30%) was slightly higher than in contemporary device trials, likely in part reflecting the high proportion (63%) of secondary prevention patients (58, 96). This suggests that, despite being a cohort of prevalent rather than new ICD patients, my study cohort may not differ significantly in terms of arrhythmic and non-arrhythmic risk, from a contemporary population of new ICD implants. In addition, none of the patients that died were in NYHA class IV heart failure on study entry, and therefore they would not have been excluded from ICD therapy by current guidelines (50).

Study follow-up was short. However, consistent with current guidelines the aim of my study was to identify patients whose chance of survival beyond 12 months was limited (50). My finding that NT-proBNP may identify patients with a low risk of appropriate ICD therapy, needs repeating in a study with longer follow-up and patients enrolled at initial implant.

Delivery of appropriate ICD therapy is not always a surrogate for preventable SCD. However, with current guidelines widening the recipient population for ICDs, the investigation of predictors of SCD in higher risk patients is difficult, as most such patients are indicated for an ICD (50). Lastly, the timing of TTE prior to study entry was variable. Although the majority of patients (71.2%) had their imaging in the 12 months prior to study entry many patients had their TTE at least a year prior to entry into the study. Both positive and negative left ventricular remodelling can take place and therefore in some patients the LVEF recorded by TTE may not

be an accurate reflection of left ventricular function at study entry. This would have tended to reduce the accuracy of LVEF as a predictor of outcomes in my analysis and therefore is an important limitation.

6.5. Conclusions

This pilot study suggests that NT-proBNP and sST2 are promising biomarkers for identifying patients with little potential to gain survival benefit from ICD therapy. They may provide a simple strategy for refining patient selection for ICD therapy. The incremental benefit of these biomarkers in addition to currently available clinical risk prediction models remains unclear. My study indicates the need for a prospective cohort study, from time of ICD implantation, powered to derive and validate algorithms to predict potential to benefit from ICD therapy.

7. PROTEOMIC PROFILING

7.1. Introduction

Heart failure is a major healthcare problem, affecting over 10 million people in Europe and America alone (231). Although contemporary medical therapy has significantly improved prognosis in patients with heart failure and asymptomatic LVSD mortality remains high (17). However, there is wide variation in mortality rates and mode of death among patient groups with LVSD (17). Though overall SCD is the commonest cardiac mode of death, in patients with more advanced disease death due to pump failure predominates (17). The ability to predict mode, as well as risk of death, in patients with LVSD is important, as the clinical and cost-effectiveness of ICD therapy depends on its use in appropriately selected patient populations (110).

Multiple studies have demonstrated that individual biomarkers, including markers of systemic vascular inflammation, myocardial stress, neurohormonal activation and myocyte injury, are powerful predictors of prognosis in patients with LVSD (111). However, such studies are limited by their evaluation of a small number of predetermined potential biomarkers. Current proteomic techniques enable the simultaneous assessment of a large number of proteins present in a sample, and allow the identification of potential candidate biomarkers by comparison of protein expression between groups of patients and controls (170). Such techniques have been successfully used to identify biomarkers in a range of cardiovascular conditions, including heart failure, peripheral vascular disease and myocardial infarction (178, 182, 183, 232). I hypothesised that an approach using untargeted proteomic profiling techniques may identify prognostic biomarkers in patients with LVSD.

The aims of this pilot study were twofold:

(i) To use surface-enhanced laser desorption ionisation time-of-flight mass spectrometry (SELDI-TOF MS) to identify potential serum biomarkers associated with systolic heart failure (SHF).

(ii) To prospectively explore the association of these biomarkers with mortality and the occurrence of ventricular arrhythmias in a cohort of patients with ICDs on the background of LVSD.

7.2. Methods

7.2.1. Study Population

All patients were recruited from those attending the Wessex Cardiothoracic Centre (Southampton University Hospital) device service. Two different patient populations were enrolled:

a) Control Patients. This group comprised consecutive patients with a permanent pacemaker and preserved LVEF. Patients with a high percentage of right ventricular pacing (>30%), or history, signs or symptoms of heart failure, were excluded. This group was chosen as controls to avoid the introduction of potential bias related to pre-analytical factors due to sample collection and patient preparation; they attended the same hospital clinic, in the same fashion (same time of day, no specific food requirements i.e. not nil by mouth), as patients with ICDs (the LVSD group).

b) Left Ventricular Systolic Dysfunction Patients. This group comprised patients with LVSD, and an ICD or cardiac resynchronisation defibrillator (CRT-D). All patients were on optimal medical therapy. None had heart failure admissions or therapy changes in the six weeks prior to enrolment. Based on New York Heart Association (NYHA) functional class and left ventricular ejection fraction (LVEF), two further subgroups of patients with LVSD were identified:

- Patients with SHF, defined as LVEF ≤40% and NYHA ≥II.
- Patients with asymptomatic LVSD, defined as LVEF ≤40% and NYHA I.

Additional exclusion criteria for both groups were pregnancy, a major co-morbid illness, or an acute coronary syndrome or surgery of any type within the preceding 6 weeks.

At study entry, baseline demographic and clinical data were recorded, a 12-lead resting ECG performed, and NYHA functional class assessed. All patients had a TTE prior to study entry. Blood was drawn from a forearm vein and collected in serum separator (serum) and EDTA tubes (plasma). Samples were collected and processed according to a standardised protocol, and all handled identically. Serum samples were allowed to clot for 30 minutes, and then centrifuged at 3000 rpm for 10 minutes. Samples were divided into aliquots and frozen within 1 hour of sampling. Samples were stored at -80°C prior to analysis and underwent no more than two freeze-thaw cycles. The study complied with the Declaration of Helsinki and was approved by the local research ethics committee (Southampton and South West Hampshire Research Ethics Committee local ethics number 08/H0502/54 and University Hospital Southampton NHS

Foundation Trust Research and Development department study number CAR0357). Written informed consent was obtained from all patients.

7.2.2. Study End Points and Follow-Up

Control patients were not followed-up. LVSD patients, who all had ICD/CRT-D devices, were followed up at 3-6 months with a hospital visit or via a remote patient management system. Device programming was at the discretion of the treating physician. Patients under remote follow-up also attended the hospital every 6 months. At each hospital visit the patient was clinically assessed and the device interrogated. The occurrence of any ICD therapy was recorded.

Appropriate ICD therapy was defined as:

(i) Antitachycardia pacing therapy (ATP) for ventricular tachycardia (VT).
(ii) Shock therapy for VT or ventricular fibrillation (VF).

Correct arrhythmia detection/discrimination was confirmed by analysis of stored electrograms by two electrophysiologists blinded to the biomarker analysis.

For the prospective part of the study, two study end-points were chosen to enable exploration of the utility of the serum proteomic biomarkers in defining outcomes. These were:

(i) All-cause mortality.
(ii) Patient survival with appropriate ICD therapy.

7.2.3. NT-proBNP Analysis

In view of its established diagnostic and prognostic role in LVSD, N-terminal pro-brain natriuretic peptide (NT-proBNP) was measured in all patients, as a comparator to the proteomic biomarkers. The NT-proBNP assay was based on a two-site non-competitive assay format, using in-house antibodies, as previously described (see section 6.2.3) (228). The lower limit of detection was 14.4 fmol/mL of unextracted plasma, and the within and between assay coefficients of variation were 2.3% and 4.8% respectively (229). There was no cross-reactivity of the assay with ANP, BNP or CNP (229).

7.2.4. SELDI-TOF MS Analysis

Serum samples were analysed on the weak cationic exchange (CM10) ProteinChip array (BioRad, California, USA), chosen as it produced more spectra peaks than other surface chemistries in a preliminary study. To aid reproducibility a BioMek3000 (Beckman Coulter, California, USA) liquid handling robot was used. Samples were analysed in duplicate on a

bioprocessor (BioRad). Samples were randomly assigned to bioprocessor wells to minimise bias. All samples were run over a 1 week period to reduce potential error due to variation in instrument performance.

Serum samples (10µL) were denatured at pH 9 for 60 mins on ice, in 90µl of U9 buffer (9 mol/L urea, 2% CHAPS, 50 mM Tris-HCl, pH 9) (BioRad). Bioprocessor wells were preincubated with 5µl of buffer (CM10 low-stringency, BioRad), and then incubated twice with 200µl of buffer at room temperature for 10 minutes. Ten microliter of the diluted serum mixture was mixed in 90µl sample buffer and applied to the bioprocessor wells. Samples were then incubated for 60 minutes at room temperature on a Micromix5 platform shaker (Diagnostic Products Corporation, California, USA), using a form of 20 and amplitude of 7. Following incubation, unbound proteins were removed by washing with 200µl of buffer twice, and 200µl of di-ionised water twice. Each washing step was performed with horizontal shaking for 10 minutes. The ProteinChips were removed from the bioprocessor and allowed to air dry for 45 minutes. Two applications of 1µl of 50% sinapinic acid in 50% acetonitrile/0.5% trifluoroacetic acid were delivered to each spot, and allowed to air dry for 15 minutes.

Time-of-flight (TOF) spectra were generated using an Enterprise 4011 mass spectrometer (BioRad). Each spot was analysed using laser settings optimised for low and high mass proteins. For the low mass range (mass range 0-20 kDa, focus mass 10 kDa, matrix attenuation 1 kDa) spectra were generated with 17 shots per position, at laser intensities of 2000, 3000 and 4000, preceded by 2 warming shots at 2200, 3300 and 4400 respectively. For the high mass range (mass range 18-200 kDa, focus mass 20 kDa, matrix attenuation 1 kDa), TOF spectra were generated with 17 shots at a laser intensity of 4000, preceded by 2 shots at 4400.

Spectra were calibrated externally using a protein standard calibration kit comprising recombinant hirudin (6.96 kDa), equine cytochrome *C* (12.23 kDa), equine myoglobin (16.95 kDa) and carbonic anhydrase (29.0 kDa) (BioRad). Following baseline subtraction, spectra were normalised to total ion current, 2-20kDa for the low mass range and 18-200kDa for the high mass range. Spectra were visually inspected and poor quality spectra removed. For each sample, one spectrum for each mass range was used, chosen in order to minimise deviation in total ion current to within 0.4-2.5 times the mean of all included patients.

Biomarker Wizard software (version 3.1, BioRad) was used to identify peaks. For the low mass range the *m/z* range between 0 and 2000 was eliminated from the analysis to avoid chemical noise relating to the energy absorbing molecules. Peaks with a signal-to-noise (S/N) ratio of ≥5 for the first pass and ≥2 for the second pass, and a valley depth greater than or equal to 3, were

considered for clustering if present in ≥10% of spectra. The mass window for each cluster was 0.3% of the peak mass. To avoid analysis of spurious peaks all peaks were visually inspected and relabelled as required prior to statistical analysis. The high mass range was run in parallel.

Every other ProteinChip contained a control sample, taken from a single pooled mixture of 50 case and control patients, to assess assay variability. Coefficients of variation (CV) for peak intensity for these spectra, derived from the pooled sample, run 34 times (five assays), was 34%. These data were obtained by averaging values for 17 randomly selected peaks, spread across the analysed mass range (2-200kDa), using the formula $CV=\sqrt{((CV_1^2+CV_2^2+CV_3^2...+CV_n^2)/n)}$, where n represents the number of included peaks.

7.2.5. Statistics

Categorical variables are expressed as percentages (numbers). Normally distributed continuous variables are expressed as mean ± standard deviation. Variables not normally distributed are expressed as median (lower quartile to upper quartile) and compared using the Mann-Whitney *U* test.

In order to identify proteomic biomarkers associated with LVSD the serum proteomic profiles were compared between patients with SHF and controls. For the analysis patients were randomly separated into two equal sized discovery and validation sets, each containing equal numbers of SHF and control patients. For each proteomic peak I calculated a p value, using the Mann-Whitney *U* test, to indicate its ability to differentiate SHF patients from controls. Peaks that were differentially expressed ($p<0.05$) in both sets were considered significant. For each of the significant peaks, Receiver Operating Characteristic (ROC) curves were constructed, to assess accuracy in distinguishing SHF from controls patients. The Jonckheere-Terpstra test was used to assess the trend in peak intensity levels across patient groups categorised by functional class.

The 6 biomarker peaks that demonstrated an association with SHF were then evaluated in a prospective study of ICD recipients, to assess their ability to predict prognosis in patients with LVSD. Univariate cox proportional hazards analyses were used to investigate biomarker predictors of the two prospective end-points (all-cause mortality and survival with appropriate ICD therapy). For the end-point of all-cause mortality, in view of the small number of patients reaching the end-point (n=11), multivariable analysis was not performed. As NT-proBNP was not normally distributed and its normality improved with log transformation, the log transformed values were used for analysis. The proportional hazards assumption was checked using Schoenfeld residuals (193).

For the end-point of all-cause mortality, for selected biomarker peaks cut-off points were chosen to identify patients with a high risk of death. Kaplan-Meier survival analysis was used for comparison between patient groups stratified according to these biomarker cut-off points, and survival curves were compared using the log-rank test.

Statistical analyses were performed on SPSS Version 17 (SPSS Inc., Chicago, IL, USA). In all analyses $p < 0.05$ was considered significant.

7.3. Results

7.3.1. Patient Characteristics

One hundred and eighty six patients were enrolled in the study (Table 7-1). These comprised 45 patients with permanent pacemakers, preserved LVEF, and no signs/symptoms of heart failure (control group), and 141 patients with ICDs on the background of LVSD. Of the 141 patients with ICDs, 78 had SHF and 47 asymptomatic LVSD.

Although all patients had a TTE prior to study entry the timing of TTEs with respect to study entry was variable. Of the 141 patients with ICDs the majority had their TTE in the 12 months prior to study entry (n=102, 72.3%), 16 patients (11.3%) had their TTE 1-2 years prior to study entry, while the remainder (n=23, 16.3%) had their TTE more than 2 years prior to study entry.

7.3.2. Proteomic Biomarkers Associated With SHF

The serum proteomic profiles were compared between the 78 SHF patients and 45 controls. Patients were randomly assigned to two sets which were analysed separately (see Methods). Biomarker wizard identified an initial 94 analysable protein peaks. Twelve peaks in the first set and 15 in the second set were differentially expressed ($p<0.05$) between SHF patients and controls. Six of these peaks were significantly different in both sets (Table 7-2). Four peaks were lower (*m/z* 4221, 5351, 5921 and 6125), and two higher (*m/z* 11834 and 14766), in SHF patients compared to controls.

The proteomic profiles were also compared between the 47 patients with asymptomatic LVSD and 45 controls. Only 2 of the 6 previously identified protein peaks (*m/z* 4221 and 11834) were significantly different between the groups (Table 7-2). However, all 6 peaks had an intermediate value between that of SHF and control patients, and for each peak there was a trend in peak intensity from controls through to asymptomatic LVSD and SHF (Table 7-2). Furthermore, there was a significant association between peak intensity and functional class (Figure 7-1). For

each of the six proteomic peaks there was a trend in peak intensity from controls, through to asymptomatic LVSD, NYHA class II SHF, and NYHA class III/IV SHF: *m/z* 4221 (p=0.001), *m/z* 5351 (p<0.001), *m/z* 5921 (p=0.004), *m/z* 6125 (p=0.03), *m/z* 11834 (p<0.001) and *m/z* 14766 (p<0.001).

ROC curves were constructed to evaluate the ability of the 6 protein peaks to differentiate between SHF and control patients. All peaks significantly distinguished between SHF patients and controls, with areas under the ROC curve ranging from 0.68-0.80 (Table 7-3). Two of the peaks (*m/z* 4221 and 11834) also differentiated between patients with asymptomatic LVSD and controls. However, none of the proteomic peaks out-performed NT-proBNP (area under the ROC curve 0.88 for SHF and 0.87 for asymptomatic LVSD).

7.3.3. Relationship of Proteomic Biomarkers To Outcomes

The ICD patients (n=141) were followed-up for a mean of 15±3 months. During this time there were 11 deaths (8%) and 43 patients (30%) experienced appropriate ICD therapy. Four patients (3%) who experienced appropriate ICD therapy subsequently died, leaving 39 patients (28%) who experienced appropriate ICD therapy and survived the study duration. Of these, 21 experienced appropriate shock therapy, while the remainder experienced only appropriate ATP.

The median values for all 6 proteomic peaks were higher in patients that died (n=11) compared to patients with event-free survival (n=91) (Table 7-4). In contrast, the levels were similar between patients that survived with appropriate ICD therapy (n=39) and those with event-free survival (n=91). An example of peak *m/z* 11834 is shown in Figure 7-2.

In univariate analyses, five of the six proteomic peaks were significant predictors of death (Table 7-5). However, none predicted survival with appropriate ICD therapy. In comparison, Log-transformed NT-proBNP predicted both mortality (p=0.001) and survival with appropriate ICD therapy (p=0.01).

7.3.4. Survival Analysis and Proteomic Biomarkers

For the ICD patients (n=141), using two of the prognostic proteomic peaks (*m/z* 11834 and *m/z* 14766) survival curves were compared between patient groups stratified according to biomarker levels using best cut-off values (see Methods section). For peak *m/z* 11834, 6 of 15 patients with a signal intensity ≥17.5 died, compared to only 5 of 126 below this level (p<0.001) (Figure 7-3). For peak *m/z* 14766, 6 of 15 patients with a signal intensity ≥5.6 died, compared to only 5 of 126 below this level (p<0.001) (Figure 7-3).

Table 7-1 Patient Characteristics At Study Entry

	Controls (n=45)	ICD patients		
		Asymptomatic LVSD (n=47)	SHF (n=78)	Overall (n=141)
Age (years)	66±14	66±11	70±10	69±10
Male Sex % (no.)	53 (24)	87 (41)	77 (59)	85 (120)
Diabetes % (no.)	9 (4)	19 (9)	20 (15)	24 (34)
History of AF % (no.)	20 (9)	30 (14)	39 (30)	36 (51)
History of hypertension % (no.)	40 (18)	36 (17)	44 (34)	46 (65)
NYHA Class % (no.)				
I	100 (45)	100 (47)	-	37 (52)
II	-	-	64 (50)	40 (57)
III	-	-	33 (26)	21 (30)
IV	-	-	3 (2)	1 (2)
Device Type % (no.)				
ICD	-	96 (45)	46 (36)	66 (93)
CRT-D	-	4 (2)	54 (42)	34 (48)
PPM single chamber	27 (12)	-	-	-
PPM dual chamber	73 (33)	-	-	-
Heart Disease Type % (no.)				
Ischaemic		72 (34)	80 (62)	77 (108)
NICM		17 (8)	18 (14)	18 (25)
Other		11 (5)	2 (2)	6 (8)
ICD indication % (no.)				
Primary Prevention	-	23 (11)	46 (36)	37 (52)
Secondary prevention	-	77 (36)	54 (42)	63 (89)
Beta-blocker % (no.)	18 (8)	83 (39)	78 (61)	91 (129)
ACE-I/ARB % (no.)	20 (9)	87 (41)	92 (72)	91 (129)
Amiodarone % (no)	2 (1)	36 (17)	28 (22)	29 (41)

Table 7-2 Association Of Proteomic Biomarkers With Systolic Heart Failure And Asymptomatic Left Ventricular Dysfunction

Biomarker	Peak Intensity			P value		
	Controls (n=45)	Asymptomatic LVSD (n=47)	SHF (n=78)	Controls vs. Asymptomatic LVSD	Controls vs. SHF	For trend (controls-asymptomatic LVSD-SHF)
m/z 4221	67.4 (47.7-90.6)	44.0 (25.4-78.5)	35.0 (19.7-73.5)	0.01	<0.001	<0.001
m/z 5351	22.1 (12.3-36.6)	15.1 (5.8-28.9)	7.6 (4.6-18.9)	0.08	<0.001	<0.001
m/z 5921	164.6 (94.3-234.0)	152.0 (82.9-152.0)	95.9 (60.9-160.1)	0.72	0.001	0.003
m/z 6125	14.2 (8.4-20.3)	12.9 (6.3-21.6)	8.2 (5.7-13.9)	0.85	0.001	0.002
m/z 11834	4.1 (3.2-5.9)	5.2 (3.8-7.5)	7.4 (5.3-10.9)	0.02	<0.001	<0.001
m/z 14766	2.5 (2.1-3.1)	2.8 (2.3-3.6)	3.5 (2.6-4.5)	0.06	<0.001	<0.001
NT-proBNP (pmol/L)	78 (20-223)	757 (247-1118)	811 (278-1440)	<0.001	<0.001	<0.001

Peak intensity data are expressed as median (lower quartile to upper quartile).
Peak intensities were compared using the Mann-Whitney U test for individual group comparisons and the Jonckheere-Terpstra test for trend.
m/z, mass/charge.

