The General Population using

Natriuretic Peptides and the Electrocardiogran

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Screening for Left Ventricular Dysfunction in the General Population using Natriuretic Peptides and the Electrocardiogram

Dr Ian Loke

Abstract

Subjects without a prior diagnosis of heart failure or left ventricular systolic dysfunction (LVSD) were randomly selected from a general population in Leicestershire (n=1360). All subjects had a transthoracic echocardiogram performed as well as a standard 12 lead electrocardiogram (ECG). Serum levels of N-ANP, BNP and N-BNP were analysed. There were twenty-eight cases of left ventricular systolic dysfunction (2.1% of the population), defined as a wall motion score index of >1.8, equivalent to an ejection fraction of ≤ 40%. Serum levels of all three cardiac peptides were significantly elevated in subjects with LVSD. The prevalence of electrocardiographic abnormalities was also significantly higher in the LVSD population. Using multivariate logistic regression analysis, ECG abnormalities, including a prolonged QRS duration, as well as N-ANP, BNP and N-BNP independently predicted LVSD. I then analyzed the performance of cardiac peptides and the ECG in diagnosing LVSD. All three cardiac peptides had high negative predictive values but low positive predictive values. The ECG performed less well as compared to cardiac peptides in diagnosing LVSD. BNP consistently performed better than N-ANP and N-BNP. Combining the ECG to any of the three cardiac peptides improved the diagnostic utility for LVSD. In an economic analysis, BNP used alone was more cost effective than other peptides, the ECG as well as a sequential application of BNP and the ECG in diagnosing LVSD.

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Author's declaration

I confirm that this thesis is a true reflection of data collected by myself. All material presented is my own work and is original in all aspects except where other sources are credited. All references were consulted personally by myself and the literature search was up to date as of July 2007.

Dr. Ian Loke

The material in my thesis has been previous published or and/or presented in the following:

1. Ng LL, Loke IW, Davies JE, Geeranavar S, Khunti K, Stone MA, Chin DT, Squire IB. Community screening for left ventricular systolic dysfunction using plasma and urinary natriuretic peptides. J Am Coll Cardiol. 2005 Apr 5;45(7):1043-50.

2. Ng LL, Loke I, Davies JE, Khunti K, Stone M, Abrams KR, Chin DT, Squire IB. Identification of previously undiagnosed left ventricular systolic dysfunction: community screening using natriuretic peptides and electrocardiography. Eur J Heart Fail. 2003 Dec;5(6):775-82.

3. Ng LL, Loke IW, O'Brien RJ, Squire IB, Davies JE. Plasma urocortin in human systolic heart failure. Clin Sci (Lond). 2004 Apr;106(4):383-8.

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Frequently Used Abbreviations

ALVSD	**Asymptomatic Left Ventricular Systolic dysfunction**
ANP	**Atrial Natriuretic Peptide**
AUC	**Area Under Curve**
N-ANP	**N-Terminal Atrial Natriuretic Peptide**
BNP	**Brain Natriuretic Peptide**
CHF	**Congestive Heart Failure**
DHF	**Diastolic Heart Failure**
ECG	**Electrocardiogram**
IHD	**Ischaemic Heart Disease**
LV	**Left Ventricular**
LVEF	**Left Ventricular Ejection Fraction**
LVH	**Left Ventricular Hypertrophy**
LVSD	**Left Ventricular Systolic Dysfunction**
NP	**Natriuretic Peptide**
N-BNP	**N-Terminal Brain Natriuretic Peptide**
ROC	**Receiver Operator Curve**
WMSI	**Wall Motion Score Index**

Units used

Levels of BNP and N-BNP are presented in my study in units of fmol.

For BNP, 1 fmol = 3.466 pg

And for, N-BNP 1fmol = 8.457 pg

Table of Contents

Figures and Tables

10 Table 4.2. Population characteristics of the upper 10^{th} centile of natriuretic peptide levels compared to the rest of the population. P values for comparison between upper 10^{th} centile and remainder of population: ¶ $P<0.05$ by Chi square test. Valvular abnormalities – severe aortic stenosis/regurgitation, mitral regurgitation/stenosis, severe tricuspid regurgitation.

11 Figures 3.2 a-c. Frequency histograms of N-ANP, BNP and N-BNP

12 Figures 4.3a, b,c. Boxplots of N-ANP, BNP and N-BNP levels in normal subjects and Table 5.1 Data presented as incidence of Major ECG abnormalities (percentage as part of the population) and mean values ±SD. Abnormalities are not mutually exclusive. P values for comparisons between LVSD and normal group: * $P<0.05$ for all, using Chi Square test, $\sum$ $P<0.05$ using Mann Whitney test.

13 Table 5.2. Logistic regression for prediction of LVSD. Variables entered forward and backward.

14 Figure 5.1. Distribution of QRS duration (msec) in population. Best fit curve shown.

15 Figure 5.2. Boxplots showing distribution of mean QRS duration in subjects with normal LV function and subjects with WMS>1.8 (LVSD). 90% and 95% confidence intervals shown. Mean values shown.

16 Figure 5.3. ROC curve for detection of LVSD by QRS duration

17 AUC 0.763 (0.65 – 0.88 95% CI, SE 0.59)

18 Table 5.3. Performance of QRS duration and ECG abnormalities in diagnosing LVSD. PPV-positive predictive value. NPV – Negative predictive value. + LR – Positive likelihood ratio. –LR – Negative predictive value.

19 Figure 5.4a, b. ROC curves for detection of LVSD by BNP and N-BNP, comparing QRS duration and logistic model combining peptide and QRS duration. Tables show area under curve (AUC).

20 Table 6.1. Demographic features of the study population free of cardiovascular disease (n=720)

21 Table 6.2 Results of univariate analysis (r_2, Spearman's Rho) of clinical and echocardiographic variables with plasma natriuretic peptides

22 Table 6.3 – Results of multiple linear regression analysis. Correlation coefficients for the correlation of clinical variables with N-ANP, BNP and N-BNP (n=720)

Chapter 1

Introduction

Heart failure – Pathophysiology,

Epidemiology and

The Case for Screening

1.1 Heart Failure: Definition and Overview

Heart failure is best defined as a clinical syndrome that consists of a variety of symptoms such as fatigue, oedema and dyspnoea, which is caused by a failure of the heart to adequately perfuse the tissues during exertion. The primary cause of this is an impaired emptying action or filling phase of the heart. The symptoms of heart failure are largely due to the unmet metabolic demands of the body. The relationship between the degree of symptoms experienced and the actual degree of left ventricular impairment is complex and very far from linear in nature. The European task force on the diagnosis and treatment of heart failure defines chronic heart failure (CHF) as "a syndrome where the patient should have the following features: symptoms of heart failure, typically breathlessness or fatigue, either at rest or on exertion, or ankle oedema and objective evidence of cardiac dysfunction at rest" [1]. A clinical response to treatment is insufficient grounds for a diagnosis. It is important to distinguish between acute heart failure, characterized by either pulmonary oedema and/or low cardiac output due to cardiogenic shock, and chronic heart failure, which is altogether a more insidious and is characterized by haemodynamic, neuroendocrine, renal and hormonal adaptive changes.

Heart failure is one of the most common causes of mortality and morbidity in the developed world [2]. It is a significant economic burden to the state and is the most common cause of hospital admission in the over 65yrs age group. Heart failure also has a major impact on the individual's quality of life [3]. Despite the advent of new therapeutic strategies and drug treatments, the incidence of heart failure is expected to rise and prognosis in severe CHF continues to be very poor.

The underlying pathology behind CHF is largely due to left ventricular systolic dysfunction (LVSD) or "pump output failure". Over the last decade, there has been

considerable interest in the category of diastolic heart failure (DHF). This is a separate entity from LVSD, having different pathophysiological process, but being difficult to diagnose accurately as there is considerable overlap.

The challenge we face in the 21st century is not only the early and precise diagnosis of heart failure but perhaps, even more significantly, to seek to make a diagnosis of cardiac dysfunction even before the onset of symptoms.

1.2 Pathophysiology of Heart Failure

It is beyond the scope of this thesis to go into a detailed discussion of this complex area. The integrity of the arterial circulation, as determined by cardiac output and peripheral arterial resistance, is closely monitored by various neurohormanal systems that act in concert to maintain a delicate balance. The trigger in this cascade of events is an event that causes a reduction in arterial filling and can be due to variety of events (Fig 1.1). Arterial underfilling is detected by a variety of means (Fig 1.2). The baro-receptor mediated increase in sympathetic tone leads to an increase in cardiac contractility, tachycardia and increased peripheral vasoconstriction. This also has local selective vasoconstriction in the kidneys. Activation of the crucial renin-angiotensin system by increased sympathetic drive and vasoactive neurohormones causes sodium and water retention by the kidneys as well as peripheral and local vasoconstriction (Fig 1.2). Other active neurohormones include vasopressin, natriuretic peptides, nitric oxide, prostacyclin, prostglandins and cytokines, such as tumour necrosis factor. The short-term consequence of these compensatory mechanisms is to restore arterial filling. However, over the long term, these neurohormonal reflexes may have deleterious effects that

include pulmonary edema, hyponatremia, increased cardiac afterload and preload and cardiac remodeling. The reduction in cardiac output leads to symptoms of CHF.

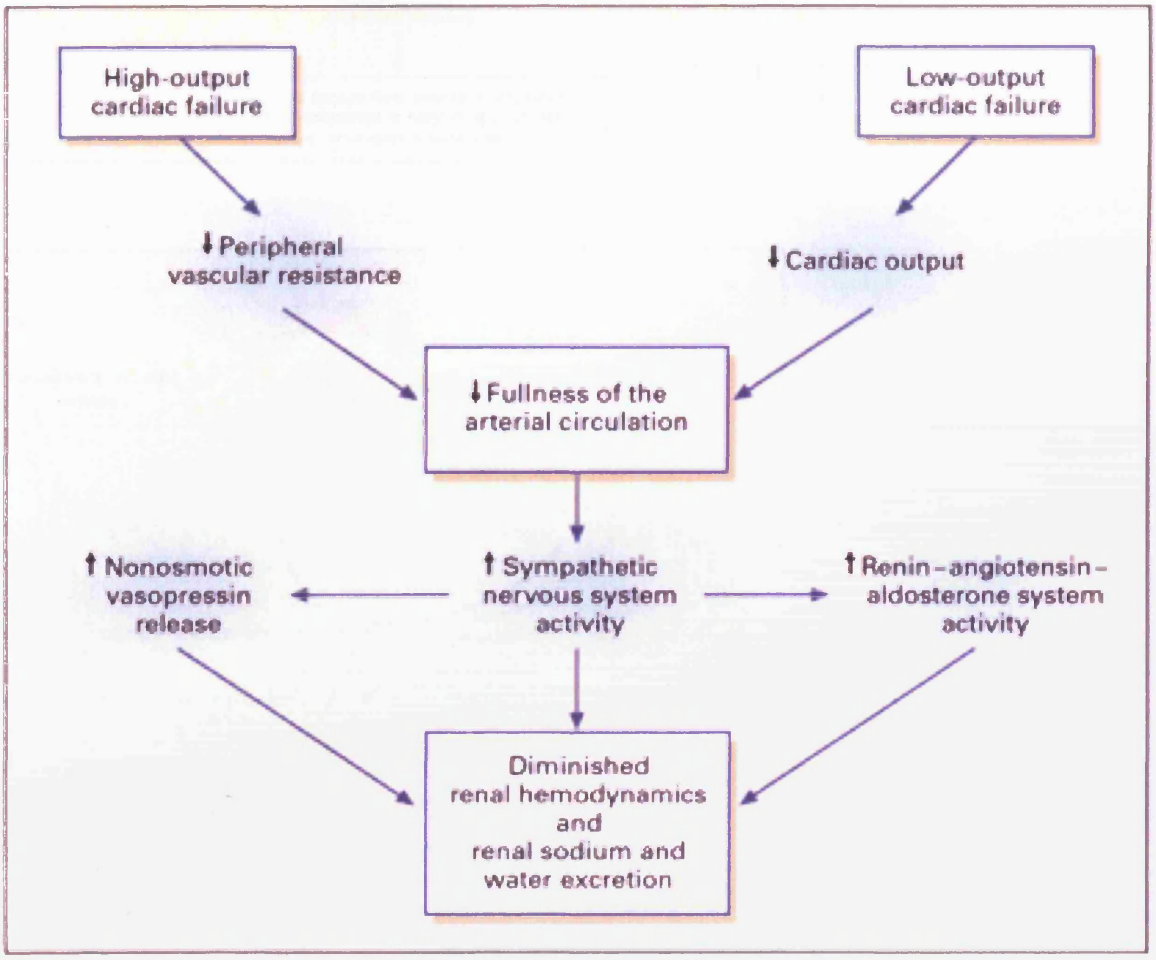
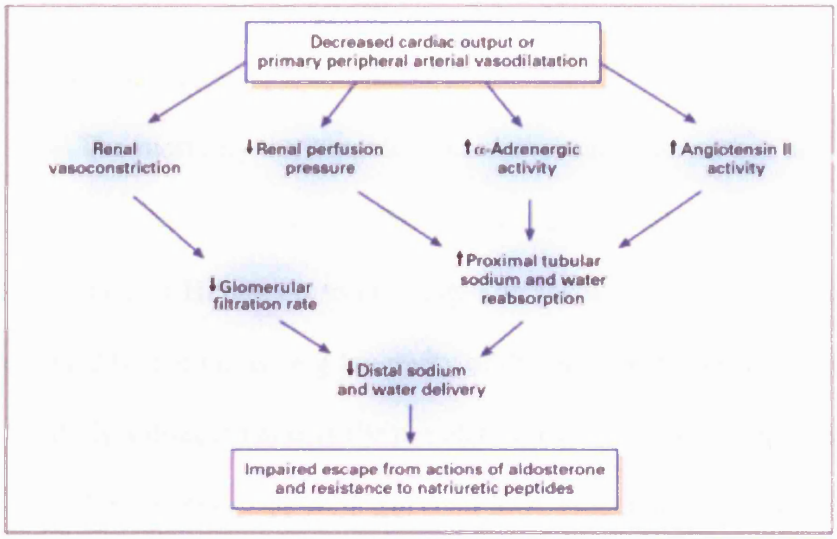


Figure 1.1. Causes and Consequences of Arterial Underfilling in Patients with Heart Failure, Including the Mechanisms Activated to Counterbalance the Underfilling

Schrier R and Abraham W. N Engl J Med 1999;341:577-585



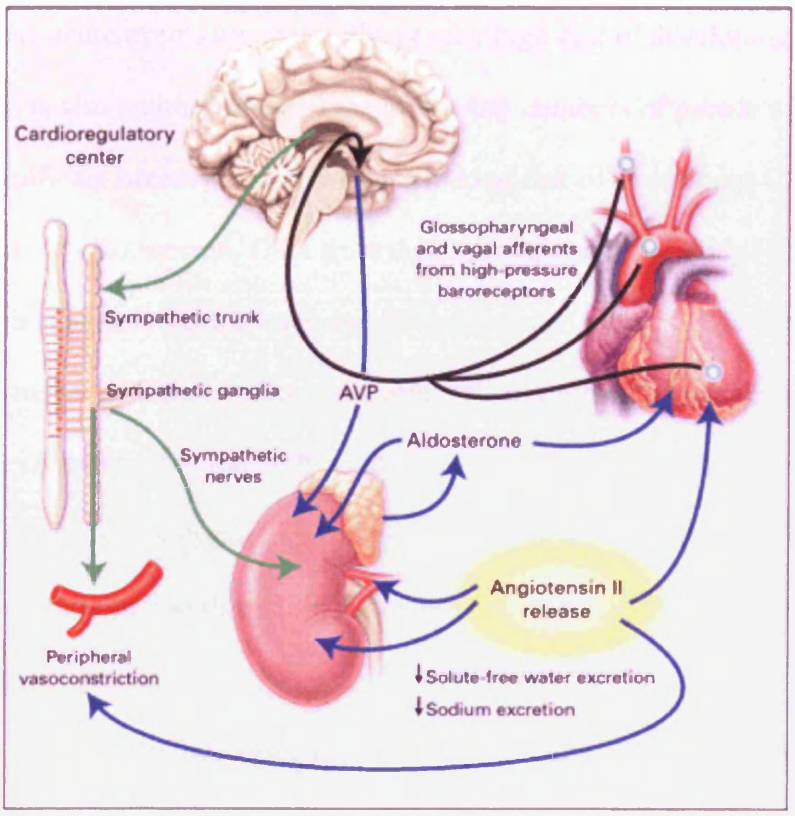


Figure 1. 2a and b. Mechanisms by Which High-Output or Low-Output Heart Failure Leads to the Activation of Neurohormonal Vasoconstrictor Systems and Renal Sodium and Water Retention Schrier R and Abraham W. N Engl J Med 1999;341:577-585

1.3. Epidemiology

The mortality attributable to cardiovascular causes has declined over the last 20-30 years mainly due to improved therapeutic measures [3, 4]. Despite that, the incidence and the prevalence of CHF are set to increase. The absolute increase in numbers can be partially explained by the increasing longevity of the population. Heart failure is far more common in the elderly subset (5) and as the population lives longer, the number of people who develop heart failure increases as well. Survival following acute coronary events, particularly in myocardial infarction has also improved drastically over the last 20 years [5]. Survivors of these acute events are, naturally, at very high risk of developing subsequent heart failure and this is also another cause of the increasing numbers of people with heart failure. CHF is a very significant health problem with a life-long risk of developing CHF estimated at 16% for men and 10% for women. Data from the Framingham heart study [6] shows that it develops in approximately 16% of men and 18% of women who have diabetes; 12% of men and 8% of women who have hypertension; and 30% of both sexes who have myocardial infarction. Over the second half of the 20th century, there has been a striking increase in the frequency of coronary artery disease and diabetes as aetiological factors for CHF, whereas the impact of hypertension and rheumatic valve disease has declined [7].

Two studies looked at trends in hospitalisation in the US and Scotland [8, 9]. Both groups reached a conclusion that the prevalence of CHF was rising despite better treatment and more that admissions and re-admissions were rising to create an "epidemic of heart failure". Investigators (8) looking again at the public health problem of CHF in the US, predicted a

future rise in the prevalence and CHF, particularly in the elderly. The authors suggested that mortality was delayed with little difference in the incidence. Subsequently, this would lead to more morbidity despite a overall reduction in annual mortality. An aging population with a much higher prevalence of the disease would accentuate that problem. Haldeman et al. looked at the US National Hospital Discharge Survey in 1999 [10]. Over 1985 to 1995, the number of hospitalizations increased from 577,000 to 871,000 (an increase of 34%) for a first-listed diagnosis of CHF and from 1.7 to 2.6 million for any diagnosis of heart failure. This study also concluded that the incidence and prevalence was set to increase in the future. This patient group is particularly at risk of re-admissions. Of 332 subjects were followed up after their index admission for CHF, 72% were re-admitted in a 19-month follow up period [11].

1.3.1 Prevalence

The changes in the definition of CHF have made the diagnosis of CHF more precise but at the same time, more difficult. The Heart of England Study [12], Glasgow MONICA [13] and the Rotterdam Studies [14] defined heart failure in their study populations on the basis of symptoms and objective evidence of LVSD, in this case using an echocardiogram to estimate an ejection fraction, valvular disease or arrhythmias such as atrial fibrillation. This would be in-line with European guidelines in the definition of CHF [1] and hence, these three studies would provide us with the most accurate figures as regards the prevalence of CHF.

The Heart of England study based in the UK West Midlands [12] studied 6286 subjects and assessed the population aged over 45years, for both symptoms as well as objective echocardiographic assessment of left ventricular function. CHF, as defined by an ejection fraction of <40% and symptoms, was found to be present in 3.2%. 1.8% was due to LVSD and

the rest, 1.4%, was due to atrial fibrillation and/or valvular disease. Only half of the subjects with definite LVSD had symptoms, giving a prevalence of symptomatic LVSD of 0.96%. In the1999 Rotterdam study [14], 5540 subjects were screened for LVSD resulting in an overall prevalence of 3.4%. Again only 40% of these were symptomatic at the time. The1997 MONICA study in Scotland studied 1540 subjects in Glasgow [13]. The prevalence of LVSD was relatively high compared to the studies in Rotterdam [14] and the West Midlands [12], being 3.9% under a 30% ejection fraction (1.5% being symptomatic and 1.4% asymptomatic) and 7.7% under an ejection fraction of 35%. This may be partially explained by the fact that a Scottish population would be at higher risk of ischaemic heart disease. The overall prevalence of CHF in the community aged over 45yrs, defined as the presence of symptoms as well as objective evidence of cardiac dysfunction would probably range between 3.2% to 7.7%.

1.3.2 Incidence

Cowie et al [15] reported that the incidence of heart failure (defined by clinical assessment as well as radiological, electrographic and echocardiographic assessment techniques) in a population if 151 000 in London was 1.3 per 1000 population per year. The incidence rate increased from 0.02 cases per 1000 population per year in those aged 25-34 years to 11.6 in those aged 85 years and over. The age adjusted incidence rate was 1.75 and the median age at presentation was 76 years. This compares to an incidence rate of 1.1 new cases per 1000 population in the Olmsted community study [16], which looked at a US population in 1981. The longest-term study looking at incidence of CHF would be the Framingham study [17], which followed 5209 patients over a course of 40 years. During the 1980s, the annual age-adjusted incidence of congestive heart failure among persons aged 45yrs and over was 7.2

cases/1,000 in men and 4.7 cases/1,000 in women. At 34 years follow up, the incidence of heart failure was approximately 2 new cases/1000 in persons aged 45-54 years, increasing to 40 new cases/1000 in men aged 85-94 years. Using similar criteria, the study of men born in 1913 reported incidence rates of "manifest" heart failure of 1.5, 4.3, and 10.2 new cases/1000 in men aged 50-54, 55-60, and 61-67 years, respectively. Both the Framingham [17] and the Olmsted Minnesota [16] studies mainly used clinical parameters to define CHF.

Increasing age is associated with a higher prevalence of CHF. Hobbs [12], using an ejection fraction of less than 40% as a cut-off found a prevalence of 0.3% in the 45-54Yrs group and 3.6% in the over 75years. These findings are in-line with the Glasgow MONICA (13) study. A cross sectional study based in the UK [18]looked at the prevalence of CHF in a general population aged 70-84 found an overall prevalence of 7.5% of mild, moderate and severe LVSD. The mean age of people with heart failure is between 70-75years of age and the prevalence in the under 60yrs age group is 1-2% compared to over 10% in the population over 80yrs. Hence, although not primary a disease of the elderly, heart failure predominantly affects the geriatric population. A Scottish group [19] projected a 52% and 16% increase in the incidence of heart failure in men and women respectively over a 20yr period, based entirely on population demographic changes.

1.3.3 Prognosis

Cowie et al [20] looked at the survival of patients with a new incidence of heart failure in London. In a cohort of 220 patients who developed heart failure over a course of 16 months, there were 90 deaths over 16-months and overall survival was just 57% at 18-months. Age was the only significant predictor of re-admissions but serum creatinine, age and functional class all

predicted mortality. A Scottish study [21] followed up 66 547 patients over a 10-year period and found crude fatality rates at 30 days and at 1, 5, and 10 years were 19.9%, 44.5%, 76.5%, and 87.6%, respectively. These two prospective observational studies found higher event rates compared to other major heart failure trials such as SOLVD [22] and CIBIS [23] (involving angiotensin converting enzyme inhibitor and beta-blocker medication respectively) but confirmed earlier studies such as the follow-up of the Framingham cohort, which found 1-year and 5-year survival rates of 57% and 25% in men and 64% and 38% in women, respectively. This can be partially explained by the fact that the clinical trials had selected younger population groups with relatively mild CHF. Features such as a poor New York Heart Association class, reduced ejection fraction and increased age are particularly associated with a relatively poor prognosis.

The mortality rates of patients admitted with heart failure are similar to patients with cancer of the bowel, breast, prostate and lung. CHF had a 5-year mortality of 25%, which was similar to bowel cancer and significantly higher than all other cancers with the notable exception of lung cancer [24].

An analysis of the number of hospital admissions in Leicestershire with a first time diagnosis of CHF showed that this number rose from 2.9 /1000 in 1993 to 4.7/1000 in 2000, a rise of 62%, mainly in the over 65yr old group [25]. This large population of 12,200 was then followed up over an average of 22 months. 1-year and 5-year survival was 57% and 27% respectively. Inpatient mortality was 20%, a finding similar to the London study conducted in 1999 [20]. This study found that Kaplan-Meyer estimates of survival showed a clear trend to improved survival over the period of 8-years, 1-year survival improving from 57% in 1993 to 76% in 2000. This was also found in a Scottish observational study [26,27], that CHF, although

increasing in incidence, has an improving survival rate. Cleland et al [28] examined three cohorts of Scottish patients in 1984, 1988 and 1992. Although the number of "first time admissions" had increased by 30% over the 3-year period, mortality declined in patients under 65 years from 53% to 41% and, for patients over 65 years, from 71% to 66%. Reports from the United States Olmstead County [29]. as well as the Framingham group also found evidence of improving mortality, although less so in women and the elderly. Importantly, this report also found an increase in incidence of CHF.

Whilst it would be tempting to ascribe this improvement in outcome to modern medical therapy, we cannot draw such an inference from these trials. A further observation from these trials is that mortality appears to be highest in the first year after the index admission for heart failure.

1.3.4 The burden of CHF

The economic burden of treating heart failure has also risen considerably over the last 2 decades. Despite the rising cost of drug treatments as well as more sophisticated systems such as biventricular pacing, the greatest cost remains that of inpatient care. An analysis of a district general hospital in North-West London serving a population of approximately 155,000 in 1991 found that heart failure accounted for 4.2% of all medical/geriatric admissions over a 12-month follow-up [30]. In a population of 151,000 subjects, More recently, a population study in based in a South London of 292,000 [20] found 332 new cases of CHF, 208 being diagnosed as inpatients. Thirty eight inpatients died on their index admission; of the remainder, 173 cases were hospitalized on 311 occasions over an average follow-up of 19 months. On a national level, assuming these studies were representative of the U.K. population, this would equate to

approximately 120,000 admissions per year. In an Italian review, 18% of the heart failure patients were admitted more than once and 5% more than twice over a 2-year period [32]. The rise in admissions is mirrored in Spain as well. Rodriguez et al [33] found that CHF was the leading cause of admissions in the over 65yrs age group, accounting for 5% of all admissions. The mean length of stay was long ranging from 10-14 days for men and 12-16 days for women. Ghali [34] reviewed hospital admissions in the United States from 1973-86 and found that approximately 1 million admissions per year were primarily due to heart failure and that this was the most common hospital-discharge diagnosis in patients over 65yrs old.

The cost of CHF to the NHS in the UK was estimated at £360 million for the year of 1990-91, over 1% of the total NHS budget [35]. Most of this, approximately 60%, was taken up by the cost of emergency and elective hospital admissions. Given that every more study since 1991 has reported increased hospitalizations and re-admission rates with only marginally reduced inpatient stay time [27, 32, 33, 34] this figure is almost certainly on the conservative side. The most recent figures [36] estimated that the direct cost of heart failure to the NHS was £716 million, almost double that of the estimate in 1990. Most of the expenditure was, as always, on cost of hospitalization, estimated at 1.83% of total NHS expenditure.

1.3.5 Aetiology of CHF

CHF in the western world in largely associated with coronary artery disease and hypertension. Cowie et al [15] found that 36% of the patients with CHF had a history of coronary artery disease. 14% had hypertension, 7% had valvular disease, 5% had atrial fibrillation alone, and 5% had other unclassified causes such as cardiomyopathies. The Glasgow MONICA studies [13] as well as the Heart of England Study [12] have confirmed this

observation that coronary artery disease is the primary cause of heart failure. Hypertension was, until the advent of anti-hypertensive treatment, the most common underlying cause of CHF. Nicholls M in the book Heart Failure in Clinical Practice [37] argues the case of hypertension being more significant a cause than is recognized. One reason is that CAD and hypertension do overlap and that as cases of unrecognized hypertension develop CHF, a reduced cardiac output would have a "pseudo-normalizing" effect on their blood pressure. Certainly, trials such as SHEP [38], investigating the effect of systolic blood pressure reduction in the elderly, would suggest that blood pressure significantly confers a strong protective effect against CHF. It is also significant that in London epidemiological survey of cases of CHF [15], of the 220 cases of new CHF, there was no obvious cause in 34%. 5143 subjects from the Framingham cohort were followed-up over an average time of 20.1years [39]. After adjusting for age and other cardiovascular risk factors, the hypertensive men had a 2-fold increase of developing CHF and the hypertensive women had a 3-fold risk increase. More recently, Fox et al [40] found that coronary artery disease, as defined by myocardial perfusion scans and angiography was the underlying cause of 52% of the 330 cases of newly diagnosed CHF. In an epidemiological survey, Davies et al further analyzed the high-risk subjects from the Heart of England study [12] and found the incidence of heart failure of LVSD was no higher in the population with uncomplicated hypertension compared to a normal population. In a multicentre study, 20,000 patients followed over a 10yr period formed the population for the review paper by Gheoghiade et al [41]. The authors accepted that coronary artery disease was the primary cause of CHF. It is probably safe to conclude that coronary artery disease and hypertension are the two most important aetiologies in the pathogenesis CHF. Other causes would include cardiomyopathies, valvular disease, arrhythmias, alcohol and other metabolic conditions.

1.4. Asymptomatic LVSD (ALVSD)

Patients undergo the transition from asymptomatic to symptomatic heart failure because of worsening LV remodeling and cardiac decompensation (figure 1.4). Patients with impaired cardiac function have a period whereby compensatory physiological mechanisms can result in little or no actual symptoms [42]. There has been increased interest in this early "pre-symptomatic" phase of heart failure because early intervention, such as drug therapy, could potentially prevent the onset of symptoms. The current American College of Cardiology/ American Heart Association practice guidelines [43] for CHF divide the disorder into 4 stages, stage A referring to subjects are high risk of CHF (e.g. hypertension, diabetes) with normal cardiac function and Stage B subjects with abnormal cardiac function but without symptoms. The term "asymptomatic" may be somewhat misleading as patients can have very mild symptoms, particularly in the elderly who may have a reduced effort tolerance due to other co-morbidity or even simple advanced years. It is perhaps preferable to consider this group as having "pre-clinical" LVSD.

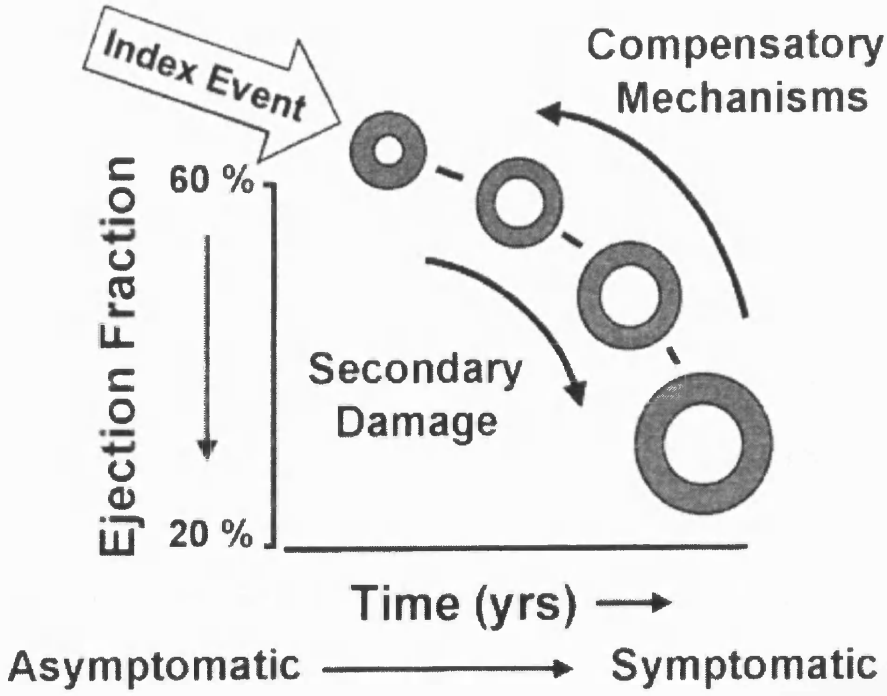


Figure 1.3 Mechanisms and Models in Heart Failure. Mann DL (*Circulation.* 1999;100:999-1008.)

As mentioned earlier, asymptomatic or pre-clinical LVSD has prevalence approximately equal to that of symptomatic LVSD. The overall prevalence ranges from 2.3% to 8% (see table), with a very similar prevalence to subjects with symptomatic LVSD. The long-term prognosis of subjects with ALVSD is not benign. Lauer et al [44] followed up 1,493 men who were free of symptomatic cardiovascular disease for a mean of 4.15 years. The study found that subclinical LV end diastolic dilatation and a low fractional shortening led to an increased incidence of cardiovascular events, 68 men having 92 cardiovascular events. An age and sex adjusted relative risk of 1.42 for a 4% reduction in fractional shortening. Vasan et al. [45] confirmed this finding in the Framingham study follow up of asymptomatic individuals with LV dilatation. The incidence of ALVSD in higher risk populations has a higher prevalence and a similarly

poorer long-term outlook. Similarly, 2384 asymptomatic hypertensive patients [46] were followed for up to 17 years (mean 6.0). LVSD, as defined by an echocardiographic ejection fraction <50%, was found in 3.6% of subjects and conferred a markedly increased risk for CHF (odds ratio, 9.99. Lastly in the SOLVD-P study [47], in which 80% of patients had a previous myocardial infarction, 30% of untreated asymptomatic subjects with ejection fraction <30% progressed to overt CHF over the course of 1 year. The Glasgow MONICA trial found that the 4 year mortality for subjects with an EF<30%, regardless of the presence or absence of symptoms, was 21% [48]. Most recently, Wang et al [49] studied a cohort of 4257 subjects from the Framingham study who underwent echocardiography. An ejection fraction of 50% (estimated visually) was used as the cut-off for LVSD and the subjects were followed up for 12 years. Subjects with a prior diagnosis of CHF were excluded. The overall prevalence of LVSD was 3%, rising with age and in men. Most cases had mild LVSD (EF 40-50%). Overall, ALVD conferred a hazard risk of developing CHF of 5.8 compared to 0.7 in subjects with normal LV function. Severe LVSD (EF<40%) had a hazard ratio of 9.6 as compared to subjects with mild LVSD having a ratio of 3.9. The mean time to first presentation in this group was 10 years suggesting a fairly long latent period. There was no significant difference between groups with or without previous myocardial infarction or valvular disease. The mortality was also increased (Hazard ratio 1.6, Confidence interval 1.1-2.4) and reduction in survival related directly with a corresponding reduction in LV function. The risk of developing CHF was similar to the annual rates in the SOLVD-P [47] study but the mortality rates in subjects with moderate-severely impaired LV functions were substantially higher (11.0% vs. 5.1%). The risk of sudden cardiac death was particularly marked. This last study is particularly enlightening as the subjects were

based in the community and were not part of any trial. The data, as such, most reflect a "real world" scenario.

In conclusion, ALVSD is a common condition with a significantly increased risk of developing CHF as well a corresponding increase in mortality due to sudden cardiac death.

Study/Location	Numbers	Age range	Mean age	LVSD definition	Prevalence	Percentage asymptomatic
McDonagh/ UK. Glasgow [48]	1640	25-74	50	EF under 30%	2.9	48
Davies/UK.	3960	Over 45	61	40 and under	1.8	47
Birmingham [12]				50% and under	5.3	61
Mosterd/Rotterdam [14]	1698	55-95	65	FS 25% and under	3.7	60
Hedberg/Sweden [91]	433	75	75	LVEF 43% and under	8.9	
				LVWMI under 1.7	6.8	46
Nielson/Denmark [87]	2158	50 and above	75	LVWMI 1.5 and under or FS under 0.26	2.9	34

Table 1.1 The prevalence of symptomatic and asymptomatic LVSD in population studies. Reproduced from The Lancet Vol 358 Aug 2001, A commentary by Petrie and McMurray

1.5 Diastolic heart failure (DHF)

So far, the discussion of CHF has been largely attributed to LVSD or output failure. Diastolic heart failure (DHF), or heart failure with preserved LVEF, is a distinct entity from LVSD. It may co-exist with LVSD and may be a precursor to symptomatic LVSD. Vasan et al [50] reviewed 31 studies of CHF, finding that various studies had reported between 13-76% of subjects with clinical CHF having a normal left ventricular systolic function. A second study in 1996 [51] followed 73 subjects from the Framingham database with CHF, 37 of which had normal ejection fraction and the rest normal LV function. During a median follow-up of 6.2 years, CHF cases with normal LVEF experienced an annual mortality of 8.7% versus 3.0% for matched control subjects. Congestive heart failure cases with reduced LVEF had an annual

mortality of 18.9% versus 4.1% for matched control subjects. As such, the authors concluded that although subjects with CHF but normal LVEF had a lower annual mortality compared to cases of CHF with reduced LVEF, mortality was still increased 4-fold over a normal population. DHF is more prevalent in women and is far more common in the elderly. Risk factors include hypertension and obesity.

The pathological process behind DHF is inherently different from LVSD. The key problem appears to be a reduced compliance leading to an elevated filling pressures and causing pulmonary oedema. Patients present with acute pulmonary oedema, often with elevated blood pressures, and clinically mimic LVSD. The gold standard of diagnosing elevated filling pressures would require an invasive cardiac catheterisation. As such, surrogate echocardiographic parameters are used. The diagnosis of DHF, as indicated by European guidelines requires a clinical presentation of acute pulmonary oedema as well as echocardiographic findings of a normal systolic function as well as various "diastolic filling parameters", e.g. the mitral e to a wave ration, the interventricular relaxation time or pulmonary flow waveforms. At present, many clinicians have taken one of two approaches. One that a "normal" systolic function in the presence of clinical CHF would imply a diagnosis of DHF. Two, that abnormal echocardiographic diastolic parameters make a diagnosis of DHF. Clearly, both these approaches are flawed.

Caruana et al [52] looked at 109 patients referred from their general practitioner with suspected CHF who had normal systolic function on echocardiography. Only 9 out of 109 (8%) did not have abnormal pulmonary function tests, high BMI or a history of ischaemic heart disease. 67% of the population had abnormal e to a wave ratios. The authors suggested that an alternative explanation for symptoms could be found in most patients with a diagnosis of DHF.

Davie et al [53] reported that the prevalence of so-called diastolic echocardiographic indices were so common so as to make it almost useless as a marker of DHF. This was also observed in a large study [54] of 647 subjects that found that the specificity of traditional echocardiographic indices of diastolic dysfunction were very dependant of cut-off values and suffered from low specificity in the general population. Gandhi et al [55] followed 38 subjects who had classical clinical and radiological acute pulmonary oedema with hypertension. Echocardiography was performed both acutely and after the index admission. He found that transient lowering of systolic function or mitral regurgitation did not cause the pulmonary oedema and concluded that diastolic filling abnormalities were the primary cause of the acute pulmonary oedema.

The existence of many subjects with dyspnoea with preserved LVEF is without doubt. Whilst some of these cases may indeed have alternative explanations for their symptoms, it is safe to conclude that DHF accounts for a significant proportion. The difficulty we have at present is that of accurate, reproducible and non-invasive diagnostic methods. The European guidelines for the diagnosis of DHF call for symptoms of acute pulmonary oedema, normal left ventricular function and abnormal diastolic filling parameters on echocardiography. Few papers have used this stringent definition. A further minefield is that of "asymptomatic DHF" – again, a stringent application of the European definition would preclude such an entity. European Society of Cardiology guidelines for the diagnosis of DHF have for their starting point clinical evidence of heart failure and normal systolic function. Hence, asymptomatic DHF is an imprecise term and the accurate diagnosis of this subgroup is fraught.

1.6 Treatment

Despite with established treatment for CHF, a recent observational large-scale European study [56] have found that the uptake of treatment, even among symptomatic patients, remains surprisingly low with only 61% receiving an ACE inhibitor and 37% a beta-blocker. The discrepancy appears greater in women and the elderly, the latter being a group where CHF is often more prevalent and severe.

1.6.1 Angiotensin Converting Enzyme (ACE) Inhibitors

Many landmark randomized case-controlled trials have established ACE inhibitors as the cornerstone of CHF treatment [47, 57, 58]. In an overview of the ACE inhibitor trials involving approximately 7000 patients enrolled in various placebo-controlled randomized trials, there has been a 20-25% reduction in all-cause mortality, and a similar reduction combined risk of death and hospitalization [59]. There have also been improvements in symptoms and cardiac function. The treatment of LVSD, symptomatic or otherwise, following a myocardial infarction has thus been well established leading the ESC to recommend that all patients CHF secondary to LVSD should have an ACE inhibitor unless contra-indicated or intolerant. The HOPE study [60] examined the use of Ramipril in subjects without CHF but having other cardiovascular disease such as cerebrovascular disease or diabetes. ACE inhibitors significantly reduce mortality, morbidity and reduce the numbers of subjects who developed clinical CHF.

1.6.2 Angiotensin 2 Receptor Blockers (ARB)

This class of drugs has similar benefits to ACE inhibitors with the advantage of having significantly less side effect of cough. Trials have established equivalent benefit to ACE inhibitors in the treatment of CHF [61] and following myocardial infarction. Some trials [61, 62, 63] have also suggested additional benefit in the combination of both ARB and ACE inhibitor.

1.6.3 Beta-blockers

The use of beta-blockers in CHF remained controversial until the early nineties. Currently, approximately 12000 subjects have been enrolled in various randomised placebo-controlled trials. Many of these trials found that the addition of a beta-blocker to conventional treatment including ACE inhibitors conferred additional symptomatic and mortality benefit to patients with moderate and severe CHF. The US Carvedilol program [64] found a 65% reduction in all-cause mortality in patients with NYHA 2-3 symptoms. Other studies such as CAPRICORN [65] have also found similarly large benefits in patients after myocardial infarctions. Indeed, initiating beta-blockers in a population with severe CHF conferred additional benefit [66]. Exner performed a post-hoc analysis in the SOLVD-P group of asymptomatic patients with LVSD and found that the addition of a beta-blocker reduced mortality and morbidity and suggested a synergistic effect with a ACE inhibitor, in this case, Enalapril.

1.6.4 Aldosterone Antagonist

Use of an Angiotensin Converting Enzyme Inhibitor does not lead to full blockade of the renin-angiotensin system. Addition of an aldosterone antagonist appears to lead to greater blockade. RALES [67] looked at the use of Spironolactone in the treatment of CHF patients with New York Heart Association Class 3 and 4 symptoms and found a very significant reduction in symptoms, admissions for CHF and mortality. Subjects with severe LVSD(EF<35%) after a myocardial infarction also derived significant benefit from the use of a new Aldosterone antagonist, Eplerenone, resulted in a 15% reduction in mortality as well as a significant reduction in hospitalizations for CHF [69]. No clear data exist for the use of aldosterone antagonists in the treatment of asymptomatic LVSD.

1.6.5 Digoxin

The use of digoxin in heart failure patients without atrial fibrillation has been extensively studied. It appears to have a benefit in reducing heart failure admission rates with little actual mortality benefit. The effect seems more pronounced in patients with severe reduction in ejection fraction [70].

1.6.6 Device treatment.

Recent large-scale trials have found significant mortality benefits involving the use of internal cardiac defibrillators in patients with severe LVSD. The SCD-HF trial [71] found a 24% relative risk reduction in all-cause mortality in patients with NYHA 2-3 ischemic and non-ischaemic cardiomyopathy over a 5 yr follow-up period. Cardiac resynchronization therapy

using bi-ventricular pacemakers in a selected population with NYHA 3-4 CHF have also resulted in an increased exercise capacity as well as a reduction in mortality [72].

1.6.7 Treatment of DHF

The only study to address this potentially large group of patients was the CHARM-Preserved trial [73]. A study population of 3023 with symptoms of CHF but preserved LV systolic function was prescribed Candesartan, an Angiotensin 2 receptor blocker. There was a small but significant reduction in CHF hospitalizations (230vs 279, p=0.017). Unlike treatment of symptomatic LVSD, the use of Candesartan did not affect mortality. It must be emphasized that this cohort of patients probably was a heterogeneous group and no real attempt was made to make a definitive diagnosis of DHF by echocardiogram Furthermore, other potential etiologies such as coronary artery disease or pulmonary disease were not excluded. The SENIORS study found that the use of Nebivolol, a beta blocker, in elderly patients with CHF and a relatively preserved LVEF, led to a reduction in hospitalizations and mortality similar to that achieved by other beta-blockers in patients with a low LVEF CHF.

1.6.8 Treatment of Asymptomatic LVSD

Although there has been evidence to show that ACE inhibitors reduce the development of CHF in post-myocardial infarction patients [74, 75], there have been surprisingly few large-scale trials specific of the individual with ALVSD. The treatment of asymptomatic LVSD was explored in the 1992 SOLVD-P study [74], which looked specifically at 4228 patients with reduced ejection fraction but not on any CHF treatment. The use of Enalapril, an ACE inhibitor, reduced the incidence of new CHF and the rate of related hospitalizations by 37%

[74]. The mortality in those hospitalized for CHF was also lower. Although there was only a non-significant trend towards a reduction in all-cause mortality, there was a significant reduction in a composite end-point of death together with first admission to hospital for CHF. SAVE [75] similarly found that treating patients with LVSD after a myocardial infarction with an ACE inhibitor, in this case, Captopril, resulted in a significant reduction in CHF hospitalization (22%) and mortality (19%) [75]. Both studies [74, 75] found that patients, whatever the level of symptoms, had a reduction in mortality and progression to overt CHF. Accordingly, both the American College of Cardiology and the European Society of Cardiology have recommended the use of ACE inhibitors in patients with reduced cardiac function, even in the absence of symptoms [76]. The CAPRICORN trial [65], which evaluated the use of Carvedilol, found a significant reduction in mortality and morbidity in patients with LVSD, both symptomatic and asymptomatic, following a myocardial infarction. There has been no large-scale trial examining the use of ACE inhibitors or beta-blockers in patients with LVSD without a prior history of myocardial infarction.

1.7 The case for screening for CHF

World Health Organization guidelines were published in 1968 [71], but are still applicable today. There are 10 conditions to justify a population-screening program.

1. The condition should be an important health problem.
2. There should be a treatment for the condition.
3. Facilities for diagnosis and treatment should be available.
4. There should be a latent stage of the disease.
5. There should be a test or examination for the condition.

6. The test should be acceptable to the population.
7. The natural history of the disease should be adequately understood.
8. There should be an agreed policy on who to treat.
9. The total cost of finding a case should be economically balanced in relation to medical expenditure as a whole.
10. Case-finding should be a continuous process, not just a "once and for all" project.

We have already established that CHF is common, disabling and considerable burden to the health service. We have also observed that the prognosis is poor, particularly after the first admission with decompensated CHF. We also know that there is a pre-symptomatic phase of clinical CHF – asymptomatic LVSD, which is just as common as the clinical entity of CHF. In addition, because of poor public perception of heart failure as found in the European SHAPE study [72], the layman would tend to present late on in the onset of symptoms.

The ideal screening test would have very high sensitivity and specificity. It would also have to be acceptable to the consumer in terms of physical comfort as well as in having any side effects. It should be simple to perform and reliable. The economic cost naturally would also come into the equation. , However, in view of the fact that the prevalence of ALVSD is low, an ideal test would also have to have a high positive predictive value and preferably a high specificity, as the number of false positive tests is likely to outnumber the number of true positives.

1.9 Diagnosis of Left Ventricular Dysfunction

The traditional method of defining CHF relied on a history of breathlessness accompanied by orthopnoea, peripheral oedema and paroxysmal nocturnal dyspnoea. Baseline investigations in the outpatient setting include 12 lead standard electrocardiograms and a chest film. The ongoing debate on a precise definition of CHF and the vague and atypical presentation of early CHF make a definitive diagnosis difficult [79]. To make a definite diagnosis of heart failure would require assessment of left ventricular function either by echocardiography or by any other imaging modality, including radioisotope scans, cardiac MRI scans or contrast injection ventriculograms. Echocardiography, the least invasive and least costly investigation, is often not generally available in the community although local provision for direct access echocardiography for the primary care provider is increasing.

1.8.1 Using signs and symptoms

A full and complete history and examination is, as always, an essential and integral component of the diagnosis of CHF. However, the symptoms and signs associated with CHF are non-specific, non-sensitive or both and are often associated with much other pathology. Remes et al. [80] investigated 88 patients diagnosed as having CHF by the primary care physician. These patients were then classed in 'definite', 'possible' and 'unlikely' to have CHF based on the Boston criteria and supplementary criteria based on clinical examination as well as a 6-month follow-up results. Of these, 34% were found to be 'unlikely' to have CHF. Obesity, unrecognized symptomatic myocardial ischaemia and pulmonary diseases were the most important conditions causing a false-positive CHF diagnosis. Making a definitive diagnosis of LVSD is important as appropriate treatment can only be targeted to the correct diagnosis, be it

pulmonary disease, heart failure or even obesity. Furthermore, pharmacological intervention for LVSD, although possessing excellent evidence for clinical benefit, has serious adverse effects, which would necessitate a definitive diagnosis prior to targeting treatment. The primary care physician manages most cases of CHF [81]. Unfortunately, it has been well documented that under-prescription of ACE inhibitors and beta-blockers managed in the community. One obstacle to appropriate prescription of heart failure pharmacological intervention is poor access to diagnostic facilities.

1.8.2 Utility of transthoracic echocardiogram

Currently the transthoracic echocardiogram is the gold standard tool for the diagnosis of LVSD. The National Clinical Institute for Clinical Excellence (NICE) has stated that echocardiography should be made available for all patients with suspected CHF [82] and similarly, the European Society of Cardiology has recommended echocardiography as the most effective means of diagnosing LVSD. Despite these guidelines, only 31% of patients referred to a medical unit for suspected CHF had this "gold-standard" investigation performed [83]. Transthoracic echocardiography is technically demanding, time-consuming and operator dependant. In particular, accurate estimation of ejection fractions or fractional shortening is only possible in 60-75% of patients. Certainly, the waiting lists and financial constraints of the N.H.S. would preclude the use of echocardiography as a population-screening tool. Published studies suggest that less than half of patients suspected of having heart failure by a primary care physician have the diagnosis confirmed by echocardiography or by any other imaging modality [80, 81]. Francis et al [84] investigated the utility of an open access echocardiography service available to 550 general practioners in Edinburgh. The service was popular with general practioners who

found that 70% of patients had their medications adjusted because of the echocardiogram report.

CHF is poorly diagnosed in the community. A cross-sectional study in England assessed 621 patients diagnosed as having CHF by their general practioners [85]. Just 50% of the population prescribed loop diuretics had a LVSD (defined as EF<40%) on echocardiography. Davie et al. looked at 259 patients with breathlessness referred by their general practitioner to an open-access echocardiography service [86]. 41 were found by echocardiography to have LVSD. A thorough history and examination were performed to assess the sensitivity, specificity, positive and negative predictive value of the different signs and symptoms as well as the history. The combination of a previous history of a myocardial infarction and a displaced apex beat (a finding in only 14%) provided the best positive and negative predictive value. Surprisingly, the combination of other physical signs, aspects of the history and medication did not give a better predictive accuracy. Neilson et al [87] found that the symptoms and signs of CHF, as defined by the modified Boston criteria, were very prevalent in the general community (11.7% in the over 80 years of age) and that only a third of this group actually had LVSD on echocardiography. The findings by Fox in 2000 were more encouraging [88]. He investigated the use of open access echocardiography in the community for patients suspected of having CHF by their general practitioners. A total of 383 patients were seen over 15 months, translating to 0.4 cases per 100,000 population head per weekday, of which 178 (46%) were considered to have possible or definite CHF at the initial consultation. The figure was reduced to 26% on subsequent specialist review of the clinical findings, history and specialist investigations. The authors found that a negative ECG and a normal CXR virtually excluded CHF.

1.8.3 The use of the 12 lead electrocardiogram

UK National Service Framework guidelines have recommended the use of 12 lead ECG as a first step in ruling out cases of LVSD [82]. Davie et al [89] investigated this in 1996. A total of 534 patients with suspected CHF were referred for open access echocardiography, of which 96 subjects were found to have LVSD. In this group,90 had at least one major ECG abnormalities. It is important to note that if the ECG were to be used to screen for CHF, it would result in 6 cases missed for every 100 screened and, of lesser importance in a screening investigation, there would have been 65 without LVSD for every 100 abnormal ECGs. (Sensitivity 90/96=94%; specificity 69/438=61%; positive predictive value 90/259=35%; negative predictive value 269/275=98%). Houghton et al [90] investigated the use of ECGs in 200 patients referred for suspected CHF in Nottingham. He found that the ECGs had a sensitivity of 89.1% and a specificity of 45.7% in predicting left ventricular systolic dysfunction. The authors concluded that the using the ECG as a filter for echocardiography would have meant that 10% of patients with LVSD would have gone undetected. The Rotterdam study [91], looking at LVSD in a cohort of 75yr old men and women in the community, found that major ECG abnormalities had a sensitivity of only 57% and concluded that echocardiography was essential in screening the general population. All the above studies have looked at the diagnosis of significant LVSD, usually defined as an ejection fraction of under 30-35%. The diagnostic accuracy of ECGs, chest x-rays and physical signs in the detection of mild to moderate LVSD is unknown. Speculatively, it is likely that the sensitivity and specificity would be worse.

McCallan et al [92] tested a sample of general practitioners in their competence in interpreting the ECG. Although overall competency was judged to be good, unequivocal acute myocardial infarction was misdiagnosed by 20% of the sample. McCrea [93] also found that only 63% of his sample correctly diagnosed an acute myocardial infarction. These studies, although looking at acute myocardial infarctions, would suggest that a significant proportion of general practitioners would not be able to interpret significant ECG changes of ischaemia. This would further degrade the usefulness of ECGs in the role of screening for CHF.

The QRS duration of the 12-lead electrocardiogram is easily obtained. This correlates well with adverse prognosis, including increased risk of sudden cardiac death. A prolonged QRS duration was found to confer significantly increased risk of developing CHF in the Framingham cohort [94]. Other studies have also shown good correlation of prolonged QRS with LVSD.

Lastly, it is important to note that all these studies have looked at patients referred with either breathlessness or a high clinical suspicion of CHF. In looking only at this group of patients, we would be excluding a similar sized population who have not been to their doctor with any symptoms. It is very likely that such group would have little or no clinical features of CHF. ECGs hence can be said to have good sensitivity but poor specificity for LVSD. Assuming competent detection of ECG abnormalities, a screening program involving the use of ECGs would result in a small proportion of false negatives and a relatively large proportion of false positives.

Another strategy for screening for ALVSD would be to target a "high-risk" population, such as subjects with diabetes, previous ischaemic heart disease history or hypertensive. Such a strategy was utilized by Baker et al investigated such a 432 subjects with a history of

hypertension, diabetes and/or ischaemic heart disease. 7.9% of the population had LVSD, as defined by a visual EF of <45%. The prevalence was 15.4% in the group with previous myocardial infarctions. Left ventricular hypertrophy on the electrocardiogram as well as a previous history of ischaemic heart disease were 2 strongest independent predictors of LVSD.

Hedberg et al [91] compared BNP and the ECG in identifying LVSD, defined as an EF<40 %, in a population-based sample of 75yr olds (n=407). The sensitivity and specificity of BNP was 93% and 55% compared to the ECGs of 97% and 79%. Positive predictive value of BNP was 13% as compared to 26% in the ECGs. Less than 1% of the subjects with a no major ECG abnormalities had LVSD. In subjects with abnormal ECGs, a higher BNP level predicted LVSD. The authors concluded that ECGs yielded fewer false positive cases compared to BNP but found that BNP has a diagnostic value in subjects with abnormal ECGs.

The case for screening for LVSD, both symptomatic and pre-symptomatic is by no means straightforward. The condition is relatively common and treatment would require long-term drug therapy as well as further costly interventions and investigations. The cost implications of treating every case of LVSD have yet to be fully evaluated in a long-term study but it has been suggested by some to be a cost-effective intervention [95], providing certain criteria are met. Clearly, the diagnostic test for the diagnosis of LVSD would necessarily have to be relatively inexpensive, easy to utilize, acceptable to both primary care physician and patient and lastly have good sensitivity and specificity. Natriuretic peptides, secreted in response to left ventricular and atrial stretch, have been proposed as diagnostic aids in the detection of LVSD.

Chapter 2

Introduction

Utility of Cardiac Natriuretic Peptides

2.1. A Historical Overview

Kisch et al [96] who detected secretory granules in the atria of guinea pigs first established the concept of the heart as an endocrine organ in the 1950's. In 1981, de Bold and colleagues observed that an infusion of atrial tissue extract caused a copious natriuresis in rats [97]. This led to the discovery of the first natriuretic peptide, atrial natriuretic peptide (ANP). Shortly after, Brain natriuretic peptide (BNP) was found in porcine brain tissue, hence the name, although this has become a misnomer, as it is mainly secreted by the cardiac ventricles. C type natriuretic peptide (CNP) was discovered in 1990 by Sudoh [98] and is expressed to a much greater extent in the central nervous system and vascular tissue. These 3 peptides exert a strong effect on the haemodynamic balance through a wide range of effects on the vasculature, heart, central nervous system and kidney. Atrial, brain and C-type natriuretic peptides are widely circulated in the plasma and exert their effect on a set of common receptors found on the surface of the target organs. C-type natriuretic peptide (CNP) appears to have less potent haemodynamic effects than ANP and CNP. All 3 peptides share a common central ring structure with variable carboxy and amino terminal tails.

Natriuretic peptides have been described as a "window to the heart" [99] as they appear to reflect haemodynamic strain and stress. As such they closely correlate left ventricular function, both systolic and diastolic as well as left ventricular mass. In addition natriuretic peptides appear to be strong prognostic markers in many situations as well as predictors of mortality and the development of cardiovascular disease, in particular heart failure. Natriuretic peptides are in widespread use in the United States, in primary care, the emergency room as well as in the secondary care setting.

2.2 Atrial Natriuretic Peptide (ANP)

Original studies in the 80's and 90's [100, 101, 102] established that ANP was mainly secreted in the cardiac atria and was secreted in response to local atrial stretch as well as to direct stimulation by hormones and various neurotransmitters such as vasopressin and endothelin. These latter stimuli have an independent effect to myocyte stretch. Regulation of ANP release seems to be on the level of release from the secretory granules. ANP is secreted as a 126 amino acid precursor protein, which is then cleaved to release a 98-amino-acid N-terminal portion as well as a 28-amino-acid carboxy-terminal fragment, which is the mature ANP. The ANP gene is mainly expressed in the atria but also in the kidney, which generates a 32-amino-acid peptide called urodilatin from the original precursor hormone. First described in 1988 [103], urodilatin has a role in the renal excretion of sodium and water. Because ANP is primarily secreted in the atrium as opposed to BNP, it has a different response to different clinical scenario. This was investigated by Yoshimura M et al [104] who analyzed levels of BNP and ANP in patients with CHF due to LV systolic dysfunction as well as in patients with mitral valves disease with preserved LV function. In this study, ANP and BNP levels both correlated well with pulmonary wedge pressure in the patients with a dilative cardiomyopathy. However, in patients with mitral valves stenosis and normal left ventricular function, ANP but not BNP correlated to wedge pressure leading the authors to conclude that the differing levels of ANP and BNP were due to the different degrees of overload in atria and ventricles.

2.3 Brain Natriuretic Peptide (BNP)

Sudoh first reported a peptide isolated in porcine brain with very similar activity to ANP and bearing a similar structure [105]. Subsequently, BNP was found in much higher concentration in the human ventricles and it is now known that this is where most of the BNP is secreted [106]. BNP, like ANP, is stored in granules in myocardial cells, which is released by an increase in end-diastolic pressure or volume resulting in myocyte stretch. Concentrations of BNP are related to left ventricular filling pressures and wall stress [107]. Again, like ANP, BNP secretion is also augmented by tachycardia, hormones such as glucocorticoids and thyroxine, and vasoactive peptides like endothelin-1 and angiotensin II. Human pro-BNP contains 108 amino acids and processing releases a 32-amino-acid mature BNP with a 76-amino-caid N-terminal portion. Both of these circulate in plasma and exert similar effects. BNP binds to receptors on various target organs and is subject to post-signaling receptor uptake by endocytosis. Target organs include the brain, kidneys, vascular endothelium and adrenal glands. Inactivation by neural endopeptidases. Only biologically active BNP is subject to cleavage and inactivation by neutral endopeptidases, a class of proteolytic enzymes found on the surface of endothelial cells, smooth muscle cells, cardiac myocytes, renal epithelium and fibroblasts. BNP is subject to clearance by normal glomerular filtration. The half life of BNP is approximately 20 minutes, which compares to N-BNP, which has a half life of 120minutes as it is only cleared by renal excretion. Both of these have been utilized as markers in cardiovascular disease states. Differences in N-BNP and BNP are listed in table 2.1.

Characteristic	BNP	N-BNP
Molecular weight	3.5 kilodaltons	8.5 kilodaltons
Hormonally active	Yes	No, inactive peptide
Genesis	Cleavage from proBNP	Cleavage from proBNP
Half-life	20mins	120mins
Clearance Mechanism	Natriuretic peptide receptors	Renal clearance
Increases with normal aging	+	+++
Cut-off for CHF diagnosis	100 pg/ml	Age<75yrs: 125pg/ml Age <75yrs: 450pg/ml

Table 2.1 Differences between BNP and N-BNP. Adapted from Silver MA et al For the BNP Consensus Panel 2004: A critical Approach for the Diagnosis, Prognosis, Screening, Treatment, Monitoring and Therapeutic Roles of Natriuretic Peptides in Cardiovascular Diseases. Congest Heart Fail 2004;5 (suppl 3):1-28

2.4 CNP

Processing of the pro-CNP molecule produces 2 different CNP molecules, one 22 amino-acids in length, the other 53 amino-acids. The former is contained within the latter's carboxy-terminal portion. CNP is mainly found in the central nervous system., anterior pituitary, endothelium and kidneys but little is known about the precise effects it exerts. Some investigators have suggested a role of CNP in behavioral modification. Compared to ANP and BNP, CNP plasma concentration is low. Kalra et al [108] recently demonstrated elevated CNP levels in the coronary sinuses of patients with stable heart failure as compared to levels in the aorta, suggesting that CNP is produced directly in the myocardium and that general circulating levels do not reflect the CNP concentrations in proximity to CNP receptors located in the myocardium. A further study has shown a corresponding increase in CNP levels with increasing severity of CHF [109].

2.5 Natriuretic Peptide receptors

Haemodynamic stress lead to increased natriuretic peptide production by atrial, ventricular and endothelial cells. Genes encoding for these enzymes are activated to cause a production of the peptides. Three natriuretic peptide receptors, A, B and C, have been identified. The A receptors binds both and A and B peptides although preferentially to ANP.B receptors bind to CNP. Both A and B receptors are linked to cGMP-dependant signaling cascade. Hence a binding of a natriuretic peptide to the receptor causes an increase in intra-cellular cGMP. Receptor C is involved in the clearance of the peptides.

2.6. Effects of Natriuretic Peptides

The actions of natriuretic peptides are often in antagonism to angiotensin and angiotensin II and generally have a cardio-protective effect. As mentioned previously, ANP is secreted in response to increased atrial stretch, which can be caused both by increased pre-load or after-load. A low-dose infusion of ANP or BNP causes a lowering of blood pressure mediated by the activation of the sympathetic nervous system, renin-angiotensin-aldosterone system and the endothelin pathway leading to a reduction in intravascular fluid due to increased hydraulic pressure in the capillary bed as well as increased venous capacitance. This is in-contrast to a high dose infusion, which increases the peripheral vascular resistance despite the overall lowering of blood pressure. Both ANP and BNP block central and peripheral sympathetic nervous system activity, even when cardiac filling pressure falls. ANP and BNP directly block secretion of renin and aldosterone and further inhibit the stimulatory effects of angiotensin II on the release of Aldosterone. In addition, BNP has direct lusitropic properties in

the myocardium The overall effect of natriuretic peptides is to improve the loading of the failing heart.

ANP and BNP have very similar actions on the kidney. Glomerular filtration is increased and sodium resorption is reduced. Natriuretic peptides have a vasodilatory effect on peripheral vasculature. ANP reduces the sympathetic outflow by a direct effect on the central nervous system and also by reducing the release of catecholamines from autonomic nerve endings. BNP and CNP have similar effects although the latter has the most potent vasodilatory effect of the three. The actions of CNP tend to be locally medicated in the vasculature as vasodilator and inhibitor of vascular cell proliferation. BNP might have anti-proliferative and anti-fibrotic effects in vascular tissue.

2.7. Utility of Natriuretic Peptides

As mentioned earlier, natriuretic peptides are a reflection of the haemodynamic stressed on the heart. Many studies have looked at the utility of these peptides in the diagnosis and management in different cardiovascular pathologies.