Table 7-3 ROC Scores For Proteomic Biomarkers To Distinguish Between Systolic Heart Failure (N=78) And Control (N=45) Patients

Biomarker	SHF vs. controls		Asymptomatic LVSD vs. controls	
	Area under the ROC curve (95% CI)	P value	Area under the ROC curve (95% CI)	P value
m/z 4221	0.69 (0.60-0.79)	<0.001	0.65 (0.54-0.77)	0.01
m/z 5351	0.75 (0.66-0.84)	<0.001	0.61 (0.49-0.72)	0.08
m/z 5921	0.68 (0.58-0.77)	0.001	0.52 (0.40-0.64)	0.72
m/z 6125	0.68 (0.58-0.78)	0.001	0.51 (0.39-0.63)	0.85
m/z 11834	0.80 (0.72-0.88)	<0.001	0.65 (0.53-0.76)	0.02
m/z 14766	0.77 (0.68-0.85)	<0.001	0.62 (0.50-0.73)	0.06
NT-proBNP	0.88 (0.82-0.94)	<0.001	0.87 (0.80-0.94)	<0.001

m/z, mass/charge.

Table 7-4 Baseline Biomarker Levels In Relation To Outcome In ICD Recipients

Biomarker	Peak Intensity		
	Event-free survival (n=91)	Survival with appropriate ICD therapy (n=39)	All-cause mortality (n=11)
m/z 4221	37.1 (21.6-73.4)	39.0 (34.5-68.5)	56.1 (26.9-78.4)
m/z 5351	9.4 (4.7-22.6)	10.0 (5.8-22.3)	10.1 (6.8-36.6)
m/z 5921	111.0 (59.4-191.9)	102.5 (73.5-182.7)	159.6 (94.5-252.7)
m/z 6125	9.9 (5.9-16.9)	9.8 (6.3-15.2)	14.3 (8.2-23.7)
m/z 11834	5.8 (4.4-9.3)	5.8 (4.4-9.3)	18.6 (6.0-29.9)
m/z 14766	3.0 (2.3-3.8)	3.0 (2.3-3.8)	5.7 (2.6-6.8)
NT-proBNP (pmol/L)	412.1 (124.6-1144.3)	820 .6 (399.3-1118.1)	2207.1 (611.3-2883.2)

Peak intensity data are expressed as median (lower quartile to upper quartile).
m/z, mass/charge.

Table 7-5 Biomarker Univariate Predictors Of All-Cause Mortality And Survival With Appropriate ICD Therapy

Biomarker	All-cause Mortality		Survival with appropriate ICD therapy	
	P value	Hazard Ratio (95% CI)	P value	Hazard Ratio (95% CI)
m/z 4221	0.51	1.00 (0.99-1.02)	0.85	1.00 (0.99-1.01)
m/z 5351	0.01	1.02 (1.00-1.03)	0.89	1.00 (0.98-1.02)
m/z 5921	0.02	1.01 (1.00-1.01)	0.50	1.00 (0.99-1.00)
m/z 6125	0.002	1.03 (1.01-1.05)	0.45	0.99 (0.95-1.02)
m/z 11834	0.002	1.04 (1.02-1.06)	0.48	1.01 (0.98-1.04)
m/z 14766	0.007	1.40 (1.10-1.80)	0.36	0.89 (0.70-1.14)
Log NT-proBNP	0.001	53.08 (4.96-568.4)	0.01	2.33 (1.20-4.53)

m/z, mass/charge.

Figure 7-1 Relationship Between Clinical Status And Biomarker Peak Intensity For Biomarkers (A) *m/z* 11834 , (B) *m/z* 14766 and (C) *m/z* 5351

Patients are grouped into controls, and for patients with LVEF ≤40%, by NYHA class (III/IV combined). The peak intensity levels are presented as box (25th percentile, median, 75th percentile) and whisker (10th and 90th percentiles) plots. Patient numbers are indicated. The Jonckheere-Terpstra test was used to assess the trend in peak intensity levels across patient groups.

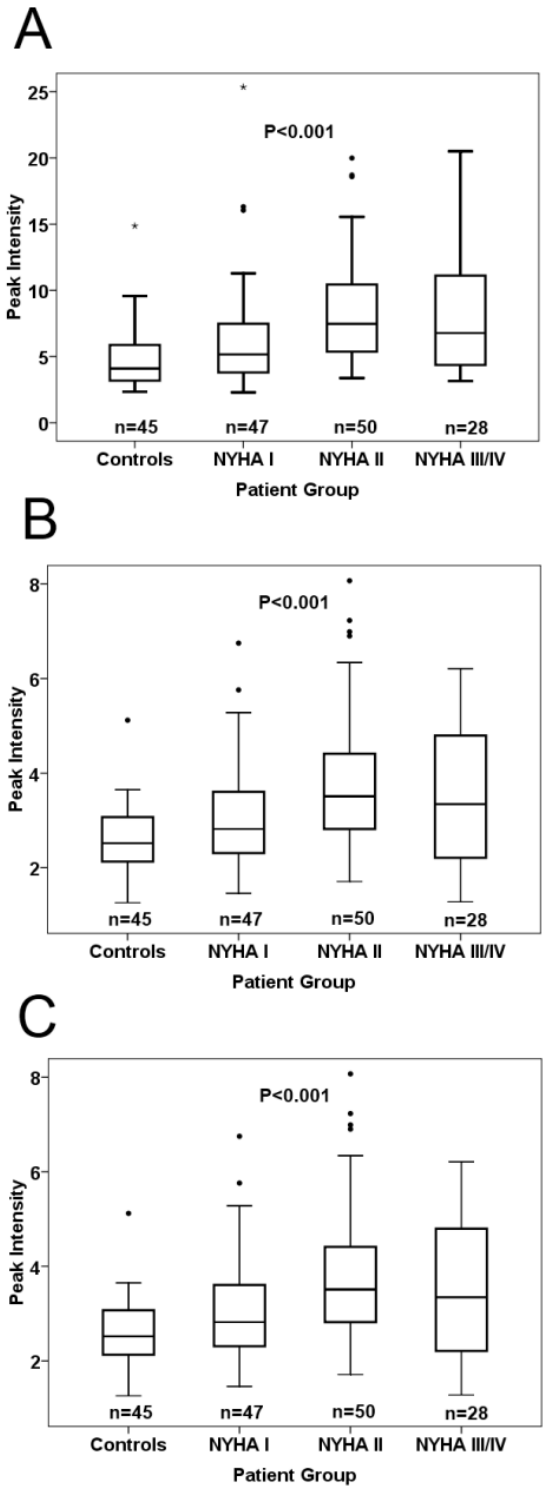


Figure 7-2 Increased Expression Of Peak *M/Z* 11834 In Patients With ICDs That Died Compared To Those That Survived

A region of mass spectra from 10 to 16 kDa has been expanded and aligned for 4 patients that died during follow-up and 4 patients that survived. Peak intensity for biomarker peak *m/z* 11834 (arrow) is higher in patients that died versus those that survived. The x-axis is the ratio of mass-to-charge (*m/z*) and the y-axis represents peak intensity.

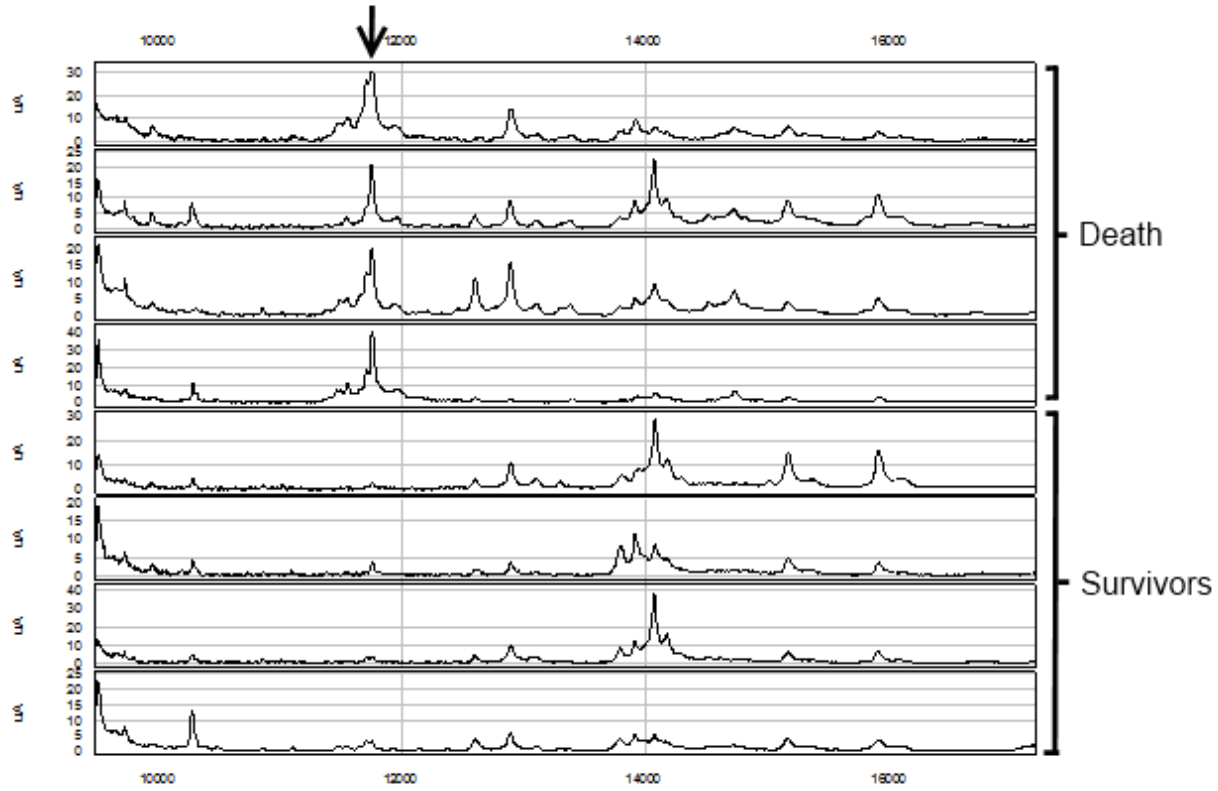
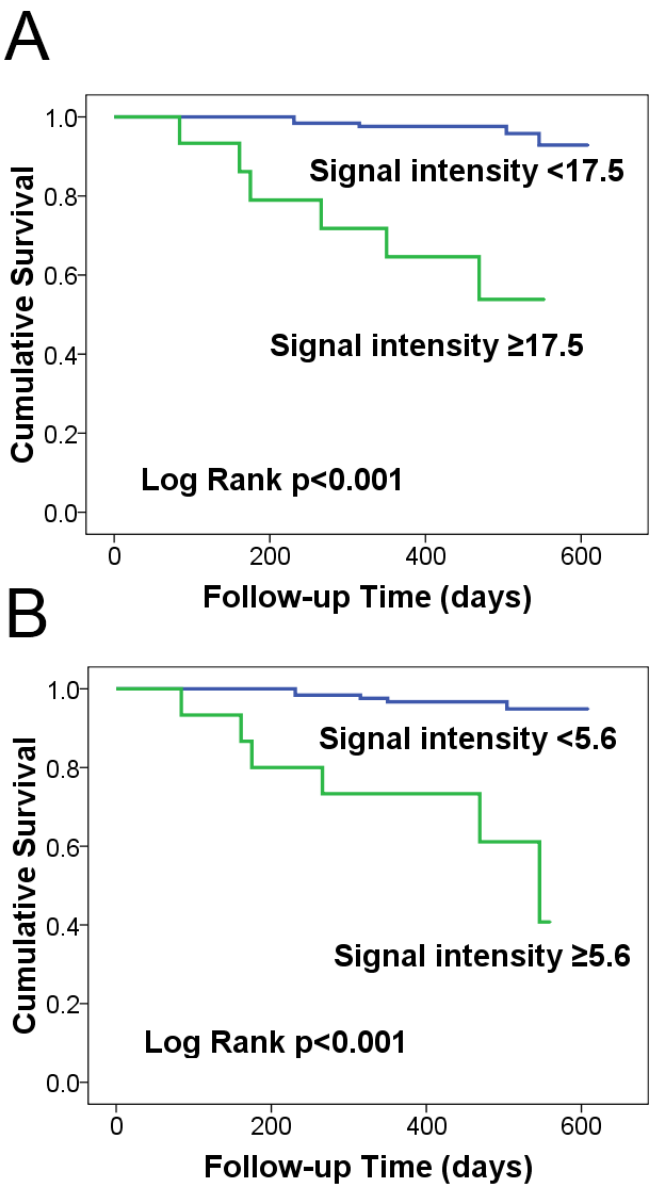


Figure 7-3 Kaplan–Meier Survival Curve Analysis For All-Cause Mortality In ICD Patients

Patient groups are stratified by: (A) *m/z* 11834 signal intensity (cut-off 17.5) and (B) *m/z* 14766 signal intensity (cut-off 5.6).



7.4. Discussion

The main findings of this pilot study are that, using proteomic techniques, I have identified serum biomarkers that are differentially expressed in patients with SHF compared to controls, and that these biomarkers predict all-cause mortality in patients with ICDs on the background of LVSD. Furthermore, although these biomarkers are associated with all-cause mortality, they do not predict the occurrence of appropriate ICD therapy. These findings demonstrate proof-of-principle, and suggest that a proteomic approach may be useful in the identification of biomarkers that predict overall mortality, as well as mode of death, in patients with LVSD. However, the study is too small, with too short a follow-up, for firm conclusions to be drawn. Furthermore, none of the proteomic biomarkers outperformed NT-proBNP in either a diagnostic or prognostic role.

My finding, that proteomic techniques can be used to identify biomarkers with diagnostic accuracy in SHF, is consistent with the results of other studies (182). Jones *et al*. evaluated the diagnostic value of plasma proteomic biomarkers in 100 patients with SHF and 100 matched controls (182). Using matrix assisted laser desorption ionisation time-of-flight mass spectrometry, a complementary proteomic technique, they identified 67 protein peaks that were differentially expressed between SHF and control patients, of which 6 had independent predictive value when used in addition to NT-proBNP.

To my knowledge this is the first study to evaluate the potential use of proteomic techniques to identify biomarkers that predict mortality in patients with LVSD. The ability to predict mode of death in patients with LVSD is important, as the efficacy and cost-effectiveness of ICD therapy depends not just on the overall risk of death, but also the relative contribution of SCD and non-sudden death. ICD therapy improves overall survival in patients at high risk of SCD by recognising and terminating life-threatening ventricular arrhythmias. However it does not prevent non-SCD, which in patients with LVSD is predominantly due to pump failure (17). Furthermore, even in patients at high SCD risk ICD therapy does not improve overall survival if the risk of non-SCD is significantly elevated (110). There is therefore significant clinical value in identifying novel markers of non-SCD risk that may identify patients with LVSD who are unlikely to benefit from ICD therapy.

In my study, 5 of the 6 proteomic peaks differentially expressed in SHF predicted all-cause mortality in patients with LVSD, but none predicted the occurrence of ventricular arrhythmias. It is interesting to speculate that this may reflect the different pathophysiological mechanisms underlying pump failure death and SCD, in patients with LVSD. Although a range of potentially

proarrhythmic structural and functional changes have been described in patients with LVSD, the precise pathophysiological processes that leads to SCD are not clearly understood (41). The identification of biomarkers associated with mode of death may help better understand the pathological processes underlying mortality in patients with LVSD.

The levels of all 5 proteomic biomarkers associated with mortality were higher in patients that died compared to those that survived. However, in the identification phase of the study the levels of 3 of these biomarkers (*m/z* 5351, 5921 and 6125) were actually lower in patients with SHF compared to controls. Although a non-linear relationship between biomarkers and disease severity in SHF has been described, my findings are new and require confirmation (233).

My results suggest that a proteomic approach may be useful in the identification of biomarkers that predict mode of death. However, to be of clinical value such biomarkers would need to add incremental prognostic accuracy in addition to the currently available clinical risk models and there are currently no data to support this possibility (109, 110).

The aim of this study was to demonstrate proof-of-principle, that a proteomic approach may be able to identify prognostic biomarkers in patients with LVSD. As such, I performed only limited proteomic profiling, using one surface chemistry (CM10) and a single set of binding conditions - an approach that has been used successfully by other investigators - in a small group of patients with a relatively short follow-up (170). I have not established the identities of the proteomic peaks, as I feel that the next step would be to perform more exhaustive proteomic profiling in an appropriately powered study, and only then identify the most discriminative peaks. My limited profiling yielded approximately 100 analysable protein peaks. However, it has been estimated that up to 900,000 plasma proteins exist, and therefore I have only analysed a very small subset of the serum proteome (234). It is likely that using a range of surface chemistries and binding conditions would identify a significantly greater number of potential biomarkers (178).

Although my coefficient of variation of the normalised peak intensities is consistent with published data it is significantly higher than that of more conventional measurement methods, such as those using antibodies (170, 235). Thus the biomarker peaks I identified are likely to have significantly better diagnostic and prognostic accuracy than suggested by the spectra, once identified and measured using more conventional methods.

7.4.1. Limitations

The study sample is small and the number of patients reaching the study end-points low. Furthermore, the follow-up in the prognostic part of the study was relatively short. My findings need repeating in a significantly larger cohort with a longer follow-up.