2.7.1. Large scale population screening

Natriuretic peptides have good negative predictive utility. As such, they can be utilized as "pre-screening" tests, prior to the use of a formal echocardiogram. The Glasgow MONICA project was the first study to evaluate the use of N-ANP and BNP in the detection of LVSD [110]. A random sample of the general population (n=1252) in North Glasgow aged between 24-75yrs of age was studied. Median N-ANP and BNP levels were significantly higher in subjects with LV systolic dysfunction, defined as an ejection fraction under 30%, (2.8ng/ml and

24 pg/ml) as compared to normal (1.3ng/ml and 7.7 pg/ml). BNP was found to be superior to N-ANP and the area under curve (AUC) of the receiver-operator-characteristic (ROC) curve was 0.882 for the population aged between 25-74yrs. Using a BNP cut-off value of 17.9 pg/ml, sensitivity was 76% and specificity 87% for the detection of LVSD defined as an ejection fraction of under 30%. The authors found that the sensitivity and specificity of both peptides was markedly improved when a relatively higher risk population, such as the elderly or cases of hypertension/ischaemic heart disease, was identified.

The Heart of England Study [111] investigated the use of N-BNP in identifying subjects with heart failure (defined by echocardiographic evidence of left ventricular dysfunction, atrial fibrillation and valvular disease) in 4 distinct groups: general population, patients with an existing label of heart failure, patients on diuretics and patients deemed at high risk of heart failure. A cut-off value of 36pmol/ml gave an AUC of 0.92, 0.8, 0.87 and 0.84 in each of the groups. The positive predictive value was naturally highest in the group with the highest incidence of heart failure (the group with a clinical label of heart failure) was 39% compared to a value of 7% in the general population. The negative predictive value was 100% in 3 groups and 97% in the group taking diuretics. The likelihood of negative result was 0 in 3 groups and 0.18 in the group taking diuretics. The authors concluded that N-BNP could be used in a screening program in high risk or symptomatic populations and that its use was most encouraging as a "rule out" test rather than a "rule in" test.

Some other studies have not been as encouraging. A large scale population screening study in the U. S in 2004 by Vasan et al. [112] examined 3177 subjects that were part of the Framingham database and found that the performance of BNP and N-BNP (assayed using the Shionigi non-competitive immunoradiometric assay) in diagnosing both elevated LV mass and

LVSD in this large contemporary screening study was "sub optimal". AUC of the ROC curves were under 0.75 for both NT-ANP and BNP. A BNP level of 46pg/ml gave high specificity (95%) but only 29% sensitivity for LVSD. These results were improved in targeted "high-risk" groups. The authors concluded that the low positive predictive values of BNP and N-ANP would make these peptides a poor screening test for LVSD. A further US study in Rochester [113] also found limited utility of BNP in screening for pre-clinical LV systolic and diastolic dysfunction. The study found that between 10-40% of the population would require echocardiograms most of which would be negative studies. More disappointingly, between 10-60% of cases of LV dysfunction would have been missed.

Despite these results, other studies have shown cost-effectiveness in using natriuretic peptides as "pre-screening" tests. Heidenreich et al [114] explored the cost-effectiveness of screening using BNP. The authors factored lifetime cost of care because of a positive result of screening as well as improved outcome (estimated as 7.9 and 1.3 quality-adjusted life years for men and women respectively). Cost-effectiveness naturally is higher in population with higher prevalence of LVSD. Consequently, screening using peptides should be targeted at the appropriate group for the greatest cost-effectiveness. The authors found that screening populations with a 1% prevalence of reduced ejection fraction would provide a health benefit of 22,300 US dollars per quality adjusted life year gained for men and 77,700 for women, a figure comparable to other health care outcomes. The authors concluded that a minimum prevalence of 1% of LVSD in the target population would allow testing to be cost-effectiveness. A similar conclusion was reached by Neilson et al [18] who performed a retrospective analysis of the Glasgow MONICA cohort. This study also suggested that screening be targeted to a "high-risk"

group to achieve cost-effectiveness. This trial achieved a 26% reduction in cost for each case of LVSD if the case was "pre-screened" with BNP test prior to formal echocardiography.

Despite the early encouraging results, BNP, or for that matter, N-BNP, have high specificity for LVSD but correspondingly low positive predictive values owing to the low prevalence of the disease and a high negative predictive value. NP have a good sensitivity for LVSD but, owing to the relatively low prevalence of the disease, a corresponding low positive predictive value. A mass population screening would lead to many "false positives" if a 100% sensitivity is to be achieved. However, in comparison to many other widely accepted screening tests, e.g. Papanicalau smears for cervical cancer (AUC 0.70), mammography for breast cancer (AUC 0.85) or prostate specific antigen for prostatic carcinoma (AUC 0.94), the accuracy of NP for the detection of LVSD is at comparable and in many cases superior.

In general, most trials [110,111,112,113] have shown high negative predictive value of natriuretic peptides and have concluded that natriuretic peptides are more useful as "rule out" tests, implying that a patient with low cardiac peptide levels was very unlikely to have LVSD. The use of NP in identifying LVSD could be targeted to high risk populations, such as hypertensive and diabetic patients. Another strategy would be to utilize a 2 step process where NP measurement could be combined with another test such as a 12 lead ECG or hand held echocardiography to improve the specificity of the test [117].

2.7.2 Diagnosis in patients suspected of having CHF

Rather than screening the general population, the Hillingdon Heart Failure Study [116] utilized BNP in the assessment of patients with suspected CHF referred to a rapid access clinic. Approximately a third of these subjects had CHF confirmed by echocardiography. The

diagnostic value of BNP was considered to be high compared to an expert cardiological opinion. The AUC was 0.96 compared to 0.79 for the cardio-thoracic ratio on a chest x-ray. A cut-off of 22pmol/ml gave a very high sensitivity of 97% and a good specificity of 84%. BNP performed better than N-ANP and ANP. Most recently, the UK natriuretic peptide study [118] studied the use of BNP, NT-BNP and ECGs in the assessment of 306 patients referred with suspected CHF. At the manufacture's recommended cut-off points, the AUC for the detection of LVSD for BNP was 0.84 (95%CI 0.79-0.89) and for N-BNP, 0,85(95% CI 0.81-0.90). N-BNP had a higher negative predictive value but a lower positive predictive value compared to BNP. An abnormal ECG did not provide any further diagnostic information beyond the NP levels.

2.7.3. Diagnostic utility in acute presentation of breathlessness

Natriuretic peptide levels correlate well with elevated pulmonary wedge pressures in decompensated left ventricular failure/pulmonary oedema [107]. As a result, several recent studies have focused on the use of peptides in the emergency care setting. One of the first to investigate this, the Breathing Not Properly trial [119], was a prospective trial of 1586 patients presenting to the emergency room with dyspnoea. The investigators used BNP in conjunction with clinical acumen in the diagnosis of acute heart failure.

Using a cut off level of 100pg/ml, BNP used alone had a sensitivity and specificity of 90% and 73% respectively, performing better than any single factor in the physical examination or patient history. When BNP was used in conjunction with clinical judgment, the accuracy of a diagnosis of heart failure was increased from 74% to 81%. BNP was particularly useful in

excluding heart failure, a threshold of 50ng/l having a negative predictive value of 96%/. Mueller et al [120] investigated the use of BNP in the evaluation of breathlessness in patients presenting to the emergency room. This was a multi-centre study involving 452 patients with 2 groups, one utilizing BNP in the decision making process of diagnosing LVSD and the other using a more traditional approach. The use of BNP resulted in a 10% reduction in the rate of hospitalisation, a reduction in the median length of stay by 3 days and a reduction in mean total cost of treatment by $1800.

A large multi-centre trial assessed the utility of N-BNP in a pooled analysis of 1256 patients presenting with acute dyspnoea to the emergency room [121]. The authors found that an age related cut-off points for a diagnosis of heart failure resulted in a sensitivity of 90% and a specificity of 84%. A single cut-off point which was independent of age gave a high negative predictive value of 98%. The study also demonstrated a strong relationship between acute levels of N-BNP and early mortality. This latter relationship was similarly observed by investigators in the REDHOT [122] study who found that BNP levels were far more significant as regards to outcomes than was physician perception of severity.

The use of natriuretic peptides in the diagnosis of acute heart failure, be it systolic or diastolic, does not achieve 100% sensitivity and specificity. Most trials have found that use of NP, in conjunction with the application of clinical skills and other investigations, e.g. ECGs or CXR's, yield the best diagnostic testing modality. Hence, it is important to note that although NP's are useful guides in the assessment of acute dyspnoea, particularly in the exclusion of CHF, it is important to consider them in conjunction with a good history, examination as well as other investigations such as the ECG and CXR. In addition, elevated peptide levels do not necessarily indicate heart failure as the primary diagnosis as other pathologies such as

pulmonary embolism, pulmonary hypertension can result in elevated peptide levels. Guidelines to that effect, incorporating peptide levels, basic investigations as well as clinical history and physical examination findings have been produced by Maisel in a review article [123], (figures 2.1a and b)

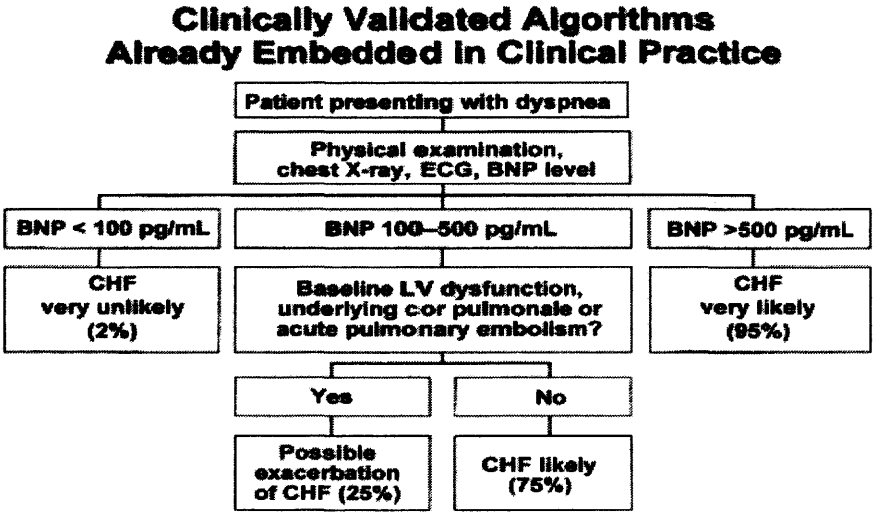


Figure 2.1a

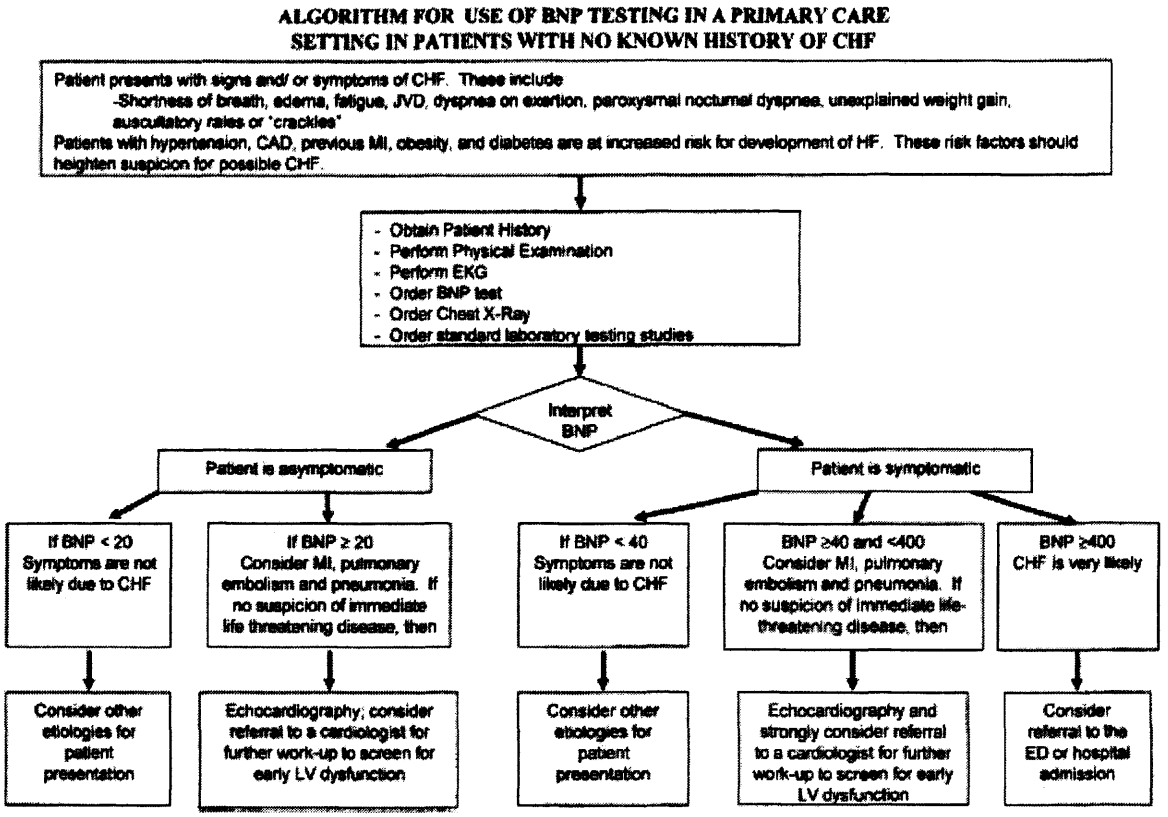


Figure 2.1a and b. Algorithm for using BNP in diagnosing CHF in patients presenting with breathlessness. CAD=coronary artery disease, ED=casualty department, EKG=electrocardiogram, HF=heart failure, JVD=jugular venous distension, LV=left ventricular, MI=myocardial infarction. Adapted from Maisel et al [123].

2.7.4 Diagnosing Diastolic heart failure (DHF).

Peptide levels have correlate with rising left ventricular end diastolic pressure as well as ventricular wall stress [124]. Despite the fact that a clear diagnosis of DHF is hard to pin down, several studies have been published looking at the utility of peptides in this field. Studies [125,126] have correlated BNP to echocardiographic abnormalities associated with DHF, both in traditional Doppler measures of left ventricular "stiffness" as well as newer indices of tissue Doppler. Perhaps more significantly, Kitzman, defining DHF as a syndrome with symptoms of congestive heart failure despite a normal systolic function, found significantly higher levels of

BNP (similar to that in patients with LVSD) compared to a normal control group. Troughton [127] recently analyzed BNP levels in 106 subjects with systolic heart failure. Newer echocardiographic measurements of diastolic function, including tissue Doppler imaging and colour m-mode in addition to more traditional measures of diastolic function such as early diastolic filling time, deceleration time and early transmitral flow were applied to grade diastolic function. Plasma BNP levels correlated independently with these newer measures of DHF. As diastolic dysfunction, particularly in patients with LVSD, is known to be a predictor of adverse prognosis, the authors suggest that this close relationship of BNP and DHF may explain the usefulness of BNP in predicting adverse outcomes. This finding was confirmed by Mottram [128] who found that BNP levels were elevated in a group of hypertensive patients with mild symptoms of heart failure compared to a control group. The BNP levels correlated well with these new measures of DHF (colour m-mode and tissue velocity). The authors did find, like earlier groups, that the levels of BNP, though elevated, were not as high as levels in LVSD. In this study, 79% of the group with DHF had levels within what would be considered normal. A German group studied the utility of N-BNP in diagnosing DHF in patients with normal systolic function [129]. Subjects underwent convention echocardiography, tissue Doppler analysis as well as invasive cardiac catheterization. N-BNP had high negative predictive value, correlated well with tissue Doppler measurements as well as left ventricular pressure measurements. Most recently, 1678 subjects from the MONICA Ausberg study [130] were assessed using BNP for DHF. BNP levels measured using a commercial assay were increased in subjects with DHF but to a lesser extent compared to subjects with increased left ventricular mass or LVSD. However, DHF was not an independent predictor of BNP levels

when LV mass was included in a multivariate analysis. ROC analysis found that BNP only had a sensitivity of 61% and a specificity of 55% for the detection of DHF.

The echocardiographic determinants of DHF are difficult to measure and furthermore, can be found as part of the normal spectrum, particularly in advancing age [54]. This has particular relevance as it has been well documented that natriuretic peptide levels rise in advancing age [135]. A further complication is using peptides in DHF is the accurate diagnosis of DHF. Some authors [52] have postulated other pathologies, such as lung disease, ischaemic heart disease and obesity, as a cause of the patient's dyspnoea, rather than DHF. It would therefore be prudent to exclude other diseases prior to making a firm diagnosis of DHF. Some of these disease states can have an effect of peptide levels and are potential confounders. In conclusion, NPs may have a role, albeit limited in the diagnosis of DHF.

2.7.5 Utility in the Hypertensive population

BNP has been found to be raised in hypertensive subjects [130,131] and levels of BNP correlated with LV mass [130, 133]. The cost and availability of echocardiography has limited it's application in routine use and it has been suggested that natriuretic peptides have a role in identifying the hypertensive patient who is at risk of having an increased LV mass [135, 136]. A recent community-based study [112] evaluated BNP and N-ANP in the detection of LVH and found that the Area under the receiver operating characteristic curve for the detection of elevated LV mass was 0.57 and 0.72 for women and men respectively. A similar large community based trial from the Olmsted county group found no significant correlation between elevated LV mass and BNP levels as analysed using the Shionogi assay but a correlation with BNP analysed by the Biosite assay [134]. In one previous study in a relatively small group of

94 subjects[137] , BNP was superior to ANP and N-ANP in the detection of increased LV mass. Nakamura et al. [138] screened 1112 subjects of which 284 had hypertension. Thirty-six of these had LVH on echocardiography. Although BNP levels in the group with LVH were significantly higher (19.4 vs. 28.2), the ROC AUC was only 0.588. Poor positive and negative predictive values (18.9 and 90.5%) led the authors to conclude that plasma BNP is of limited use in this setting.

2.7.6 Utility in Valve Disease

N-BNP has been correlated with progression of aortic valve stenosis [140] and also predicts symptomatic deterioration [141]. Bergler-Klein [142] investigated the use of BNP, N-BNP and N-ANP in 130 patients with severe aortic stenosis. All NPs were associated with decreasing left ventricular function and increasing pre-operative NYHA class. A multifactorial analysis found that all 3NPs were independent predictors of postoperative survival. BNP appears to predictive value in the timing of aortic valve surgery as it correlates closely with symptoms. Low levels of BNP correlated with low mortality, helping the physician to make a decision on the timing of surgery more accurately. Low NP levels are also associated with a better post-operative outcome. N-BNP levels have also been found to correlate with severity of mitral regurgitation [143] and mitral stenosis [144].

2.7.7 Prognostic information in different populations

2.7.7.1. Asymptomatic population

In a large population study of asymptomatic 3346 persons without heart failure, the Framingham Offspring Study group [145] found that, after controlling for traditional risk

factors, each increment of 1 standard deviation of log N-BNP was associated with a 27% increase in the risk of death (all cause mortality, p=0.009) over a mean follow up period of 5.2yrs. There was also an increased risk of a first cardiovascular event (28%, p=0.03), heart failure (77% p<0.001), atrial fibrillation (66% p<0.001) and first stroke or transient ischaemic attack (53% p=0.002). The association was strongest with heart failure and atrial fibrillation. This relationship remained statistically significant even after correcting for echocardiographic variables of systolic function and left ventricular mass. There was no association with N-BNP levels with an increased risk of ischaemic heart disease. The levels of N-BNP associated with an adverse prognosis were considerably lower that what are cut off levels used for the detection of systolic heart failure. Similar findings were observed in a Danish study [146], which found that N-BNP was the single strongest predictor of mortality, and the 1st cardiovascular event, beyond that of the presence of traditional risk factors.

2.7.7. 2. Heart failure population

The original Glasgow MONICA cohort was followed up 5 years after the initial diagnosis of LVSD was made [48]. The four-year all cause mortality rate in the whole cohort was 4.9% (80 deaths). The levels of BNP and N-ANP differed significantly between the survivors and the group that died. Multivariate analysis found that N-ANP, BNP, male sex, ejection fraction and increasing age were independent predictors of mortality. N-BNP appeared to have better utility at predicting mortality in a population with advanced CHF, as compared to LVEF, oxygen consumption and heart failure scoring system [147]. Conversely, reduction of peptide levels in patients with CHF correlate with improved mortality [148].

2.7.7.3 Acute coronary syndrome population

Natriuretic peptides appear to give prognostic information in all the different categories of acute coronary syndrome. Serum levels of BNP rise rapidly in the first 24hrs following an MI and then stabilize. There is a further rise in patients with large ST elevation infarcts, perhaps with greater areas of infracted muscle and dysfunction 5 days later. BNP is released rapidly from myocyte stores even in the absence of myocyte necrosis. This has been shown in patients following an uncomplicated angioplasty as well as in exercise-induced ischaemia. This may make BNP more useful than traditional biomarkers of cardiac necrosis as BNP levels would reflect more the haemodynamic stress than the amount of cardiac cell necrosis.

The prognostic utility of BNP in a large (n=2525) heterogeneous group of subjects with acute coronary syndromes as part of the OPUS-TIMI 16 trial group [149]. 825 had ST elevation myocardial infarction, 565 with MI without ST elevation and 1133 with unstable angina. BNP was collected 40hrs (mean) after admission. BNP was an independent predictor of death at 30 days and at 10 months as well as recurrent MI and heart failure. This relationship was consistent across all 3 groups of ACS. When dived into quartiles by BNP levels, the adjusted odds ratio for death at 10 months was 3.8 (CI 1.1-13.3), 4.0 (CI 1.2-13.7) and 5.8 (1.7-19.7) for the 2^{nd}, 3^{rd} and 4^{th} quartile respectively. After adjusting for all independent predictors of death, a BNP level of 80 pg/ml was associated with a higher risk of death at 10months. BNP was also shown be an excellent predictor of long-term mortality in patients with acute coronary syndrome [150]. This predictive value was over and above that achieved by conventional risk stratification, including left ventricular dysfunction.

In a population of 666 patients with acute ST elevation myocardial infarction followed up over period of 3 years [151], both BNP and N-BNP, together with LV ejection fraction were independent predictors of death, heart failure and new myocardial infarction. The AUC of BNP with a cut of value of 30 pmol/L and N-BNP cut-off 162 pmol/L for detection of heart failure and death was 0.81 for both. The authors found that a combination of either BNP or N-BNP with LV ejection fraction substantially improved risk stratification than either alone.

In conclusion, we can say that in patient populations of acute coronary syndromes, including both ST and non-ST elevation myocardial infarctions, chronic heart failure and populations with systolic dysfunction, elevated serum levels of natriuretic peptides are an independent predictor of increased cardiovascular mortality and in some cases morbidity in both the medium and long term.

2.7.8 Guide to therapy

Natriuretic peptide levels are indirect markers of LV end diastolic pressure [152]. Peptide levels fall when patients with decompensated heart failure are treated aggressively with diuretic and vasodilators, this reduction correlating with a reduction of intracardiac filling pressures. Angiotensin converting enzyme inhibitors and aldosterone antagonists have also been shown to reduce BNP concentrations [153, 154]. In patients with CHF, elevated levels of NP indicates reduced exercise tolerance and increased symptoms. Patients also have a worse prognosis [155]. Several studies [157, 158] have found that BNP, N-BNP are independent predictors of mortality in patients with CHF. It may be that high NP levels are indicative of ongoing haemodynamic strain that is untreated by medial therapy and not clinically apparent

until decompensation occurs. Treatment with beta-blocker therapy leads to a reduction in peptide levels. [156]

It has been suggested by BNP consensus panel [159] that BNP has a role in the acute management of heart failure. Because of the short half of the peptide, treatment of the fluid overloaded patient with high left atrial and left ventricular end diastolic pressure would lead to a rapid reduction of BNP. Thus, BNP measurements over a 24-48hr period would predict response to therapy and indicate a point where the patient could be safely discharged. In addition, continuing high levels of BNP would suggest further escalation of treatment options. Several studies [160, 161 have found that high pre-discharge levels of natriuretic peptide levels predict risk of recurrent admission with further CHF as well as increased mortality. Conversely, low pre-discharge peptide levels suggest a better prognosis [162]. The REDHOT trial [122] followed patients admitted from the emergency room with decompensated CHF. A BNP level of >200pg/ml was a better predictor of 90 day events than physician perceived severity leading the authors to suggest that BNP was a better discriminator of risks.

Similarly, it has been suggested that natriuretic peptides may provide additional information in the outpatient treatment of chronic heart failure. Troughton et al [163] used N-BNP levels in addition to clinical assessment as a guide to therapy in a small group of patients with heart failure (n=69). The group with BNP directed therapy were more likely to be on higher doses of angiotensin converting enzyme inhibitors as well as aldosterone antagonists. In a relatively short follow period of 10mths, this group experienced significantly fewer events, including death and readmission with further episodes of decompensated heart failure, compared to the group which had therapy directed only by clinical assessment. Another group found that using therapy targeted at BNP level reduction appeared to lead to a greater reduction

in heart rate, BNP levels and increased renin activity levels as compared to a more traditional approach [164]. Treatment targeted to reduce natriuretic peptide levels may lead to better outcomes.

2.8 Physiological factors that influence natriuretic peptides.

Apart from haemodynamic stresses on the heart, several other factors influence the levels of NP. Redfield et al [34] explored the effect of age, gender on 2,041 subjects without cardiovascular disease. Increasing age, female gender and left atrial volume correlated independently with increasing BNP levels. LV mass did not correlate. The authors suggested that reference ranges for the use of NP should be gender and age specific and the normal ranges for different age groups and gender would be significantly different. The effect of age and gender was confirmed by Maisel et al. in the Breathing Not Properly trial, [165] that investigated the utility of BNP in patients presenting with acute dyspnoea. Apart from increasing age being associated with higher levels of BNP, the authors found that there was a significant difference among racial groups. As expected, the influence of physiological factors differs from peptide to peptide. Age was found to be a greater influence in BNP and N-BNP compared to N-ANP although the overall effect was small compared to the effect of impaired LV function. Lastly, increased body mass index has also been linked to higher levels of N-BNP [166, 167].

2.9 Choice of Natriuretic Peptides

Both ANP and BNP levels rise with haemodynamic strain of the left ventricle and, in the case of ANP, the left atrium. However, ANP, N-ANP, BNP and N-BNP respond differently

to stress and as such, perform differently in the diagnosis of LVSD. The variable effect of race, gender and age may also explain some of the differences. Early studies such as the Glasgow MONICA [110] group reported that N-ANP ROC AUC were inferior to the ROC AUC of BNP. A head-to-head comparison by [168] also found that N-ANP performed worst compared to N-BNP and BNP in the identification of LVSD. This group found that BNP and N-BNP performed similarly. Mueller T, et al [169] found that N-BNP appeared to perform better in the early identification of mild LVSD as opposed to BNP that performed better in the diagnosis of severe LVSD. BNP and N-BNP have been found in many trials to perform similarly [170,171, 172] but minor differences have been shown in some trials. The UK NP study [118] found slightly better negative predictive values using N-BNP as opposed to BNP (0.97 vs. 0.87). Richards et al [172] compared N-BNP and BNP. The 2 peptides correlated closely and reassuringly, found that the effect of age and gender was similar in both peptides.

BNP, being relatively unstable at room temperature is usually measured by a point of care test. N-BNP is a more stable compound and as such, can be stored for up to 72 hours before centrifuge to obtain the serum. Commercial assays allow samples to be posted from primary care surgeries for analysis at a central laboratory, a more convenient method in the context of a screening program. Because of a marked difference in the half-life of BNP and N-BNP, BNP is theoretically more representative of the real-time physiological situation. This may be an advantage in situations where acute haemodynamic changes are being monitored but perhaps les useful in a more static situation. Lastly, N-BNP, being primarily cleared via the kidneys, could theoretically be more influenced be increasing age as glomerular filtration declines with advancing age.

2.10 Choice of Assays

There are many commercial assay systems available (table 2.2). Although the results correlate well, there are significant differences in their performance [173]. Maisel, in his editorial on the use of natriuretic peptides [174], commented that cut-offs for different assays have to be established and that hospital laboratories should be consistent with their use of any one assay. Because of the effect of renal clearance on N-BNP, these assays have age-specific cut-off values for CHF. Obviously, the Biosite assay (Roche diagnostics) for BNP has advantages, as it is a bedside rapid assay that would lend itself to use in the emergency room setting as opposed to the Bayer and Roche assay which process the assays in batches. Table 2.2 assesses the concordance of the Bayer BNP test versus the Biosite BNP test. Both assays perform similarly using a threshold of 100pg/ml to diagnose LVSD.

Table II. Characteristics of BNP Assays

Vendor	Platform	Technology	Marker	Imprecision	Dynamic Range	Cutoff (pg/mL)
Abbott Laboratories, Abbott Park, IL	AxSYM	Microparticle enzyme immunoassay	BNP	Total %CV range: 6.5–9.4	0–4000	100
Bayer HealthCare Diagnostics, Tarrytown, NY	ADVIA Centaur ACS:180	Direct chemiluminescent sandwich immunoassay	BNP	Total %CV range: 2.3–4.7	0–5000	100
Biosite, Inc., San Diego, CA	Triage BNP	Single use fluorescence immunoassay device	BNP	Total %CV range: 9.9–12.2	0–5000	100
Biosite, Inc., San Diego, CA	Beckman Coulter: Access, Access 2, Synchron LXI, UniCel DXI	Two-site chemiluminescent immuno-enzymatic assay	BNP	Total %CV range: 2.1–6.7	0–5000	100
Roche Diagnostics, Indianapolis, IN	Elecsys	Electrochemiluminescent immunoassay	NT-proBNP	Total %CV range: 3.6–5.8	0–35,000	<75 yr: 125 >75 yr: >450

BNP=B-type natriuretic peptide; CV=co-efficient of variation; NT=N-terminus; Imprecision is listed in total %CV from vendor package insert.

Table 2.2 Characteristics of different BNP assays. Adapted from Tang WH et al, Clin Cornerstone. 2005;7 Suppl 1:S18-24.

2.11 Therapeutic Use of BNP

Nesiritide, a form of BNP has been used in the setting of decompensated heart failure. Phase 1 trials found that BNP infusions decreased pulmonary capillary wedge pressure and improved cardiac index and urinary flow rate in a dose dependant manner. Early trials by Colucci et al [175] found improved haemodynamic status and clinical status when compared to standard agents. This finding was similarly observed in the VMAC trial [176], which assessed the additive benefit of Nesiritide versus intravenous nitrate therapy on top of standard care. The use of Nesiritide appears to be associated with lower blood pressure but less ventricular ectopy as compared to the use of ionotropes such as dobutamine. On the strength of early trials, nesiritide gained a license in the treatment of decompensated heart failure. However, further work suggested that there was an association with worsening renal function [177]. Further pooled analysis found that the use of Nesiritide was associated with an increase in the 30-day mortality [178]. Clearly further large-scale trials are needed.

2.12 Conclusions

1. Heart failure is a common and disabling condition with significant mortality, morbidity and economic impact. It is also poorly recognized and is often diagnosed late in the course of the disease despite there being an early asymptomatic or presymptomatic phase, which is amenable to therapy.
2. The gold standard diagnosis of LVSD requires a quantification of left ventricular function, usually by echocardiography. This is not a practical means of screening the general population.
3. Natriuretic peptides are secreted by the heart in response to increased wall tension. Peptide levels are elevated in subjects in CHF and LVSD.
4. The 12 lead ECG is also frequently abnormal in patients in LVSD.

What questions are there to be answered?

1. What is the true prevalence of undiagnosed LVSD in the general population?
2. What is the utility of cardiac peptide hormones and the ECG in screening an unselected general population for LVSD?
3. Can we maximize the utility of screening (e.g. by screening high-risk groups only or using the ECG and peptides together)?

Chapter 3

Methods

3.1 Introduction

Study patients were recruited from the general population in Leicestershire. Demographic detail was obtained and subjects underwent echocardiography in order to identify cases of LVSD. Systolic function was quantified using calculations of ejection fraction as well as a sixteen-segment wall motion scoring system. Plasma serum was obtained and levels of N-ANP, BNP and N-BNP were measured using immunoluminetric assays. I then assessed the utility of the various peptides and well as a standard 12 lead ECG in diagnosing LVSD.