Although I have found that specific proteomic peaks have diagnostic and prognostic value in patients with LVSD, the actual identity of these proteins is not known. This is an important issue in proteomic research as there is a significant risk of false positive results with the multiple biomarker peaks being analysed (166). Identification of the protein peaks enables demonstration of biological plausibility, thereby strengthening the conclusions that can be drawn, as well as potentially enabling insight to be gained into the underlying pathophysiology. In addition, the study sample is small and the number of patients reaching the end-points low, which again, may potentially increase the risk of false positive discovery. However, the protein peaks were identified in two separate randomly selected sets of SHF patients and controls, and then evaluated in a separate prospective study using a separate prognostic end-point (all cause mortality). These factors suggest that, despite the study limitations, the protein peaks I identified are less likely to be false positive findings.

Delivery of appropriate ICD therapy is not always a surrogate for preventable SCD. However, with current guidelines widening the recipient population for ICDs, the investigation of predictors of SCD in higher risk patients is difficult, as most such patients are indicated for an ICD. (50). Lastly, the timing of TTE prior to study entry was variable. Although the majority of patients (72.3%) had their imaging in the 12 months prior to study entry many patients had their TTE at least a year prior to entry into the study. Both positive and negative left ventricular remodelling can take place and therefore in some patients the LVEF recorded by TTE may not be an accurate reflection of left ventricular function at study entry.

7.5. Conclusions

In this pilot study I have used proteomic techniques to identify serum biomarkers that are differentially expressed in patients with SHF and predict all-cause mortality, but not appropriate ICD therapy, in patients with ICDs on the background of LVSD. These results provide proof-of-principle, and suggest that a proteomic approach may be useful in the identification of biomarkers that predict overall mortality, as well as mode of death, in patients with LVSD.

8. LEFT VENTRICULAR SCAR TISSUE

8.1. Introduction

Assessment of LVEF has been used historically as the discriminant test to define SCD risk in patients with CAD (50). However its predictive accuracy is weak (93). Thus, many patients who receive ICD therapy in the light of current guidelines informed by LVEF assessment will never benefit from the device.

Late gadolinium enhancement cardiac magnetic resonance imaging (LGE-CMR) can accurately and reproducibly identify areas of myocardial injury, enabling discrimination between subendocardial and transmural scar (186, 187). The amount, as well as the transmural extent, of myocardial scar tissue on LGE-CMR has been shown to predict overall mortality in patients with CAD independently of reduced LVEF (188, 189, 236).

The mechanistic role of myocardial scar in the genesis of VA that may underlie arrhythmic SCD is well established (43). The aim of this study was to assess whether the extent of LV scar, quantified by LGE-CMR, is associated with the occurrence of appropriate ICD therapy in ICD recipients with CAD. It tested the hypothesis that LV scar burden, quantified by LGE-CMR, may be more strongly associated with the occurrence of VA, as a surrogate for arrhythmic SCD, than LVEF.

8.2. Methods

8.2.1. Study Population

The study was conducted in a retrospective observational manner, at the Wessex Cardiothoracic Unit, a regional cardiothoracic centre serving a population of approximately 2.8 million people. The study population consisted of consecutive patients with CAD who had undergone LGE-CMR prior to ICD implantation over a 4 year period (2006-2009).

8.2.2. Definition of CAD

CAD was defined as ≥70% stenosis in at least 1 epicardial coronary vessel on angiography and/or history of MI or coronary revascularisation. All patients underwent diagnostic coronary angiography with images reviewed by a Consultant cardiologist.

8.2.3. ICD Details

All patients received an ICD according to national guidelines (72). Patients also meeting criteria for cardiac resynchronisation therapy (CRT) received a combined cardiac resynchronisation (CRT-D) device (237). Patients received a CRT-D device (Consulta, Concerto, [Medtronic,

Minneapolis, Mn., USA]; Cognis [Boston Scientific, Natick, Mass., USA]; Contak Renewal [Boston Scientific, formerly Guidant]; Ovatio [Sorin Group, Milan, Italy]; Atlas [St. Jude Medical, St. Paul, Minn., USA)]), or a dual or single chamber ICD (Secura, Virtuoso [Medtronic]; Teligen [Boston]; Ovatio [Sorin]; Atlas [St Jude Medical]).

8.2.4. CMR Data Acquisition

All patients were scanned on a dedicated 1.5-T Avanto MRI system (Siemens Medical Systems, Erlangen, Germany). After initial localiser sequences, a stack of steady-state free precession cine images were acquired in the short axis plane from the level of the mitral valve annulus to the LV apex. Following this, 0.15 mmol/kg gadobenate dimeglumine (Multihance, Bracco SpA, Milan, Italy) was administered intravenously. Short axis LGE images were acquired using a 3D segmented inversion recovery fast gradient echo sequence (3D IR turboFLASH) in two breath holds. An appropriate time to inversion was selected to null the normal myocardium.

8.2.5. CMR Analysis

Ejection fraction and volumes were analysed on commercially available post-processing software. Short-axis cine images were used to measure end-diastolic volume, end-systolic volume and LVEF by standard methods. Papillary muscles were regarded as part of the ventricular cavity.

Scar analysis was performed using semi-automated software developed at Southampton as a plugin to the open-source DICOM viewer OsiriX (OsiriX Project, Geneva, Switzerland) (Figure 8-1) (238). Endocardial and epicardial LV myocardial borders were manually delineated on the short axis LGE-CMR images.

For each patient the maximum signal intensity (SI) within an infarct region in each image of the LV stack was automatically determined, and scar was defined as myocardium with a signal intensity ≥50% of the maximum SI. Scar was automatically segmented and any areas identified as scar by the software but not deemed to be scar by the user were excluded manually. Three complementary aspects of scar were quantified: (1) the amount of scar, quantified as a percentage of the total LV myocardial volume; (2) the total scar surface area (including epicardial and endocardial surfaces); (3) the transmural extent of scar. For the transmural scar assessment, scar transmurality was split into quartiles (1-25%, 26-50%, 51-75% and 76-100%), and the number of segments of myocardium, based on a standard American Heart Association 17-segment model, with each quartile of scar, were quantified (239). CMR analysis was performed blinded to the clinical outcomes.

In order to evaluate intra- and inter-observer agreement for the scar analysis methodology, the percent scar assessment was repeated in 15 patients by the same observer and 15 patients by a second observer, blinded to the results of the initial analysis.

8.2.6. Study Follow-up and End-point

All patients were followed up every 3-6 months with a hospital visit or via a remote patient management system. Patients under remote follow-up also attended the hospital every 6 months. At each hospital visit the patient was clinically assessed and the device interrogated. The occurrence of any ICD therapy was recorded. Appropriate ICD therapy was defined as:

(i) Antitachycardia pacing therapy (ATP) for VT.
(ii) Shock therapy for VT or VF.

Correct arrhythmia detection/discrimination was confirmed by analysis of stored electrograms by two electrophysiologists blinded to the CMR analysis.

Appropriate ICD therapy was chosen as the study end-point, as a surrogate for the potential to benefit from ICD therapy, including SCD risk reduction. However, I recognise that appropriate ICD therapy does not equate to SCD prevention in all patients, and that in some patients, prevention of SCD does not translate into a significant increase in life expectancy, as some patients will subsequently suffer death from pump failure or another cause (101, 221).

8.2.7. Statistics

Categorical variables are expressed as percentages (numbers). Normally distributed continuous variables are expressed as mean ± standard deviation, and compared using Student's *t*-test. Variables not normally distributed are expressed as median (lower quartile to upper quartile).

The association between clinical, electrocardiographic and CMR variables, and the study end-point, were assessed in univariate Cox proportional hazards analyses. Since the aim of the study was to explore the association between the extent of LV scar and appropriate ICD therapy, a multivariable model was constructed with number of transmural scar segments as the scar variable, and amiodarone use, any previous pre-ICD revascularisation, and LVEF, as the covariables. These covariables were chosen based on the univariate analysis results, as well as previous studies, and were necessarily limited due to the small number of patients that received appropriate ICD therapy (n=19). In view of the strong correlation between percent scar and number of transmural scar segments (Pearson correlation 0.8, $p<0.001$), only the scar variable with the strongest association with the study end-point (number of transmural scar segments) was used in the multivariable model. The proportional hazards assumption was checked by

plotting the Schoenfeld residuals against rank time, and fitting a smooth curve with 95% confidence bands, as well as plotting log(-log[survival probability]) against time for different variables, to ensure that the curves were parallel (193, 240). Unadjusted and adjusted hazard ratios (HR) with their corresponding 95% confidence intervals (CI) are reported.

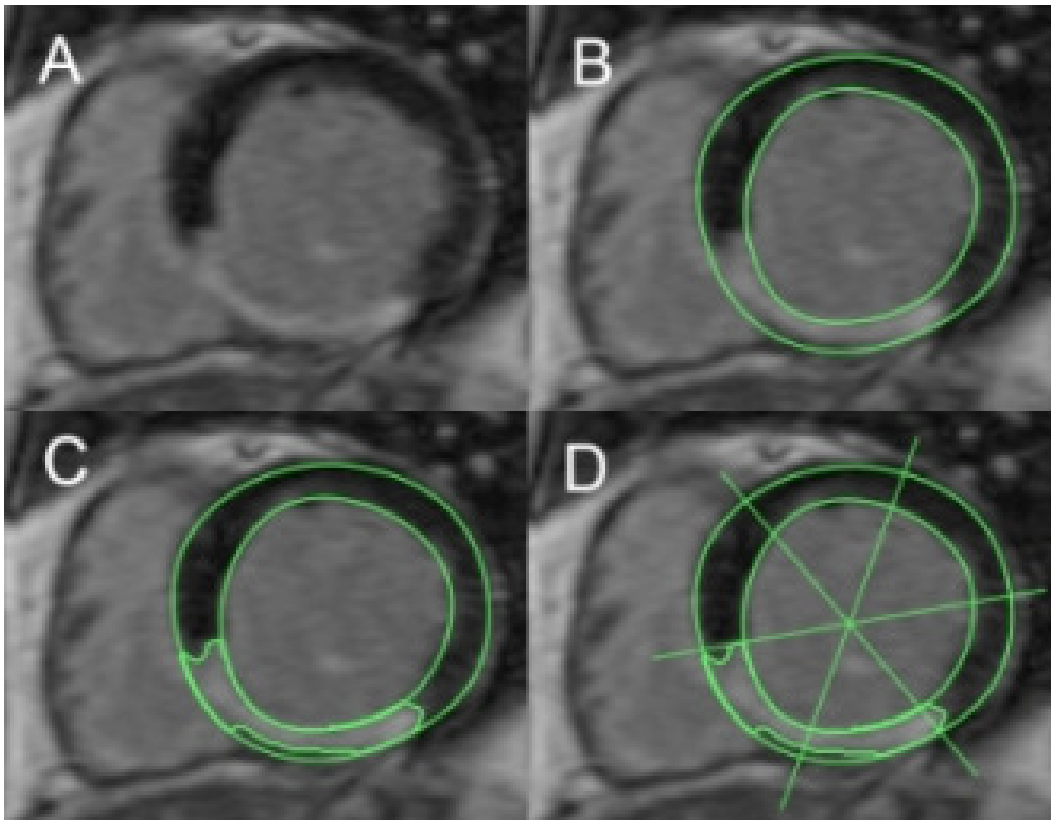
To explore the relationship between VA burden and the extent of scar, the VA rate (number of appropriate ICD therapies per year) for each patient was calculated, and the association between arrhythmia rate and scar variables (percent scar and number of transmural scar segments) was assessed using Spearman's rank correlation.

For scar variables with a significant association with the study end-point (percent scar and number of transmural scar segments), the study population was divided into two groups, based on the observed median value for each variable, and event rates were analysed by the method of Kaplan-Meier. Differences in event rate between groups over time were compared using the log-rank test.

Intra-observer and inter-observer agreement for scar quantification measurements were calculated using the intraclass correlation coefficient for absolute agreement.

Statistical analyses were performed on SPSS Version 17 (SPSS Inc., Chicago, IL, USA). In all analyses a p value of <0.05 was considered significant**.**

Figure 8-1 Scar Analysis In A Patient With A Previous MI
(A) A Short-axis LGE-CMR image has been loaded onto customised software. (B) The left ventricular epi- and endocardium have been outlined manually. (C) The maximum signal intensity within the infarct region has been determined, and the infarct core, defined as signal intensity ≥50%, automatically detected. (D) The image has been segmented according to the American Heart Association 17-segment model, to enable transmurality assessment.



8.3. Results

8.3.1. Patient Characteristics

During the study period there were 257 new ICD implants for CAD, of which 64 (25%) had a LGE-CMR prior to device implantation and were included in the study. The characteristics of patients who had a LGE-CMR (n=64) and were included in the study were broadly similar to those who did not have a LGE-CMR (n=193) (Table 8-1). However, patients that did not have LGE-CMR were significantly more likely to have had a previous MI than patients that did have a scan (92 vs. 77%, p=0.003).

Baseline demographics of the 64 patients included in the study are shown in Table 8-1. There was a balanced distribution of primary and secondary indication patients (48% vs. 52% respectively). Patients were on optimal medical therapy for heart failure (beta-blockade in 86% and ACE-inhibition/angiotensin receptor blockade in 88%). The ICD VT treatment zone lower setting was similar in patients who did, and did not, experience the study end-point (147±26 bpm vs. 149±23 bpm respectively; p=0.83).

In all patients LGE-CMR was performed to guide the need for potential revascularisation prior to ICD implantation. This included an assessment of myocardial viability in all patients, as well as an assessment of ischaemic burden in the majority of patients (73%, n=47).

8.3.2. Assessment of Ischaemic Burden

Of the 47 patients who had an assessment of ischaemic burden performed as part of their LGE-CMR 15 had evidence of reversible ischaemia, and in 9 of these it was considered clinically appropriate to perform surgical/percutaneous revascularisation prior to ICD implantation.

8.3.3. Clinical Outcomes

During a mean follow-up of 19±10 months, 19 (30%) patients received appropriate ICD therapy and 5 (8%) patients died. The distribution of appropriate ICD therapies was:

- No episodes of appropriate ICD therapy – 45 patients
- Appropriate ICD therapy for VT (rate <182bpm) only – 10 patients
- Appropriate ICD therapy for fast VT (rate ≥182bpm) (230) – 8 patients
- Appropriate ICD therapy for VF – 1 patient

For the 19 patients that received appropriate ICD therapy, the median number of episodes per patient was 3, and the median rate of appropriate ICD therapies per patient was 2.1 therapies per year. Seven patients were treated with shock therapy, and the remaining 12 with ATP only.

8.3.4. MRI Variables

In the study population median LVEF was 30% (22 to 39%), with a range of 11-78%. The mean end-systolic volume was 192±96 mls, and mean end-diastolic volume 269±97 mls. Fifty-eight patients (91%) had evidence of scar tissue on the late enhancement images. The mean percent scar was 14±10%, mean scar surface area 89±60 cm^2, and mean number of myocardial segments with transmural scar 2.3±2.1.

The intraclass correlation coefficient for percent scar quantification was 0.91 for intra-observer agreement and 0.89 for inter-observer agreement ($p<0.001$ for both), demonstrating high reproducibility.

8.3.5. Relationship of Scar Indices to Outcomes

In univariate analyses, variables significantly associated with the study end-point were percent scar (HR per 10% increase 1.75; 95% CI 1.09-2.81; p=0.02), number of transmural scar segments (HR per segment 1.40; 95% CI 1.15-1.70; p=0.001), amiodarone use (HR 0.12; 95% CI 0.02-0.97; p=0.04), and any previous pre-ICD revascularisation (HR 0.30; 95% CI 0.09-0.95; p=0.04) (Table 8-2). The associations with both percent scar (HR per 10% 1.80; 95% CI 1.07-3.02; p=0.03) and number of transmural scar segments (HR per segment 1.41; 95 CI 1.15-1.74; p=0.001) remained significant when the 6 patients without myocardial scar were excluded. Notably, LVEF (p=0.86), QRS width (p=0.13), and scar surface area (p=0.15) were not associated with the study end-point.

After adjustment for multiple covariates (amiodarone use, any previous pre-ICD revascularisation, and LVEF) (see Methods section), number of transmural scar segments remained strongly associated with the occurrence of appropriate ICD therapy (HR per segment 1.48; 95% CI 1.18-1.84; p=0.001) (Table 8-2).

8.3.6. Relationship of Scar Indices to Ventricular Arrhythmia Burden

There was a significant association between the amount of scar, quantified as both percent scar (Spearman's rank correlation 0.33, p=0.008) and number of transmural scar segments (Spearman's rank correlation 0.45, $p<0.001$), and burden of ventricular arrhythmias, expressed as the rate of appropriate ICD therapy (number of therapies per year).

8.3.7. MRI scar and Kaplan-Meier Analysis

Survival curves were compared between patient groups stratified by scar indices (percent scar and number of transmural scar segments). For percent scar, patients were separated into two groups based on the median value (12.6%). Using Kaplan-Meier analysis, appropriate ICD therapy occurred in 15 of 33 patients with >12.6% scar, compared to only 4 of 31 patients with ≤12.6% scar (p=0.02) (Figure 8-2).

For number of transmural scar segments, patients were again separated into two groups based on the median value (2 segments). Using Kaplan-Meier analysis, appropriate ICD therapy occurred in 15 of 33 patients with ≥2 segments of transmural scar, compared to only 4 of 31 patients with <2 segments (p=0.016) (Figure 8-2).

Table 8-1 Clinical characteristics of all new ICD implants during the study period, presented on the basis of whether they had a LGE-CMR prior to ICD implantation

	All new ICD Implants (n=257)		
	LGE-CMR (n=64)	No LGE-CMR (n=193)	P value
Age (years)	66±11	69±9	0.06
Male Sex % (no.)	80 (51)	87 (168)	0.16
History of AF % (no.)	27 (17)	34 (65)	0.35
Diabetes % (no.)	27 (17)	27 (53)	1.0
Hypertension % (no.)	47 (30)	36 (70)	0.14
Previous MI % (no.)	77 (49)	92 (177)	0.003
Previous PCI % (no.)	20 (13)	18 (35)	0.71
Previous CABG % (no.)	33 (21)	44 (85)	0.14
Any previous pre-ICD revascularisation % (no.)	44 (28)	56 (109)	0.08
Device Type % (no.)			
ICD single chamber	12 (8)	18 (35)	0.34
ICD dual chamber	50 (32)	47 (91)	0.77
CRT-D	38 (24)	35 (67)	0.76
ICD VT treatment zone lower setting (bpm)	148±24	149±19	0.65
Resting heart rate (bpm)	64±13	67±11	0.09
QRS width (ms)	122±31	125±29	0.44
ICD indication % (no.)			
Primary Prevention	48 (31)	42 (82)	0.47
Secondary prevention	52 (33)	58 (111)	-
Beta-blocker % (no.)	86 (55)	74 (142)	0.06
ACE-I/ARB % (no.)	88 (56)	85 (165)	0.84
Amiodarone % (no.)	23 (15)	27 (52)	0.63
Creatinine (µmol/l)	111±35	121±36	0.05
Haemoglobin (g/dl)	130±16	132±18	0.43

PCI, percutaneous intervention; CABG, coronary artery bypass grafting.

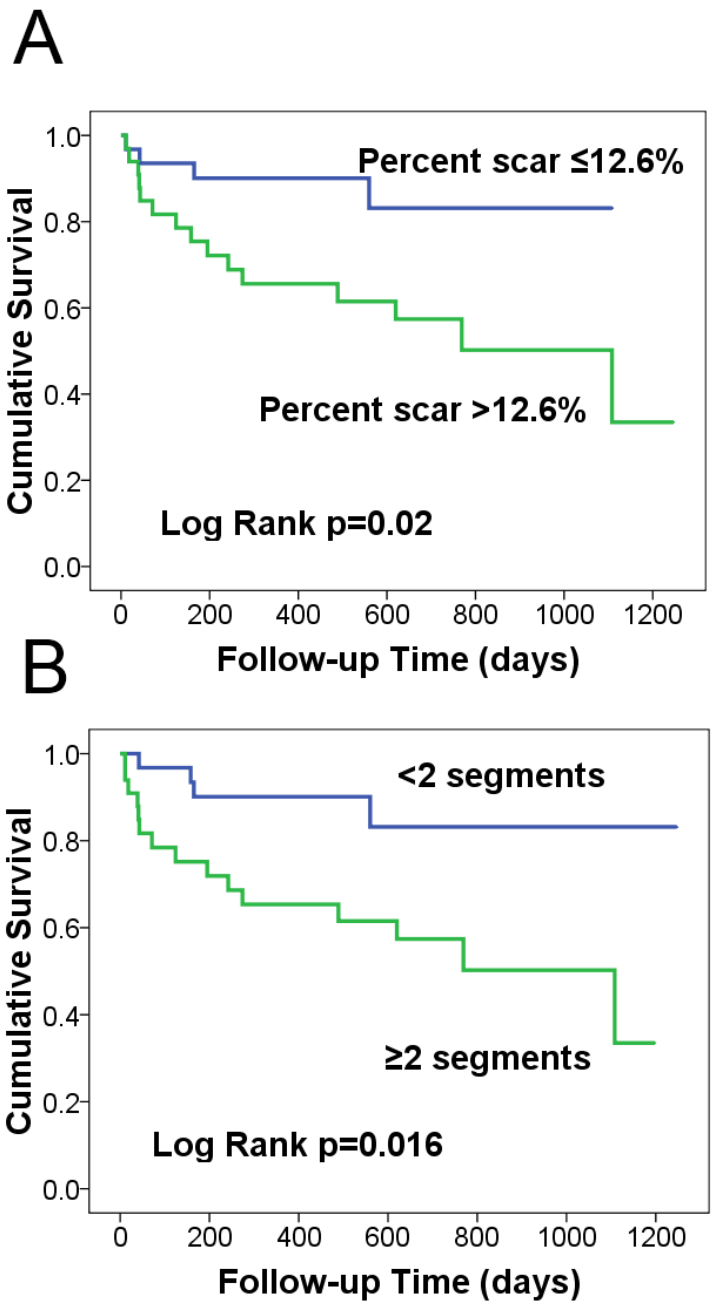
Table 8-2 Cox Proportional Hazards Analyses Demonstrating The Association Of Clinical, Biochemical, ECG And CMR Variables, And The Occurrence Of Appropriate ICD Therapy

	Univariate Analysis		Multivariable Analysis	
	P value	Hazard Ratio (95% CI)	P value	Hazard Ratio (95% CI)
Clinical, biochemical and ECG variables				
Age (per year)	0.26	0.98 (0.94-1.02)		
Diabetes	0.60	0.77 (0.29-2.03)		
History of AF	0.20	0.45 (0.13-1.53)		
Amiodarone use	0.04	0.12 (0.02-0.97)	0.04	0.09 (0.01-0.98)
Creatinine (per 10 µmol/l increase)	0.06	0.86 (0.73-1.00)		
Haemoglobin (per g/dl increase)	0.41	1.01 (0.98-1.04)		
QRS width (per 10 ms increase)	0.13	0.88 (0.75-1.04)		
Any previous pre-ICD revascularisation	0.04	0.30 (0.09-0.95)	0.09	0.42 (0.15-1.14)
CMR variables				
LVEF (per 10% decrease)	0.86	1.03 (0.76-1.39)	0.32	0.84 (0.72-1.19)
LVEDV (per 10 ml increase)	0.47	1.02 (0.97-1.06)		
Scar mass (per 10g increase)	0.07	1.31 (0.98-1.73)		
Percent scar (per 10% increase)	0.02	1.75 (1.09-2.81)		
Scar surface area (per 10 cm^2 increase)	0.15	1.06 (0.98-1.14)		
Number of affected segments by transmurality in a 17-segment model:				
1% to 25%	0.48	0.67 (0.22-2.04)		
26% to 50%	0.77	0.95 (0.68-1.34)		
51% to 75%	0.15	0.72 (0.45-1.13)		
76% to 100%	0.001	1.40 (1.15-1.70)	0.001	1.48 (1.18-1.84)

LVEDV, left ventricular end-diastolic volume.

Figure 8-2 Kaplan–Meier Survival Curves For The Occurrence Of Appropriate ICD Therapy

Patient groups are stratified by (A) Percent left ventricular scar (cut-off >12.6%); (B) Number of left ventricular segments with transmural scar (cut-off ≥2 segments).



8.4. Discussion

In a small, retrospective, observational pilot study, I have shown that indices of LV scar, quantified by LGE-CMR, are associated with the occurrence of appropriate ICD therapy in patients with CAD, independently of LVEF. In an analysis including clinical, biochemical and CMR variables, the number of transmural scar segments had the strongest association with the occurrence of appropriate ICD therapy. Furthermore, the burden of VA was significantly associated with scar burden.

These findings are consistent with those of previous studies. The amount of myocardial scar, identified by LGE-CMR, has been shown to predict all-cause mortality in a range of patient groups, including patients with a previous MI, patients with an ischaemic cardiomyopathy, and patients with vascular risk factors but without clinical evidence of a prior MI (186-188, 236, 241). As SCD accounts for a large proportion of deaths in post-MI and heart failure patients, it is plausible that scar quantification predicts the occurrence of VA (17, 37, 77). Scar quantification has also been shown to predict inducibility of VA at EPS, and thus identifies patients with the substrate for sustained VT. Bello *et al*. quantified scar using LGE-CMR in 48 patients with CAD and reduced LVEF undergoing EPS (242). Eighteen of these patients had inducible monomorphic VT, and infarct mass (as a percentage of the LV) ($p=0.009$) and infarct surface area ($p=0.002$) were the strongest predictors of inducibility.

The mechanistic relationship between ventricular scar and arrhythmogenesis is well established and post-mortem studies have suggested that scar burden may reflect susceptibility to VT (43, 243-245).

In my study, although percent scar was associated with the occurrence of appropriate ICD therapy, the strongest association was with the number of myocardial segments with full-thickness (75-100%) scar. The transmural extent of scar, as assessed by LGE-CMR, has been shown to predict both the long-term improvement in contractile function following MI and the response to revascularisation (246, 247). Interestingly, scar transmurality also predicts mortality in patients with a previous MI. Roes *et al*. quantified myocardial scar with LGE-CMR in 231 patients with a healed MI (189). Over a mean follow-up of 1.7 years 19 patients died. The amount of transmural scar, defined as extending from 51-100% of the LV wall thickness, was a significant predictor of death ($p=0.003$). These findings were confirmed by Kwon *et al*. in 349 patients with CAD and significantly reduced LVEF (188). During a mean follow-up of 2.6 years there were 56 events (51 deaths and 5 cardiac transplantations), and the amount of transmural

scar, defined as the number of segments in a 17-segment model with scar covering 51-100% of the LV wall thickness, was significantly higher in patients with an event (p=0.004).

Although the majority of previous LGE-CMR studies have used a binary approach to classify myocardium as scar or normal (remote) myocardium, a few studies have used a more graduated approach (248-250). It has been suggested that the border zone around an infarct (peri-infarct zone), which has an intermediate LGE-CMR signal intensity between the bright scar and dark remote myocardium, contains an admixture of normal and viable myocardium (248). The quantification of this peri-infarct zone by LGE-CMR has been found to predict mortality, inducibility of VA, and the occurrence of appropriate ICD therapy (248-250). However, there are a number of possible mechanisms that may contribute to the intermediate signal intensities found in this "peri-infarct" zone, and the histological extent of viable and scar tissue in comparison to the LGE-CMR findings needs validation (251).

Several methods are available for the measurement of LGE-CMR scar, ranging from a simple visual assessment, to quantitative assessment by planimetry of hyperenhanced areas (184). However, these methods can be time consuming, and are relatively operator-dependent. More recently, semi-automated methods have been developed in an attempt to improve objectivity. However, these also have limitations (184). One problem with the semi-automated quantification of myocardial infarction by LGE-CMR is the lack of a standard definition of scar. Many studies have defined scar as having a mean signal intensity of more than a multiple of standard deviations (usually 2 or 3) above an area of remote user-defined viable myocardium (241, 248). However, using this definition in my dataset resulted in a large overestimation of infarct size, which in part may be due to suboptimal signal suppression of remote myocardium, or image artifacts. Instead scar was defined as myocardium with a SI $\geq$50% of the maximum SI within an infarct region. This definition has been used by other researchers, and has been shown to correlate most accurately with infarct size in post-mortem studies (250, 252). There may be considerable clinical benefit in the development of accurate tools to enable 3-dimensional modelling of scar tissue for quantification.

Although LGE-CMR scar quantification is associated with outcomes independently of LVEF, it is unclear whether it gives incremental prognostic information in addition to other available complementary risk stratification tools such as EPS, the presence of NSVT on ambulatory monitoring and the assessment of cardiac sympathetic dennervation (50, 253). In addition, a consistent problem with most risk stratification tests designed to guide ICD use is their lack of specificity for SCD prediction. Although indicative of raised SCD risk, most risk stratification tools are also strong predictors of non-SCD mortality, which in the post-MI population is often

due to pump failure (81). No studies have evaluated the relationship of LGE-CMR scar quantification to non-SCD mortality, and its specificity remains unknown.

8.4.1. Limitations

This study has limitations. First, it is an observational study, and has all the limitations inherent in such a study design (254). Second, I included only a small number of patients, with a short follow-up. The results need confirming in a larger cohort with a longer follow-up.

Third, during the 4-year study period only 25% of new ICD implants for patients with CAD had a LGE-CMR prior to device implantation. Although the baseline demographics of ICD recipients who did and did not have a LGE-CMR were broadly similar, LGE-CMR is an expensive investigation and there may well be some selection bias related to local referral patterns not adequately captured by this baseline demographic data. In addition, patients with both primary and secondary prevention indications were included, and therefore the study cohort does not fully reflect the patient population (primary prevention patients) in whom risk stratification tests are most needed. Both of these factors may limit the generalisability of the results. Fourth, in this study the median LVEF, at 30%, is relatively high, likely reflecting the high proportion of secondary prevention patients (52%) included. Consequently there may be a relative underrepresentation of low LVEF patients in the study. This is a significant limitation, and, in view of the small number of patients included in the study, may limit the generalisability of my results to the low LVEF population.

Fifth, nine patients had revascularisation between their LGE-CMR and ICD implantation. Although this revascularisation would not have reduced the amount of LV scar, it may have altered the left ventricular volumes and LVEF, as well as potentially increased the amount of LV scar, compared to the values measured by the CMR and subsequently included in my analysis. Although I included “any previous pre-ICD revascularisation” as a variable in the multivariable analysis, this would not have adjusted for any changes in the variables between the LGE-CMR and ICD implantation, and as a consequence may have impacted on my results.

Lastly, although I have demonstrated that scar quantification using my methodology is reproducible, it is unclear how well it correlates with infarct size in my dataset. This problem is a general limitation of the methodology of scar quantification using semi-automated tools, and would benefit from a standardised scar definition, as well as further anatomical validation in additional datasets. However this study is hypothesis generating, as to my knowledge this is the first study to show correlation between scar burden and ventricular arrhythmia burden.

8.5. Conclusions

In this single centre pilot study of patients with CAD and ICDs, the extent of LV scar, characterised by LGE-CMR, is strongly associated with the occurrence of spontaneous ventricular arrhythmias. Its association is independent of LVEF. I hypothesise that LGE-CMR may be a valuable risk stratification tool to guide optimal ICD use. However, its specificity for SCD and its incremental prognostic value when used in addition to other available risk stratification tests are unknown, and need to be evaluated by an appropriately powered study using improved tools for automated and accurate scar quantification.

9. SUMMARY DISCUSSION

9.1. Summary Of Key Findings

9.1.1. The Epidemiology of ICD Use

My thesis addressed two important issues with respect to the epidemiology of ICD use in England and Wales. Firstly, based on the revised NICE guidelines, what is the actual requirement for ICD therapy for the primary prevention of SCD post-MI, and secondly, whether the prescription of ICD therapy is influenced by clinical setting.

My results suggest that there is significant underuse of ICD therapy in England and Wales. My study estimated that, using NICE guidance updated in 2006, the incidence of indications for ICD therapy for the primary prevention of SCD due to CAD was 29-39/million/year. A recent estimate of the incidence of ICD secondary prevention indications based on current NICE guidance was 76/million/year (69). Taken together these imply a combined ICD indication incidence in the region of 105-115/million/year. At the time of the study the overall new ICD implant rate in England and Wales was 40/million/year (2005). An increase in device use to achieve the estimated implantation rate based on my findings would have significant cost implications. Using the published NICE costs of £20102 per device, which includes follow-up appointments and replacement/repair costs (though based on 2004 costs), but not the potential cost saving of a reduction in cardiac events, would mean an extra cost of approximately £74-86 million ($152-177 million, 103-120 million Euro) per year to the NHS (199).

As well as significant under-provision of ICD therapy, there is also considerable regional variation in implant rates in England and Wales (73). The National Device Survey for 2006, demonstrated a nearly three-fold difference in new ICD implantation rates between the lowest (26/million) and highest (73/million) implanting regions (63). In order to improve ICD uptake it is important to understand the reasons underlying this regional variation.

My results suggest that the prescription of ICD therapy is significantly influenced by clinical setting. In my study, implant rates were approximately two-fold higher in areas whose local hospital was a regional cardiothoracic centre, than those whose local hospital was a DGH. The difference in implant rates was most significant with respect to CAD primary prevention indications, where overall implant rates were 2-3 times higher in an area served by a regional centre.

Over the 4-year study period the average annual ICD implant rate was 103/million in the area served by the Regional Cardiothoracic Centre and 48-49/million in the area served by a DGH.

My results, as well as those of other investigators, have estimated that the implantation rate based on published guidelines should be between 105-504/million/year (69, 70). It is therefore likely that these differences reflect an underuse in DGHs rather than an overuse in the regional centre, and in fact may actually represent an underuse in the regional centre as well, though not as great as in the DGHs. This is supported by the significantly higher implant rates in other European countries and North America.

The reasons underlying these findings are probably multifactorial. However an important issue is likely to be the limitations and complexity of current SCD risk stratification tools. LVEF estimation alone, while widely available and simple to apply, has low sensitivity and specificity for SCD. In contrast other risk stratification tests, such as EPS, microvolt T-wave alternans and tests of autonomic function, are complex, their availability limited and their role in selection of patients for ICD therapy unclear.

These observations set the scene for the development of new clinical processes to identify patients with most potential to benefit from ICD therapy.

9.1.2. The Role of Biomarkers To Guide the Use of ICD Therapy

My thesis evaluated the potential role of biomarkers in the selection of patients for ICD therapy in three different areas. First, the accuracy of the most studied individual biomarker, BNP (or NT-proBNP), in predicting SCD or the occurrence of ventricular arrhythmias. Second, the potential use of a proteomic approach, using SELDI-TOF MS, to identify prognostic biomarkers in patients with LVSD. Third, the value of biomarkers in identifying patients with greatest potential to gain benefit from ICD therapy.

The results of the meta-analysis suggest that BNP is a powerful indicator of SCD/VA risk, with a predictive power that is independent of reduced LVEF. In an analysis, including data on over 4500 patients, BNP predicted both the occurrence of SCD in patients without ICDs and the occurrence of VA in patients with devices. These results are consistent with findings from a previous systematic review, that demonstrated that BNP predicts overall mortality in patients with heart failure or asymptomatic LVSD, and laboratory studies, that have shown that myocardial stretch, of which BNP is a measure, results in several pro-arrhythmic electrophysiological changes (128, 216, 217).

Using proteomic techniques, I identified serum biomarkers that were differentially expressed in patients with systolic heart failure compared to controls, and in a prospective study demonstrated that these biomarkers predicted all-cause mortality in patients with ICDs on the

background of LVSD. Furthermore, although these proteomic biomarkers were associated with all-cause mortality, they were not associated with the occurrence of appropriate ICD therapy. These findings demonstrate proof-of-principle, and suggest that a proteomic approach may be useful in the identification of biomarkers that predict overall mortality, as well as mode of death, in patients with LVSD.

In a prospective study of ICD recipients, I found that both NT-proBNP and sST2 identified patients with advanced heart failure whose short-term mortality risk, even with an ICD, was high. The identification of patients whose heart failure is too advanced, and risk of non-sudden death too high, to gain significant benefit from an ICD, is an important aspect of the refinement of the application of ICD therapy. Although previous studies have found that biomarkers can predict mortality in patients with advanced LVSD, this is the first study to demonstrate that they may be able to identify patients whose mortality risk is too high to benefit from an ICD (111).

In addition to their poor predictive accuracy for, and lack of specificity for SCD, many currently available risk stratification tools have further limitations. Despite being the most widely used risk stratification test in clinical practice, the accurate assessment of LVEF is inaccurate with poor reproducibility (220). EPS, which is still used to guide ICD use in NICE guidelines, is invasive and only available in larger centres (72). Other tests, such as MTWA, require specialist equipment and expertise to perform.

In contrast, the use of biomarkers in a model to select patients for ICD therapy has potential benefits in comparison to these tools. Their measurement is cheap, reproducible, straightforward and non-invasive. Furthermore, rather than necessarily dichotomising risk as high or low, as many tests such as EPS or MTWA do, biomarkers can expresses risk as a continuum and this too may be advantageous. In addition, in patients with acquired heart disease, risk is a dynamic rather than static quantity, and the non-invasive nature of biomarker assessment lends itself to repeated measurement over time.

9.1.3. The Role of LGE-CMR In Patient Selection

My thesis evaluated the association of the extent of LV scar, quantified by LGE-CMR, and the occurrence of appropriate ICD therapy. In my study, LV scar quantification by LGE-CMR, was strongly associated with the occurrence of appropriate ICD therapy, as a surrogate for SCD, in patients with CAD. Its predictive accuracy was independent of LVEF. Furthermore, the burden of ventricular arrhythmias was significantly associated with scar burden, with patients with a greater extent of LV scar having an increased frequency of ventricular arrhythmias during follow-up.