3.2 Recruitment

This study complied with the Declaration of Helsinki and approved by the local Leicester Research Ethics Committee. All subjects gave written informed consent prior to undergoing physical examination, echocardiography and blood sampling. The screening population was selected from twenty-one general practices in Leicestershire Health Authority area, population approximately 1 million, which were stratified by their list size and deprivation index (based on their Jarman score). Randomly selected Caucasian men (45-80 years) and women (55-80 years) were sent an invitation to be screened for heart failure. Patients were excluded if there was a prior confirmed diagnosis of heart failure. This was ascertained from general practice records that indicated previously formal assessment of left ventricular function, generally initiated by hospital medical contact. Patients in whom screening was considered inappropriate, for example housebound or terminally ill patients were also excluded. This exclusion process was initiated by the general practitioner and was further checked by a member of the research team before patients were contacted by post. Practice

records yielded the past medical history, ischaemic heart disease (defined as myocardial infarction and/or angina), hypertension, diabetes, smoking status and prescribed cardiovascular medication.

3.3 Patient screening

Patients attended the Leicester Royal Infirmary for a full transthoracic echocardiogram performed by myself (accredited by the British Society of Echocardiography). A standard 12-lead electrocardiogram, height and weight measurements and blood sampling for standard electrolyte measurements and natriuretic peptide assays were also obtained. A questionnaire was then filled out by the patient about their symptoms – namely dyspnoea, orthopnoea and peripheral oedema.

3.4 Echocardiographic measurements

Transthoracic echocardiography was performed in all patients using an Agilent Sonos 5500 instrument (Philips Medical Systems). Scans were performed with subjects in the standard left lateral decubitus position. Images were acquired and downloaded onto a digital database where further measurements were performed "off-line".

The following echocardiographic parameters were assessed

1. Left ventricular systolic function – by LVEF and WMSI
2. Left ventricular mass and other chamber dimensions
3. Valvular function – by qualitative assessment as well as Doppler assessment
5. Diastolic function - peak early transmitral flow velocity (E), atrial flow velocity (A), E/A ratio and deceleration time of the mitral E wave were obtained from the digitised images.

3.4.1 Quantification of left ventricular systolic function

Accurate quantification of left ventricular volume and systolic function is assessed by a variety of techniques including invasive angiography, radionuclide angiography and magnetic resonance imaging. It is important to use a single method of assessment as different imaging techniques result in significant inter-modality variability. Transthoracic echocardiography is currently the most commonly applied imaging modality in the practice of cardiology to calculate left ventricular systolic function. Echocardiographic assessment of LV function is non-invasive, safe and most acceptable. However, assessing left ventricular function by echocardiography is subject to inter-observer variation. Furthermore, ultrasound penetration in tissues is also very much affected by the subject body habitus and this means that accurate determination of function may not always be achievable in all subjects. However, these disadvantages are minor compared to the ease of use and acceptability of echocardiography, particularly in a large-scale screening program such as ours. There are different ways of forming an echocardiographic quantitative assessment of systolic function, all with their individual advantages and disadvantages.

3.4.1.1. M-mode echocardiography

The M-mode scan is a one-dimensional ultrasound scanning of the cardiac structures. It was developed in the early 1970s and immediately applied in practice for left ventricular function assessment because of its simple algorithm and non-invasiveness. Ejection fraction was estimated as a percentage derived from the mid left ventricular diameters in end-systole and end-diastole and expressed as fractional shortening. However, serious errors are made in

estimating function in patients with segmental wall motion abnormalities and asymmetric ventricles. Despite that, this method has been widely utilized in very large-scale population studies including the Framingham series [44, 45].

3.4.1.2. Simpson's apical biplane method (biplane method of discs)

Two-dimensional sectional echocardiography, with the ability of imaging of the heart in tomographic views, considerably improved the accuracy of left ventricular volume measurement. Of the different mathematical models, modified biplane Simpson's rule provided more accurate data in both symmetric and asymmetric left ventricles. Software-based algorithms for automatic endocardial border detection and on-line calculation of left ventricular volume and ejection fraction have been developed. As a result, two-dimensional echocardiography has become a routine examination for left ventricular volume and function assessment but the assumptions about left ventricular geometry remain a limitation. Naturally, the technique is further influenced by the technical image quality of the scan. This method utilizes the following equation - $\frac{\text{LVd MOD Vol} - \text{LVs MOD Vol}}{\text{LVd MOD Vol}} \times 100$ and requires an accurate estimation of the end diastolic and end-systolic left ventricular volume. Systolic function is expressed as an ejection fraction (EF), which is the percentage of the volume of blood expelled by the left ventricle and the left ventricular end diastolic volume. In addition to being inaccurate due to geometrical assumptions of left ventricular dimensions ,suboptimal image quality can mean that an accurate ejection fraction is not obtained in as many as 30-40% of a study population. When achievable, the EF does correlate well with the gold standard of left ventricular function, gated radionuclide ventriculography [179].

3.4.1.3 The Sixteen-segment wall motion score index (WMSI)

This simple method of assessment of systolic function is least limited by asymmetry of the left ventricular cavity and is often obtainable in most of the population as it less affected by technical limitations compared to the modified Simpson's method. This has previously been utilized in large-scale trials [181, 185], which found close correlation between LVEF and wall motion scores as well as good reproducibility. A large-scale population study in Sweden [91] found that not only was wall motion reproducible and was also obtained in over 95% of subjects compared to 68% in the case of LVEF calculated by Simpson's rule. Wall motion scoring was also diagnostic of low LVEF, as analyzed by using ROC analysis (AUC 0.998 for EF <40%). Despite the various methods at disposal to quantify systolic function, which the best means of calculating left ventricular function can be a best guess visual estimate by an experienced operator [180].

In my study, left ventricular function was independently quantified by three investigators using two techniques, the Simpson's modified biplane measurement of the ejection fraction and the 16-segment wall motion score. Recommendations from the American Society of Echocardiography [183] were followed to calculate the EF using Simpson's method. Images were initially obtained "live", then transferred to a digitalized system, and subsequently analyzed "off-line". The stop frame model and the reference ECG were used to identify the left ventricular end diastolic endocardial borders at the peak of the R wave. The end systolic endocardial borders were measured near the end of the T wave at the maximum inward motion of the left ventricle. In tracing the cross sectional echocardiographic endocardial borders, minor irregularities due to decreased visual integrity of the endocardium were interpolated from the real time and slow motion images. The sixteen segment model of the left ventricle, based on the

American Society of Echocardiography model [183], was scored according to an assessment of endocardial incursion of each individual segment (scored as 1=normal, 2=hypokinesis, 3=akinesis and 4=dyskinesis) and dividing the total by the number of segments scored. I also assessed the relationship between LVWMI and LVEF in our population. Both EF and WMSI was calculated 3 times for each case and the final result was a mean of the three.

3.4.3 Measurement of left ventricular mass and valvular function

Left ventricular internal dimension (LVID) as well as interventricular septal thickness (IVST) and posterior wall thickness (PWT) were obtained by averaging digital M-mode or 2-D long axis images over 3 cardiac cycles at end-diastole, using guidelines from the American Society of Echocardiography [183]. LV mass was calculated from the formula (g) = $0.8[1.04(LVID + IVST + PWT)^3 - LVID^3] + 0.6$ [13]. This was then corrected for body surface area to give the left ventricular mass index (LVMI).

The left atrium was also measured at its maximum diameter. The aortic valve, mitral valve, tricuspid valve and pulmonary valve were also assessed by qualitative as well as quantitative methods using standard guidelines from the American Society of Echocardiography [183].

3.5 ECG analysis

The 12-lead ECG was obtained using a Hewlett-Packard automated machine following a twenty-minute bed-rest to reduce anxiety induced adrenergic effects. Each 12-lead ECG was coded independently by two consultant physicians using the Minnesota criteria [184]. If there was disagreement between the results, a new coding was reached in consensus. The coding was

based on the Minnesota coding system. If the coding differed between the physicians, a new coding occurred in consensus.

3.6 Laboratory methods

Because of the possible influence of postural change on natriuretic peptide levels, all subjects were venesected after 20-30 min bed-rest during which they had their echocardiogram performed. Following that, 20 ml of peripheral venous blood was drawn into pre-chilled sodium/EDTA (1.5 mg/ml of blood) tubes containing 500 international units/ml aprotinin. After centrifugation at 1500 g at 4 °C for 15 min, plasma was separated and stored at −70 °C until assayed. Subjects also provided a urine sample, and 20 ml was collected into tubes with the above additives, centrifuged and stored as above. Twenty milliliters of blood was transferred to chilled tubes containing 500 i.u/ml aprotinin (Trasylol, Bayer, Newbury, Berks, U.K.) and EDTA (1.5 mg/ml). After centrifugation, plasma was stored at-70 °C until assay. All samples were analyzed within 2 months of venesection.

Plasma specimens were defrosted and 1 ml was acidified with an equal volume of 1% TFA. After centrifugation, the supernatant was loaded on to C18 extraction columns. After two washes (3 ml each) with 0.1% TFA, the peptides were eluted with 2 ml of 0.1% TFA containing 60%acetonitrile. The eluates were then dried in a centrifugal evaporator. The dried eluates were reconstituted in 1 ml of the ILMA buffer (see below) containing 0.1%Triton X-100 and assayed immediately.

3.6.1 BNP and N-ANP assays

Competitive immunoluminometric assays for BNP and N-ANP were based on commercially available antibodies from Peninsular Laboratories Inc. (Belmont, CA) and

Phoenix Pharmaceuticals Inc. (Belmont, CA), respectively, following extraction on C columns. Biotinylated tracer 18 peptides were purified on reverse-phase C HPLC. 18 Streptavidin labeled with methyl-acridinium ester was used to detect bound biotinylated tracer w18x. Unextracted plasma was assayed for N-BNP using a non-competitive immunoluminometric assay. The lower limit of detection of N-ANP, BNP and N-BNP was 3.4 fmol/ml, 2.0 fmol/ml and 5.7 fmol/ml, respectively. There was no cross reactivity between assays. The N-BNP was an "in-house" assay and as such, I will go into this in greater detail in the following section.

3.5.2 N-BNP non-competitive immunoluminometric assay

3.5.2.1 Materials

Peptides corresponding to amino acids 37±49 (SLEPLQESPRPTG) and amino acids 65±76 (RKMVLYTLRAPR) of the preproBNP sequence, representing the midsection and C-terminus respectively of NT-proBNP were synthesized in theMRC Toxicology Unit, Leicester University. Both of these peptides were further purified by HPLC on preparative C18 columns with an acetonitrile gradient, resulting in greater than 98%purity. The pure peptides were confirmed to be of the correct predicted molecular mass using matrix-assisted laser desorption mass spectrometry, and are referred to hence as NT1 and NT2 respectively. Protein A±Sepharose CL4B gel was obtained from Pharmacia, Herts., U.K. The horseradish-peroxidase-conjugated anti-rabbit IgG and the enhanced chemiluminescence kits were obtained from Amersham International, Bucks., U.K. The methyl acridinium ester [4-(2-succinimidyloxycarbonyl ethyl)- phenyl-10-methylacridinium 9-carboxylate fluorosulphonate] was a gift from Drs Stuart Woodhead and Ian Weeks, Molecular Light Technology Ltd, Cardiff, U.K. The paramagnetic particles coated with goat antirabbit IgG were from Metachem

Diagnostics Ltd, Northampton, U.K. The C18 plasma extraction columns and the peptides ANP, BNP, C-type natriuretic peptide (CNP) and proBNP(22±46) were obtained from Peninsula Laboratories, Merseyside, U.K. The nitrocellulose (0.2 lm) for peptide blotting using tricine gels was from Schleicher and Schuell, Dassel, Germany. The peptide molecular mass markers, tricine and all other reagents of Analar grade were obtained from Sigma Chemical Co. Ltd, Poole, Dorset, U.K.

3.6.2.2 Production of antibodies

The NT1 and NT2 peptides were conjugated to haemocyanin with the hetero-bifunctional cross-linker e-maleimidocaproic acid N-hydroxysuccinimide ester. Haemocyanin was dissolved in buffer (10 mg/ml in 100 mmol}l Na_2HPO% buffer, pH 8) and 1 ml was rapidly mixed with 400 nmol of e-maleimidocaproic acid N-hydroxysuccinimide ester (in 10 ml of dimethylformamide). After 30 min, the derivatized haemocyanin was gel-filtered on a Sephadex G25 column conditioned with 100 mmol/ml Na_2HPO% buffer, pH 7.4. The haemocyanin was then mixed with 1 mg of NT1 or NT2 and the conjugation reaction proceeded for 3 h at room temperature. The conjugates were then dialysed with PBS for 3 days at 4 °C, with four changes of the PBS. Two rabbits were injected subcutaneously with antigens (1 mg) emulsified with complete Freund's adjuvant. After a month, booster injections (1 mg) were given intravenously every 2 weeks and the antisera obtained after 3 months. The IgG fraction was obtained by Protein A±Sepharose chromatography. Antibody G172 reacted with the peptide NT1 and G185 with NT2.

3.5.2.3 Peptide labelling with the methyl acridinium ester

The peptides NT1 and NT2 were dissolved in 100 mmol/l Na_2HPO% buffer, pH 8, at of 200 µmol/l and 100 µl was pipetted into an Eppendorf tube. Five micrograms of the methyl acridinium ester was dissolved in 5 µl of dimethylformamide and mixed with 20 nmol of the peptide to be labelled. After incubation at room temperature for 30 min in the dark, 100 µl of a lysine quench solution (10 mg/ml in 100 mmol/l Na_2HPO% buffer, pH 8) was added and incubated for another 5 min. The labelled peptide solution was acidified with an equal volume of 1% trifluoroacetic acid (TFA). An aliquot of this was then injected on to a 3.9 mm¨150 mm Deltapak C_{18} 300 Å column, mounted within a HPLC system (Waters, Watford, Herts, U.K.) consisting of a Waters 600S controller, 626 pump and 486 tuneable absorbance detector set at 215 nm to detect ultraviolet absorbance of the peptide peaks. The column was equilibrated with 0.1% TFA and a gradient of acetonitrile from 0 to 55% (at a rate of 2%/min) was used to elute peptides. NT1 and NT2 were eluted at 35 and 35.5% acetonitrile respectively. Their respective methyl acridinium esters were eluted at 45 and 47% acetonitrile and the recovery of these labelled tracers amounted to 27.5 ± 2.8% of the total label used. The hydrophobicity of the labelled peptides after derivatization with the methyl acridinium ester facilitated the separation from unlabelled peptide. These fractions were collected and used for development of the ILMAs. Fractions collected at other times (corresponding to 215 nm absorbance peaks) were inactive. The labeled peptides were stored in the dark at -70 °C in the 0.1% TFA buffer, being stable for over 6 months. One preparation produced enough tracer for about 10000 experiments.

Twenty milliliters of blood was transferred to chilled tubes containing 500 i.u./ml aprotinin (Trasylol4, Bayer, Newbury, Berks, U.K.) and EDTA (1.5 mg}ml). After

centrifugation, plasma was stored at -70 °C until assay. All samples were analyzed within 2 months of venesection. Plasma specimens were defrosted and 1 ml was acidified with an equal volume of 1% TFA. After centrifugation, the supernatant was loaded on to C18 extraction columns. After two washes (3 ml each) with 0.1% TFA, the peptides were eluted with 2 ml of 0.1% TFA containing 60%acetonitrile. The eluates were then dried in a centrifugal evaporator. The dried eluates were reconstituted in 1 ml of the ILMA buffer containing 0.1%Triton X-100 and assayed immediately.

3.5.2.4 ILMA for N-BNP

The ILMA buffer consisted of 1.5 mmol/l NaH_2PO%, 8 mmo/l Na_2HPO%, 140 mmo/l NaCl, 1 mmol/l EDTA, 1 g/l BSA and 0.1 g/l azide. Wash buffer was composed of 1.5 mmol/ml NaH_2PO%, 8 mmol/l Na_2HPO%, 140 mmol/l NaCl, 0.5 g/l Tween 20, 1 g/l gelatin and 0.1 g/l azide. On day 1 of the assay, 100 µl of assay buffer containing 20 ng of the antibodies G172 or G185 was pipetted into tubes and incubated overnight at 4 °C with 100 ll of peptide standards in the range 1±2000 fmol per tube. All samples and standards were assayed in duplicate. One hundred microlitres of assay buffer containing about 10^6 relative light units (RLU) of the labelled peptide NT1 or NT2 was then added and tubes again incubated overnight at 4 °C. On day 3, 10 µl (10 µg) of paramagnetic particles coated with goat anti-rabbit IgG was added to tubes to recover the immunoprecipitates. The particles with attached immunoprecipitates were washed three times with the wash buffer described above (2 ml for each wash) and the particles recovered each time using a magnetized tube rack, allowing the wash solutions to drain adequately. After the last wash, 100 µl of distilled water was added to the tubes and the particles resuspended by vortexing the tubes. Readings of the

chemiluminescence from the immunoprecipitates were then obtained on a Luminoportable luminometer (Stratec Electronic GMBH, Birkenfeld, Germany). In order to initiate chemiluminescence of the label, the fist injection was 100 µl of 100 mmol/l HNO_3 containing 0.05% hydrogen peroxide, followed 4 s later by an injection of 100 µl of 250 mmol/l NaOH containing 0.25% cetyltriethylammonium bromide. The detergent optimized the light emission from the label. Chemiluminescence was measured over 2 s after the second injection and expressed as RLU. Standard curves were obtained and non-linear least squares fitting performed using an algorithm with a Rodbard 4 parameter equation.

3.6.2.5 Peptide blotting using tricine/SDS/polyacrylamide gels

Five to ten milliliters of plasma was acidified with an equal volume of 0.1% TFA, incubated on ice for 30 min and then centrifuged. The supernatants were then loaded on to C18 columns (containing 1 g of the sorbent material). After five washes with 4 ml each of 0.1%TFA, the peptides were eluted with 4 ml of 60%acetonitrile in 0.1% TFA. The samples were dried in a centrifugal evaporator. Samples were then dissolved in 100 µl of gel sample buffer [consisting of 200 mmol/l Tris (pH 6.8), 20 g/l SDS, 0.4 g/l Coomassie Blue, 40%(by vol.) glycerol and 10 mmol/ml dithiothreitol] and boiled for 5 min. They were then loaded on to a 16.5% tricine/SDS/polyacrylamide gel. This consisted of a 16.5% resolving gel, a 10% spacer gel and a 5% stacking gel, made as described previously [186]. Gels were run at a constant voltage (90 V) over 16 h when the current fell from an initial 30 mA to 10 mA. Coloured peptide markers were loaded to enable calibration of the observed bands. The peptides were then blotted on to reinforced nitrocellulose (0.2 lm), and blocked overnight in 1% dried milk powder in Tris-buffered saline (TBS, composed of 20 mmol/l Tris, 135 mmol/l NaCl)

containing 0.1% Tween 20. Detection was achieved by incubating for 1 h at room temperature with 5 μg/ml G185 (specific for NT2 epitope on NT-proBNP) in TBS-Tween. After 10 washes in TBS-Tween, the second antibody (horseradish- peroxidase-conjugated anti-rabbit IgG) was added at 1 μg/ml in TBS-Tween and incubated at room temperature for 1 h. After 10 further washes, the blot was developed using the Amersham enhanced chemiluminescence kit according to the instruction manual. Blots were exposed on to pre-flashed x-ray films to visualize the immunoreactive bands.

3.7 Statistical Analysis

Statistical analysis was performed using SPSS version 13.0 (SPSS Inc, IL) and Stata (Release 7.0). Factors and covariates related to LVSD in univariate analysis ($P<0.1$) were entered in logistic regression analysis to develop prognostic indices for the probability of having LVSD, adjusting where necessary for co-morbidity. The same analysis was performed stepwise (both forward and backward) to assess reliability of the predictors. Binary logistic regression analysis was performed with SPSS with the stated variables, including a constant in the model and probability for entry or removal set at $p< 0.05$ and $p>0.10$, respectively. The resulting prognostic indices were validated using a jack-knifing method [187] to correct for development and validation of the models on the same data. The resulting bias-corrected indices were used to construct receiver-operator-characteristic (ROC) curves. The area under the curves (AUC) and their associated Standard Errors (SE) were estimated using the non-parametric method of Hanley and McNeil [188]. Comparison of AUC of competing ROCs was performed using the method of DeLong et al [189] which adjusts for the fact that the different AUCs were estimated using the same patients. A ROC AUC of 1.0 would indicate a test with

perfect discrimination between subjects with and without the disease, whereas an AUC under 0.5 indicates a test with no discriminatory power. Different optimal cut-off levels for different peptides were assessed. Statistical analysis included sensitivities, specificities, positive and negative predictive values and likelihood ratios (LR) [190]. A positive predictive value (Figure 3.1) is the proportion of subjects with a positive test who have the disease and a negative predictive value gives the number of subjects with negative tests that are truly disease free. The tests give us a better picture as the utility of a diagnostic test and are particularly useful as the prevalence of the condition is factored into the equation. The Likelihood Ratio (LR) is the likelihood that a given test result would be expected in a patient with the target disorder compared to the likelihood that that same result would be expected in a patient without the target disorder. This, too, varies less with disease prevalence compared to sensitivities and specificities.

$$PPV = \frac{\text{Sensitivity} \times \text{prevalence}}{\text{Sensitivity} \times \text{prevalence} + (1 - \text{specificity}) \times (1 - \text{prevalence})}$$

$$NPV = \frac{\text{Specificity} \times (1 - \text{prevalence})}{(1 - \text{Sensitivity}) \times \text{prevalence} + \text{specificity} \times (1 - \text{prevalence})}$$

$$\text{Positive likelihood ratio (LR+)} = \text{sensitivity} / (1-\text{specificity})$$

$$\text{Negative likelihood ratio (LR-)} = (1-\text{sensitivity}) / \text{specificity}$$

Figure 3.1 Statistical calculations for utility of diagnostic tests. PPV – positive predictive value, NPV – negative predictive value

3.8 Summary

- Subjects were randomly selected from general practices.
- The screening procedure consisted of an echocardiogram, 12 lead electrocardiogram, symptom questionnaire and a analysis of renal function and cardiac peptide levels.
- Left ventricular function was assessed by the sixteen-segment wall motion analysis as well as calculation of ejection fraction by Simpson's method.
- BNP and N-ANP were analyzed by commercially available competitive immunoluminometric assays. N-BNP was analyzed by a non-competitive in-house immunoluminometric assay.

Chapter 4

Characteristics of the Study Population

4.1 Study uptake

Screening invitations were accepted by 1360 of 2392 patients approached, an overall uptake of 57% (men 56%, women 58%, P = NS). The number of participants with analyzable ECG and echo, and available blood and urine sample was 1308(Table 1). There was no difference between the general practice mean Jarman scores of those who accepted (+7.14) and those who declined screening (+8.08). There were a slightly larger proportion of men who attended for screening (56.7%). Mean age was 63yrs (range of 45-80yrs). Other population demographics are as stated in table.

4.2 Medical History and Prescribed medication (Table 4.1)

Only 32 patients had a history of a previous myocardial infarction and 90 had a diagnosis of angina. A relatively large proportion of the study population (23.7%, n=310) had a diagnosis of hypertension although this was well controlled (mean systolic BP 139, diastolic 78mmHg). All of these were on medication prescribed by their general practitioner. Diabetes was found in 63 subjects (4.8%). The data in the group with normal left ventricular function was compared to that in the group with LVSD. The latter group was significantly older (mean 68 vs. 53yrs, $p<0.05$) and was more likely to have a diagnosis of angina and/or a previous history of having had a myocardial infarction. The proportion of patients with hypertension, diabetes or a smoking history was not significantly different in the 2 groups. A loop diuretic (Furosemide) was prescribed in 174 subjects, of which only 44 (25.3%) were on an Angiotensin Converting Enzyme inhibitor. The prescription of a diuretic implies the presence of oedema and would suggest that at least a proportion would have undiagnosed LVSD.

	All	No LVSD	LVSD
Men/women	742/566 (56.7/43.3)	720/560 (56.2/41.7)	22/6 (78.6/21.4)†
Age (yrs), mean (range)	63 (45 to 80)	63 (45 to 80)	68 (51 to 80)‡
Practice Jarman score, mean (range)	6.96 (-16.0-41.4)	63 (45 to 80)	68 (51-80)
Body mass index (kg/m^2)	26.7 ± 4.4	26.7 ± 4.4	27.2 ± 5.3
Systolic BP	135 ± 19	135 ± 19	138 ± 19
Diastolic BP	78 ± 12	78 ± 12	79 ± 14
Current smoker	254 (19.4)	247 (19.3)	7 (25)
Plasma Creatinine	89.5 ± 30.3	89.3 ± 30.5	99.9 ± 18.3 §
Creatinine clearance	77.8 ± 22.4	77.9 ± 22.4	71.4 ± 21.6
Medical History			
Myocardial infarction	32 (2.4)	26 (2.0)	6 (21.4)
Angina	90 (6.9)	82 (6.4)	8 (28.6)¶
Hypertension	310 (23.7)	302 (23.6)	8 (28.6)
Diabetes mellitus	63 (4.8)	61 (4.8)	2 (7.1)
ECG abnormalities			
AF	17	15	2
ECG LVH	124	121	3
Pathological Q waves	46	40	6
Left bundle branch block	23	13	10
Left axis deviation	57	56	1
Prescribed therapy			
ACE inhibitor/ARB	115 (8.8)	109 (8.5)	6 (21.4)
Loop diuretic	36 (2.7)	34 (2.7)	2 (7.1)
Other diuretic	165 (12.6)	163 (12.7)	2 (7.1)
Beta-blocker	147 (11.2)	143 (11.2)	4 (14.3)
Nitrate	51 (3.9)	44 (3.4)	7 (25) ¶
Calcium channel blocker	128 (9.8)	122 (3.4)	6 (21.4)†
Natriuretic Peptides, median (range)			
Plasma N-BNP (fmol/ml)	44.5 (5.7 to 1,230.2)	42.2 (5.7 to 1,166.4)	360 (5.7 to 1,230.2)§
Plasma N-ANP (fmol/ml)	401.57 (5.21-4115.4)	397.15 (5.21 to 4115.4)	742.85 (179.8 to 2992.96) §
Plasma BNP (fmol/ml)	20.29 (2.0-506.92)	19.94 (2.0 to 276.63)	74.79 (19.23 to 506.92) §
Average EF	57.49 ±10.48	59.51	32.11
Mean QRS (msec)	93.54 (53.62 to 184.00)	92.66 (53.62 to 160.71)	110.64 (74.01 to 167.23)
Mean QT	380.51 (284.07 to 507.86)	381.51 (298.79 to 507.86)	383.84 (287.07 to 461.02)

Table 4.1. Population characteristics. Data are presented as the number (%) of patients or mean value ± SD, unless specified otherwise. *Valvular abnormalities include moderate/severe mitral regurgitation or aortic stenosis. P values for comparisons between LVSD and no LVSD groups: †p _ 0.05 and ¶p < 0.001 (chi-square test); ‡p< 0.005 and §p < 0.001 (Mann-Whitney test). Legend: ACE =angiotensin-converting enzyme; AF= atrial fibrillation; ARB=angiotensin receptor blocker; ECG LVH = electrocardiographic left ventricular hypertrophy; LVSD = left ventricular systolic dysfunction; N-ANP= N-terminal atrial natriuretic peptide, BNP = Brain natriuretic peptide, N-BNP = N-terminal pro-brain natriuretic peptide.

4.3 Measurements of left ventricular function

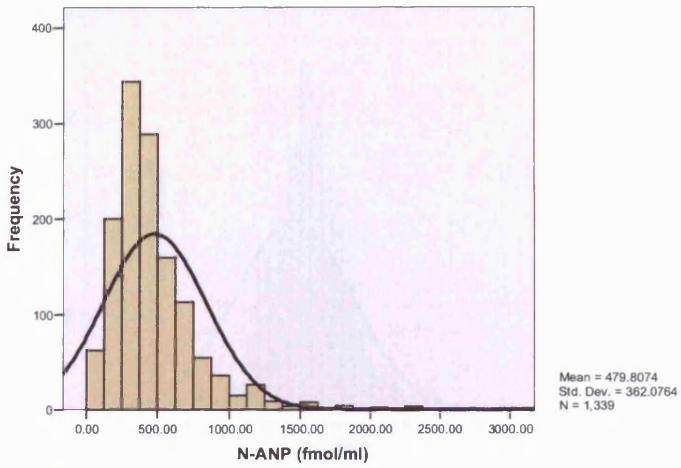
LVEF was obtainable in only 1058 (79%) of the subjects whereas the 16-segment LV wall motion score index (WMSI) was computed from 1331 (99.4%) of the population. There was a strong correlation between these parameters ($r = 0.80$, $P<0.0005$, Figure 1) and as such we used the WMSI to define our population with LVSD. Severe LVSD was defined as WMSI of ≥ 2.equivalent to EF <35%, , Moderate LVSD was defined as an LVEF ≤40%, a figure that has been used in other large scale epidemiological surveys [12, 112]. This correlated to a WMSI ≥ 1.8.

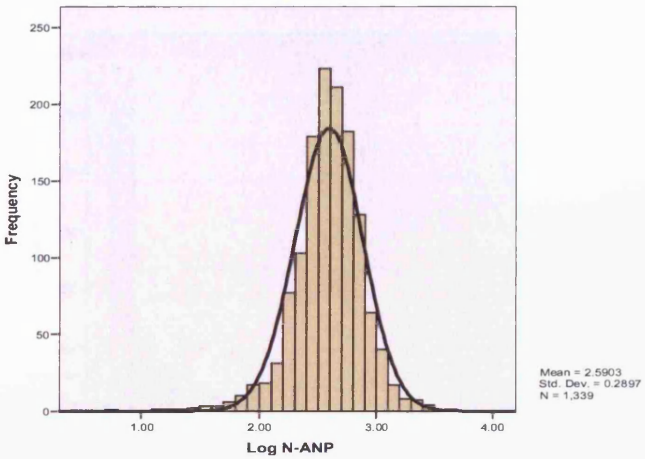
4.4 Natriuretic peptides levels

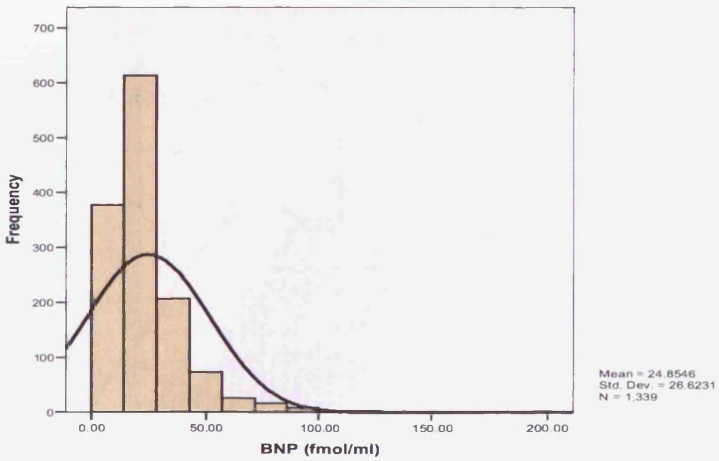
Natriuretic peptide levels were not distributed normally as analyzed by their skewness and Kurtosis errors (Figure 3.2a-c). Therefore, natriuretic peptide levels were normalized by log transformation before statistical analysis. Seventeen individuals (1.28%) had severe LVSD, and an additional 11(0.98%) had moderate LVSD. In total, there were 28 subjects with moderate to severe LVSD. Plasma concentration of each natriuretic peptide was higher in those with LVSD than in those with preserved systolic function (see figure 3.3a-c): median N-ANP(range) 943(288–3020) fmol/ml *vs.* 385(5-4115) fmol/ml ($p<0.0005$); BNP 92.9(19.0-501.2) fmol/ml *vs.* 17.1 (2.0-275.4) fmol/ml ($p<0.0005$); N-BNP 301(38-1230) fmol/ml *vs.* 36(5-1174) fmol/ml, ($p<0.0005$). Table 4.2 explores the differences in the population over the 90th centile of N-ANP, BNP and N-BNP. Differences in the population were also significant ($P<0.001$) using Chi-square analysis. Prevalence of LVSD (both severe and moderate to severe), valvular disease and ECG abnormalities were significantly higher in the group with highest N-ANP, BNP and N-BNP. Hypertension and IHD, but not diabetes was more frequent in the groups with levels of peptides in the top 10th centile.

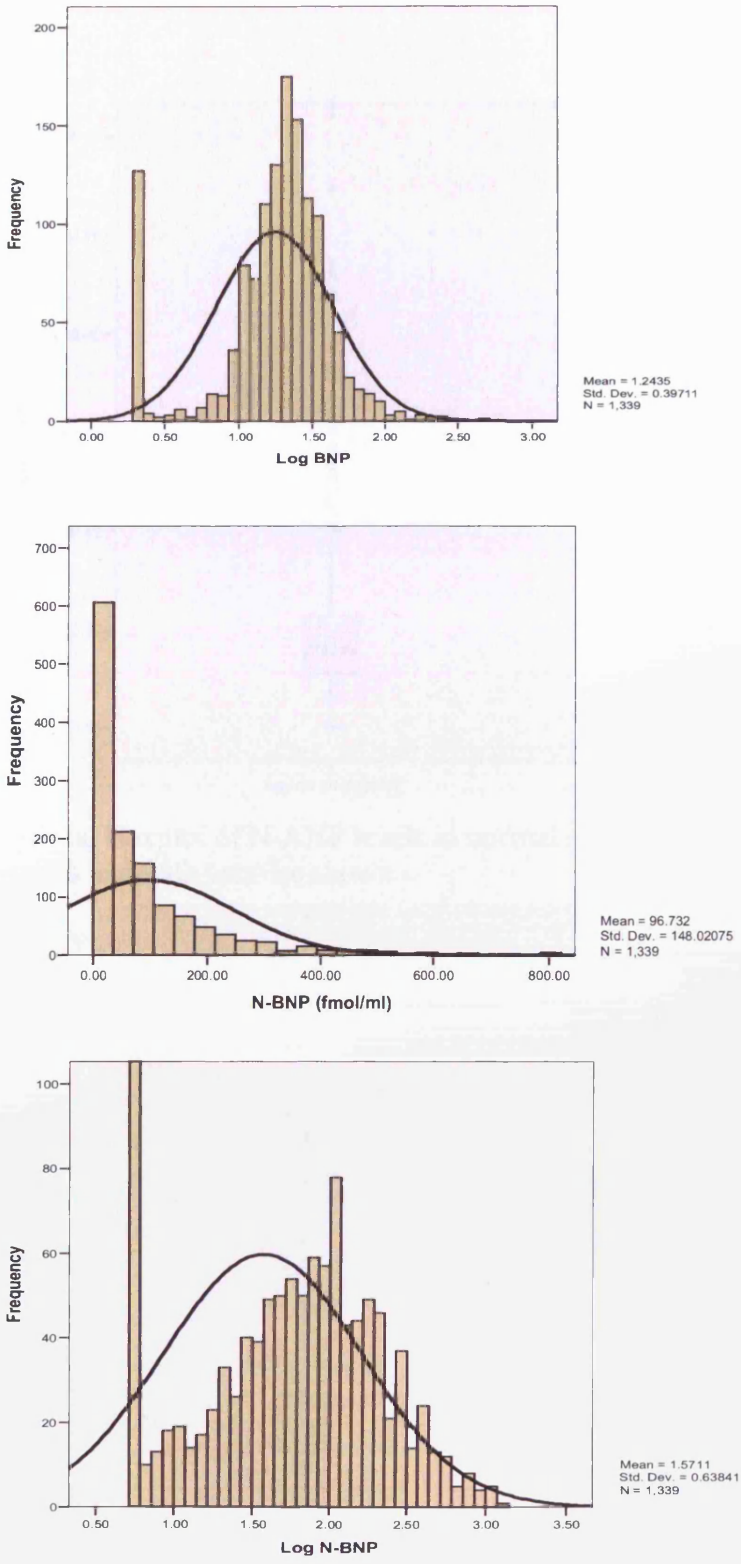
No. of cases (% of population in parenthesis)		IHD	Hyper-tension	Diabetes	Severe LVSD	Mod-severe LVSD	ECG abnormalities	Valvular disease
N-BNP	10^{th} centile (n=135)	¶32 (24)	¶53 (39)	8 (6)	¶10 (7)	¶16 (12)	¶59 (44)	¶5 (4)
	Remainder (n=1204)	73 (6)	272 (6)	56 (5)	7 (0.6)	12 (1)	158 (13)	4 (0.3)
N-ANP	10^{th} centile (n=135)	¶31 (24)	66 (49)	9 (5)	¶9 (7)	¶12 (9)	¶57 (44)	¶5 (4)
	Remainder (n=1204)	74 (6)	259 (21)	55 (5)	8 (0.6)	16 (1.3)	160 (13)	4 (0.3)
BNP	10^{th} centile (n=135)	¶31 (24)	¶54 (39)	15 (11)	¶14 (10)	¶25 (19)	¶57 (44)	¶5 (4)
	Remainder (n=1204)	74 (6)	271 (6)	49 (4)	3 (0.2)	3 (0.2)	160 (13)	4 (0.3)

Table 4.2. Population characteristics of the upper 10^{th} centile of natriuretic peptide levels compared to the rest of the population. P values for comparison between upper 10^{th} centile and remainder of population: ¶ $P<0.05$ by Chi square test. Valvular abnormalities – severe aortic stenosis/regurgitation, mitral regurgitation/stenosis, severe tricuspid regurgitation.


Frequency
400
300
200
100
0
0.00
500.00
1000.00
1500.00
2000.00
2500.00
3000.00
N-ANP (fmol/ml)
Mean = 479.8074
Std. Dev. = 362.0764
N = 1,339


Frequency
250
200
150
100
50
0
1.00
2.00
3.00
4.00
Log N-ANP
Mean = 2.5903
Std. Dev. = 0.2897
N = 1,339


Frequency
700
600
500
400
300
200
100
0
0.00
50.00
100.00
150.00
200.00
BNP (fmol/ml)
Mean = 24.8546
Std. Dev. = 26.6231
N = 1,339



Figures 3.2 a-c. Frequency histograms of N-ANP, BNP and N-BNP and their log transformation histograms

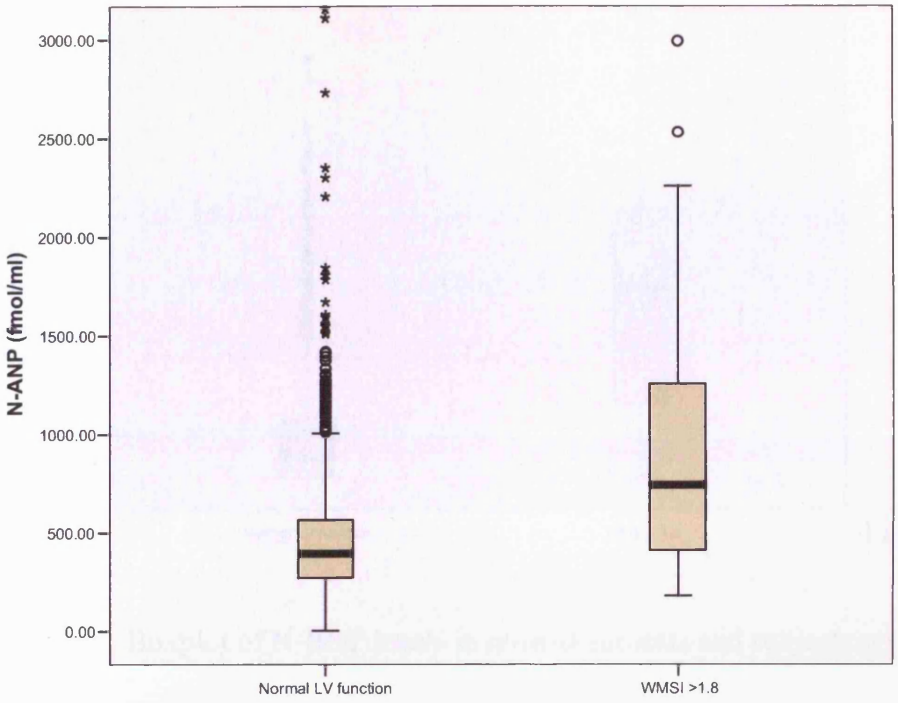


Fig 3.3a. Boxplot of N-ANP levels in normal subjects and subjects with LVSD. Medians, 95th centiles and 99th centiles shown

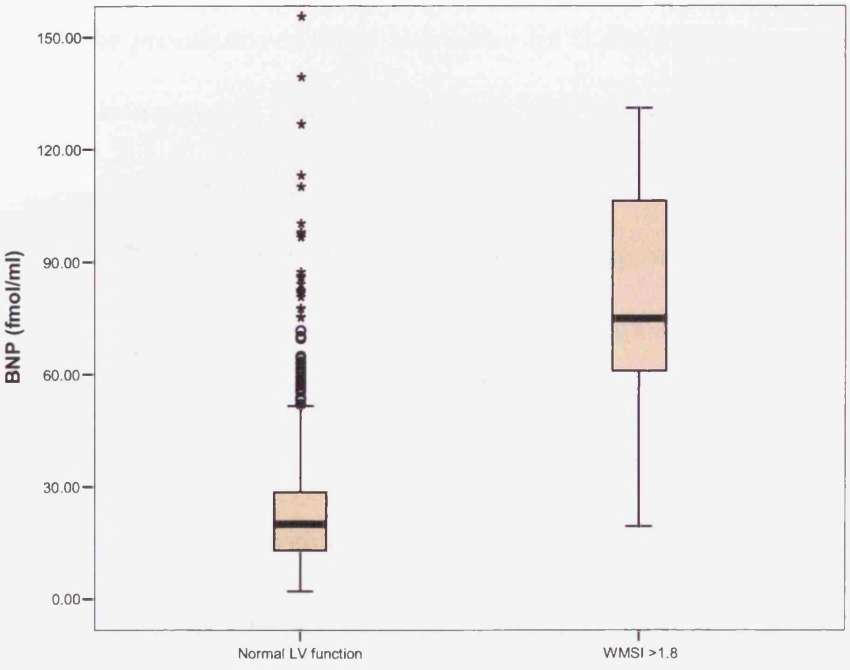


Fig 3.3b Boxplot of, BNP levels in normal subjects and subjects with LVSD. Medians, 95th centiles and 99th centiles shown

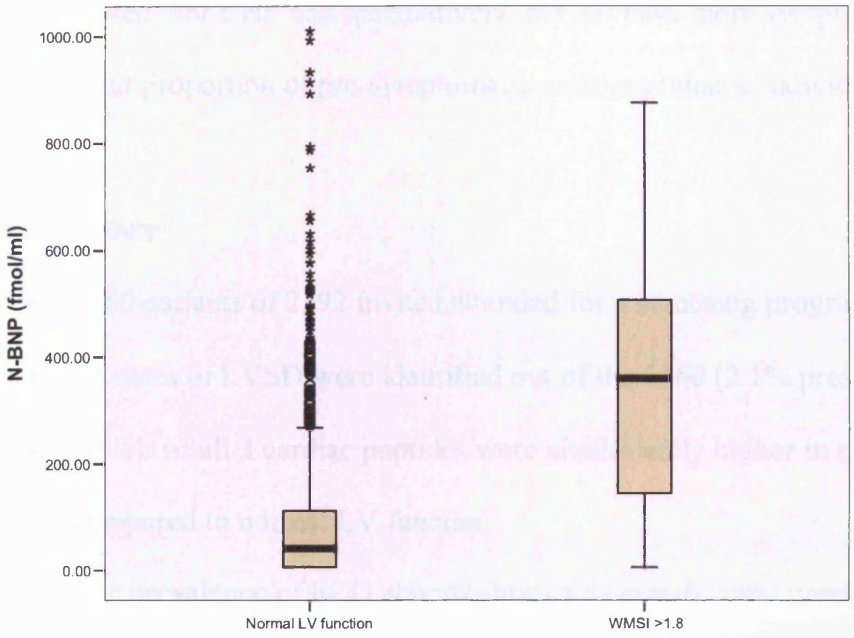


Fig 4.3c

Figures 4c. Boxplot of N-BNP levels in normal subjects and subjects with LVSD. Medians, 95th centiles and 99th centiles shown.

4.5 Differences in the population with LVSD compared to normal subjects

The prevalence of major and minor ECG abnormalities was 75% in the group with LVSD as compared to 40.6% of the group with normal LV function (ECGs will be discussed in more detail in Chapter 5).There was no significant difference in renal function (as measured by creatinine clearance) and body mass index. Subjects with LVSD were more likely to have ischaemic heart disease (defined as a history of angina or previous myocardial infarction). Neither hypertension nor diabetes mellitus was more common in the group with LVSD. Of the 28 cases, 13 (46.4%) did not have a history of IHD, hypertension or diabetes. In prescribed medication, the group with LVSD had relatively more prescriptions for nitrate and calcium channel blocker therapy. Prescription for Angiotensin Converting Enzyme (ACE) inhibitors or diuretics was not higher in the group with LVSD, suggesting that this population was truly

"undiagnosed" for CHF and speculatively, did not have more symptoms of oedema, suggesting a significant proportion of pre-symptomatic or asymptomatic individuals.

4.6 Summary

- 1360 patients of 2392 invited attended for a screening program
- 28 cases of LVSD were identified out of the 1360 (2.1% prevalence)
- Levels of all 3 cardiac peptides were significantly higher in subjects with LVSD compared to normal LV function
- The prevalence of ECG abnormalities was significantly greater in the subjects with LVSD.

Chapter 5

Screening for Left Ventricular Systolic Dysfunction using the 12 lead Electrocardiogram

5.1 Introduction

Obtaining a 12 lead-electrocardiogram requires little technical training and hence, can be performed by non-medical personnel. It is non-invasive, acceptable to patients and routinely available in primary care facilities. Interpretation of the ECG by general practitioners can be subject to significant inter-observer variability [96] but is generally quite good, with the vast majority being able to identify major ECG abnormalities [95]. Several studies have looked at the utility of the 12 lead ECG in identifying subjects suspected of CHF [91, 191, 192] but the use of the ECG in screening the general population for LVSD has been less explored. I obtained 12 lead ECGs in 1360 subjects from the general population who had echocardiography to identify cases of LVSD. The utility of the ECG in identifying cases of LVSD was then explored in more detail.

5.2 Defining ECG abnormalities

The study ECGs were coded using the well-validated and widely utilized Minnesota criteria [184]. Major abnormality in the electrocardiogram was defined as the presence of Minnesota codes 1.1 to 1.2, 4.1 to 4.4, 5.1 to 5.3, 7.1 to 7.2, 8.3, or 9.2 (i.e., abnormal Q-wave, ST-segment depression, T-wave inversion, left bundle branch block [LBBB], right bundle branch block [RBBB], atrial fibrillation, or ST-segment elevation). Minor abnormality was defined as Minnesota codes 1.3, 2.1, 3.1 to 3.2, 6.1 to 6.3, 7.3, or 7.6 (i.e., borderline Q-wave, left axis deviation, high R-wave amplitude, atria-ventricular block, incomplete LBBB, or incomplete RBBB). Left ventricular hypertrophy was defined as the joint occurrence of code 3.1 (i.e., high R-wave amplitude) in combination with either codes 4.1 to 4.4 (i.e., ST-segment depression) or 5.1 to 5.3 (i.e., T-wave inversion). ECGs were analysed for the presence of

major (pathological Q wave, left bundle branch block, left ventricular hypertrophy, atrial flutter/fibrillation) and minor abnormalities (left axis deviation, right bundle branch block, poor R-wave progression, atrial hypertrophy, non-specific ST segment change, sinus bradycardia or tachycardia). Left ventricular hypertrophy was defined by the Lyon-Sokolov criteria. Further analysis was performed using a computer program to calculate the maximum, minimum and mean QT, QT dispersion and QRS duration.

5.3 Prevalence of ECG abnormalities (Table 5.1)

There were 19 subjects with major ECG abnormalities out of the 28 subjects with LVSD (67.86%). A further 7 subjects had minor ECG abnormalities and 2 (7.1%) had entirely normal ECGs. The breakdown of the various abnormalities is in table 4. LBBB and the presence of Q waves made up 72.7% of all major abnormalities. The mean QRS duration, 110.64msec, was significantly longer in the group with LVSD (figure 4.2). There was no significant difference in the QT, QTc or QT dispersion in the 2 groups. In the population with normal LV function (n=1280), 792 had entirely normal ECGs, 197 had major abnormalities and 314 had minor ECG abnormalities.

	Sum of ECG abnormalities (percentage of population)								Mean values			
	AF	Q	T inversion	LBBB	LVH	LAD	ST	Poor R	QRS	QTc	QT dis	HR
Normal (n=1303)	*15 (1.13)	*40 (3)	*0	*13 (0.9)	*121 (9.1)	*56 (4.2)	*41 (3.1)	*39 (2.9)	$^{\Sigma}$93.8	414.9	71.8	72
LVSD (n=28)	2 (7.1)	6 (21.4)	1 (3.6)	10 (35.7)	3 (10.7)	1 (3.6)	1 (3.6)	1 (3.6)	116.2	427.4	91.3	75.2

Table 5.1 Data presented as incidence of Major ECG abnormalities (percentage as part of the population) and mean values ±SD. Abnormalities are not mutually exclusive. P values for comparisons between LVSD and normal group: * $P<0.05$ for all, using Chi Square test, $\sum$ $P<0.05$ using Mann Whitney test. (Legend: AF-Atrial fibrillation, LBBB-Left bundle branch block, LVH-Left ventricular hypertrophy, LAD-Left axis deviation, ST – ST segment abnormalities, Poor R – Poor R wave progression, QT dis- QT dispersion, HR-heart rate)

5.4 Statistical Analysis

Potential factors or covariates included in logistic regression analysis for univariate determinants of LVSD were plasma natriuretic peptide, age, gender, plasma creatinine, major ECG abnormality, QRS duration, minor ECG abnormality, body mass index, and past history of IHD, diabetes or hypertension. Neither a history of hypertension nor diabetes predicted LVSD. Factors significant on univariate analysis ($p<0.1$) were plasma peptide level, gender, major ECG abnormality, QRS interval, IHD history, and plasma creatinine. These were entered into a multivariate logistic regression analysis. Independent predictive factors for LVSD for all peptides were peptide level, presence of major and minor ECG abnormalities, QRS duration and IHD history (Table 5.2). Mann Whitney test did not show any significant statistical difference in heart rate or QT dispersion between the normal group and the group with LVSD.

	Odds ratio	P value
Log N-BNP	8.59	<0.005
Gender (Male)	3.42	0.011
Any ECG abnormality	8.79	0.004
IHD	2.60	0.029
Log N-ANP	10.67	0.001
Gender (Male)	2.65	0.042
Any ECG abnormality	11.27	0.001
IHD	3.83	0.002
Log BNP	290.49	<0.001
Gender (Male)	2.93	0.041
Any ECG abnormality	5.78	0.024
IHD	NS	NA

Table 5.2 Multivariate analysis for variables independently associated with LVSD. Logistic models considered separately for each natriuretic peptide.

5.5.1 Utility of ECG abnormalities

Using the presence of major ECG abnormalities to "pre-screen" the population for LVSD prior to echocardiography would result in 1116 (82.06%) out of a population of 1360 being excluded. Of these 1116 patients, 9 would had LVSD, meaning that 32% of all 28 cases of LVSD would be inadvertently "lost" before a definitive investigation, in this case the echocardiogram, could be utilised, giving a false negative rate of only 0.8% . Of the 214 subjects with major ECG abnormalities, 19 would have LVSD. The presence of major ECG abnormalities has a sensitivity of 67.86%, specificity of 84.96% and positive predictive value 8.82 for detecting LVSD (Table 5.2). Using major and minor ECG abnormalities to detect LVSD had a sensitivity of 92.86% but a corresponding reduction of specificity to 60.86% and a positive predictive value of 3.1%. However, using ECG abnormalities to "pre-screen" the population would result in only 8% of the total cases of LVSD being "lost" prior to the echocardiogram as 2 of the 28 (12%) individuals with LVSD had an ECG was completely

normal. However a completely normal ECG was good at excluding LVSD, having a negative predictive value of 99.2. The ROC AUC for the detection of LVSD by major ECG abnormalities and a combination of major and minor ECG abnormalities was 0.764 and 0.769 respectively.

5.5.2 Utility of the QRS duration

Using a continuous variable in the detection of a LVSD would allow the selection of a cut-off value of most appropriate sensitivity and specificity. The QRS duration, which had a normal distribution in our population (Figure 5.1), was significantly greater in the group with LVSD compared to the group with normal systolic function (Figure 5.2), using the Mann-Whitney test ($P<0.005$). From the logistic regression analysis (Table 5.2), we showed that the QRS interval was an independent predictor of LVSD. However, the AUC of the ROC curve (Figure 5.3) for the detection of LVSD was relatively poor at 0.763, especially when compared to the performance of natriuretic peptides. A QRS interval of 84.18msec had 93% sensitivity and 19% specificity, PPV 2.1%, NPV 99.22% for LVSD. A QRS interval of 89.75msec had a sensitivity of 82%, specificity of 39% for LVSD, PPV 2.8%, NPV 99%. A QRS of 100msec had a sensitivity of 67.9%, specificity of 73.7%, PPV 5.25% and NPV 99.1% (Table 5.3).

Natriuretic peptides and QRS duration were independent predictors of LVSD. I assessed the whether adding natriuretic peptide to QRS duration in a logistic model would increase diagnostic yield. ROC curve were then constructed (Figure 5.4a, b). AUC of the BNP and N-BNP were higher than QRS AUC for the detection of LVSD. Furthermore, the combined logistical model of BNP and QRS duration and QRS and N-BNP levels did not improve AUC

above that of the AUC for BNP and N-BNP alone. QRS duration, however, performed very well as a "rule-out" test, having similar negative predictive values to natriuretic peptides.

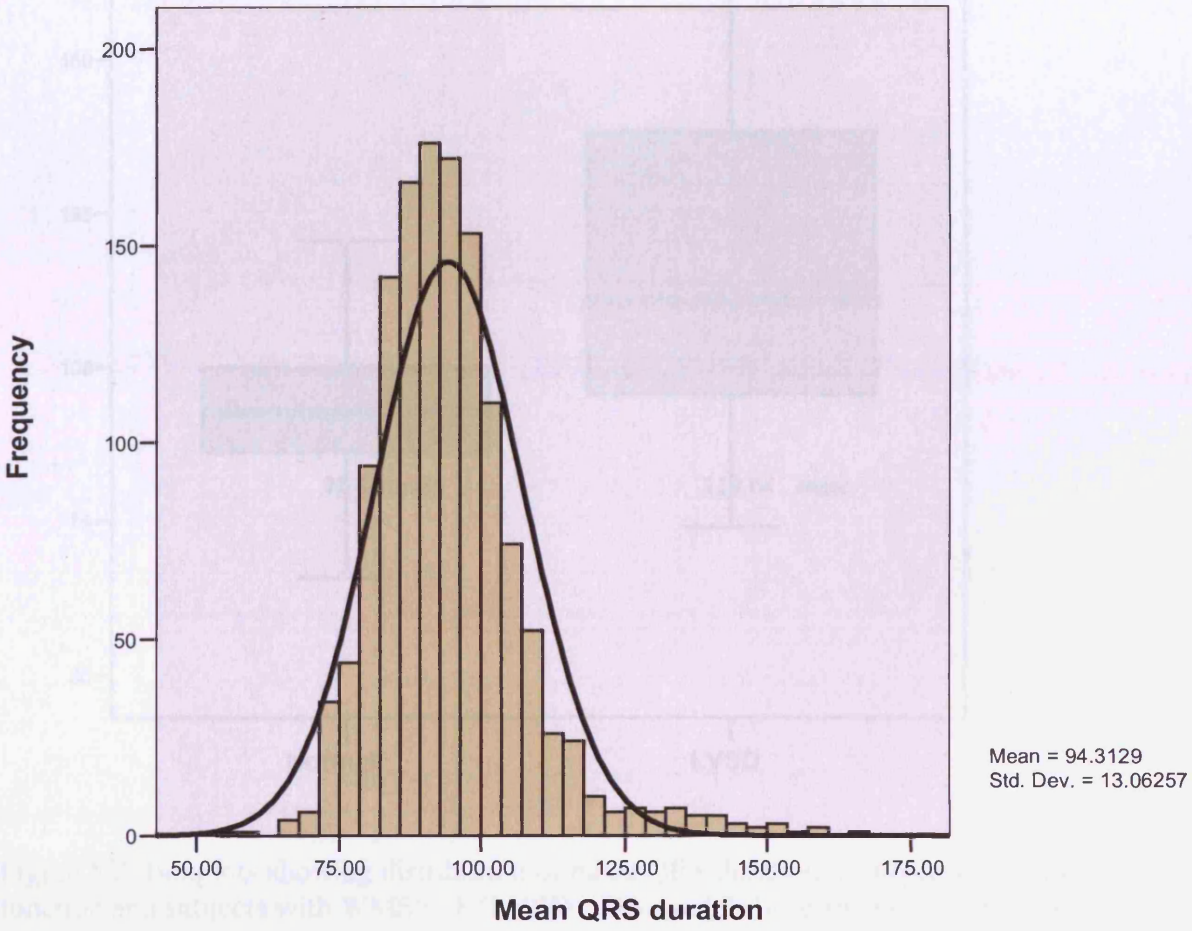


Figure 5.1. Distribution of QRS duration (msec) in population. Best fit curve shown.

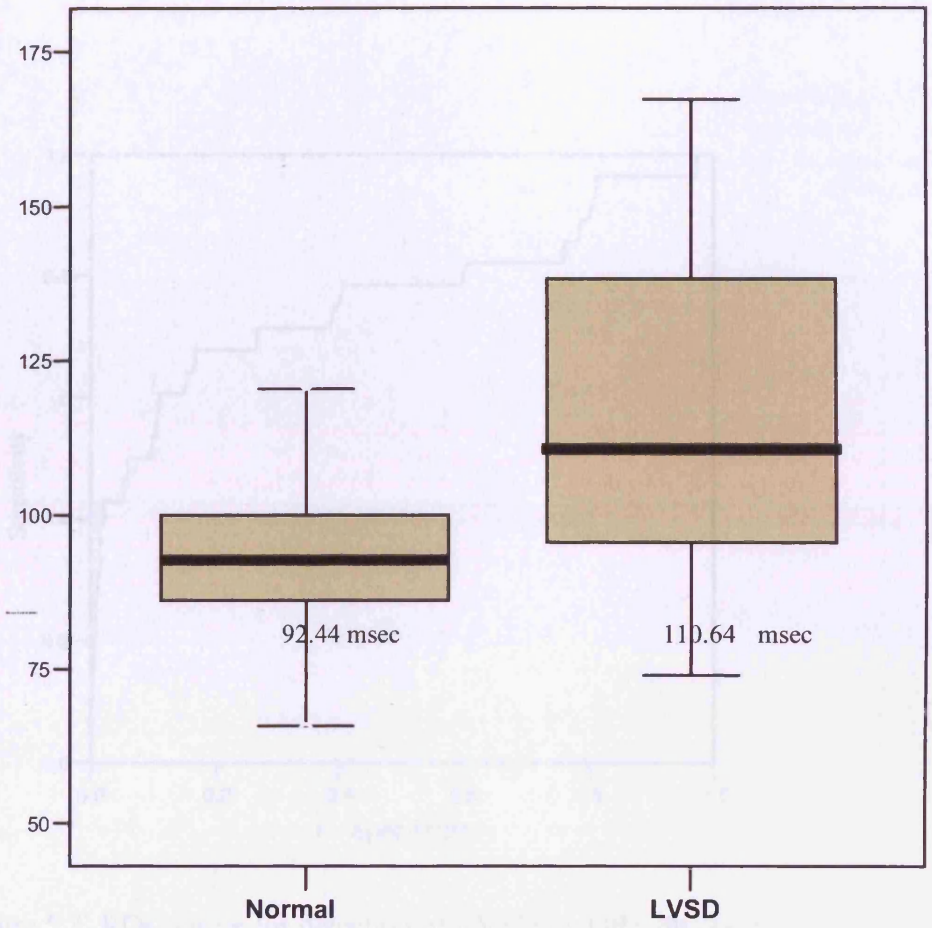


Figure 5.2. Boxplots showing distribution of mean QRS duration in subjects with normal LV function and subjects with WMS>1.8 (LVSD). 90% and 95% confidence intervals shown. Mean values shown.

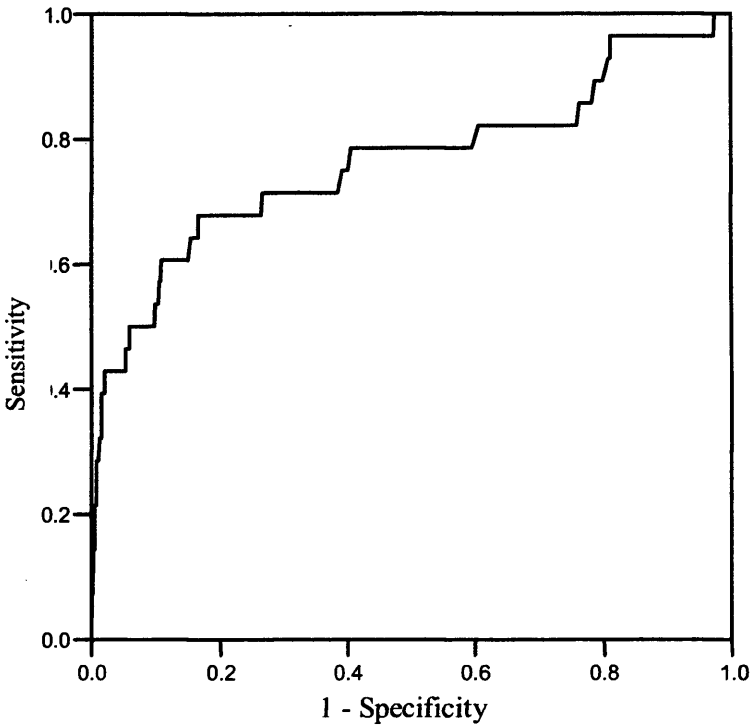
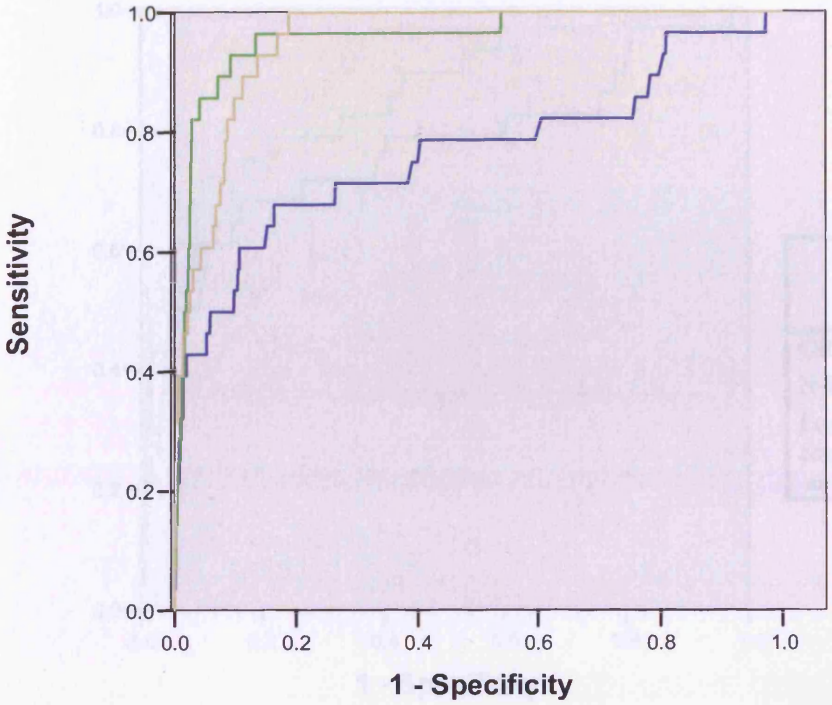


Figure 5.3. ROC curve for detection of LVSD by QRS duration
AUC 0.763 (0.65 – 0.88 95% CI, SE 0.59)

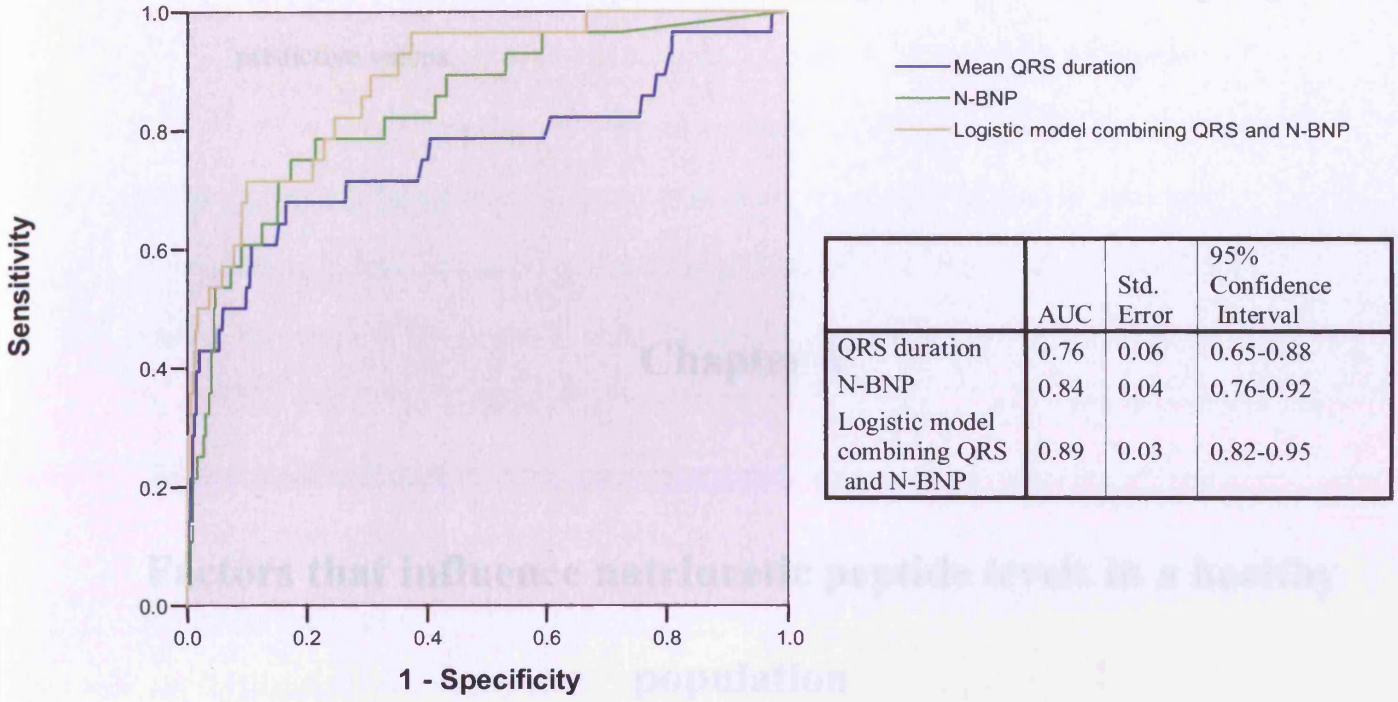
	Intervals (msec)	Sensitivity (%)	Specificity (%)	PPV	NPV	+ LR	- LR
QRS Duration	74	100	2.6		NA	1.02	NA
	84.18	93	19	2.1	99.22	1.15	0.37
	89.75	82	39	2.8	99	1.34	0.46
	100	67.9	73.7	5.25	99.1	2.58	0.44
Major ECG abnormalities		67.86	84.96	8.82	99.20	4.52	0.38
Major + minor ECG abnormalities		92.86	60.86	4.84	99.75	2.37	0.12

Table 5.3. Performance of QRS duration and ECG abnormalities in diagnosing LVSD. PPV-positive predictive value. NPV – Negative predictive value. + LR – Positive likelihood ratio. – LR – Negative predictive value.