My findings are consistent with those of previous studies in patients with CAD that have demonstrated an association between the amount of LV scar, identified by LGE-CMR, and both all-cause mortality and inducibility of ventricular arrhythmias at EPS (186-188, 236, 241, 242). They are also consistent with the mechanistic role of ventricular scar in the genesis of ventricular arrhythmias (43, 243-245).

9.2. Unresolved Questions And Future Research Recommendations

9.2.1. The Epidemiology of ICD Use

Since my studies were performed there has been a gradual increase in ICD implantation rates in the UK. The new ICD implant rate in England has risen from just over 40/million in 2005 to just over 60/million in 2008 and 2009 (255). However the implant rate is still significantly lower than elsewhere in Europe and there remains significant regional variation in ICD use (255). Furthermore, this is not just a UK problem, as the underuse and regional disparity are present throughout Europe and in some parts of North America (64, 66, 202).

It has been suggested that much of the underutilisation of ICD therapy relates to three factors: shortage of electrophysiologists, difficult or confusing financial circumstances, and poorly developed local referral strategies and care pathways (64). While research is unlikely to have an impact on the first two factors, understanding where the 'block' to patients being referred for ICD therapy is important. Though my results suggest that clinical setting has a significant impact on implant rates in the area served by Southampton University Hospitals, it is unclear to what degree this is a universal rather than a local problem. Furthermore, it is unclear if the problem relates to patients not being referred by general physicians, or from primary care, to cardiologists, or whether cardiologists are not referring patients on to the local implanting centre.

In order to improve implant rates and reduce regional disparity of ICD use in the UK, it is essential to better understand these factors, as well as the degree to which they impact on implant rates.

9.2.2. The Role of Biomarkers To Guide the use of ICD Therapy

The results of my studies suggest that in patients with LVSD individual biomarkers can predict all-cause mortality (NT-proBNP and sST2) and the occurrence of ventricular arrhythmias (NT-proBNP) in ICD recipients, as well as SCD (NT-proBNP) in patients without devices. As such they may be useful in identifying patients who are likely to gain most benefit from ICD therapy.

However, there are a number of unresolved issues that need addressing before they could be considered for clinical, rather than research use.

My work is the first to explore the use of biomarkers to identify patients whose risk of death is too high to gain significant benefit from ICD therapy. My findings need repeating in a larger cohort with longer follow-up, and patients enrolled at the time of initial device implantation, both to confirm my results, as well as establish the optimum biomarker cut-off values. Though my results suggest that the biomarkers NT-proBNP and sST2 provide prognostic information that is independent of age and creatinine, two of the individual markers that have been used in previous studies, it is unclear how they compare to the clinical risk models evaluated by Levy *et al*. and Goldenberg *et al.* (109, 110). For the biomarkers to be of clinical value they need to add incremental prognostic information in addition to these clinical risk models, and this needs to be established with further work. Furthermore, it is likely that future risk stratification models to select patients for ICD therapy will combine multiple tests that provide complementary information regarding sudden and non-sudden death risk, and therefore biomarkers should be evaluated in combination with multiple other tests, including established ones, such as LVEF estimation, and evolving ones, such as the assessment of cardiac sympathetic denervation (253).

My results also suggest that an approach using proteomic techniques may be useful in the identification of biomarkers that predict overall mortality, as well as mode of death, in LVSD. However my study provided only proof-of-principle and the findings need repeating in a larger cohort, with more exhaustive proteomic profiling and identification of the differentiating protein peaks.

9.2.3. The Role of LGE-CMR In Selection of Patients for ICD Therapy

The results of my study suggest that scar quantification, by LGE-CMR, may be a valuable risk stratification tool to guide optimal ICD use. However, there are a number of unresolved issues that need addressing prior to its more widespread clinical use.

First, its specificity for SCD, versus non-sudden death, is unknown. This is a universal problem with currently available SCD risk stratification tools, and it is unclear whether scar quantification by LGE-CMR has similar limitations.

Second, although in my dataset, as well as in other studies, LGE-CMR scar quantification predicts outcomes independently of LVEF, it is unclear whether it gives incremental prognostic information in addition to other available complementary risk stratification tools, or whether it could be used in combination with other tests.

Third, there is currently no standardised definition of myocardial scar on LGE-CMR. Previous studies have used differing methods of scar quantification, with varying definitions of scar. The methods available for the quantification of LV scar by LGE-CMR range from a simple visual assessment, to quantitative assessment by planimetry of hyperenhanced areas, and semi-automated detection of scar using specifically designed software, as used in my study (184). Although semi-automated scar detection is likely to be more objective than other methods of scar quantification its main limitation is a lack of a standard definition of scar. If LGE-CMR scar quantification is to become a useful clinical tool for the risk stratification of SCD, more standardisation of scar quantification methodology is needed.

Future research needs to evaluate LGE-CMR scar quantification prospectively, in conjunction with other contemporary risk stratification tools. Such a study would need to be appropriately powered to predict both sudden and non-sudden cardiac death, and use improved tools for automated and accurate scar quantification. A prospective, multicentre trial, evaluating the potential role of LGE-CMR-guided ICD therapy, is currently enrolling patients. The DETERMINE study is designed to test the hypothesis that ICD therapy improves survival in patients with a previous MI, LVEF >35%, but ≥10% LV scar on LGE-CMR (256). The study aims to enrol 1,550 patients over 36 months, and should answer some of these unresolved questions.

9.3. Limitations

Each study included in this thesis has specific limitations, and these are described in detail in the relevant chapters. However, there are also some general limitations common to more than one of the studies.

First, all of the studies, other than the meta-analysis, have been performed at a single institution. This is an important limitation, as it questions whether it is possible to generalise the results of my research to the wider population.

Second, three of the studies (Chapters 3, 4 and 8) were retrospective cohort studies. While such a study design is a relatively efficient way to address a research question it has important limitations. In comparison to prospective studies, retrospective analyses have significant risk of bias and confounding, and the results of the retrospective analyses included in my thesis are primarily hypothesis generating, rather than giving definitive answers to the research questions posed.

Third, other than the meta-analysis the studies included relatively small numbers of patients, limiting their statistical power. The prospective biomarker studies (Chapters 6 and 7) and the retrospective LGE-CMR study (Chapter 8) were in areas where there had been previously little published work, and as such were designed as pilot studies to demonstrate a proof-of-principle. As detailed earlier in this section, my findings need repeating in larger, prospective studies.

9.4. Conclusions

Despite well established national guidance, there is both significant underuse of ICD therapy and great regional variation in ICD prescription rates in England and Wales. The reasons for this are likely to be multifactorial, however in some areas clinical setting may have an important impact on implantation rates. Despite the availability of a great range of risk stratification tests, the selection of patients for ICD therapy remains a challenge, and there is significant need for better tools to guide ICD use. It is likely that future risk stratification models will combine multiple tests that provide complementary information regarding sudden and non-sudden death risk. Both serum/plasma biomarkers and the assessment of left ventricular scar by LGE-CMR may be valuable tools in such models. However, the incremental benefit of these tests in addition to currently available clinical risk prediction models remains unclear. My research indicates the need for prospective studies, powered to derive and validate risk stratification algorithms, incorporating biomarkers and LGE-CMR scar quantification, to predict potential to benefit from ICD therapy.

10. APPENDIX: DISSEMINATION OF FINDINGS

10.1.Publications

Scott PA, Gorman S, Andrews NP, Roberts PR, Kalra PR. Estimation of the requirement for implantable cardioverter defibrillators for the primary prevention of sudden cardiac death post-myocardial infarction based on UK national guidelines (2006). *Europace* 2008;**10**:453-7. http://europace.oxfordjournals.org/content/10/4/453.long

Scott PA, Barry J, Roberts PR, Morgan JM. Brain natriuretic peptide for the prediction of sudden cardiac death and ventricular arrhythmias: a meta-analysis. *European Journal of Heart Failure* 2009;**11**:958-66.
http://eurjhf.oxfordjournals.org/content/11/10/958.long

Scott PA, Turner NG, Chungh A, Morgan JM, Roberts PR. Varying implantable cardioverter defibrillator referral patterns from implanting and non-implanting hospitals. *Europace* 2009;**11**:1048-51.
http://europace.oxfordjournals.org/content/11/8/1048.long

Scott PA, Townsend PA, Ng LL, Zeb M, Harris S, Roderick PJ, Curzen NP, Morgan JM. Defining Potential To Benefit From Implantable Cardioverter Defibrillator Therapy – The Role of Biomarkers. *Europace* 2011;**13**:1419-27.

Scott PA, Morgan JM, Carroll N, Murday DC, Roberts PR, Peebles CR, Harden SP, Curzen NP. The Extent of Left Ventricular Scar Quantified By Late Gadolinium Enhancement MRI Is Associated With Spontaneous Ventricular Arrhythmias In Patients With Coronary Artery Disease And Implantable Cardioverter Defibrillators. *Circulation: Arrhythmia and Electrophysiology* 2011;**4**:324-30.

Scott PA, Zeidan B, Ng LL, Zeb M, Curzen NP, Townsend PA, Morgan JM. Proteomic Profiling To Identify Prognostic Biomarkers In Heart Failure. Submitted.

10.2.Presentations

Scott PA, Gorman S, Kalra PR. Estimation of the requirement for implantable cardiac defibrillator post myocardial infarction based on nice primary prevention guidelines (2006). Heart Rhythm Congress, Oct 2008.

Scott PA, Turner NG, Chungh A, Barry J, Yue AM, Roberts PR, Morgan JM. The Effect of Hospital Type on ICD Implantation Rates. Heart Rhythm Society, May 2009.

Scott PA, Barry J, Roberts PR, Morgan JM. A meta-analysis of Brain Natriuretic Peptide for the prediction of sudden cardiac death and ventricular arrhythmias. Heart Rhythm Society, May 2009.

Scott PA, Barry J, Roberts PR, Morgan JM. A meta-analysis of Brain Natriuretic Peptide for the prediction of sudden cardiac death and ventricular arrhythmias. Europace, June 2009.

Scott PA, Zeb M, Yue AM, Curzen NP, Townsend PA, Ng LL, Roberts PR, Morgan JM. The Use of Biomarkers to Refine Patient Selection For ICD therapy. Heart Rhythm Congress, Oct 2010.

Scott PA, Carroll N, Murday D, Zeb M, Yue AM, Roberts PR, Peebles C, Harden S, Morgan JM, Curzen NP. Scar Quantification Assessed by Contrast-Enhanced MRI Predicts Spontaneous Ventricular Arrhythmias In patients with Coronary Artery Disease and Implantable Cardioverter Defibrillators. Heart Rhythm Congress, Oct 2010.

Scott PA, Carroll N, Murday D, Zeb M, Yue AM, Roberts PR, Peebles C, Harden S, Morgan JM, Curzen NP. Scar Quantification Assessed by Contrast-Enhanced MRI Predicts Spontaneous Ventricular Arrhythmias In patients with Coronary Artery Disease and Implantable Cardioverter Defibrillators. Heart Rhythm Society, May 2011.

11. REFERENCES

1. Hunt SA, Abraham WT, Chin MH, Feldman AM, Francis GS, Ganiats TG, et al. 2009 Focused Update Incorporated Into the ACC/AHA 2005 Guidelines for the Diagnosis and Management of Heart Failure in Adults: A Report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines Developed in Collaboration With the International Society for Heart and Lung Transplantation. *Journal of the American College of Cardiology* 2009; **53**: e1-e90.

2. Dickstein K, Cohen-Solal A, Filippatos G, McMurray JJ, Ponikowski P, Poole-Wilson PA, et al. ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure 2008 The Task Force for the Diagnosis and Treatment of Acute and Chronic Heart Failure 2008 of the European Society of Cardiology. Developed in collaboration with the Heart Failure Association of the ESC (HFA) and endorsed by the European Society of Intensive Care Medicine (ESICM). *Eur J Heart Fail* 2008; **29**: 2388-2442.

3. Levy D, Kenchaiah S, Larson MG, Benjamin EJ, Kupka MJ, Ho KK, et al. Long-term trends in the incidence of and survival with heart failure. *N Engl J Med* 2002; **347**: 1397-1402.

4. Rosamond W, Flegal K, Friday G, Furie K, Go A, Greenlund K, et al. Heart disease and stroke statistics--2007 update: a report from the American Heart Association Statistics Committee and Stroke Statistics Subcommittee. *Circulation* 2007; **115**: e69-171.

5. Stewart S, Jenkins A, Buchan S, McGuire A, Capewell S, McMurray JJ. The current cost of heart failure to the National Health Service in the UK. *Eur J Heart Fail* 2002; **4**: 361-371.

6. Hogg K, Swedberg K, McMurray J. Heart failure with preserved left ventricular systolic function: epidemiology, clinical characteristics, and prognosis. *Journal of the American College of Cardiology* 2004; **43**: 317-327.

7. Wang TJ, Levy D, Benjamin EJ, Vasan RS. The epidemiology of "asymptomatic" left ventricular systolic dysfunction: implications for screening. *Ann Intern Med* 2003; **138**: 907-916.

8. Ho KK, Anderson KM, Kannel WB, Grossman W, Levy D. Survival after the onset of congestive heart failure in Framingham Heart Study subjects. *Circulation* 1993; **88**: 107-115.

9. Goldberg RJ, Ciampa J, Lessard D, Meyer TE, Spencer FA. Long-term survival after heart failure: a contemporary population-based perspective. *Arch Intern Med* 2007; **167**: 490-496.

10. Gottdiener JS, McClelland RL, Marshall R, Shemanski L, Furberg CD, Kitzman DW, et al. Outcome of congestive heart failure in elderly persons: influence of left ventricular systolic function. The Cardiovascular Health Study. *Ann Intern Med* 2002; **137**: 631-639.

11. McDonagh TA, Cunningham AD, Morrison CE, McMurray JJ, Ford I, Morton JJ, et al. Left ventricular dysfunction, natriuretic peptides, and mortality in an urban population. *Heart* 2001; **86**: 21-26.

12. Kober L, Bloch Thomsen PE, Moller M, Torp-Pedersen C, Carlsen J, Sandoe E, et al. Effect of dofetilide in patients with recent myocardial infarction and left-ventricular dysfunction: a randomised trial. *Lancet* 2000; **356**: 2052-2058.

13. Kober L, Torp-Pedersen C, Carlsen JE, Bagger H, Eliasen P, Lyngborg K, et al. A clinical trial of the angiotensin-converting-enzyme inhibitor trandolapril in patients with left ventricular dysfunction after myocardial infarction. Trandolapril Cardiac Evaluation (TRACE) Study Group. *N Engl J Med* 1995; **333**: 1670-1676.

14. Pfeffer MA, Braunwald E, Moye LA, Basta L, Brown EJ, Jr., Cuddy TE, et al. Effect of captopril on mortality and morbidity in patients with left ventricular dysfunction after myocardial infarction. Results of the survival and ventricular enlargement trial. The SAVE Investigators. *N Engl J Med* 1992; **327**: 669-677.

15. The SOLVD Investigators. Effect of enalapril on mortality and the development of heart failure in asymptomatic patients with reduced left ventricular ejection fractions. *N Engl J Med* 1992; **327**: 685-691.

16. Sharpe N, Murphy J, Smith H, Hannan S. Preventive treatment of asymptomatic left ventricular dysfunction following myocardial infarction. *Eur Heart J* 1990; **11 Suppl B**: 147-156.

17. Mozaffarian D, Anker SD, Anand I, Linker DT, Sullivan MD, Cleland JG, et al. Prediction of mode of death in heart failure: the Seattle Heart Failure Model. *Circulation* 2007; **116**: 392-398.

18. Kannel WB, Plehn JF, Cupples LA. Cardiac failure and sudden death in the Framingham Study. *American Heart Journal* 1988; **115**: 869-875.

19. Mehta PA, Dubrey SW, McIntyre HF, Walker DM, Hardman SM, Sutton GC, et al. Mode of death in patients with newly diagnosed heart failure in the general population. *Eur J Heart Fail* 2008; **10**: 1108-1116.

20. Poole-Wilson PA, Uretsky BF, Thygesen K, Cleland JG, Massie BM, Ryden L. Mode of death in heart failure: findings from the ATLAS trial. *Heart* 2003; **89**: 42-48.

21. Ezekowitz JA, Armstrong PW, McAlister FA. Implantable Cardioverter Defibrillators in Primary and Secondary Prevention: A Systematic Review of Randomized, Controlled Trials. *Ann Intern Med* 2003; **138**: 445-452.

22. Ezekowitz JA, Rowe BH, Dryden DM, Hooton N, Vandermeer B, Spooner C, et al. Systematic review: implantable cardioverter defibrillators for adults with left ventricular systolic dysfunction. *Ann Intern Med* 2007; **147**: 251-262.

23. Senni M, Tribouilloy CM, Rodeheffer RJ, Jacobsen SJ, Evans JM, Bailey KR, et al. Congestive heart failure in the community: a study of all incident cases in Olmsted County, Minnesota, in 1991. *Circulation* 1998; **98**: 2282-2289.

24. Mosterd A, Cost B, Hoes AW, de Bruijne MC, Deckers JW, Hofman A, et al. The prognosis of heart failure in the general population: The Rotterdam Study. *Eur Heart J* 2001; **22**: 1318-1327.

25. Narang R, Cleland JG, Erhardt L, Ball SG, Coats AJ, Cowley AJ, et al. Mode of death in chronic heart failure. A request and proposition for more accurate classification. *Eur Heart J* 1996; **17**: 1390-1403.

26. Goldman S, Johnson G, Cohn JN, Cintron G, Smith R, Francis G. Mechanism of death in heart failure. The Vasodilator-Heart Failure Trials. The V-HeFT VA Cooperative Studies Group. *Circulation* 1993; **87**: VI24-31.

27. Morgan JM, Cowan JC, Camm AJ, McComb JM. Sudden cardiac death: opportunities for prevention. *Heart* 2006; **92**: 721-723.

28. Zipes DP, Wellens HJJ. Sudden Cardiac Death. *Circulation* 1998; **98**: 2334-2351.

29. Hinkle LE, Jr., Thaler HT. Clinical classification of cardiac deaths. *Circulation* 1982; **65**: 457-464.

30. Pratt CM, Greenway PS, Schoenfeld MH, Hibben ML, Reiffel JA. Exploration of the Precision of Classifying Sudden Cardiac Death : Implications for the Interpretation of Clinical Trials. *Circulation* 1996; **93**: 519-524.

31. Kuller L, Lilienfeld A, Fisher R. An epidemiological study of sudden and unexpected deaths in adults. *Medicine (Baltimore)* 1967; **46**: 341-361.

32. de Vreede-Swagemakers JJ, Gorgels AP, Dubois-Arbouw WI, van Ree JW, Daemen MJ, Houben LG, et al. Out-of-hospital cardiac arrest in the 1990's: a population-based study in the Maastricht area on incidence, characteristics and survival. *J Am Coll Cardiol* 1997; **30**: 1500-1505.

33. Myerburg MDRJ, Interian A, Mitrani MDRM, Kessler MDKM, Castellanos MDA. Frequency of Sudden Cardiac Death and Profiles of Risk. *The American Journal of Cardiology* 1997; **80**: 10F-19F.