	AUC	Std. Error	95% Confidence Interval
QRS duration	0.76	0.06	0.65-0.88
BNP	0.96	0.02	0.92-0.99
Logistic model combining QRS and BNP	0.95	0.01	0.93-0.97

Figure 5.4a



	AUC	Std. Error	95% Confidence Interval
QRS duration	0.76	0.06	0.65-0.88
N-BNP	0.84	0.04	0.76-0.92
Logistic model combining QRS and N-BNP	0.89	0.03	0.82-0.95

Figure 5.4a, b. ROC curves for detection of LVSD by BNP and N-BNP, comparing QRS duration and logistic model combining peptide and QRS duration. Tables show area under curve (AUC).

5.6 Summary

Major ECG abnormalities were significantly more common in the patients with LVSD. Major ECG abnormalities had some utility in diagnosing LVSD, albeit at low positive predictive value

- QRS duration was significantly prolonged in patients with LVSD but had limited utility in diagnosing LVSD because of poor specificity and positive predictive value. Combination with natriuretic peptides did not improve diagnostic utility.

- Both QRS duration and ECG abnormalities were good "rule out" test with high negative predictive values.

Chapter 6

Factors that influence natriuretic peptide levels in a healthy population

6.1 Introduction

Natriuretic peptides have been found to be useful biomarkers in a wide variety of cardiovascular conditions. However, the performances of different peptides vary considerably and are subject to the effect of confounders such as gender, age, body mass index and physiological factors such as heart rate. Assessing the effect of these variables on various cardiac peptides would guide us as to the necessity of age and gender specific reference ranges. In addition, it would potentially allow us to select a peptide that is potentially least affected by physiological confounders.

6.2 Patient selection

The association of IHD, hypertension, valvular disease and LVSD with elevated NP levels is well described [110, 134, 140]. In addition, there is also an effect of cardiovascular medication such as beta-blockers and IHD on NP levels [154, 156]. Therefore, in order to have a representative normal healthy group, subjects were excluded for the following reasons (not mutually exclusive): left ventricular systolic dysfunction, defined as an ejection fraction of-50% or left ventricular wall motion index score of)1 (n=114); previous myocardial infarction or angina (n=105); diabetes (n=64); history of hypertension (n=325); current use of diuretic (n= 210), ACE inhibitor (n=118), calcium channel blocker (n=134) or nitrate (n=53); atrial fibrillation (n=18); significant valvular disease, defined as an echocardiographic quantification of any valvular disease (mitral or aortic regurgitation and mitral and aortic stenosis) graded as moderate and above (n=26); any other significant cardiac pathology on echocardiography such as right ventricular dilatation, elevated pulmonary artery pressure or congenital heart disease (n=3). Individuals with major ECG abnormalities including left bundle branch block (n=23),

left ventricular hypertrophy (n=124) and pathological Q waves (n=47) were excluded. This left 720 healthy individuals without evidence of cardiovascular disease (Table 6.1).

	Female (n=303)	Male (n=417)
Age (years): mean (range)	64.09 (55–79)	59.71 (46–80)
Creatinine (μmol/l): mean±S.D.	77.7±11.42	92.45±12.82*
BMI (kg/m²): mean±S.D.	26.13±4.5	26.27±3.78
Systolic BP (mmHg): mean±S.D.	132.8±18.09	130.72±17.24
Diastolic BP (mmHg): mean±S.D.	75.52±11.86	78.15±12.1*
Pulse pressure (mmHg): mean±S.D.	57.29±15.11	52.63±13.64*
Natriuretic peptides: median (range)		
N-ANP (fmol/ml)	421.9 (35.83–1377.23)	365.28 (15.9–1554.66)
BNP (fmol/ml)	22.01 (2–87.35)	17.57 (2–64.81)*
N-BNP (fmol/ml)	82.37 (5.7–991.90)	46.2 (5.7–932.83)*

*P<0.05 cf. female.

Table 6.1. Demographic features of the study population (n=720)

6.3 Results

6.3.1 Statistical analysis

Univariate analysis was performed to investigate the relationship of clinical parameters with N-ANP, BNP and N-BNP. The considered parameters were age, gender, body surface area (BSA), body mass index, serum creatinine, heart rate and blood pressure. For each natriuretic peptide, factors associated in univariate analysis (P<0.1) were entered in logistic regression analysis for the prediction of each peptide. For multivariate analysis, a p value of <0.05 was considered significant. BNP, N-BNP and N-ANP were log-normally distributed and analyses were performed after transformation of the data. Variables were entered in a hierarchical as well as a stepwise method and collinearity diagnostics, the Durbin–Watson calculation and casewise diagnostics to 3 S.D. were performed to check the regression model. A general linear model (univariate) was used to confirm the correlations. In order to check the results obtained, the study population was randomly split into two equal groups and the same statistical analyses were run in each. The results were very similar. As expected, left ventricular ejection fraction

(LVEF) could be calculated in only a proportion of the cases. Thus, multivariate analyses were repeated for this cohort of patients for whom both clinical and all echocardiographic parameters (LVEF, LA size, LV mass index, mitral A wave deceleration time and interventricular relaxation time) were available.

6.3.1.1 Univariate analysis

A number of clinical variables were associated with plasma natriuretic peptide levels (Table 6.2). Levels of all three peptides were higher in females, increased with age and correlated negatively with heart rate and body surface area. Whilst the observed correlations with either systolic or diastolic blood pressure were weak, there was a consistent, positive correlation of pulse pressure for each peptide. In this healthy population, the correlation of echocardiographic parameters with natriuretic peptide levels was weak. However, in the 582 individuals in whom LVEF could be measured, there was a positive correlation of this parameter with plasma levels of each natriuretic peptide. Body mass index did not correlate with peptide levels.

		Log N-ANP	Log BNP	Log N-BNP
Clinical parameters				
Age	r_s	0.264	0.157	0.372
	P value	<0.001	<0.001	<0.001
Gender*	r_s	0.198	0.151	0.286
	P value	<0.001	<0.001	<0.001
Heart rate	r_s	−0.138	−0.107	−0.071
	P value	<0.001	0.004	0.057
Body surface area	r_s	−0.136	−0.077	−0.188
	P value	<0.001	0.041	<0.001
Log BMI	r_s	−0.052	0.032	−0.034
	P value	0.165	0.391	0.370
Systolic BP	r_s	0.107	0.089	0.073
	P value	0.005	0.019	0.055
Diastolic BP	R_s	0.022	−0.018	−0.089
	P value	0.562	0.643	0.019
Pulse pressure	R_s	0.130	0.123	0.173
	P value	0.001	0.001	<0.001
Echocardiographic parameters				
LVEF (n=582)	R_s	0.128	0.107	0.059
	P value	0.002	0.010	0.154
LA	R_s	0.017	−0.001	−0.018
	P value	0.657	0.983	0.645
LVIDD	R_s	−0.07	−0.006	−0.098
	P value	0.084	0.882	0.015
LV mass index	R_s	−0.047	−0.078	−0.006
	P value	0.262	0.063	0.887

* Peptide levels higher in females.

Table 6.2 Results of univariate analysis (r_2, Spearman's Rho) of clinical and echocardiographic variables with plasma natriuretic peptides

6.3.1.2. Multivariate analysis—clinical parameters

The results of multivariate regression analysis are shown in Table 6.3 and, for the cohort of 582 in whom LVEF was measurable, in Table 6.4. As can be seen from the tables, the factors retaining independent predictive value for each natriuretic peptide differed. Only gender (levels higher in females) and heart rate (inversely correlated with plasma natriuretic peptide levels) were independently predictive of all three peptides. Age remained predictive of plasma N-ANP and N-BNP but not BNP. The median values, together with the 5–95% predictor intervals for plasma levels of each of the natriuretic peptide levels, plotted against age, are

shown in Fig. 6.1. The observed relationships indicate a 16% increase in ANP levels and 74% increase in N-BNP levels for each 10 years of age, but no statistically significant increase in BNP. With regard to heart rate, an increase of 10 bpm would correspond to a reduction of 9% in N-ANP or BNP and a 15% reduction in N-BNP.

6.3.1.3 Multivariate analysis—clinical and echocardiographic parameters

Echocardiographic estimation of LVEF was possible in 582 (81%) of the 720 subjects. For this cohort LVEF was included as a covariate in multivariate analysis. LVEF correlated positively with both N-ANP and BNP, but not N-BNP (Table 6.4). Indeed LVEF displaced gender as an independent predictor of plasma BNP. Age and gender remained predictive of plasma N-ANP and NBNP. LA size, pulse pressure and LV mass had no significant influence on plasma natriuretic peptide levels in the models. Only 6.3% and 4% of the variation in N-ANP and BNP, respectively, was explained by the considered variables compared to 16.5% of the variation in N-BNP.

	Log N-ANP	Log BNP	Log N-BNP
Age	6.48×10^{-3} ($P<0.001$)	NA ($P=0.405$)	2.4×10^{-2} ($P<0.001$)
Gender	7.88×10^{-2} ($P<0.001$)	9.72×10^{-2} ($P=0.001$)	0.23 ($P<0.001$)
Heart rate	-4.1×10^{-3} ($P<0.001$)	-4.33×10^{-3} ($P=0.001$)	-7.14×10^{-3} ($P<0.001$)
Pulse pressure	NA ($P=0.488$)	NA ($P=0.565$)	3.05×10 ($P=0.041$)
Systolic BP	1.77×10 ($P=0.001$)	2.14×10^{3} ($P=0.01$)	NA ($P=0.153$)

Table 6.3 – Results of multiple linear regression analysis. Correlation coefficients for the correlation of clinical variables with N-ANP, BNP and N-BNP (n=720)

	Log N-ANP	Log BNP	Log N-BNP
Age	6.17×10^{-3} ($P<0.001$)	NA ($P=0.385$)	2.12×10^{-2} ($P<0.001$)
Gender	7.47×10^{-2} ($P=0.002$)	NA ($P=0.081$)	0.239 ($P<0.001$)
Heart rate	-3.4×10^{-3} ($P=0.001$)	-3.53×10^{-3} ($P=0.018$)	-6.84×10^{-3} ($P<0.001$)
Pulse pressure	NA ($P=0.585$)	3.02×10^{-3} ($P=0.017$)	3.6×10^{-3} ($P=0.033$)
Systolic BP	1.7×10^{-3} ($P=0.011$)	NA ($P=0.349$)	NA ($P=0.908$)
LVEF	4.21×10^{-3} ($P=0.043$)	7.45×10^{-3} ($P=0.021$)	NA

Table 6.4 – Results of multiple linear regression analysis. Correlation coefficients for the correlation of clinical and echocardiographic variables with N-ANP, BNP and N-BNP (n=582)

6.4 Summary

In a population without cardiovascular disease,

- N-ANP and N-BNP, but BNP correlates independently with increasing age.
- Female gender is independently associated with increasing levels of BNP, N-ANP and N-BNP
- Increasing heart rate was inversely related to all 3 cardiac peptides
- Echocardiographic LV ejection fraction but not LA size nor LV mass correlates independently with N- ANP and BNP but not N-BNP.

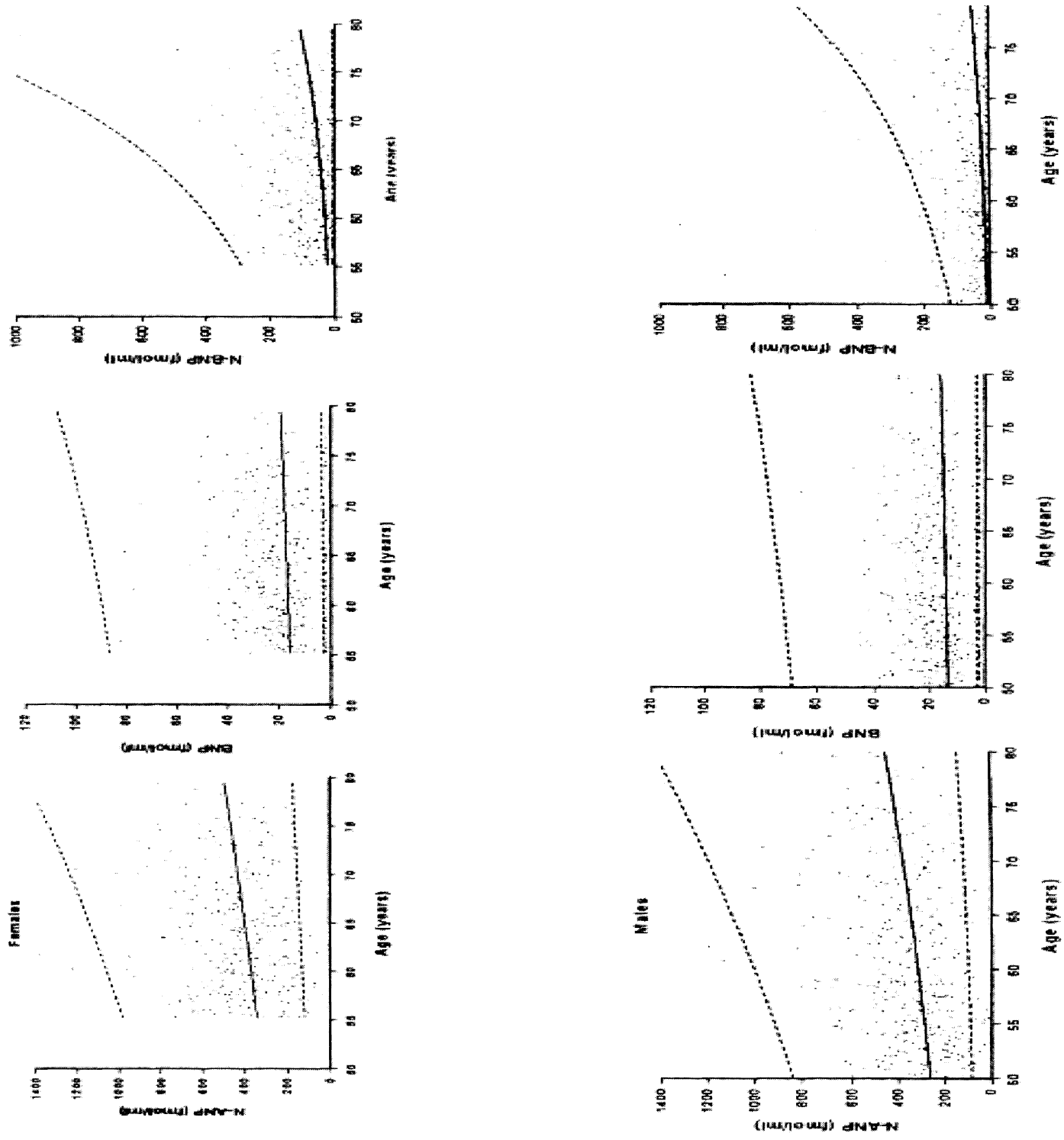


Figure 6.1 Median (bold line) and 5-95% confidence intervals (dotted lines) for plasma concentration of N-ANP, BNP and N-BNP

Chapter 7

Screening for Left Ventricular Systolic Dysfunction using Plasma Natriuretic Peptides

7.1 Introduction

Having identified 28 (2.1%) patients in our screening cohort of 1360 with LVSD (defined by a WMSI of ≥1.8, equivalent to an ejection fraction of ≤40%), the utility of N-ANP, BNP and N-BNP in identifying these cases was then assessed. Plasma concentration of each natriuretic peptide was significantly higher in those with LVSD compared to those with normal systolic function (see Figures 7.3a-c). All 3 peptide levels correlated closely with each other (Figures 7.1a-c) with significance levels of $P<0.001$ for all 3 peptides (Table 7.1). However, there appears to be poor correlation at the lower limit of detection, suggesting that at low levels of any one of each of the 3 NP's does not necessarily correlate with a low level of another peptide. In addition, examination of the boxplots (Figures 3.3a, b, c) reveals overlapping of the ranges of the normal population with the cases of LVSD because of significant scatter of the peptide levels in the presumably "normal" population.

		Log N-ANP	Log BNP	Log N-BNP
Log N-ANP	Pearson Correlation	NA	0.66	0.53
	Sig. (2-tailed)		<0.005	<0.005
Log BNP	Pearson Correlation	0.66	NA	0.39
	Sig. (2-tailed)	<0.005		<0.005
Log N-BNP	Pearson Correlation	0.53	0.39	NA
	Sig. (2-tailed)	<0.005	<0.005	

Table 7.1 Correlation between natriuretic peptides

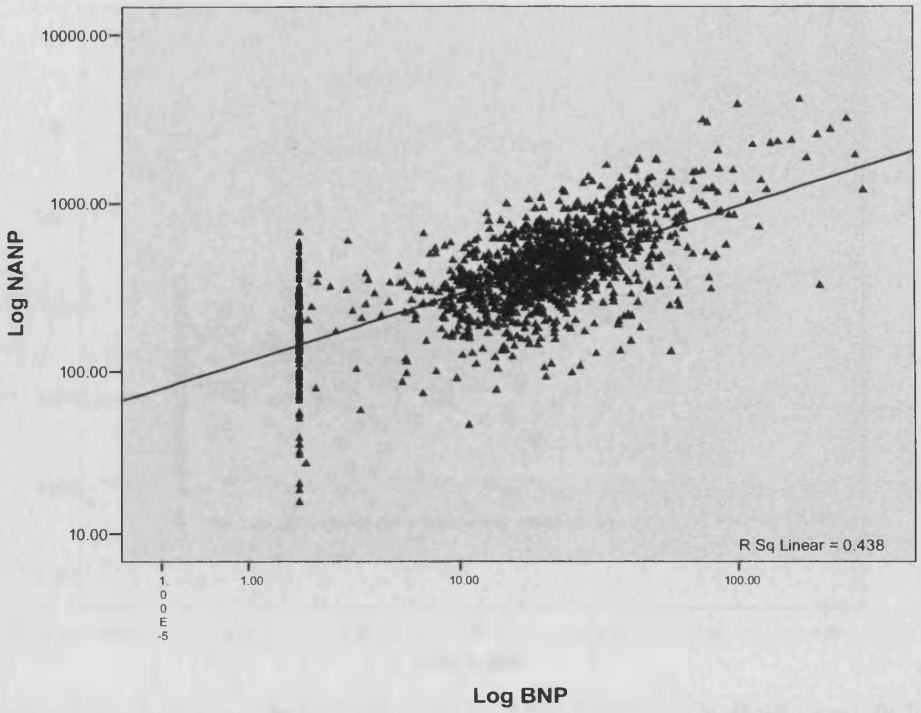


Fig 7.1a

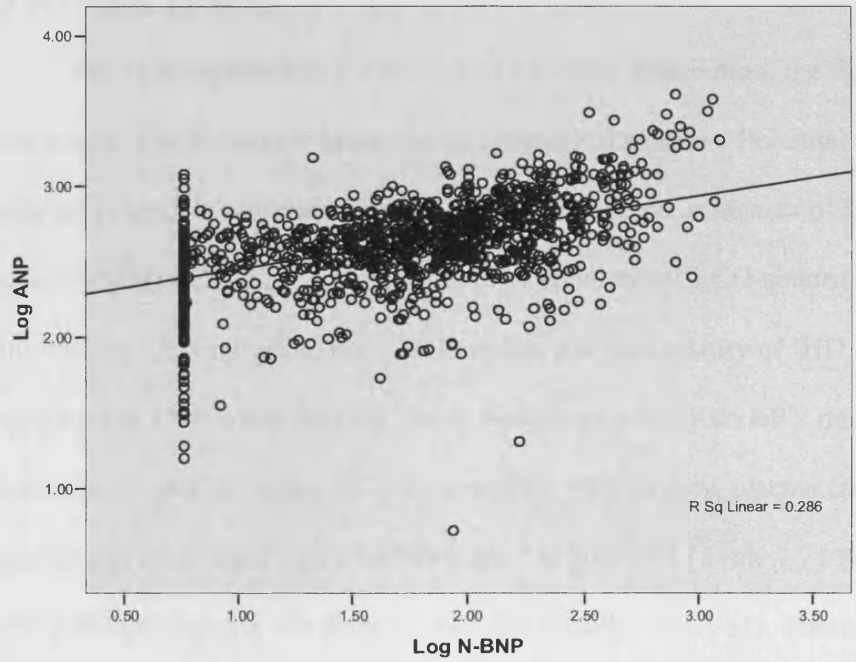


Fig 7.1b

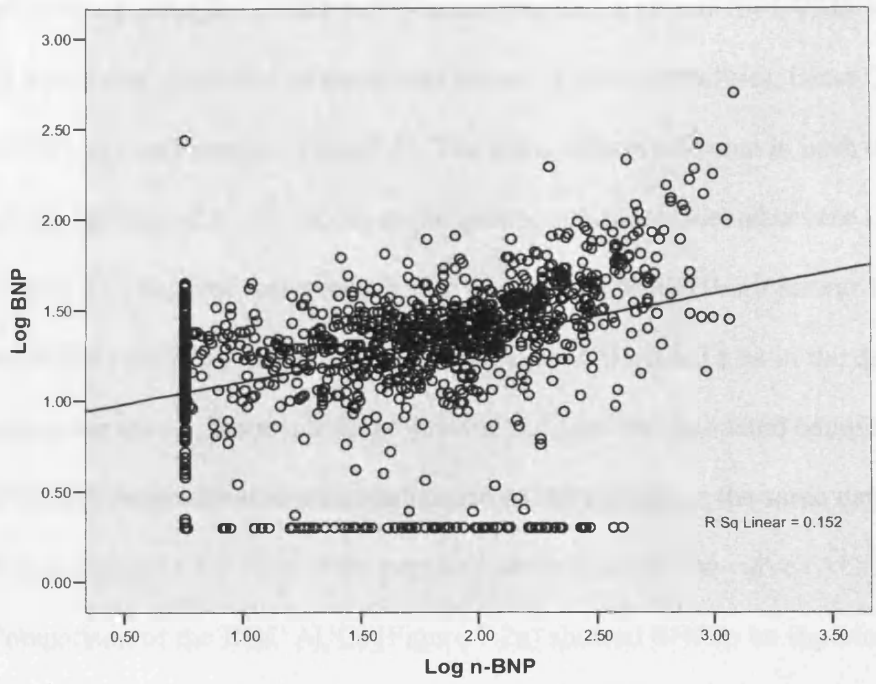


Figure 7.1a, b, c. Correlations between N-ANP, BNP and N-BNP. Best-fit line shown.

7.2 Statistical analysis

Because peptide levels were not in a normal distribution, the figures underwent a logarithmic transformation before further statistical analysis. Potential factors or covariates included in logistic regression analysis for univariate determinants of LVSD were plasma natriuretic peptide, age, gender, plasma creatinine, major ECG abnormality, minor ECG abnormality, QRS duration, body mass index, and past history of IHD, diabetes or hypertension. Univariate analysis, using Spearman's and Kendall's methods, found that plasma peptide level, gender, major ECG abnormality, IHD history, plasma creatinine were significantly associated with a LVSD with P values <0.1 (Table 7.2). Surprisingly, neither a history of hypertension nor diabetes was associated with LVSD. These factors were then entered into multivariate logistic regression analysis. Because peptide levels correlated closely with each other, logistic regression was performed for each individual peptide with other

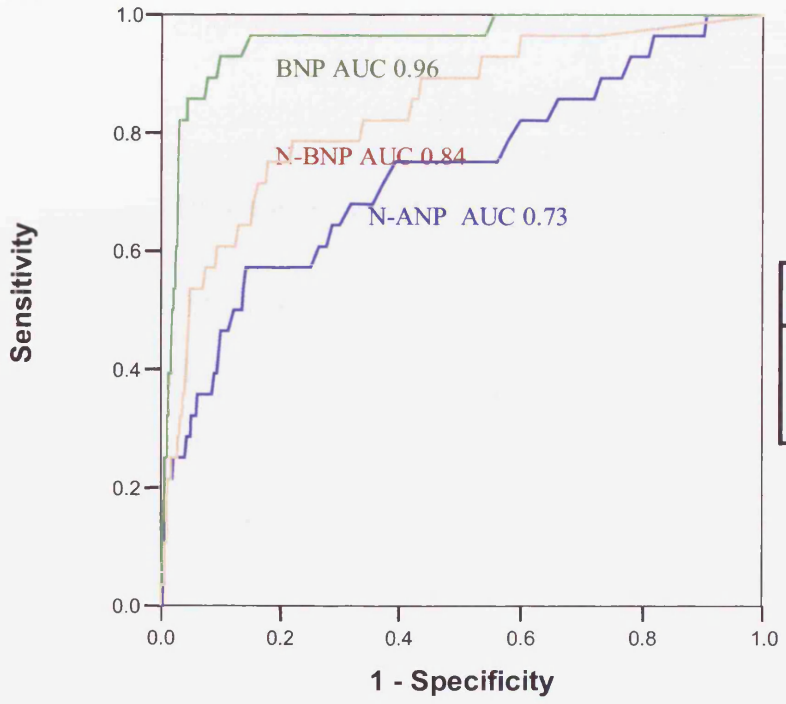
predictive factors for LVSD. Independent predictive factors for LVSD were peptide levels (of all 3 peptides), presence of major and minor ECG abnormalities, mean QRS duration, history of IHD, age and gender (Table 7.3). The same factors held true in both the group with severe LVSD (WMSI ≥2.0, EF ≤35%) as the group with moderate and severe LVSD (WMSI ≥1.8, EF ≤ 40%).The analysis was performed in a stepwise fashion (both forward and backward) to assess the reliability of the predictors. Because of potential bias in the development of the regression model, the resulting prognostic indices were validated using a 'jack-knifing' method to correct for development and validation of the models on the same data. ROC curves were then constructed for each of the peptides and area under the curve (AUC) were calculated. Comparison of the ROC AUCs (Figure 7.2a) showed BNP to be superior to N-ANP (P<0.006) or N-BNP (P<0.03) in the detection of all cases of LVSD. BNP was also superior to N-ANP (P<0.001) and N-BNP (P<0.003) for detection of severe LVSD (Figure 7.2b), defined as WMSI ≥2.0.

Variable	Coefficient Factor (P value)
Log N-ANP	0.10 (<0.05)*
Log BNP	0.19 (<0.05)*
Log N-BNP	0.14 (<0.05)*
Age	0.065 (<0.05)*
Creatinine	0.065 (<0.05)*
Gender	0.084 (<0.05)*
IHD	0.17 (<0.05)*
Hypertension	0.015 (0.59)
Diabetes	0.017 (0.55)
BMI	0.008 (0.71)
Major ECG	0.21 (<0.05)*
Major/minor ECG	0.16 (<0.05)*
Heart Rate	0.028 (0.21)

Table 7.2 Univariate Correlations with LVSD (WMSI>1.8). *Significant correlations p<0.1

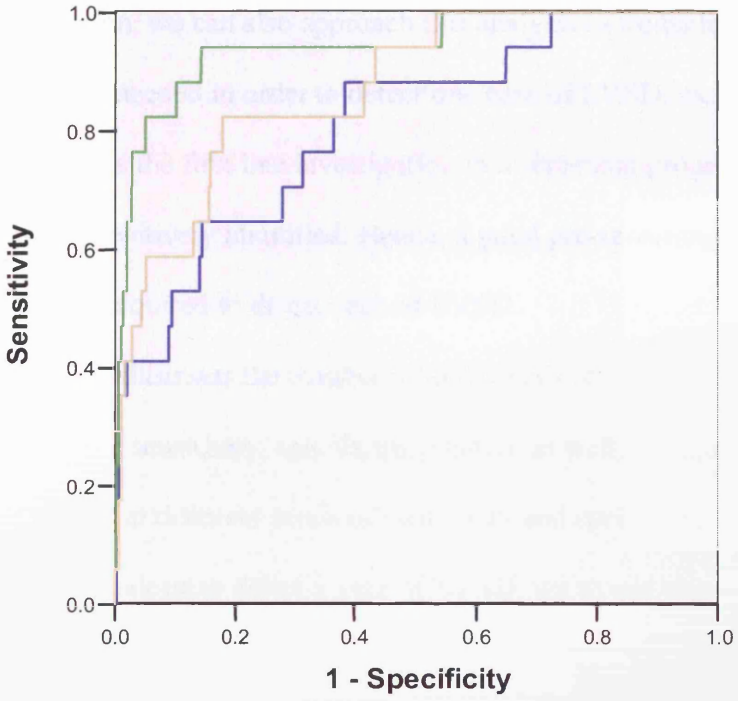
	Odds ratio	P value
Log N-BNP	8.59	<0.005
Gender (Male)	3.42	0.011
Any ECG abnormality	8.79	0.004
IHD	2.60	0.029
Log N-ANP	10.67	0.001
Gender (Male)	2.65	0.042
Any ECG abnormality	11.27	0.001
IHD	3.83	0.002
Log BNP	290.49	<0.001
Gender (Male)	2.93	0.041
Any ECG abnormality	5.78	0.024
IHD	NS	NA

Table 7.3 Multivariate analysis for variables independently associated with LVSD. Logistic models considered separately for each natriuretic peptide.



Peptides	Area under curve	95% Confidence Interval
N-ANP	0.73	0.63-0.84
BNP	0.96	0.92-0.99
N-BNP	0.84	0.76-0.92

Figure 7.2a. Receiver Operator Curves (ROC) for the identification of WMSI>1.8 using natriuretic peptides. Area under the curve figures are as stated.



	Area under curve	Std. Error	95% Confidence Interval
N-ANP	0.81	0.05	0.70-0.92
BNP	0.94	0.03	0.88-1.0
N-BNP	0.87	0.04	0.79-0.95

Figure 7.2b ROC curve for detection of severe LVSD (WMSI >2.0)

7.3 Selection for optimal cut-off value for NP

In an ideal situation, a screening test should aim for high sensitivity enabling a high pick-up rate, arguably, even at the expense of a reduction in specificity. A range of natriuretic peptides values gives us greater flexibility in deciding a "cut-off" value to maximize our sensitivity and specificity for the diagnosis of LVSD. Previous trials looking at the performance of natriuretic peptides in detecting LVSD have often found relatively high negative predictive value but low positive predictive value, also, in part, due to a low incidence of LVSD in the

population [110, 111, 112, 113]. This finding extended to my study. In order to study the utility of the screening test using different cut-off values, positive and negative predictive values as well as positive and negative likelihood ratios were also calculated, in addition to sensitivity and specificity. The intention being to pre-screen the population prior to a gold standard test of an echocardiogram, we can also approach this analysis by considering the number of echocardiograms needed in order to detect one case of LVSD, assuming that utilizing an echocardiogram as the first line investigation in a screening program would result in all cases of LVSD being positively identified. Hence, a good pre-screening test would reduce the number of scans required to detect each of LVSD.