34. Wilde AA. Channelopathies in children and adults. *Pacing Clin Electrophysiol* 2008; **31 Suppl 1**: S41-45.

35. Myerburg RJ, Kessler KM, Castellanos A. Sudden cardiac death. Structure, function, and time-dependence of risk. *Circulation* 1992; **85**: I2-10.

36. Zipes DP, Camm AJ, Borggrefe M, Buxton AE, Chaitman B, Fromer M, et al. ACC/AHA/ESC 2006 Guidelines for Management of Patients With Ventricular Arrhythmias and the Prevention of Sudden Cardiac Death: A Report of the American College of Cardiology/American Heart Association Task Force and the European Society of Cardiology

Committee for Practice Guidelines (Writing Committee to Develop Guidelines for Management of Patients With Ventricular Arrhythmias and the Prevention of Sudden Cardiac Death). *Journal of the American College of Cardiology* 2006; **48**: e247-e346.

37. de Luna AB, Coumel P, Leclercq JF. Ambulatory sudden cardiac death: Mechanisms of production of fatal arrhythmia on the basis of data from 157 cases. *American Heart Journal* 1989; **117**: 151-159.

38. Huikuri HV, Castellanos A, Myerburg RJ. Sudden Death Due to Cardiac Arrhythmias. *N Engl J Med* 2001; **345**: 1473-1482.

39. Josephson M, Wellens HJ. Implantable defibrillators and sudden cardiac death. *Circulation* 2004; **109**: 2685-2691.

40. Huikuri HV, Raatikainen MJ, Moerch-Joergensen R, Hartikainen J, Virtanen V, Boland J, et al. Prediction of fatal or near-fatal cardiac arrhythmia events in patients with depressed left ventricular function after an acute myocardial infarction. *Eur Heart J* 2009; **30**: 689-698.

41. Tomaselli GF, Zipes DP. What Causes Sudden Death in Heart Failure? *Circ Res* 2004; **95**: 754-763.

42. Mehta D, Curwin J, Gomes JA, Fuster V. Sudden death in coronary artery disease: acute ischemia versus myocardial substrate. *Circulation* 1997; **96**: 3215-3223.

43. Haqqani HM, Marchlinski FE. Electrophysiologic substrate underlying postinfarction ventricular tachycardia: Characterization and role in catheter ablation. *Heart Rhythm* 2009; **6**: S70-S76.

44. Stevenson WG. Ventricular scars and ventricular tachycardia. *Trans Am Clin Climatol Assoc* 2009; **120**: 403-412.

45. Zeppenfeld K, Schalij MJ, Bartelings MM, Tedrow UB, Koplan BA, Soejima K, et al. Catheter ablation of ventricular tachycardia after repair of congenital heart disease: electroanatomic identification of the critical right ventricular isthmus. *Circulation* 2007; **116**: 2241-2252.

46. de Bakker JM, van Capelle FJ, Janse MJ, Tasseron S, Vermeulen JT, de Jonge N, et al. Slow conduction in the infarcted human heart. 'Zigzag' course of activation. *Circulation* 1993; **88**: 915-926.

47. Jouven X, Desnos M, Guerot C, Ducimetiere P. Predicting sudden death in the population: the Paris Prospective Study I. *Circulation* 1999; **99**: 1978-1983.

48. Mirowski M, Reid PR, Mower MM, Watkins L, Gott VL, Schauble JF, et al. Termination of malignant ventricular arrhythmias with an implanted automatic defibrillator in human beings. *N Engl J Med* 1980; **303**: 322-324.

49. Glikson M, Friedman PA. The implantable cardioverter defibrillator. *The Lancet* 2001; **357**: 1107-1117.

50. Epstein AE, Dimarco JP, Ellenbogen KA, Estes NA, 3rd, Freedman RA, Gettes LS, et al. ACC/AHA/HRS 2008 guidelines for Device-Based Therapy of Cardiac Rhythm Abnormalities: executive summary. *Heart Rhythm* 2008; **5**: 934-955.

51. The Antiarrhythmics versus Implantable Defibrillators (AVID) Investigators. A comparison of antiarrhythmic-drug therapy with implantable defibrillators in patients resuscitated from near-fatal ventricular arrhythmias. *N Engl J Med* 1997; **337**: 1576-1583.

52. Wever EFD, Hauer RNW, van Capelle FJL, Tijssen JGP, Crijns HJGM, Algra A, et al. Randomized Study of Implantable Defibrillator as First-Choice Therapy Versus Conventional Strategy in Postinfarct Sudden Death Survivors. *Circulation* 1995; **91**: 2195-2203.

53. Kuck KH, Cappato R, Siebels J, Ruppel R. Randomized comparison of antiarrhythmic drug therapy with implantable defibrillators in patients resuscitated from cardiac arrest : the Cardiac Arrest Study Hamburg (CASH). *Circulation* 2000; **102**: 748-754.

54. Connolly SJ, Gent M, Roberts RS, Dorian P, Roy D, Sheldon RS, et al. Canadian implantable defibrillator study (CIDS) : a randomized trial of the implantable cardioverter defibrillator against amiodarone. *Circulation* 2000; **101**: 1297-1302.

55. Lee DS, Green LD, Liu PP, Dorian P, Newman DM, Grant FC, et al. Effectiveness of implantable defibrillators for preventing arrhythmic events and death: A Meta-Analysis. *J Am Coll Cardiol* 2003; **41**: 1573-1582.

56. Nanthakumar K, Epstein AE, Kay GN, Plumb VJ, Lee DS. Prophylactic implantable cardioverter-defibrillator therapy in patients with left ventricular systolic dysfunction: A pooled analysis of 10 primary prevention trials. *J Am Coll Cardiol* 2004; **44**: 2166-2172.

57. Desai AS, Fang JC, Maisel WH, Baughman KL. Implantable defibrillators for the prevention of mortality in patients with nonischemic cardiomyopathy: a meta-analysis of randomized controlled trials. *Jama* 2004; **292**: 2874-2879.

58. Moss AJ, Zareba W, Hall WJ, Klein H, Wilber DJ, Cannom DS, et al. Prophylactic implantation of a defibrillator in patients with myocardial infarction and reduced ejection fraction. *N Engl J Med* 2002; **346**: 877-883.

59. Bardy GH, Lee KL, Mark DB, Poole JE, Packer DL, Boineau R, et al. Amiodarone or an implantable cardioverter-defibrillator for congestive heart failure. *N Engl J Med* 2005; **352**: 225-237.

60. Camm AJ, Nisam S. The Utilization of the Implantable Defibrillator--a European Enigma. *Eur Heart J* 2000; **21**: 1998-2004.

61. Ector H, Rickards AF, Kappenberger L, Linde C, Vardas P, Oto A, et al. The World Survey of Cardiac Pacing and Implantable Cardioverter Defibrillators: calendar year 1997--Europe. *Pacing Clin Electrophysiol* 2001; **24**: 863-868.

62. Bernstein AD, Parsonnet V. Survey of cardiac pacing and implanted defibrillator practice patterns in the United States in 1997. *Pacing Clin Electrophysiol* 2001; **24**: 842-855.

63. Network Devices Survey Group. Pacemakers and Implantable Defibrillators: UK National Survey 2006 (2007). www.devicesurvey.com.

64. Camm A, Nisam S. European utilization of the implantable defibrillator: has 10 years changed the 'enigma'? *Europace* 2010; **12**: 1063-1069.

65. Sans S, Kesteloot H, Kromhout D, on behalf of the Task F. The burden of cardiovascular diseases mortality in Europe: Task Force of the European Society of Cardiology on Cardiovascular Mortality and Morbidity Statistics in Europe. *Eur Heart J* 1997; **18**: 1231-1248.

66. Shah B, Hernandez AF, Liang L, Al-Khatib SM, Yancy CW, Fonarow GC, et al. Hospital Variation and Characteristics of Implantable Cardioverter-Defibrillator Use in Patients With Heart Failure: Data From the GWTG-HF (Get With The Guidelines-Heart Failure) Registry. *Journal of the American College of Cardiology* 2009; **53**: 416-422.

67. Sanders GD, Hlatky MA, Owens DK. Cost-Effectiveness of Implantable Cardioverter-Defibrillators. *N Engl J Med* 2005; **353**: 1471-1480.

68. Buxton M, Caine N, Chase D, Connelly D, Grace A, Jackson C, et al. A review of the evidence on the effects and costs of implantable cardioverter defibrillator therapy in different patient groups, and modelling of cost-effectiveness and cost-utility for these groups in a UK context. *Health Technol Assess* 2006; **10**: iii-iv, ix-xi, 1-164.

69. Plummer CJ, Irving RJ, McComb JM. The incidence of implantable cardioverter defibrillator indications in patients admitted to all coronary care units in a single district. *Europace* 2005; **7**: 266-272.

70. Plummer CJ, Irving RJ, McComb JM. Implications of national guidance for implantable cardioverter defibrillation implantation in the United Kingdom. *Pacing Clin Electrophysiol* 2003; **26**: 479-482.

71. Plummer CJ, McComb JM. An audit of the implications of implementing NICE guidance on the use of implantable cardioverter-defibrillators. *Heart* 2003; **89**: 787-788.

72. National Institute for Health and Clinical Excellence. Implantable cardioverter defibrillators for arrhythmias (2006). http://www.nice.org.uk/nicemedia/pdf/TA095.

73. Cunningham AD, Plummer CJ, McComb JM, Lord SW, Cunningham MW, Toussaint JM, et al. The implantable cardioverter-defibrillator: postcode prescribing in the UK 1998-2002. *Heart* 2005; **91**: 1280-1283.

74. Nichol G, Thomas E, Callaway CW, Hedges J, Powell JL, Aufderheide TP, et al. Regional Variation in Out-of-Hospital Cardiac Arrest Incidence and Outcome. *JAMA* 2008; **300**: 1423-1431.

75. Sanz G, Castaner A, Betriu A, Magrina J, Roig E, Coll S, et al. Determinants of prognosis in survivors of myocardial infarction: a prospective clinical angiographic study. *N Engl J Med* 1982; **306**: 1065-1070.

76. Bigger JT, Jr., Fleiss JL, Kleiger R, Miller JP, Rolnitzky LM. The relationships among ventricular arrhythmias, left ventricular dysfunction, and mortality in the 2 years after myocardial infarction. *Circulation* 1984; **69**: 250-258.

77. Solomon SD, Zelenkofske S, McMurray JJV, Finn PV, Velazquez E, Ertl G, et al. Sudden Death in Patients with Myocardial Infarction and Left Ventricular Dysfunction, Heart Failure, or Both. *N Engl J Med* 2005; **352**: 2581-2588.

78. Grimm W, Christ M, Bach J, Muller HH, Maisch B. Noninvasive arrhythmia risk stratification in idiopathic dilated cardiomyopathy: results of the Marburg Cardiomyopathy Study. *Circulation* 2003; **108**: 2883-2891.

79. Anderson KP, DeCamilla J, Moss AJ. Clinical significance of ventricular tachycardia (3 beats or longer) detected during ambulatory monitoring after myocardial infarction. *Circulation* 1978; **57**: 890-897.

80. Grimm W, Christ M, Maisch B. Long runs of non-sustained ventricular tachycardia on 24-hour ambulatory electrocardiogram predict major arrhythmic events in patients with idiopathic dilated cardiomyopathy. *Pacing Clin Electrophysiol* 2005; **28 Suppl 1**: S207-210.

81. Makikallio TH, Barthel P, Schneider R, Bauer A, Tapanainen JM, Tulppo MP, et al. Prediction of sudden cardiac death after acute myocardial infarction: role of Holter monitoring in the modern treatment era. *Eur Heart J* 2005; **26**: 762-769.

82. Bourke JP, Richards DA, Ross DL, Wallace EM, McGuire MA, Uther JB. Routine programmed electrical stimulation in survivors of acute myocardial infarction for prediction of spontaneous ventricular tachyarrhythmias during follow-up: results, optimal stimulation protocol and cost-effective screening. *J Am Coll Cardiol* 1991; **18**: 780-788.

83. Iesaka Y, Nogami A, Aonuma K, Nitta J, Chun Y-H, Fujiwara H, et al. Prognostic significance of sustained monomorphic ventricular tachycardia induced by programmed ventricular stimulation using up to triple extrastimuli in survivors of acute myocardial infarction. *The American Journal of Cardiology* 1990; **65**: 1057-1063.

84. Nogami A, Aonuma K, Takahashi A, Nitta J, Chun Y-H, Iesaka Y, et al. Usefulness of early versus late programmed ventricular stimulation in acute myocardial infarction. *The American Journal of Cardiology* 1991; **68**: 13-20.

85. Daubert JP, Zareba W, Hall WJ, Schuger C, Corsello A, Leon AR, et al. Predictive Value of Ventricular Arrhythmia Inducibility for Subsequent Ventricular Tachycardia or Ventricular Fibrillation in Multicenter Automatic Defibrillator Implantation Trial (MADIT) II Patients. *Journal of the American College of Cardiology* 2006; **47**: 98-107.

86. Buxton AE, Lee KL, DiCarlo L, Gold MR, Greer GS, Prystowsky EN, et al. Electrophysiologic Testing to Identify Patients with Coronary Artery Disease Who Are at Risk for Sudden Death. *N Engl J Med* 2000; **342**: 1937-1945.

87. Poll DS, Marchlinski FE, Buxton AE, Josephson ME. Usefulness of programmed stimulation in idiopathic dilated cardiomyopathy. *The American Journal of Cardiology* 1986; **58**: 992-997.

88. Meinertz T, Treese N, Kasper W, Geibel A, Hofmann T, Zehender M, et al. Determinants of prognosis in idiopathic dilated cardiomyopathy as determined by programmed electrical stimulation. *The American Journal of Cardiology* 1985; **56**: 337-341.

89. Shamim W, Francis DP, Yousufuddin M, Varney S, Pieopli MF, Anker SD, et al. Intraventricular conduction delay: a prognostic marker in chronic heart failure. *International Journal of Cardiology* 1999; **70**: 171-178.

90. Gehi AK, Stein RH, Metz LD, Gomes JA. Microvolt T-wave alternans for the risk stratification of ventricular tachyarrhythmic events: a meta-analysis. *J Am Coll Cardiol* 2005; **46**: 75-82.

91. Chow T, Kereiakes DJ, Onufer J, Woelfel A, Gursoy S, Peterson BJ, et al. Does Microvolt T-Wave Alternans Testing Predict Ventricular Tachyarrhythmias in Patients With Ischemic Cardiomyopathy and Prophylactic Defibrillators?: The MASTER (Microvolt T Wave Alternans Testing for Risk Stratification of Post-Myocardial Infarction Patients) Trial. *Journal of the American College of Cardiology* 2008; **52**: 1607-1615.

92. Goldberger JJ, Cain ME, Hohnloser SH, Kadish AH, Knight BP, Lauer MS, et al. American Heart Association/American College of Cardiology Foundation/Heart Rhythm Society Scientific Statement on Noninvasive Risk Stratification Techniques for Identifying Patients at Risk for Sudden Cardiac Death: A Scientific Statement From the American Heart Association Council on Clinical Cardiology Committee on Electrocardiography and Arrhythmias and Council on Epidemiology and Prevention. *Heart Rhythm* 2008; **5**: e1-e21.

93. Buxton AE, Lee KL, Hafley GE, Pires LA, Fisher JD, Gold MR, et al. Limitations of Ejection Fraction for Prediction of Sudden Death Risk in Patients With Coronary Artery Disease: Lessons From the MUSTT Study. *Journal of the American College of Cardiology* 2007; **50**: 1150-1157.

94. Buxton AE, Ellison KE, Lorvidhaya P, Ziv O. Left ventricular ejection fraction for sudden death risk stratification and guiding implantable cardioverter-defibrillators implantation. *J Cardiovasc Pharmacol* 2010; **55**: 450-455.

95. Stecker EC, Vickers C, Waltz J, Socoteanu C, John BT, Mariani R, et al. Population-Based Analysis of Sudden Cardiac Death With and Without Left Ventricular Systolic Dysfunction: Two-Year Findings from the Oregon Sudden Unexpected Death Study. *Journal of the American College of Cardiology* 2006; **47**: 1161-1166.

96. Germano JJ, Reynolds M, Essebag V, Josephson ME. Frequency and Causes of Implantable Cardioverter-Defibrillator Therapies: Is Device Therapy Proarrhythmic? *The American Journal of Cardiology* 2006; **97**: 1255-1261.

97. Theuns DA, Schaer BA, Soliman OI, Altmann D, Sticherling C, Geleijnse ML, et al. The prognosis of implantable defibrillator patients treated with cardiac resynchronization therapy: comorbidity burden as predictor of mortality. *Europace* 2011; **13**: 62-69.
98. Koller MT, Schaer B, Wolbers M, Sticherling C, Bucher HC, Osswald S. Death without prior appropriate implantable cardioverter-defibrillator therapy: a competing risk study. *Circulation* 2008; **117**: 1918-1926.
99. Bristow MR, Saxon LA, Boehmer J, Krueger S, Kass DA, De Marco T, et al. Cardiac-resynchronization therapy with or without an implantable defibrillator in advanced chronic heart failure. *N Engl J Med* 2004; **350**: 2140-2150.
100. Kadish A, Dyer A, Daubert JP, Quigg R, Estes NA, Anderson KP, et al. Prophylactic defibrillator implantation in patients with nonischemic dilated cardiomyopathy. *N Engl J Med* 2004; **350**: 2151-2158.
101. Hohnloser SH, Kuck KH, Dorian P, Roberts RS, Hampton JR, Hatala R, et al. Prophylactic use of an implantable cardioverter-defibrillator after acute myocardial infarction. *N Engl J Med* 2004; **351**: 2481-2488.
102. Tung R, Zimetbaum P, Josephson ME. A Critical Appraisal of Implantable Cardioverter-Defibrillator Therapy for the Prevention of Sudden Cardiac Death. *Journal of the American College of Cardiology* 2008; **52**: 1111-1121.
103. Cuculich PS, Sanchez JM, Kerzner R, Greenberg SL, Sengupta J, Chen J, et al. Poor prognosis for patients with chronic kidney disease despite ICD therapy for the primary prevention of sudden death. *Pacing Clin Electrophysiol* 2007; **30**: 207-213.
104. Bunch TJ, Hohnloser SH, Gersh BJ. Mechanisms of sudden cardiac death in myocardial infarction survivors: insights from the randomized trials of implantable cardioverter-defibrillators. *Circulation* 2007; **115**: 2451-2457.
105. Goldenberg I, Moss AJ, Hall WJ, McNitt S, Zareba W, Andrews ML, et al. Causes and consequences of heart failure after prophylactic implantation of a defibrillator in the multicenter automatic defibrillator implantation trial II. *Circulation* 2006; **113**: 2810-2817.
106. Pellegrini CN, Lee K, Olgin JE, Turakhia MP, Tseng ZH, Lee R, et al. Impact of advanced age on survival in patients with implantable cardioverter defibrillators. *Europace* 2008; **10**: 1296-1301.
107. Lee DS, Tu JV, Austin PC, Dorian P, Yee R, Chong A, et al. Effect of Cardiac and Noncardiac Conditions on Survival After Defibrillator Implantation. *Journal of the American College of Cardiology* 2007; **49**: 2408-2415.
108. Chan PS, Nallamothu BK, Spertus JA, Masoudi FA, Bartone C, Kereiakes DJ, et al. Impact of age and medical comorbidity on the effectiveness of implantable cardioverter-defibrillators for primary prevention. *Circ Cardiovasc Qual Outcomes* 2009; **2**: 16-24.

109. Goldenberg I, Vyas AK, Hall WJ, Moss AJ, Wang H, He H, et al. Risk Stratification for Primary Implantation of a Cardioverter-Defibrillator in Patients With Ischemic Left Ventricular Dysfunction. *Journal of the American College of Cardiology* 2008; **51**: 288-296.
110. Levy WC, Lee KL, Hellkamp AS, Poole JE, Mozaffarian D, Linker DT, et al. Maximizing Survival Benefit With Primary Prevention Implantable Cardioverter-Defibrillator Therapy in a Heart Failure Population. *Circulation* 2009; **120**: 835-842.
111. Braunwald E. Biomarkers in Heart Failure. *N Engl J Med* 2008; **358**: 2148-2159.
112. Anker SD, von Haehling S. Inflammatory mediators in chronic heart failure: an overview. *Heart* 2004; **90**: 464-470.
113. Rauchhaus M, Doehner W, Francis DP, Davos C, Kemp M, Liebenthal C, et al. Plasma Cytokine Parameters and Mortality in Patients With Chronic Heart Failure. *Circulation* 2000; **102**: 3060-3067.
114. Deswal A, Petersen NJ, Feldman AM, Young JB, White BG, Mann DL. Cytokines and Cytokine Receptors in Advanced Heart Failure : An Analysis of the Cytokine Database from the Vesnarinone Trial (VEST). *Circulation* 2001; **103**: 2055-2059.
115. Ungvari Z, Gupte SA, Recchia FA, Batkai S, Pacher P. Role of oxidative-nitrosative stress and downstream pathways in various forms of cardiomyopathy and heart failure. *Curr Vasc Pharmacol* 2005; **3**: 221-229.
116. Anker SD, Doehner W, Rauchhaus M, Sharma R, Francis D, Knosalla C, et al. Uric acid and survival in chronic heart failure: validation and application in metabolic, functional, and hemodynamic staging. *Circulation* 2003; **107**: 1991-1997.
117. Tang WHW, Brennan M-L, Philip K, Tong W, Mann S, Van Lente F, et al. Plasma Myeloperoxidase Levels in Patients With Chronic Heart Failure. *The American Journal of Cardiology* 2006; **98**: 796-799.
118. Kameda K, Matsunaga T, Abe N, Hanada H, Ishizaka H, Ono H, et al. Correlation of oxidative stress with activity of matrix metalloproteinase in patients with coronary artery disease: Possible role for left ventricular remodelling. *Eur Heart J* 2003; **24**: 2180-2185.
119. Mocatta TJ, Pilbrow AP, Cameron VA, Senthilmohan R, Frampton CM, Richards AM, et al. Plasma concentrations of myeloperoxidase predict mortality after myocardial infarction. *J Am Coll Cardiol* 2007; **49**: 1993-2000.
120. Cicoira M, Rossi A, Bonapace S, Zanolla L, Golia G, Franceschini L, et al. Independent and additional prognostic value of aminoterminal propeptide of type III procollagen circulating levels in patients with chronic heart failure. *Journal of Cardiac Failure* 2004; **10**: 403-411.
121. Schrier RW, Abraham WT. Hormones and Hemodynamics in Heart Failure. *N Engl J Med* 1999; **341**: 577-585.

122. Latini R, Masson S, Anand I, Salio M, Hester A, Judd D, et al. The comparative prognostic value of plasma neurohormones at baseline in patients with heart failure enrolled in Val-HeFT. *Eur Heart J* 2004; **25**: 292-299.
123. Horwich TB, Patel J, MacLellan WR, Fonarow GC. Cardiac Troponin I Is Associated With Impaired Hemodynamics, Progressive Left Ventricular Dysfunction, and Increased Mortality Rates in Advanced Heart Failure. *Circulation* 2003; **108**: 833-838.
124. Peacock WFIV, De Marco T, Fonarow GC, Diercks D, Wynne J, Apple FS, et al. Cardiac Troponin and Outcome in Acute Heart Failure. *N Engl J Med* 2008; **358**: 2117-2126.
125. Daniels LB, Maisel AS. Natriuretic peptides. *J Am Coll Cardiol* 2007; **50**: 2357-2368.
126. Yasue H, Yoshimura M, Sumida H, Kikuta K, Kugiyama K, Jougasaki M, et al. Localization and mechanism of secretion of B-type natriuretic peptide in comparison with those of A-type natriuretic peptide in normal subjects and patients with heart failure. *Circulation* 1994; **90**: 195-203.
127. Nakagawa O, Ogawa Y, Itoh H, Suga S, Komatsu Y, Kishimoto I, et al. Rapid transcriptional activation and early mRNA turnover of brain natriuretic peptide in cardiocyte hypertrophy. Evidence for brain natriuretic peptide as an "emergency" cardiac hormone against ventricular overload. *J Clin Invest* 1995; **96**: 1280-1287.
128. Doust JA, Pietrzak E, Dobson A, Glasziou P. How well does B-type natriuretic peptide predict death and cardiac events in patients with heart failure: systematic review. *Bmj* 2005; **330**: 625.
129. Rehman SU, Mueller T, Januzzi Jr JL. Characteristics of the Novel Interleukin Family Biomarker ST2 in Patients With Acute Heart Failure. *Journal of the American College of Cardiology* 2008; **52**: 1458-1465.
130. Weinberg EO, Shimpo M, De Keulenaer GW, MacGillivray C, Tominaga S, Solomon SD, et al. Expression and regulation of ST2, an interleukin-1 receptor family member, in cardiomyocytes and myocardial infarction. *Circulation* 2002; **106**: 2961-2966.
131. Sabatine MS, Morrow DA, Higgins LJ, MacGillivray C, Guo W, Bode C, et al. Complementary Roles for Biomarkers of Biomechanical Strain ST2 and N-Terminal Prohormone B-Type Natriuretic Peptide in Patients With ST-Elevation Myocardial Infarction. *Circulation* 2008; **117**: 1936-1944.
132. Bootcov MR, Bauskin AR, Valenzuela SM, Moore AG, Bansal M, He XY, et al. MIC-1, a novel macrophage inhibitory cytokine, is a divergent member of the TGF-beta superfamily. *Proc Natl Acad Sci U S A* 1997; **94**: 11514-11519.
133. Kempf T, Eden M, Strelau J, Naguib M, Willenbockel C, Tongers J, et al. The Transforming Growth Factor-{beta} Superfamily Member Growth-Differentiation Factor-15 Protects the Heart From Ischemia/Reperfusion Injury. *Circ Res* 2006; **98**: 351-360.

134. Xu J, Kimball TR, Lorenz JN, Brown DA, Bauskin AR, Klevitsky R, et al. GDF15/MIC-1 functions as a protective and antihypertrophic factor released from the myocardium in association with SMAD protein activation. *Circ Res* 2006; **98**: 342-350.
135. Kempf T, Horn-Wichmann R, Brabant G, Peter T, Allhoff T, Klein G, et al. Circulating concentrations of growth-differentiation factor 15 in apparently healthy elderly individuals and patients with chronic heart failure as assessed by a new immunoradiometric sandwich assay. *Clin Chem* 2007; **53**: 284-291.
136. Kempf T, von Haehling S, Peter T, Allhoff T, Cicoira M, Doehner W, et al. Prognostic Utility of Growth Differentiation Factor-15 in Patients With Chronic Heart Failure. *Journal of the American College of Cardiology* 2007; **50**: 1054-1060.
137. Foley PWX, Stegemann B, Ng K, Ramachandran S, Proudler A, Frenneaux MP, et al. Growth differentiation factor-15 predicts mortality and morbidity after cardiac resynchronization therapy. *Eur Heart J* 2009; **30**: 2749-2757.
138. Berger R, Huelsman M, Strecker K, Bojic A, Moser P, Stanek B, et al. B-type natriuretic peptide predicts sudden death in patients with chronic heart failure. *Circulation* 2002; **105**: 2392-2397.
139. Bayes-Genis A, Vazquez R, Puig T, Fernandez-Palomeque C, Fabregat J, Bardaji A, et al. Left atrial enlargement and NT-proBNP as predictors of sudden cardiac death in patients with heart failure. *Eur J Heart Fail* 2007; **9**: 802-807.
140. Tigen K, Karaahmet T, Kahveci G, Tanalp AC, Bitigen A, Fotbolcu H, et al. N-terminal pro brain natriuretic peptide to predict prognosis in dilated cardiomyopathy with sinus rhythm. *Heart Lung Circ* 2007; **16**: 290-294.
141. Watanabe J, Shinozaki T, Shiba N, Fukahori K, Koseki Y, Karibe A, et al. Accumulation of risk markers predicts the incidence of sudden death in patients with chronic heart failure. *Eur J Heart Fail* 2006; **8**: 237-242.
142. Winkler K, Wanner C, Drechsler C, Lilienthal J, Marz W, Krane V. Change in N-terminal-pro-B-type-natriuretic-peptide and the risk of sudden death, stroke, myocardial infarction, and all-cause mortality in diabetic dialysis patients. *Eur Heart J* 2008.
143. Pascual-Figal DA, Ordoñez-Llanos J, Tornel PL, Vázquez R, Puig T, Valdés M, et al. Soluble ST2 for Predicting Sudden Cardiac Death in Patients With Chronic Heart Failure and Left Ventricular Systolic Dysfunction. *Journal of the American College of Cardiology* 2009; **54**: 2174-2179.
144. Tapanainen JM, Lindgren KS, Makikallio TH, Vuolteenaho O, Leppaluoto J, Huikuri HV. Natriuretic peptides as predictors of non-sudden and sudden cardiac death after acute myocardial infarction in the beta-blocking era. *J Am Coll Cardiol* 2004; **43**: 757-763.

145. Albert CM, Ma J, Rifai N, Stampfer MJ, Ridker PM. Prospective Study of C-Reactive Protein, Homocysteine, and Plasma Lipid Levels as Predictors of Sudden Cardiac Death. *Circulation* 2002; **105**: 2595-2599.

146. Korngold EC, Januzzi JL, Jr., Gantzer ML, Moorthy MV, Cook NR, Albert CM. Amino-terminal pro-B-type natriuretic peptide and high-sensitivity C-reactive protein as predictors of sudden cardiac death among women. *Circulation* 2009; **119**: 2868-2876.

147. Blangy H, Sadoul N, Dousset B, Radauceanu A, Fay R, Aliot E, et al. Serum BNP, hs-C-reactive protein, procollagen to assess the risk of ventricular tachycardia in ICD recipients after myocardial infarction. *Europace* 2007; **9**: 724-729.

148. Budeus M, Reinsch N, Wieneke H, Sack S, Erbel R. The prediction of ICD therapy in multicenter automatic defibrillator implantation trial (MADIT) II like patients: a retrospective analysis. *Indian Pacing Electrophysiol J* 2008; **8**: 80-93.

149. Klein G, Lissel C, Fuchs AC, Gardiwal A, Oswald H, Desousa M, et al. Predictors of VT/VF-occurrence in ICD patients: results from the PROFIT-Study. *Europace* 2006; **8**: 618-624.

150. Klingenberg R, Zugck C, Becker R, Schellberg D, Heinze G, Kell R, et al. Raised B-type natriuretic peptide predicts implantable cardioverter-defibrillator therapy in patients with ischaemic cardiomyopathy. *Heart* 2006; **92**: 1323-1324.

151. Manios EG, Kallergis EM, Kanoupakis EM, Mavrakis HE, Kambouraki DC, Arfanakis DA, et al. Amino-terminal pro-brain natriuretic peptide predicts ventricular arrhythmogenesis in patients with ischemic cardiomyopathy and implantable cardioverter-defibrillators. *Chest* 2005; **128**: 2604-2610.

152. Nagahara D, Nakata T, Hashimoto A, Wakabayashi T, Kyuma M, Noda R, et al. Predicting the need for an implantable cardioverter defibrillator using cardiac metaiodobenzylguanidine activity together with plasma natriuretic peptide concentration or left ventricular function. *J Nucl Med* 2008; **49**: 225-233.

153. Yu H, Oswald H, Gardiwal A, Lissel C, Klein G. Comparison of N-terminal pro-brain natriuretic peptide versus electrophysiologic study for predicting future outcomes in patients with an implantable cardioverter defibrillator after myocardial infarction. *Am J Cardiol* 2007; **100**: 635-639.

154. Kanoupakis EM, Manios EG, Kallergis EM, Mavrakis HE, Goudis CA, Saloustros IG, et al. Serum markers of collagen turnover predict future shocks in implantable cardioverter-defibrillator recipients with dilated cardiomyopathy on optimal treatment. *J Am Coll Cardiol* 2010; **55**: 2753-2759.

155. Verma A, Kilicaslan F, Martin DO, Minor S, Starling R, Marrouche NF, et al. Preimplantation B-type natriuretic peptide concentration is an independent predictor of future appropriate implantable defibrillator therapies. *Heart* 2006; **92**: 190-195.

156. Arab S, Gramolini AO, Ping P, Kislinger T, Stanley B, van Eyk J, et al. Cardiovascular Proteomics: Tools to Develop Novel Biomarkers and Potential Applications. *Journal of the American College of Cardiology* 2006; **48**: 1733-1741.
157. Gerszten RE, Wang TJ. The search for new cardiovascular biomarkers. *Nature* 2008; **451**: 949-952.
158. Monteoliva L, Albar JP. Differential proteomics: an overview of gel and non-gel based approaches. *Brief Funct Genomic Proteomic* 2004; **3**: 220-239.
159. Merchant M, Weinberger SR. Recent advancements in surface-enhanced laser desorption/ionization-time of flight-mass spectrometry. *Electrophoresis* 2000; **21**: 1164-1177.
160. Petricoin EF, Ardekani AM, Hitt BA, Levine PJ, Fusaro VA, Steinberg SM, et al. Use of proteomic patterns in serum to identify ovarian cancer. *Lancet* 2002; **359**: 572-577.
161. Papadopoulos MC, Abel PM, Agranoff D, Stich A, Tarelli E, Bell BA, et al. A novel and accurate diagnostic test for human African trypanosomiasis. *The Lancet* 2004; **363**: 1358-1363.
162. Adam BL, Qu Y, Davis JW, Ward MD, Clements MA, Cazares LH, et al. Serum protein fingerprinting coupled with a pattern-matching algorithm distinguishes prostate cancer from benign prostate hyperplasia and healthy men. *Cancer Res* 2002; **62**: 3609-3614.
163. Li J, Zhang Z, Rosenzweig J, Wang YY, Chan DW. Proteomics and bioinformatics approaches for identification of serum biomarkers to detect breast cancer. *Clin Chem* 2002; **48**: 1296-1304.
164. Poon TCW, Sung JJY, Chow SM, Ng EKW, Yu ACW, Chu ESH, et al. Diagnosis of Gastric Cancer by Serum Proteomic Fingerprinting. *Gastroenterology* 2006; **130**: 1858-1864.
165. Diamandis EP. Mass Spectrometry as a Diagnostic and a Cancer Biomarker Discovery Tool: Opportunities and Potential Limitations. *Mol Cell Proteomics* 2004; **3**: 367-378.
166. Ransohoff DF. Lessons from controversy: ovarian cancer screening and serum proteomics. *J Natl Cancer Inst* 2005; **97**: 315 - 319.
167. Check E. Proteomics and cancer: running before we can walk? *Nature* 2004; **429**: 496-497.
168. Baggerly KA, Morris JS, Edmonson SR, Coombes KR. Signal in noise: evaluating reported reproducibility of serum proteomic tests for ovarian cancer. *J Natl Cancer Inst* 2005; **97**: 307-309.
169. Ransohoff DF. Rules of evidence for cancer molecular-marker discovery and validation. *Nat Rev Cancer* 2004; **4**: 309-314.
170. Agranoff D, Fernandez-Reyes D, Papadopoulos MC, Rojas SA, Herbster M, Loosemore A, et al. Identification of diagnostic markers for tuberculosis by proteomic fingerprinting of serum. *The Lancet* 2006; **368**: 1012-1021.

171. Gravett MG, Novy MJ, Rosenfeld RG, Reddy AP, Jacob T, Turner M, et al. Diagnosis of Intra-amniotic Infection by Proteomic Profiling and Identification of Novel Biomarkers. *JAMA* 2004; **292**: 462-469.

172. Mohri Y, Mohri T, Wei W, Qi YJ, Martin A, Miki C, et al. Identification of macrophage migration inhibitory factor and human neutrophil peptides 1-3 as potential biomarkers for gastric cancer. *Br J Cancer* 2009; **101**: 295-302.

173. Zhang Z, Bast RC, Yu Y, Li J, Sokoll LJ, Rai AJ, et al. Three biomarkers identified from serum proteomic analysis for the detection of early stage ovarian cancer. *Cancer Res* 2004; **64**: 5882 - 5890.

174. Trocme C, Marotte H, Baillet A, Pallot-Prades B, Garin J, Grange L, et al. Apolipoprotein A-I and platelet factor 4 are biomarkers for infliximab response in rheumatoid arthritis. *Ann Rheum Dis* 2009; **68**: 1328-1333.

175. Tuñón J, Martín-Ventura JL, Blanco-Colio LM, Lorenzo Ó, López JA, Egido J. Proteomic Strategies in the Search of New Biomarkers in Atherothrombosis. *Journal of the American College of Cardiology*; **55**: 2009-2016.

176. Mateos-Cáceres PJ, García-Méndez A, López Farré A, Macaya C, Núñez A, Gómez J, et al. Proteomic analysis of plasma from patients during an acute coronary syndrome. *Journal of the American College of Cardiology* 2004; **44**: 1578-1583.

177. Brea D, Sobrino T, Blanco M, Fraga M, Agulla J, Rodriguez-Yanez M, et al. Usefulness of haptoglobin and serum amyloid A proteins as biomarkers for atherothrombotic ischemic stroke diagnosis confirmation. *Atherosclerosis* 2009; **205**: 561-567.

178. Wilson AM, Kimura E, Harada RK, Nair N, Narasimhan B, Meng X-Y, et al. β2-Microglobulin as a Biomarker in Peripheral Arterial Disease: Proteomic Profiling and Clinical Studies. *Circulation* 2007; **116**: 1396-1403.

179. Vahitha BA-S, Gideon AP, Ali JO, Simon RG, Dinah R, Graham WT, et al. Identification of plasma protein biomarkers associated with idiopathic pulmonary arterial hypertension. *PROTEOMICS* 2006; **6**: 2286-2294.

180. Abdul-Salam VB, Paul GA, Ali JO, Gibbs SR, Rahman D, Taylor GW, et al. Identification of plasma protein biomarkers associated with idiopathic pulmonary arterial hypertension. *Proteomics* 2006; **6**: 2286-2294.