Table 7.4 illustrates the number of individuals requiring echocardiography to detect 1 case of LVSD, the sensitivity, specificity, positive as well as negative and positive predictive values of all 3 NP at different levels of sensitivity and specificity If relying on echocardiography alone to detect 1 case of LVSD, we would need to scan 48.57 subjects. Using a cut off of 175.8 fmol/ml for NANP achieved 100% sensitivity but would exclude just 125 subjects from the original population of 1360 and result in needing 44.1 scans to detect 1 case of LVSD. A cut off of 18.8 fmol/ml (65.3 pg/ml) for BNP, again achieved 100% sensitivity but excluded 585 (43%) of our population, requiring 27.7 scans to detect 1 case of LVSD. N-BNP was unable to achieve 100% sensitivity. At a sensitivity level of 96.4%, a cut off level of 966.05 mol/ml for N-ANP excluded 243 (17.9%) subjects. However, using BNP at a sensitivity of 96.5%, a cut-off value 35.9 fmol/ml would exclude 1119 subjects, requiring just fewer than 9 scans to detect each 1 case of LVSD. A cut-off value of 26.0 fmol/ml (219.9 pg/ml) for N-BNP excluded 533(39%) subjects with a sensitivity of 96.4%. Positive predictive values were low for all 3 NPs, reflecting a low prevalence of the condition. High negative predictive values

confirmed the utility of NP as a test of exclusion. The likelihood ratio is the ratio of the probability of the specific test result in people who do have the disease to the probability in people who do not. A ratio >10 in the event of a positive test indicates good "rule in" and <1 in event of a negative test, a good "rule out" test. The likelihood ratio, in event of a negative test using BNP, would be zero in the situation of 100% sensitivity, and 0.042 and 0.12 for BNP and N-BNP respectively at a sensitivity of 96.2%. Likelihood ratio, in event of a positive test, would be 1.8 and 1.1 for BNP and N-ANP at 100% sensitivity, and 6.56, 1.18 and 1.61 for BNP N-ANP and N-BNP respectively at a sensitivity of 96.2%. It would appear that peptide values, used alone, would be excellent rule out tests but less good at ruling in.

Using different cut-off values resulted in different sensitivities and specificities. BNP generally performed better than N-BNP and N-ANP, the latter generally performing worst, whichever cut-off value was selected. Using the 95th centile as a cut off (i.e. 1118.81fmol/ml for N-ANP, 57.81fmol/ml for BNP and 384.15fmol/ml for N-BNP) achieved improved specificities but reduced sensitivities (Table 7.5). All three cardiac peptides had high negative predictive values. BNP again performed best of the three peptides. Using this as a pre-screening test resulted in missing five cases of LVSD, a false negative rate of 17.9%. However, the number of scans needed to detect one case of LVSD was just 2.96.

Test modality (Peptide cut off values in pM fmol/ml)	No. of scans to detect 1 case of LVSD	Sensitivity	Specificity (%)	PPV (%)	NPV (%)	+LR	-LR
Echo alone	48.6	100	100	NA			
N-ANP (175.79)	44.1	100	9.5	2.3	99.2	1.1	NA
BNP (18.84)	27.9	100	44.7	3.7	99.8	1.8	NA
N-ANP + ECG Logistic model	44.4	100	8.6	2.3	99.8	1.1	NA
BNP + ECG Logistic model	12.7	100	73.9	9.6	99.9	3.83	NA
N-ANP (966.05)	39.9	96.4	18.5	2.5	99.6	1.18	0.19
BNP (35.89)	8.6	96.4	85.3	12.3	99.9	6.56	0.04
N-BNP (26)	29.5	96.4	40.4	3.4	99.8	1.62	0.09
Major ECG abnormalities	8.7	67.9	85.0	8.8	99.2	4.51	0.38
Major and Minor ECG abnormalities	19.1	92.9	60.9	4.8	99.8	2.37	0.18
Sequential – ECG, then BNP (19.23)	12.3	92.9	74.7	7.3	99.8	3.67	0.10

Table 7.4 Diagnostic utility of NP at maximal sensitivity and comparing to other test modalities. (PPV- Positive predictive vale, NPV- Negative predictive value, LR+ Positive likelihood ratio, LR- Negative likelihood ratio)

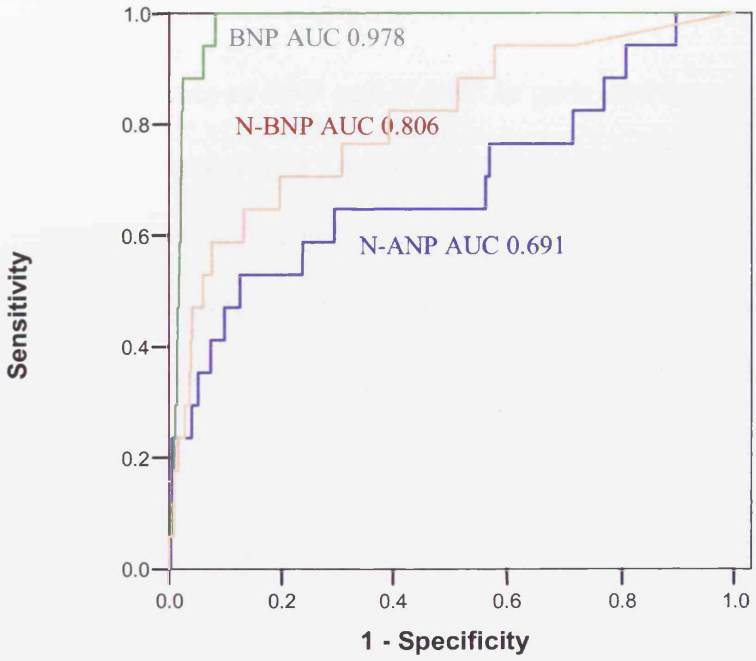
NP (cut-off fmol/ml)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	LR+	LR-
N-ANP 1118.81	28.57	98.43	28.89	98.47	18.2	0.73
BNP 57.81	82.14	99.6	81.50	99.62	205.35	0.18
N-BNP 26.0	42.86	98.74	42.19	99.76	34.0	0.58

Table 7.5 Diagnostic utility of NP using 95th centile as cut-off
(PPV Positive predictive vale, NPV Negative predictive value, LR+ Positive likelihood ratio, LR- Negative likelihood ratio)

7.4 Screening in high risk populations

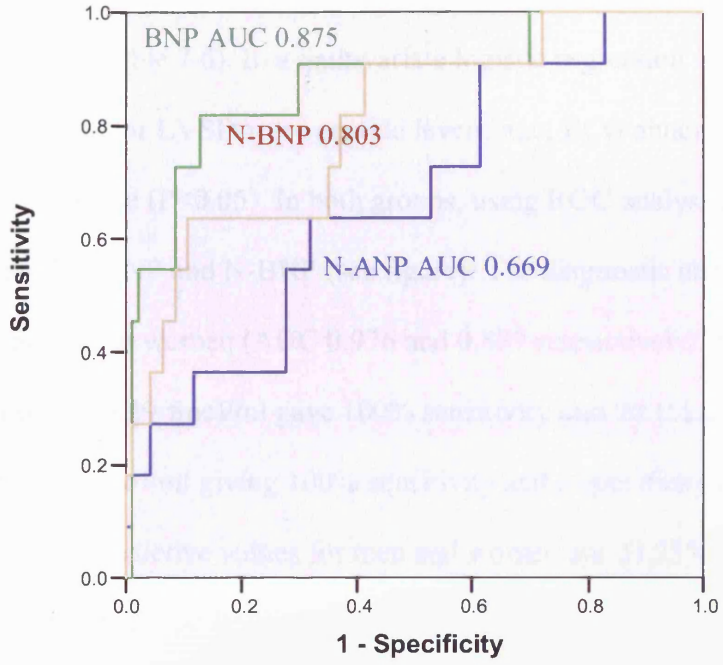
The positive predictive value of NP would be increased by targeting the screening at groups with a higher prevalence of the disease state, namely higher risk groups. In this study, neither diabetes nor hypertension was an independent predictor of LVSD. Patients with a

history of IHD had higher prevalence of LVSD. Of the 1204 patients without IHD, 17 had LVSD (1.41%) compared to 11 cases in 94 patients with IHD (11.7%), the difference being statistically significant using Mann-Whitney test ($p<0.05$). Using logistic regression analysis, N-ANP, BNP and N-BNP were independent predictors of LVSD in both groups of patients. ROC curve analysis was performed in both groups (see fig 5.3a, b) and showed that BNP performed best in both groups, although AUC was better in the group without IHD (AUC 0.978 *cf.* 0.875). A cut off value of 19.19fmol/ml gave 100% sensitivity, a specificity of 29.8% and positive predictive value of 15.9%. Sensitivity 91% correlated with specificity 70.2% and a positive predictive value of 28.8%. This compares to a positive predictive value of 2.2% when screening the population as a whole.



	Area under curve	Std. Error	95% Confidence Interval
N-ANP	0.69	0.08	0.54-0.84
BNP	0.98	0.01	0.97-0.99
N-BNP	0.81	0.06	0.69-0.92

Figure 7.3b ROC curve showing detection of LVSD by NP in patients *without* IHD history (n=17)



	Area under curve	95% Confidence Interval
N-ANP	0.73	0.63-0.84
BNP	0.96	0.92-0.99
N-BNP	0.84	0.76-0.92

Figure 7.3a ROC curve showing detection of LVSD by NP in patients *with* IHD history (n=11)

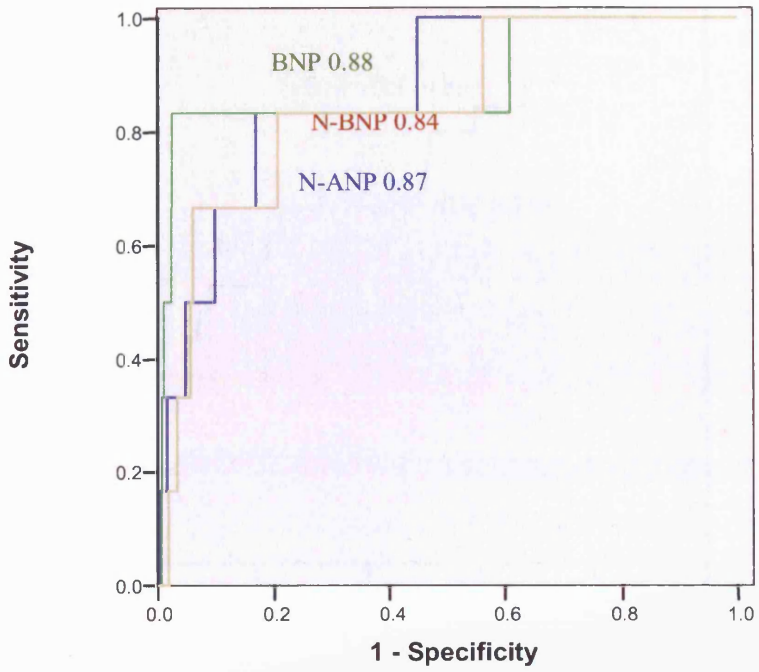
7.5 Performance of BNP and N-BNP in male and female population

I have previously shown that gender has an independent effect on peptide levels in the normal population (chapter 6). This observation has been observed in studies in normal healthy populations as well as subjects with cardiac disease [198]. Several studies [111, 112] have used male and female specific cut off values for the diagnosis of LVSD and found that BNP and N-BNP have different cut-off values and utility in men and women. I performed a univariate regression analysis was performed with the same variables in male and female groups. Age, a history of ischaemic heart disease, ECG abnormalities and all 3 peptides was associated with WMSI >1.8 ($P<0.1$). A multivariate logistic model was then calculated using these variables using a forward and backward (stepwise) regression method and ROC curves were constructed.

There were six cases of LVSD in 583 women (1.03%) and 22 in 756 men (2.91%). Levels of peptides were significantly different between men and women (Chi-square test $P<0.05$) (Table 7.6). In a multivariate logistic regression for each of the 3 peptides, independent predictors for LVSD were peptide levels, age, ECG abnormalities and a history of ischemic heart disease ($P<0.05$). In both groups, using ROC analysis, the AUC for BNP, was superior to that of N-ANP and N-BNP (see figure). The diagnostic utility of BNP was superior in men compared to women (AUC 0.976 and 0.887 respectively). In the male group, a BNP cut-off value of 36.39 fmol/ml gave 100% sensitivity and 90.1% specificity. This compares to a level of 19.23 fmol/ml giving 100% sensitivity and a specificity of 38.9% in the female group. Positive predictive values for men and women are 23.23% and 1.7% respectively (Table 5.5). It would appear that BNP performed better in diagnosing LVSD in men as compared to women. However, it must be emphasized that the sample size, particularly in the women, was very small and as such, this significantly limits the power to demonstrate true gender differences.

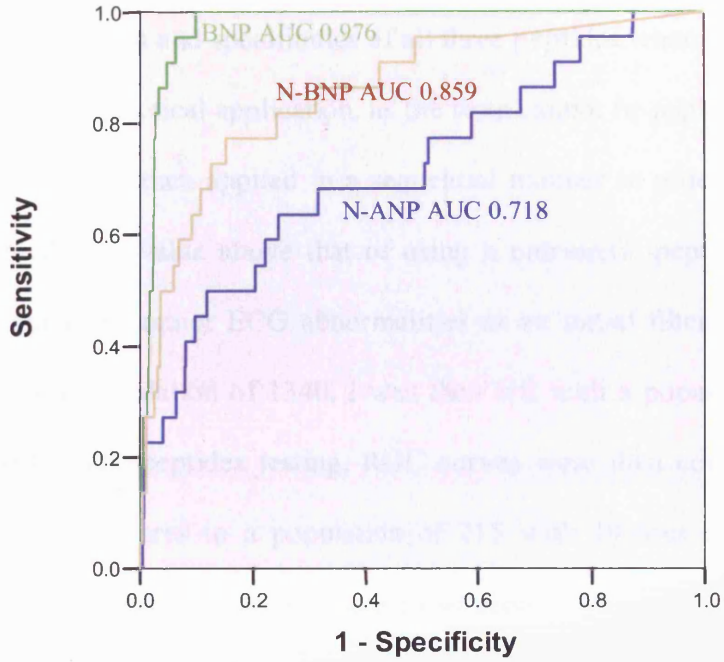
	Male (n=756)	Female (n-583)
LVSD Prevalence (%)	2.91	1.03
Mean age	61.5 +/- 8.5	66.03 +/- 6.82
N-ANP; median (range)	364.9 (`5.9-4115.4)	450.7 (5.2-3151.1)
BNP	19.1 (2-506.9)	22.1 (2-243.4)
N-BNP	27.3 (5.7-1230.3)	73.3 (5.7-1160.7)
Performance of BNP in detecting LVSD at optimal cut-off (36.39 and 19.23fmol/ml for men and women respectively)		
Sensitivity (%)	100	100
Specificity (%)	90.1	38.9
PPV (%)	23.23	1.71
NPV (%)	99.3	98.4
Number to echo to diagnose 1 case LVSD	3.4	59.37

Table 7.6. Peptide levels in men (shaded) and women. Diagnostic utility of BNP in diagnosing LVSD.



Peptide	Area under curve	95% Confidence Interval
N-ANP	0.87	0.75-0.99
BNP	0.89	0.71 – 2.0
N-BNP	0.84	0.69-1.0

Figure 7.4a ROC curve for detection of LVSD in women (n=6)



	Area under curve	Std. Error	95% Confidence Interval
N-ANP	0.72	0.06	0.6-0.84
BNP	0.98	0.006	0.96-0.99
N-BNP	0.86	0.04	0.77-0.94

Figure 7.4b. ROC curves for detection of WMSI>1.8 in men (n=22)

7.6 Combining ECG and Natriuretic Peptides in screening for LVSD

As the positive predictive value of all three peptides when used alone was low, I then investigated different statistical models to see if specificity would be improved whilst maintaining high sensitivity. The ECG's utility in the detection of LVSD was discussed in the previous chapter. The presence of major ECG abnormalities, QRS duration and all three natriuretic peptides independently predicted the presence of LVSD. Applying the ECG together with natriuretic peptides in a logistic model improved detection of LVSD significantly. At 100% sensitivity, specificity of BNP improved from 44.7 to 73.9. PPV rose from 3.73 to 9.64%.

Although a logistic model combining the ECG and BNP, N-BNP and N-ANP improved sensitivities and specificities of all three peptides when used alone (Figures 7.5a, b, c), this has limited practical application, as the tests cannot be applied in a simultaneous manner. The two tests were then applied in a sequential manner in order to see if this could increase positive predictive value above that of using a natriuretic peptides test alone. Using the presence of major and minor ECG abnormalities as an initial filter, thus removing 795 subjects from the initial population of 1340, I was then left with a population of 545 subjects, which then had natriuretic peptides testing. ROC curves were then constructed for this group (Figure 7.6b). This compares to a population of 215 with 19cases of LVSD if we used just major ECG abnormalities as the first step in screening. Using major ECG abnormalities excluded more subjects compared to the use of all ECG abnormalities (84% c.f. 59%) This latter strategy resulted in two missed cases of LVSD, both of which had entirely normal ECGs. A further 202 cases were excluded with a BNP cut-off value of 19.23 fmol/ml from the 536 (Figure 7.6a), leaving 334 subjects from the original 1360 to have echocardiograms. An overall sensitivity of 92.9% (26 cases diagnosed out of a total of 28 cases of LVSD) and 74.7% specificity was achieved. Positive and negative predictive values were 7.3% and 99.8% respectively.

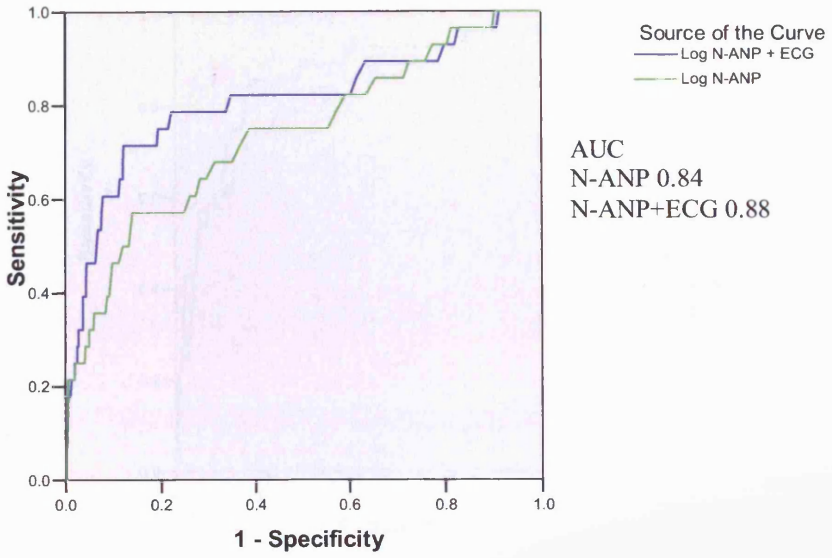


Figure 7.5a. ROC AUC for detection of LVSD by N-ANP and combination with ECG

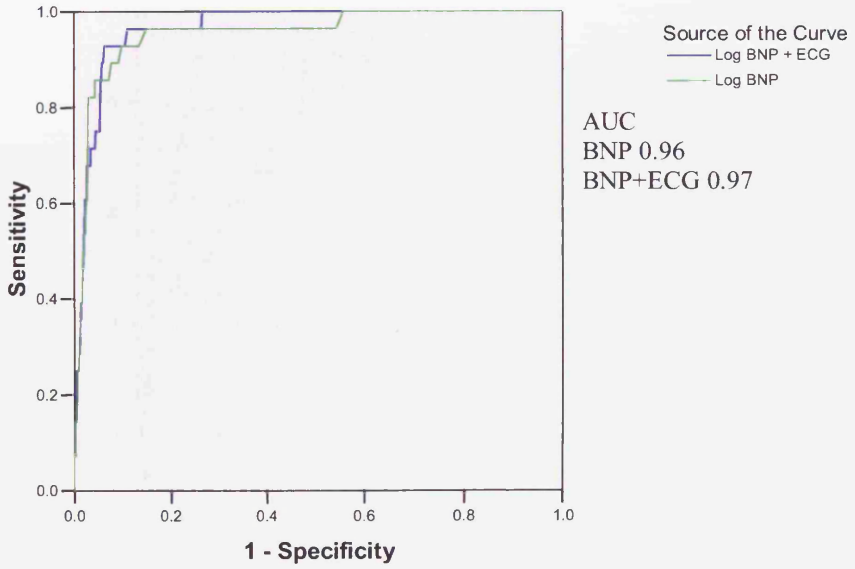
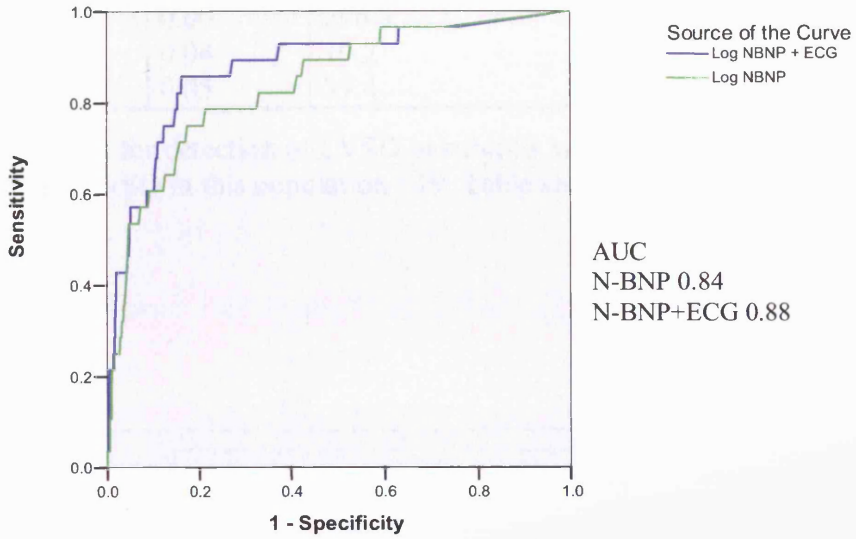


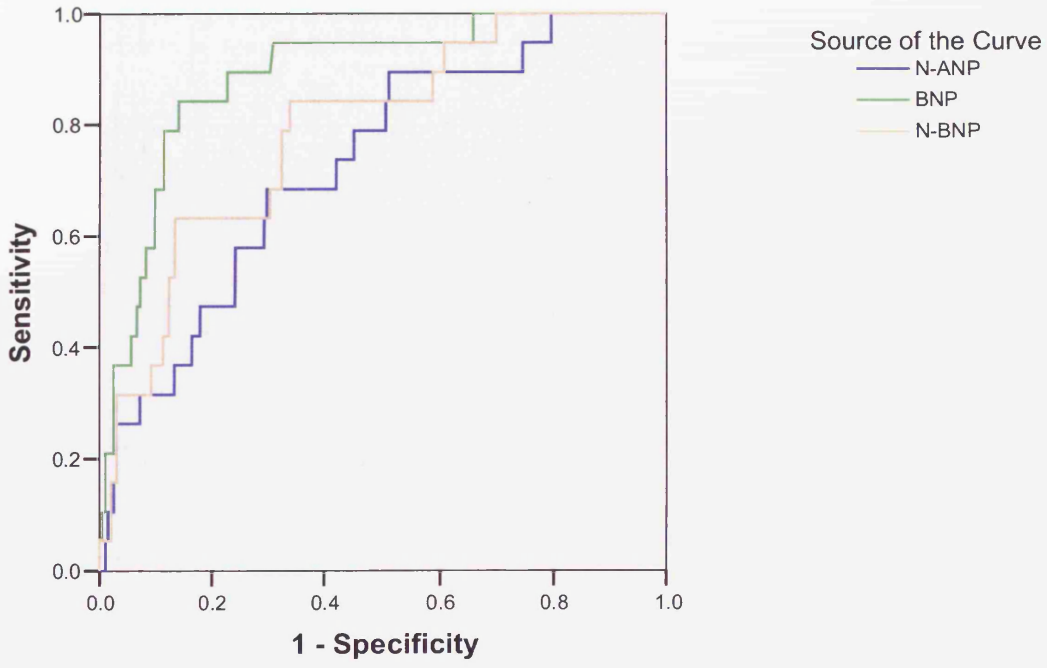
Fig 7.5b. ROC.

Figure 7.5b AUC for detection of LVSD by N-ANP and combination with ECG



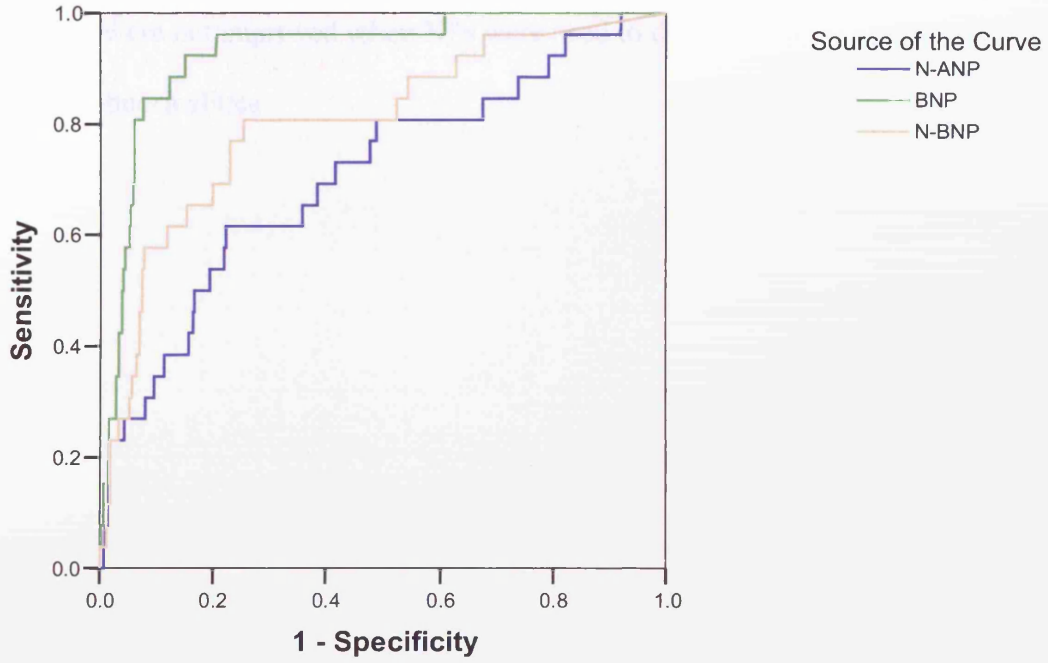
AUC
N-BNP 0.84
N-BNP+ECG 0.88

Figure 7.5c. ROC AUC for detection of LVSD by N-ANP and combination with ECG



Test Result Variable(s)	AUC	Std. Error	Cut-off value for 100% sensitivity (fmol/ml)	Specificity (%)	95% Confidence Interval
N-ANP	0.73	0.06	286.4	20	0.62-0.84
BNP	0.89	0.04	19.2	34	0.82-0.96
N-BNP	0.79	0.05	37.4	30	0.69-0.89

Figure 7.6a ROC curve for detection of LVSD in subjects with major ECG abnormalities (n=215). Cases with LVSD in this population =19. Table shows AUC of peptides and utility at 100% sensitivity.



	AUC	Std. Error	Cut off value for 100% sensitivity	Specificity (%)	95% Confidence Interval
N-ANP	0.71	0.06	177.3	8	0.6-0.82
BNP	0.93	0.02	19.23	39	0.9-0.98
N-BNP	0.81	0.05	NA	NA	0.7-0.90

Figure 7.6b ROC curve for detection of LVSD in subjects with major and minor ECG abnormalities (n=536). Cases with LVSD in this population =26. Table shows diagnostic utility of peptides at 100% sensitivity.

7.7 Conclusions

- N-ANP, BNP and N-BNP were independent predictors of LVSD. All three peptides achieved high negative predictive values but low positive predictive values.
- Performance of all three peptides was improved by screening a higher risk population – subjects with history of ischaemic heart disease, abnormal ECGs and male gender.
- BNP performed better than N-ANP and N-BNP in the detection of LVSD in all subgroups.
- ROC AUC were not improved when NPs were used to detect LVSD in a population with ECG abnormalities.

Chapter 8

Discussion

8.1 Summary

Heart failure, a common and serious condition in the community, has significant mortality and morbidity [2, 3, 4]. There is a well-recognized latent phase of "pre-clinical" heart failure where the subject can be relatively asymptomatic. This asymptomatic or pre-symptomatic phase is associated with an adjusted hazard ratio of 7.8 of developing heart failure and 1.6 of increasing mortality [49]. The common causes of LVSD, ischaemic heart disease and hypertension, have an insidious onset and patients commonly present later on in the disease process with relatively severe symptoms. Early identification of this latent phase allows early treatment with established medical therapy, which can delay the onset of symptoms as well as improve prognosis [47]. The United States Preventative Services Task Force advocates three minimum criteria for assessing the efficacy of screening: (1) demonstration of the burden of suffering; (2) an accurate screening test and (3) evidence that early detection and treatment is effective. Congestive Heart Failure (CHF), and its precursor, asymptomatic or presymptomatic LVSD meet these criteria. Although, currently, there is no UK program to screen the general population for this common disease, UK National Service Framework as well as National Institute of Clinical Excellence guidelines do call for an early diagnosis of symptomatic patients. The diagnosis of CHF, even in the presence of symptoms is difficult and studies have shown that up to a third of patients labeled as CHF in primary care do not have LVSD when subject to more detailed investigation [80, 84, 86]. Despite its limitations, the transthoracic echocardiogram remains the gold standard for the assessment of left ventricular function. However utilizing the echocardiogram in a population wide screening program is not practical owing to the cost and time required to perform each scan. Natriuretic peptides and the 12 lead electrocardiogram have been recommended in the recent national guidelines by the National

Institute of Excellence [82] as strategies to rule out heart failure prior to further investigation in the diagnosis of LVSD.

8.2 Study Aims

- To test the utility of plasma levels of 3 natriuretic peptides and the standard 12 lead electrocardiogram in screening a unselected general population without a prior diagnosis of congestive cardiac failure for LVSD.
- To assess means of improving the diagnostic utility of these tests.

8.3 Population

The study population was randomly drawn from the primary care setting with patients offered a chance for screening for heart failure. Cases of known LVSD/CHF were specifically excluded, making this study the first large scale screening study, which assessed the different methods of detecting previously unknown cases of LVSD. The total number of cases with analyzable ECGs and echocardiograms was 1360.

8.4 Defining LVSD

Systolic function has traditionally been defined by an estimate of ejection fraction, a measure of the change in left ventricular volume. Left ventricular volume estimate is prone to error and is often not easily measured due to the technical limitations of the technique. Most studies dependant on LVSD find that up to an accurate EF estimate is not obtained in up to a third of subjects, regardless of the technique used. I used Simpson's rule of discs to estimate the ejection fraction as well as a well-validated 16-segment wall motion scoring system. These two

methods correlated extremely closely with each other and confirm what has been found in other studies [91, 185,186]. Previous studies employing echocardiography derived ejection fraction assessments (by Biplane method or fractional shortening measurements) [110, 111, 112] have excluded up to a third of subjects because an accurate estimation of ejection fraction was not obtained because of technical limitations. In my study, an accurate ejection fraction was only obtained in 65% of the study population. In comparison, the WMSI was measured in over 99% of the screening population, which allowed analysis of virtually all of the study population. A wall motion score of ≥ 1.8 (equivalent to ejection fraction $\leq 40\%$) was used to define LVSD.