181. Willingale R, Jones DJ, Lamb JH, Quinn P, Farmer PB, Ng LL. Searching for biomarkers of heart failure in the mass spectra of blood plasma. *Proteomics* 2006; **6**: 5903-5914.

182. Jones DJL, Willingale R, Quinn PA, Lamb JH, Farmer PB, Davies JE, et al. Improving the Diagnostic Accuracy of N-Terminal B-Type Natriuretic Peptide in Human Systolic Heart Failure by Plasma Profiling Using Mass Spectrometry. *Journal of Proteome Research* 2007; **6**: 3329-3334.

183. Pinet F, Beseme O, Cieniewski-Bernard C, Drobecq H, Jourdain S, Lamblin N, et al. Predicting left ventricular remodeling after a first myocardial infarction by plasma proteome analysis. *Proteomics* 2008; **8**: 1798-1808.

184. Kim HW, Farzaneh-Far A, Kim RJ. Cardiovascular Magnetic Resonance in Patients With Myocardial Infarction: Current and Emerging Applications. *Journal of the American College of Cardiology* 2009; **55**: 1-16.

185. Pereira RS, Prato FS, Wisenberg G, Sykes J, Yvorchuk KJ. The use of Gd-DTPA as a marker of myocardial viability in reperfused acute myocardial infarction. *Int J Cardiovasc Imaging* 2001; **17**: 395-404.

186. Wu E, Judd RM, Vargas JD, Klocke FJ, Bonow RO, Kim RJ. Visualisation of presence, location, and transmural extent of healed Q-wave and non-Q-wave myocardial infarction. *The Lancet* 2001; **357**: 21-28.

187. Mahrholdt H, Wagner A, Holly TA, Elliott MD, Bonow RO, Kim RJ, et al. Reproducibility of chronic infarct size measurement by contrast-enhanced magnetic resonance imaging. *Circulation* 2002; **106**: 2322-2327.

188. Kwon DH, Halley CM, Carrigan TP, Zysek V, Popovic ZB, Setser R, et al. Extent of Left Ventricular Scar Predicts Outcomes in Ischemic Cardiomyopathy Patients With Significantly Reduced Systolic Function: A Delayed Hyperenhancement Cardiac Magnetic Resonance Study. *JACC: Cardiovascular Imaging* 2009; **2**: 34-44.

189. Roes SD, Kelle S, Kaandorp TAM, Kokocinski T, Poldermans D, Lamb HJ, et al. Comparison of Myocardial Infarct Size Assessed With Contrast-Enhanced Magnetic Resonance Imaging and Left Ventricular Function and Volumes to Predict Mortality in Patients With Healed Myocardial Infarction. *The American Journal of Cardiology* 2007; **100**: 930-936.

190. Cheong BYC, Muthupillai R, Wilson JM, Sung A, Huber S, Amin S, et al. Prognostic Significance of Delayed-Enhancement Magnetic Resonance Imaging: Survival of 857 Patients With and Without Left Ventricular Dysfunction. *Circulation* 2009; **120**: 2069-2076.

191. Southampton City Primary Care Trust. Hampshire & Isle of Wight local health comparisons 2008: Chapter 9 Circulatory disease. http://www.southamptonhealth.nhs.uk/aboutus/publichealth/hi/**.**

192. Zamora J, Abraira V, Muriel A, Khan K, Coomarasamy A. Meta-DiSc: a software for meta-analysis of test accuracy data. *BMC Med Res Methodol* 2006; **6**: 31.

193. Schoenfeld D. Partial residuals for the proportional hazards regression model. *Biometrika* 1982; **69**: 239-241.

194. Hohnloser SH, Klingenheben T, Zabel M, Schöpperl M, Mauß O. Prevalence, characteristics and prognostic value during long-term follow-up of nonsustained ventricular tachycardia after myocardial infarction in the thrombolytic era. *Journal of the American College of Cardiology* 1999; **33**: 1895-1902.

195. Senges JC, Becker R, Schreiner KD, Bauer A, Weretka S, Siegler K, et al. Variability of Holter electrocardiographic findings in patients fulfilling the noninvasive MADIT criteria. Multicenter Automatic Defibrillator Implantation Trial. *Pacing Clin Electrophysiol* 2002; **25**: 183-190.

196. Wichterle D, Simek J, Camm J, Malik M. Predictive characteristics of holter-based postinfarction risk stratifiers appear superior to electrophysiological testing. *Pacing Clin Electrophysiol* 2005; **28 Suppl 1**: S182-186.

197. Maggioni AP, Zuanetti G, Franzosi MG, Rovelli F, Santoro E, Staszewsky L, et al. Prevalence and prognostic significance of ventricular arrhythmias after acute myocardial infarction in the fibrinolytic era. GISSI-2 results. *Circulation* 1993; **87**: 312-322.

198. Network Devices Survey Group. Pacemakers and Implantable Defibrillators: A Two Year National Survey for 2003 and 2004 (2007). http://www.devicesurvey.com/.

199. National Institute for Health and Clinical Excellence. Arrhythmia - implantable cardioverter defibrillators: Analysis of cost impact (2006). http://guidance.nice.org.uk/TA95/CostingReport/xls/English.

200. Buxton AE, Lee KL, Fisher JD, Josephson ME, Prystowsky EN, Hafley G. A randomized study of the prevention of sudden death in patients with coronary artery disease. Multicenter Unsustained Tachycardia Trial Investigators. *N Engl J Med* 1999; **341**: 1882-1890.

201. Cleland JG, Tageldien A, Maarouf N, Hobson N. Patients with heart failure who require an implantable defibrillator should have cardiac resynchronisation routinely. *Heart* 2008; **94**: 963-966.

202. Birnie DH, Sambell C, Johansen H, Williams K, Lemery R, Green MS, et al. Use of implantable cardioverter defibrillators in Canadian and US survivors of out-of-hospital cardiac arrest. *Cmaj* 2007; **177**: 41-46.

203. Berwick DM. Disseminating Innovations in Health Care. *JAMA* 2003; **289**: 1969-1975.

204. DerSimonian R, Laird N. Meta-analysis in clinical trials. *Control Clin Trials* 1986; **7**: 177-188.

205. Egger M, Smith GD, Phillips AN. Meta-analysis: principles and procedures. *Bmj* 1997; **315**: 1533-1537.

206. Deville WL, Buntinx F, Bouter LM, Montori VM, de Vet HC, van der Windt DA, et al. Conducting systematic reviews of diagnostic studies: didactic guidelines. *BMC Med Res Methodol* 2002; **2**: 9.

207. Lijmer JG, Bossuyt PM, Heisterkamp SH. Exploring sources of heterogeneity in systematic reviews of diagnostic tests. *Stat Med* 2002; **21**: 1525-1537.

208. Higgins JP, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses. *Bmj* 2003; **327**: 557-560.

209. Begg CB, Mazumdar M. Operating characteristics of a rank correlation test for publication bias. *Biometrics* 1994; **50**: 1088-1101.
210. Christ M, Sharkova J, Bayrakcioglu S, Herzum I, Mueller C, Grimm W. B-type natriuretic peptide levels predict event-free survival in patients with implantable cardioverter defibrillators. *Eur J Heart Fail* 2007; **9**: 272-279.
211. Konstantino Y, Kusniec J, Reshef T, David-Zadeh O, Mazur A, Strasberg B, et al. Inflammatory biomarkers are not predictive of intermediate-term risk of ventricular tachyarrhythmias in stable CHF patients. *Clin Cardiol* 2007; **30**: 408-413.
212. Vrtovec B, Okrajsek R, Golicnik A, Ferjan M, Starc V, Schlegel TT, et al. Atorvastatin Therapy May Reduce the Incidence of Sudden Cardiac Death in Patients With Advanced Chronic Heart Failure. *Journal of Cardiac Failure* 2008; **14**: 140-144.
213. Gardiwal A, Yu H, Oswald H, Luesebrink U, Ludwig A, Pichlmaier AM, et al. Right ventricular pacing is an independent predictor for ventricular tachycardia/ventricular fibrillation occurrence and heart failure events in patients with an implantable cardioverter-defibrillator. *Europace* 2008; **10**: 358-363.
214. Simon T, Becker R, Voss F, Bikou O, Hauck M, Licka M, et al. Elevated B-type natriuretic peptide levels in patients with nonischemic cardiomyopathy predict occurrence of arrhythmic events. *Clin Res Cardiol* 2008; **97**: 306-309.
215. Berger R, Huelsmann M, Strecker K, Moertl D, Moser P, Bojic A, et al. Neurohormonal risk stratification for sudden death and death owing to progressive heart failure in chronic heart failure. *Eur J Clin Invest* 2005; **35**: 24-31.
216. Reiter MJ. Effects of mechano-electrical feedback: potential arrhythmogenic influence in patients with congestive heart failure. *Cardiovasc Res* 1996; **32**: 44-51.
217. Franz MR, Cima R, Wang D, Profitt D, Kurz R. Electrophysiological effects of myocardial stretch and mechanical determinants of stretch-activated arrhythmias. *Circulation* 1992; **86**: 968-978.
218. Bailey JJ, Berson AS, Handelsman H, Hodges M. Utility of current risk stratification tests for predicting major arrhythmic events after myocardial infarction. *Journal of the American College of Cardiology* 2001; **38**: 1902-1911.
219. Marcos RdS, Carlos AM, FÃ¡bio TR, AntÃ´nio MNF, Antonio LPR. Non-sustained ventricular tachycardia as a predictor of sudden cardiac death in patients with left ventricular dysfunction: A meta-analysis. *European Journal of Heart Failure* 2008; **10**: 1007-1014.
220. McGowan JH, Cleland JGF. Reliability of reporting left ventricular systolic function by echocardiography: A systematic review of 3 methods. *American Heart Journal* 2003; **146**: 388-397.

221. Ellenbogen KA, Levine JH, Berger RD, Daubert JP, Winters SL, Greenstein E, et al. Are implantable cardioverter defibrillator shocks a surrogate for sudden cardiac death in patients with nonischemic cardiomyopathy? *Circulation* 2006; **113**: 776-782.

222. Moayyedi P. Meta-analysis: Can we mix apples and oranges? *Am J Gastroenterol* 2004; **99**: 2297-2301.

223. Scott PA, Barry J, Roberts PR, Morgan JM. Brain natriuretic peptide for the prediction of sudden cardiac death and ventricular arrhythmias: a meta-analysis. *Eur J Heart Fail* 2009; **11**: 958-966.

224. Kanoupakis EM, Manios EG, Kallergis EM, Mavrakis HE, Goudis CA, Saloustros IG, et al. Serum markers of collagen turnover predict future shocks in implantable cardioverter-defibrillator recipients with dilated cardiomyopathy on optimal treatment. *J Am Coll Cardiol*; **55**: 2753-2759.

225. Streitner F, Kuschyk J, Veltmann C, Ratay D, Schoene N, Streitner I, et al. Role of proinflammatory markers and NT-proBNP in patients with an implantable cardioverter-defibrillator and an electrical storm. *Cytokine* 2009; **47**: 166-172.

226. Khan SQ, Ng K, Dhillon O, Kelly D, Quinn P, Squire IB, et al. Growth differentiation factor-15 as a prognostic marker in patients with acute myocardial infarction. *Eur Heart J* 2009; **30**: 1057-1065.

227. Anand IS, Latini R, Florea VG, Kuskowski MA, Rector T, Masson S, et al. C-Reactive Protein in Heart Failure: Prognostic Value and the Effect of Valsartan. *Circulation* 2005; **112**: 1428-1434.

228. Ng LL, Pathik B, Loke IW, Squire IB, Davies JE. Myeloperoxidase and C-reactive protein augment the specificity of B-type natriuretic peptide in community screening for systolic heart failure. *American Heart Journal* 2006; **152**: 94-101.

229. Omland T, Persson A, Ng L, O'Brien R, Karlsson T, Herlitz J, et al. N-Terminal Pro-B Type Natriuretic Peptide and Long-Term Mortality in Acute Coronary Syndromes. *Circulation* 2002; **106**: 2913-2918.

230. Wilkoff BL, Williamson BD, Stern RS, Moore SL, Lu F, Lee SW, et al. Strategic Programming of Detection and Therapy Parameters in Implantable Cardioverter-Defibrillators Reduces Shocks in Primary Prevention Patients: Results From the PREPARE (Primary Prevention Parameters Evaluation) Study. *Journal of the American College of Cardiology* 2008; **52**: 541-550.

231. Cleland JG, Khand A, Clark A. The heart failure epidemic: exactly how big is it? *Eur Heart J* 2001; **22**: 623-626.

232. Zeidan BA, Cutress RI, Murray N, Coulton GR, Hastie C, Packham G, et al. Proteomic analysis of archival breast cancer serum. *Cancer Genomics Proteomics* 2009; **6**: 141-147.

233. Sharma R, Francis DP, Pitt B, Poole-Wilson PA, Coats AJ, Anker SD. Haemoglobin predicts survival in patients with chronic heart failure: a substudy of the ELITE II trial. *Eur Heart J* 2004; **25**: 1021-1028.

234. Anderson L. Candidate-based proteomics in the search for biomarkers of cardiovascular disease. *J Physiol* 2005; **563**: 23-60.

235. Yasui Y, Pepe M, Thompson ML, Adam BL, Wright GL, Jr., Qu Y, et al. A data-analytic strategy for protein biomarker discovery: profiling of high-dimensional proteomic data for cancer detection. *Biostatistics* 2003; **4**: 449-463.

236. Wu E, Ortiz JT, Tejedor P, Lee DC, Bucciarelli-Ducci C, Kansal P, et al. Infarct size by contrast enhanced cardiac magnetic resonance is a stronger predictor of outcomes than left ventricular ejection fraction or end-systolic volume index: prospective cohort study. *Heart* 2008; **94**: 730-736.

237. National Institute for Health and Clinical Excellence. Cardiac resynchronisation therapy for the treatment of heart failure (2007). http://www.nice.org.uk/Guidance/TA120.

238. Rosset A, Spadola L, Ratib O. OsiriX: an open-source software for navigating in multidimensional DICOM images. *J Digit Imaging* 2004; **17**: 205-216.

239. Cerqueira MD, Weissman NJ, Dilsizian V, Jacobs AK, Kaul S, Laskey WK, et al. Standardized myocardial segmentation and nomenclature for tomographic imaging of the heart. A statement for healthcare professionals from the Cardiac Imaging Committee of the Council on Clinical Cardiology of the American Heart Association. *Int J Cardiovasc Imaging* 2002; **18**: 539-542.

240. Bradburn MJ, Clark TG, Love SB, Altman DG. Survival analysis Part III: multivariate data analysis -- choosing a model and assessing its adequacy and fit. *Br J Cancer* 2003; **89**: 605-611.

241. Kwong RY, Chan AK, Brown KA, Chan CW, Reynolds HG, Tsang S, et al. Impact of Unrecognized Myocardial Scar Detected by Cardiac Magnetic Resonance Imaging on Event-Free Survival in Patients Presenting With Signs or Symptoms of Coronary Artery Disease. *Circulation* 2006; **113**: 2733-2743.

242. Bello D, Fieno DS, Kim RJ. Infarct morphology identifies patients with substrate for sustained ventricular tachycardia. *J Am Coll Cardiol* 2005; **45**: 1104 - 1108.

243. Bolick D, Hackel D, Reimer K, Ideker R. Quantitative analysis of myocardial infarct structure in patients with ventricular tachycardia. *Circulation* 1986; **74**: 1266 - 1279.

244. Miller JM, Harken AH, Hargrove WC, Josephson ME. Pattern of endocardial activation during sustained ventricular tachycardia. *J Am Coll Cardiol* 1985; **6**: 1280-1287.

245. de Bakker JM, van Capelle FJ, Janse MJ, Wilde AA, Coronel R, Becker AE, et al. Reentry as a cause of ventricular tachycardia in patients with chronic ischemic heart disease: electrophysiologic and anatomic correlation. *Circulation* 1988; **77**: 589-606.

246. Choi KM, Kim RJ, Gubernikoff G, Vargas JD, Parker M, Judd RM. Transmural extent of acute myocardial infarction predicts long-term improvement in contractile function. *Circulation* 2001; **104**: 1101-1107.

247. Kim R, Wu E, Rafael A. The use of contrast-enhanced magnetic resonance imaging to identify reversible myocardial dysfunction. *N Engl J Med* 2000; **343**: 1445 - 1453.

248. Yan AT, Shayne AJ, Brown KA, Gupta SN, Chan CW, Luu TM, et al. Characterization of the Peri-Infarct Zone by Contrast-Enhanced Cardiac Magnetic Resonance Imaging Is a Powerful Predictor of Post-Myocardial Infarction Mortality. *Circulation* 2006; **114**: 32-39.

249. Schmidt A, Azevedo CF, Cheng A, Gupta SN, Bluemke DA, Foo TK, et al. Infarct Tissue Heterogeneity by Magnetic Resonance Imaging Identifies Enhanced Cardiac Arrhythmia Susceptibility in Patients With Left Ventricular Dysfunction. *Circulation* 2007; **115**: 2006-2014.

250. Roes SD, Borleffs CJW, van der Geest RJ, Westenberg JJM, Marsan NA, Kaandorp TAM, et al. Infarct Tissue Heterogeneity Assessed With Contrast-Enhanced MRI Predicts Spontaneous Ventricular Arrhythmia in Patients With Ischemic Cardiomyopathy and Implantable Cardioverter-Defibrillator. *Circ Cardiovasc Imaging* 2009; **2**: 183-190.

251. Klocke FJ, Wu E, Lee DC. "Shades of gray" in cardiac magnetic resonance images of infarcted myocardium: can they tell us what we'd like them to? *Circulation* 2006; **114**: 8-10.

252. Amado LC, Gerber BL, Gupta SN, Rettmann DW, Szarf G, Schock R, et al. Accurate and objective infarct sizing by contrast-enhanced magnetic resonance imaging in a canine myocardial infarction model. *Journal of the American College of Cardiology* 2004; **44**: 2383-2389.

253. Boogers MJ, Borleffs CJW, Henneman MM, van Bommel RJ, van Ramshorst J, Boersma E, et al. Cardiac Sympathetic Denervation Assessed With 123-Iodine Metaiodobenzylguanidine Imaging Predicts Ventricular Arrhythmias in Implantable Cardioverter-Defibrillator Patients. *Journal of the American College of Cardiology* 2010; **55**: 2769-2777.

254. Jepsen P, Johnsen SP, Gillman MW, SÃ¸rensen HT. Interpretation of observational studies. *Heart* 2004; **90**: 956-960.

255. Network Devices Survey Group. Heart Rhythm Devices: UK National Clinical Audit 2009 (2010). www.devicesurvey.com**.**

256. Kadish AH, Bello D, Finn JP, Bonow RO, Schaechter A, Subacius H, et al. Rationale and design for the Defibrillators to Reduce Risk by Magnetic Resonance Imaging Evaluation (DETERMINE) trial. *J Cardiovasc Electrophysiol* 2009; **20**: 982-987.