8.5 Prevalence of LVSD in study population

The prevalence of LVSD, both symptomatic and asymptomatic in the study was 2.1%. Bearing in mind that this was a screening study in the general population, this number is roughly in line with that seen in other similar studies in Glasgow (2.9%) [13], Birmingham UK (1.8%) [12], the United States (2.9%) [145] and Rotterdam (3.7%) [14], none of which excluded known cases of heart failure (see table 1.1). Using a self-administered questionnaire, approximately half of our population with LVSD felt that their exercise tolerance was not significantly impaired. Again, this roughly accords with what is known about the incidence and prevalence of asymptomatic or pre-symptomatic LVSD, which ranges widely in different studies from 0.9% to 12.9% [95], depending on the trial design, population characteristics and definitions. Similarly, the ECHOES study in Birmingham UK [12] as well as the MONICA study in Glasgow found that approximately half of the population with LVSD (1.8% and 1.4% respectively) did not report significant symptoms. A surrogate marker of symptoms of fluid overload may be the prescription of diuretics by the general practitioner. 171 patients (12.57%)

in my study population were prescribed diuretics, of which only two (7.14%) had LVSD. Again, this would suggest that a substantial proportion of the subjects with LVSD had little in the way of symptoms. This would also suggest that cases of fluid overloaded were inappropriately prescribed diuretic therapy. Of the 28 cases of LVSD in my study, only 6 were prescribed ACE inhibitors and four were prescribed diuretic therapy, suggesting that the condition was either under-treated or truly asymptomatic in primary care.

Men made up the majority of cases of LVSD in this study (78.6%), mirroring the Birmingham and Glasgow studies [12, 13]. Similarly, most other population studies have largely found that increasing age and male gender are associated with increasing incidence of LVSD, both symptomatic or asymptomatic [20, 13, 49,87]. Ischaemic heart disease and hypertension have been found associated with LVSD [15, 13] and are presumed to be the aetiology for the LVSD. In my study, IHD, but not hypertension nor diabetes mellitus, was associated with LVSD. However, the mean blood pressure in the study was 135/78, suggesting either the patients had only mild hypertension and/or their BP control was good. Speculatively, such patients would not be at particularly high risk of LVSD/CHF.

8.6 Utility of the 12-lead electrocardiogram

The advantages of using the 12-lead electrocardiogram are evident. The investigation is easily obtained in most settings, including the primary care setting, without the need for specialist equipment or technician support. Obtaining an ECG is non-invasive and acceptable to most patients. The cost is also low, depending on the machine used. Analysis of the ECG would require specialist interpretation for subtle abnormalities but major abnormalities are generally, but not always, well detected by primary care physicians.

8.6.1 Using major and minor ECG abnormalities

Using the Minnesota coding criteria, the incidence of major ECG abnormalities in the study population was 16.3% (n=217). 40.5% (n=539) had major or minor ECG abnormalities. This compares with ECG being abnormal in 75% and 38% in the population based studies in the Glasgow MONICA [110] and Birmingham [12] studies respectively, neither of which excluded known cases of LVSD and CHF (Table 8.1). The incidence of ECG abnormalities in patients referred to rapid access heart failure clinics had incidences of abnormal ECGs ranging from 25.8% [118] to 49% [89]. The presence of major ECG abnormalities, as defined by the Minnesota criteria, was 67.86% sensitive and 84.96% specific in the diagnosis of LVSD. This compares to a sensitivity of 92.86 and specificity of 60.86% when both major and minor ECG abnormalities are considered. Positive predictive value of the 2 tests was 8.82 and 4.42 respectively.

	Cases of LVSD/ Population	Prevalence of abnormalities (%)	Sensitivity	Specificity	PPV	NPV	+LR	-LR
Major Abnormalities	28/1360	16.3	67.86	84.96	8.82	98.8	4.51	0.38
Major and minor		40.5	92.86	60.86	4.42	99.2	2.37	0.12
Galasko [208]*	62/1205		92	78	19	99	4.18	0.1
UK-NP [118]	104/306	25.8	81	60	51	86	2.02	0.32
Hedberg [91]*	28/407	26	96	79	26	100	4.57	0.05
Davie [89]	16/534	49	94	61			2.41	0.1
Nakamura [181] *	39/1098	28	100	74	12.5		3.84	NA
Goudie [207]	41/150	67	94	58	47	96	2.24	0.1

Table 8.1. Diagnostic utility of the ECG in detecting LVSD. * denotes population screening trial. Current study highlighted. (PPV: Positive predictive value, NPV: Negative predictive value, LR+: Positive likelihood ratio, LR- : Negative likelihood ratio)

A meta-analysis of 4 large-scale trials [191] (n=1419) that utilized the ECG in screening symptomatic patients referred for open access echocardiography with suspected heart failure. The overall mean sensitivity was 86% (range 73-94%) and mean specificity was 58% (range 20-61%). The best sensitivity in the 4 trials was found by Davie et al. [89] who achieved 94% with a corresponding specificity of 61%. This study found 96 cases of LVSD out a total study population of 534. 90 of the 96 had major ECG abnormalities with the remaining 6 cases having minor ECG abnormalities. In the analysis of all 1419 cases, the area under the ROC curve was 0.84 (95% CI: 0.33 to 1.00). The prevalence of LVSD in the 4 trials ranged from 18% to 36%, reflecting a high-risk population which was already considered by the referring

primary care physician to be at risk of having heart failure. The authors found that using the ECG alone would have failed to identify between 6-27% of cases in a population that was already felt by the primary care physician to be at high risk of having LVSD.

Few large-scale studies have assessed the utility of the ECG in detecting LVSD in the community. Nakamura et al screened 1098 Japanese subjects for cardiac disease [192], defining this as LVEF <40%, significant valvular disease, arrhythmia, including atrial fibrillation. The ECG was abnormal in all 39 subjects with cardiac disease, although none had EF<40%. In this study, the ECG, perhaps unsurprisingly, was sensitive and specific for the presence of valvular disease and arrhythmia. Hedberg [91] conducted a Swedish screening study looking at LVSD in a population of 75yr old patients. Again, the ECG achieved high sensitivity and specificity. These results are surprisingly good, particularly when compared to other studies that have assessed the ECG in populations with suspected CHF (see table). However, neither study is entirely applicable to the general population as the former did not actually have subjects with LVSD and the latter looked only at 75yr olds, a relatively high-risk group. Most recently, Galasko et al. screened a UK general population (n=1205) and found 64 cases of LVSD. An abnormal ECG was 92% sensitive and 78% specific for LVSD. These results are comparable to that found in my study when using the presence of major and minor ECG abnormalities.

The results in my study showed good sensitivity but relatively poorer specificity, although having good negative predictive value. This may be due to characteristics of the population involved. I had already excluded known cases of LVSD and as such this would be a relatively "low-risk" population, but one that would be exactly representative of a target screening population. This is confirmed by the relatively low incidence of LVSD (2.1%) in my trial which would be indicative of that in a true general population-screening program.

Choosing to use both major and minor ECG abnormalities has advantages and disadvantages. Despite improving sensitivity, specificity reduced from 84.96% to 60.86%. Positive predictive value reduced from 8.82 to 4.82. My study confirms a high negative predictive value of major and minor abnormalities in the 12 lead ECG, suggesting that it's main role would be as a "rule-out" test. In my population, only 2 cases out of 28 subjects (12%) had entirely normal ECGs.

8.6.2 Comparing Natriuretic Peptides and ECG

A UK study specifically utilized ECGs and BNP as screening tests prior to referral for echocardiography of symptomatic patients [193]. The sensitivities of the ECG for LVSD, heart failure, LVH and valvular disease were 97%, 95%, 76%, and 69%, respectively. Using standard reference cut-off values, the corresponding figures for BNP were 86%, 82%, 59%, and 48%, respectively. The authors found that in screening for LVSD, both BNP and the ECG performed similarly, if anything the advantage being with the ECG. Similarly, Hedberg et al [91] compared the ECG against BNP in screening 405 randomly selected 75yr olds. The incidence of major ECG abnormalities was 25.8%. In this study BNP, using a cut-off of 28pg/ml had a sensitivity of 93%, worse than that of the ECG, which was 96%. Specificities of BNP and ECG were 38% and 79% respectively. The results for these 2 trials have the best results in the literature for the utility of the ECG, particularly in having improved sensitivity. The UK Natriuretic Peptide study [118] found that classifying ECGs into "abnormal" or "normal" had a 81% sensitivity for LVSD. 53% of the total population had an abnormal ECG. In a logistic regression analysis, which included natriuretic peptides, the authors found that ECG abnormalities were independent predictors of LVSD but did not perform as well as N-BNP or BNP. An abnormal ECG did not add to the predictive value of N-BNP. Lastly Galasko et al [210] found that using there was little difference in cost-effectiveness in using ECG or N-BNP

as a pre-screening test. In this study, the ECG and N-BNP had a sensitivity/specificity of 92/78% and 80/88% respectively. In my study, using the presence of major and minor ECG abnormalities to screen for LVSD would result in 7.1% of cases of LVSD being lost, achieving a sensitivity/specificity of 92.9/60.9%. Using optimal cut-off values rather than manufacturer guidelines, BNP achieved a sensitivity of 96.4% and a specificity of 85.3% for LVSD. All these trials [91, 113, 193, and 208] chose to use standard assays and, followed the manufacturer's guidelines for the cut-off values. Speculatively, adjusting the cut-off value to maximise either sensitivity or specificity would lead to better results.

8.6.3 Difficulties in ECG interpretation

Two experienced cardiovascular physicians analyzed the ECGs in the present study. Their diagnostic acumen in detecting ECG abnormalities can be assumed to be superior to the general practitioner. This has been demonstrated previously by McCallan [95], who tested a sample of general practitioners in their competence in interpreting the ECG. Although overall competency was judged to be good, unequivocal acute myocardial infarction was misdiagnosed by 20% of the sample. McCrea [96] also found that only 63% of his sample correctly diagnosed an acute myocardial infarction. These studies, although looking at acute myocardial infarctions, would suggest that a significant proportion of general practitioners would not be able to interpret significant ECG changes of ischaemia. This is likely to further degrade the usefulness of ECGs in the role of screening for CHF, particularly if we had to detect minor abnormalities.

8.6.4 Diagnostic utility of the mean QRS duration for LVSD

The presence of a major ECG abnormality has relatively good sensitivity but poor specificity for LVSD. An ordinal value (either a normal or an abnormal ECG) is naturally unlikely to achieve 100% sensitivity, a feature that is particularly desirable in a screening study. The QRS duration is easily measured and is often by of an automated computerized analysis package found on many standard ECG machines. Applying a routinely measured parameter would also remove difficulties in the interpretation of the ECG. The Framingham population analysis [194] found that incomplete bundle branch block (QRS <120msec) and complete bundle branch block (>120msec) was associated with a 1.4 and 1.7 fold increase risk of CHF respectively. Studies have also found that the presence of a prolonged QRS duration in the presence of LVSD is associated as well as worsening prognosis [195, 196]. A New York study [94] population consisted of patients with suspected heart failure. The mean QRS duration in the group with an abnormal EF was 102msec. A German study on 128 cases of suspected LVSD [93] found that a QRS duration of >0.1, >0.11 or >0.12 s was highly specific (63, 90 and 98%) but less sensitive (84, 81 and 75%) for the prediction of LVSD. Mean QRS in the group with LVSD was 129msec versus 96msec in the group with normal LV function. A QRS cut-off value of 106 ms was moderately sensitive (65%) but very specific (87%) for the prediction of LVSD, whereas a BNP cut-off value of >84 pg mL^{-1} was highly sensitive (89%) but only modestly specific (58%).

In the present study, mean QRS duration in the LVSD cohort was 110msec, which is lower compared to the two previous studies [93, 94]. This may reflect a relatively healthier screening general population compared to a symptomatic cohort suspected of having LVSD.

Univariate and multivariate logistic regression analysis showed mean QRS duration to be an independent predictor of LVSD, along with NP levels. ROC AUC was 0.76 (95% CI 0.65-0.88). QRS duration of 84.18 had a sensitivity of 93%, specificity of 19% and positive and negative predictive value of 2.1 and 99 respectively. A QRS of 100msec had a sensitivity of 67.9%, specificity of 73.7%, PPV 5.25% and NPV 99.1%. Unlike a previous studies [93, 197], this study found that a combination of the QRS duration and BNP/N-ANP did not improve the diagnostic utility of either peptide for LVSD.

These results compare unfavorably to other studies [93, 94,197]. This may be due to a different population – comparing subjects with suspected CHF to a general screening population with relatively few symptoms would naturally lead to lower positive predictive values because of a lower prevalence of LVSD. Despite that, the QRS duration has significant utility as a "rule-out" test.

8.6.5 Conclusions

Despite the advantages in using the 12 lead ECG as a screening tool, the presence of major abnormalities in the 12 lead ECG is sub-optimal in diagnosing LVSD, failing to diagnose up to a third of all case with LVSD. The inclusion of minor abnormalities results in an improved sensitivity but we would have to assume skilful interpretation of the ECG. Using the mean QRS duration, although possessing high sensitivity, has a poor specificity and low likelihood ratio. The standard 12-lead ECG has good sensitivity and high negative predictive value. This utility was found to be superior to the use of N-ANP but not BNP and BNP in the detection of LVSD. The mean QRS duration had high negative predictive values but poor positive likelihood ratio.

8.7 Diagnosing LVSD using N-ANP, BNP and N-BNP.

Natriuretic peptides, being secreted by cardiac chambers in response to stretch are indirect determinants of pressure within the cardiac chambers. Peptides have been proposed as diagnostic aids in identifying subjects with LVSD. However, the levels of cardiac peptides are also influenced by factors such as age and gender. I performed a sub study of our original population, looking at the variables influencing peptide levels in the normal population, exclusions being LVSD, arrhythmias such as AF, significant valvular disease, hypertension, previous ischaemic heart disease and diabetes. A comparison of the effect of different variables on BNP, N-BNP and N-ANP was made.

8.7.1 Factors influencing natriuretic peptide levels in a population without overt cardiovascular disease

8.7.1.1 Effect of Gender

I observed potentially important differences in the determinants of individual natriuretic peptides and confirmed previous reports, in similar healthy populations, of higher BNP levels in women [135, 198] and extended this finding to N-ANP and N-BNP. Indeed female gender was the strongest independent predictor of natriuretic peptide levels in healthy individuals, plasma levels of BNP being approximately 25% higher in females. This compares to the 32 and 80% higher levels reported previously with alternative assay systems [135]. Higher BNP levels in women taking HRT as well as higher ANP in premenopausal women [199], suggest an influence of oestrogen. The consistent finding of higher BNP levels in females confirms the

need for gender-specific reference ranges for BNP, as suggested by Redfield [135]. We observed a lack of effect of age on plasma BNP, contrasting with a clear effect on levels of N-ANP and N-BNP. Previous reports indicated a positive correlation of age and plasma BNP [135,198,199,200]. There are a number of possible reasons for the differences between studies. Differences in the age-range of study populations may be relevant, as may the considered covariates.

8.7.1.2 Effect of Heart Rate and Blood Pressure

Heart rate, not previously considered as a covariate [135, 198], predicted levels of all three peptides. Interestingly, in this healthy population the correlation is of lower natriuretic peptide levels with higher heart rate, an increase in heart rate of 10 bpm corresponding to a reduction of 15% in N-BNP and 9% in N-ANP or BNP. The consistency of the observed relationship between heart rate and all three natriuretic peptides suggests that the association is real. There have been no prior reports of an association between heart rate and plasma BNP, although a reduction in heart rate by introduction of a beta-blocker has been found to increase BNP levels [201]. It may be that synthesis or secretion of natriuretic peptides may be dependent upon diastolic duration or filling pressure in the healthy individual. This would be of relevance only if alterations in heart rate resulted in significant intra-individual fluctuations in plasma natriuretic peptide levels. Our study in patients free of cardiovascular pathology does not inform us as to the relationship between heart rate and peptide level in pathological conditions. Plasma levels of both ANP and BNP increase in response to exercise in patients with coronary artery disease and normal LV function [202, 203] and in patients with LVH [204].Other reports have indicated little change [205] or significant increase [206] in plasma BNP levels during

exercise in patients with heart failure. Further studies to determine the relationship between plasma natriuretic peptide and heart rate are required, both in healthy populations and in cohorts in whom the assay of BNP may be clinically applicable. In contrast to a previous study [136], we observed a weak correlation of both systolic blood pressure and pulse pressure with at least some natriuretic peptide moieties (Table 3). Once again, the intra-individual influence of changes in blood pressure on natriuretic peptide levels has not been explored.

8.7.1.3 Effect of Age

Previous studies of BNP in the identification of patients with LVSD reported the sensitivity and specificity of partition values [110, 111] without reference to age. Redfield et al. [135] observed increasing plasma BNP levels with age using both the Shionogi and Biosite BNP assays and concluded, justifiably, that the use of either assay in clinical practice requires age- and gender specific reference ranges. Similar findings were made by Galasko et al, who found that age and female gender were independently associated with N-ANP levels in a normal population [207]. N-BNP commercial assays now have a gender and age corrected normal ranges. In the present study, increasing age was independently associated with an increase in N-ANP and N-BNP but not BNP levels, hence suggesting that correction for both age and gender may not be necessary, depending on the assay system used. This contrasts with a study that compared the effect of age on N-ANP, BNP and N-BNP levels in patients with CHF, which found greatest associated elevated BNP and N-BNP with increasing age. The least effect was, in contrast, on N-ANP levels.

8.7.1.4 Effect of LV ejection fraction

As with heart rate, LVEF does not appear to have been considered as a potential cofactor influencing plasma natriuretic peptide levels in healthy populations [135, 198] we observed an independent, though weak, association of LVEF with plasma levels of two of the peptides, namely N-ANP and BNP. In both cases, the correlation was positive, i.e. higher peptide levels with higher ejection fraction, the converse of the situation in LVSD and heart failure. To our knowledge, this is a novel finding. In contrast to previous studies [135, 198] we found no correlation between echocardiographic parameters and plasma natriuretic peptide levels. It may be relevant that in our study, unlike some others [135], all scans and analyses were performed by a single operator. Differing associations between natriuretic peptide levels and echocardiographic parameters in healthy populations as opposed to cohorts with cardiac pathology may, however, be important in the understanding of mechanisms of control of the natriuretic peptide systems in health and in disease. The associations of natriuretic peptide levels with higher LVEF and lower heart rate in our healthy population, the converse of the situation in heart failure, suggests profound differences in the mechanisms of control of these peptide systems in health and disease.

8.7.1.5 Conclusion

This study in healthy individuals confirms previous observations [134, 198] on the effects of gender and age on N-ANP, BNP and N-BNP. Several studies [135, 198] have advocated age and gender specific normal ranges of different peptides. My study confirms the higher levels of all 3 peptides in normal women. Advancing age was correlated with increased N-ANP and N-BNP levels but not with BNP. This has been previously observed and is

reflected in commercial assay recommended normal ranges: unlike N-BNP assays, BNP assays do not have an age specific cut-offs. Despite this, the actual effect of age and gender is relatively small and may have little actual clinical effect.

8.7.2 Screening for previously undiagnosed LVSD using natriuretic peptides

8.7.2.1 Performance of the different cardiac peptides

This was one of the first prospective studies to compare the utility of various natriuretic peptides in combination with ECGs for the identification of previously undiagnosed LVSD. The levels of BNP, N-BNP and N-ANP correlated closely with each other. The plasma levels of N-ANP, BNP and N-BNP were significantly higher in the LVSD subjects compared to the normal population. ROC AUC analysis of the performance of the 3 NP's in detecting LVSD showed that BNP performed best and N-ANP worst.

The current study clearly demonstrates the superiority of BNP over N-ANP and N-BNP in the diagnosis of LVSD. I studied the performance of 3 NP's with different cut-off values: both the 95th centile, manufacturers guidelines were used as well as optimal values at 100% and 96.4% sensitivity. The performance of BNP remained superior to that of N-BNP at all cut-off values and both of these peptides performing better than N-ANP. This superiority of BNP over N-BNP and N-ANP was maintained at different cut-off values, in men and women and also in screening a high-risk group. This superiority was particularly marked when comparing the performance of BNP against N-ANP, although negative predictive values were high, confirming the observation of all major similar studies that peptides are excellent "rule out" tests (see table 8.2). The superiority of BNP has not been consistently observed in other, albeit few, studies that have performed "head to head" comparative trials of the different natriuretic

peptides. Hobbs et al. [111] compared BNP against N-BNP, finding that BNP performed better (AUC 0.88 vs. 0.76). However, the recent UK NP study utilized both N-BNP and BNP in the diagnosis of 306 patients referred with suspected LVSD. The AUC of the ROC of BNP and N-BNP was 0.84 and 0.85 respectively. Similarly, there was no observed difference in the utility of BNP and N-BNP in the Framingham cohort [112]. The Olmstead County screening study [113] found that N-BNP performed better than BNP in the male population although there was no significant difference in the female group. N-ANP, although consistently found to be elevated in cases with LVSD, performs less well against BNP or N-BNP. This was demonstrated in the Glasgow MONICA cohort [110], the Framingham cohort [112] as well as in a Japanese cohort [138].

This has implications in the debate over which natriuretic peptide to use, as both BNP and N-BNP are commercially available as diagnostic tests. Although there are differences in the performance of different peptides, the ultimate utility of each individual peptide would be influenced by several factors - the precise condition to be identified, be it LVSD or diastolic dysfunction, the target population characteristics, e.g. age, gender racial group as well as the actual peptide assay to be used. It may be that, at the end of the day, the decision on which peptide marker is used is based on issues of practicality regarding the actual assays performances rather than the relative diagnostic performance of the peptides.

Studies	Cases of LVSD/total population	Testing modality and cut off value	AUC	Sensitivity	Specificity	PPV	NPV
Sweden [109]	28/407	BNP>28pg/ml	NR	93	55	13	99
		ECG	NR	96	79	26	100
UK Natriuretic Peptide study	104/306	N-BNP >125 (95th centile) ECG	NR	98 81	35	46	96
		BNP>100 (95^{th} centile)	NR	79	72	59	87
		ECG	NR	91	60	86	51
Birmingham UK [110] *	33/607	N-BNP	NR	100	70	7	100
MONICA* North Glasgow [111]	27/1252	BNP >17.9 pg/ml N-ANP >1.76 ng/ml	0.882 0.723	77	87	16	98
Framingham* [112]	179/3177	Men :BNP >46 (mod-severe LVSD) Women: BNP >34	0.72 (men) 0.56 (women)	65 50	86 93	4.1 4	98 100
Olmstead, Minnesota US. [113]	37/1869	BNP >66 pg/ml (EF<40%) N-BNP > 228	0.89 0.94	81.1 86.5	81.1 86	NR	NR
Durham GP UK, Fuat 2006	114/297	BNP > 40pg/ml N-BNP > 150pg/ml ECG	0.79 0.81	92 94 82	38 40 58	49 48 55	88 92 83
London, UK * [210]	30/1392		NR	80	88	20	99
Current* study	***28/1360***	***BNP>65.3 pg/ml N-BNP***	***0.96***	***100***	***44.7***	***3.73***	***99.95***

Table 8.2. Diagnostic utility of cardiac peptides and ECG in identifying LVSD in various trials. (*) trials are general population screening studies. Other trials involve patients referred with suspected LVSD/CHF. (PPV: Positive predictive vale, NPV: Negative predictive value, AUC – area under curve of Receiver Operator Curves, NR – not reported in study)

8.7.2.2 The use of gender specific cut-off values

The effect of gender on peptide levels is well recognized and several studies [135, 210] that have used cardiac peptides to diagnose LVSD have proposed gender specific normal ranges. I had previously shown that female gender had a small but statistically significant effect on all 3 peptides in a healthy population, although to different degrees. In the study population, significantly more men had LVSD compared to women (2.9% vs. 1.03%, $p<0.05$). In the diagnosis of LVSD, BNP performed best out of all 3 peptides in both men and women. The AUC for ROC curves for diagnosis of LVSD by all 3 peptides in male and female groups were similar to that in the population as a whole, specifically BNP AUC was being 0 .96 and 0.98 in men and women respectively. This finding was also seen in the Olmstead county group, which did not find any significant differences in the performance of NP in detecting LVSD in men and women. However, the Framingham group, although generally having lower ROC AUCs, found better performance in the male group In the present study, because of a higher prevalence of LVSD, the positive predictive value increased from 3.73 in the population as a whole to 23.2 in the male group. Conversely, the female group positive predictive value reduced to 1.7. The cut off values in my study were surprisingly higher in men compared to women (36 vs. 19 fmol/ml), although this was optimized for 100% sensitivity. This analysis is limited because of a relatively small sample size, particularly in the female group, which had only 6 cases of LVSD. The results, however, suggest that having gender specific cut-off values resulted in an improvement in the diagnostic utility of BNP in the male group.

8.7.2.3 Identifying the best cut-off value is best in identification of LVSD

The selection of an "optimal" cut-off value is also influenced by the need to maximize either sensitivity, specificity or to compromise on both. It has been suggested that high specificity and positive predictive value, the "rule-in" strategy is important in a screening test to reduce the numbers of false positive results [214]. This would reduce expensive follow-up test as well as the anxiety related to false positive results. However, the utility of natriuretic peptides is probably best as a "pre-screening" test, prior to echocardiography. In this situation, a high sensitivity may well prove to be more useful as the aim of pre-screening would be to reduce the number of actual scans required to make a definitive diagnosis.

The optimal cut-off value of any of the 3 NPs is influenced not only by clinical variables, but also by the assay utilized [173]. Although peptide levels correlate closely between different assays, small but significant differences have been reported [173, 135]. As such although commercial manufacturers of the assays have given guidelines for the utility of peptides in varying clinical scenarios, these cut-off values cannot be directly applied to the different assays that were utilized in my study. Some studies [110, 113, 210] have used the 95th centile as a cut-off. In my study, using the 95^{th} centile of BNP and N-BNP as the cut-off would result in 1 in 5 cases and 3 in 5 cases of LVSD being undiagnosed respectively.

8.7.2.4 Screening a high-risk subgroup – subjects with a history of IHD

The positive predictive value of BNP in our study was relatively low at 2.2%. In part, this represents a low prevalence of LVSD in our screening population, particularly as we specifically excluded patients with a prior diagnosis of CHF. Thus, our approach is likely to reflect the true predictive value of natriuretic peptides in such a screening program. In order to

improve the PPV, we may apply the screening test to high-risk groups: subjects with suspected heart failure or with high-risk profiles, e.g. previous myocardial infarction, hypertension or diabetes, as suggested by some authors [192, 115, 210]. 122 subjects of the original 1360 had a past of current history of a myocardial infarction or angina. The incidence of LVSD in the IHD subgroup was significantly higher (11.7 vs. 1.4%, $p<0.005$ by Mann-Whitney test).

Surprisingly, despite higher prevalence of LVSD in the high-risk sub-groups, AUC did not significantly improve. Positive predictive value increased from 2.2% in the whole population, to 15.9 % in this high-risk group with 100% sensitivity (28.8% with 91% sensitivity). These results are similar to the ECHOES group who found that the diagnostic utility of N-BNP did not improve despite the use in a high-risk group with a higher incidence of LVSD. Moreover, in focusing on this high-risk group, 17 other subjects without LVSD from the general population would have been excluded from the start, 60.1% of the total number of cases. It could also be argued that subjects with IHD justify the need of an echocardiogram without the need for a "pre-screening" test, as valuable information, apart from systolic function, would be gained from performing an echocardiogram. It may be that the use of medication such as Angiotensin Converting Enzyme inhibitors (20% versus 7.9%) and beta-blockers (41.9% versus 8.8%) would influence the result, as these variables are known to impact of peptide levels [153, 154, 156].

8.8 Screening for diastolic dysfunction

The accurate diagnosis of this condition is difficult and some authors [52, 53] have suggested that other causes of breathlessness, such as lung disease, cardiac ischaemia or obesity, need to be actively excluded before making a confident diagnosis of DHF. The definitive diagnosis is made with invasive monitoring which is simply not practical in a large-

scale trial. Many of the echocardiographic parameters that are used to diagnose DHF can be difficult to acquire consistently and are also not specific for DHF, as some are related to advancing years. In addition, DHF often occurs together with a degree of systolic dysfunction. The European Society of Cardiology has defined DHF as subjects who have symptoms of CHF and documented normal LV systolic function. In addition, subjects should have echocardiographic parameters suggestive of impaired relaxation. This definition would make asymptomatic DHF a contradiction in terms.

It has been found that cardiac peptides levels in isolated DHF are generally elevated but not to the extent of systolic heart failure [124, 125]. Galasko et al defined DHF using the European Society guidelines and found that N-BNP had a poor sensitivity of 29% and specificity of 86% [210]. Similarly, Redfield [113] found that the sensitivity of peptides in diagnosing DHF in a screening population was only 40%. Neither of these trials actively excluded other causes of dyspnoea, such as respiratory disease or ischaemic heart disease. Speculatively, because DHF causes a relatively smaller elevation of natriuretic peptides compared to systolic dysfunction, a greater overlap with normal values would reduce the diagnostic utility of peptides in diagnosing DHF.

The effect of DHF in our analysis is unknown. Like the previous 2 studies, other alternative causes of dyspnoea were not actively excluded. In addition, the mitral valve E to A wave ratio, A wave deceleration time as well as the interventricular relaxation time measured in the population, are crude measures of diastolic dysfunction and would not be sufficient to make a confident diagnosis of diastolic heart failure. Speculatively, the presence of cases of DHF mixed in with the "normal" population would degrade rather than augment the performance of the peptide assays in detecting systolic heart failure.

8.9 Cost-effectiveness of pre-screening for LVSD

Heidenreich et al [115] found that a prevalence of 1% for LVSD would make a screening program using peptides prior to echocardiography cost effective screening program. Our overall prevalence of 2.1% would imply that screening our population with NP followed by echocardiography would be cost-effective. The AUCs in my study compare favourably to other accepted screening tests, specifically prostate specific antigen in screening for prostatic carcinoma (AUC 0.94),22, mammography for breast carcinoma (AUC 0.85)23 and Papanicolau smears for cervical carcinoma (AUC 0.70)

A strategy to target patients with LVSD history would be naturally more cost-effective but would also inevitably fail to diagnose a significant number of cases. In my study, 13 of the 28 cases of LVSD (46%) did not have a history of IHD, diabetes or hypertension. However, I did not survey other risk factors such as family history of IHD, hyperlipidaemia or alcohol consumption. In the Hillingdon epidemiological study, 30% of cases of LVSD did not have a history of IHD, diabetes or hypertension [12].

It could be argued that men would be a "high-risk" subgroup of the general population. Certainly, the AUC of BNP in my study was significantly better in men compared to women (0.98 vs. 0.88). The number of echocardiograms needed to diagnose a single case of LVSD was 3.4 for men compared to59.4 in women, suggesting screening men with BNP would be very cost-effective. However, this analysis could potentially be skewed by the small number of women with LVSD (n=6).

Galasko's recent study [210] estimated the cost of each ECG, N-BNP analysis and echocardiogram at 16.50, 22.50 and 150 € (Euros) respectively. That particular trial explored various strategies to assess the different cost implications for screening the population for LVSD. However, the authors used a commercially available kit for analysis of N-BNP (Roche Biosite) and applied gender specific 95th centile values as the cut-off. Hence, the sensitivity and specificity varied between tests and applying a "cost per case diagnosed" across all the tests makes accurate comparison difficult. However, the authors suggest that pre-screening with either ECG or N-BNP make substantial cost savings, a finding supported by Neilson et al [115], who analysed data from the Glasgow MONICA study. I deliberately maximized the sensitivity of the tests to make appropriate comparisons between screening strategies. The cost of a commercially available assay (Bayer) was also very similar to the Roche kit and I applied the same coast of each assay to the analysis. Using BNP to pre-screen prior to echocardiography achieved a cost saving of just under 30%, maintaining 100% pick-up rate. N-ANP did not reduce costs. However, to pick up 96.2% (i.e. missing just 1 case in 38), using BNP or N-BNP resulted in cost savings of 69.1% and 22.6% respectively. This compares to a saving of 60.1% and 45.6 % using major ECG abnormalities and major and minor ECG abnormalities. However, this strategy resulted in 22.1 and 7.1% cases being undiagnosed. My study suggests that BNP, used as a pre-screening test prior to formal echocardiography, is more cost effective than N-ANP or N-BNP. In addition, it outperforms the ECG both in diagnostic accuracy as well as in cost-effectiveness. Galasko et al. performed a cost-effectiveness analysis for diagnosing LVSD in the community. Both the ECG and N-BNP performed similarly with a sensitivity/specificity of 92/78% and 80/88% respectively. The cost per case of LVSD diagnosed was also similar (1614 vs. 1501 Euros). In my analysis, using BNP alone proved

more cost-effective than applying the ECG and BNP in a sequential manner. This sequential testing was more effective than using either N-BNP or ECG abnormalities alone.

Any screening program would have to agree on an acceptable "false negative and positive" rate and adjust the cut-off accordingly. Naturally, cost-efficiency would be best if the target population had a higher prevalence of LVSD, such as patients with a history of IHD or even limiting screening to men over the age of 65yrs.Ultimately, what determines the cost-effectiveness of the screening test would be the acceptable number of cases of LVSD allowed to go undetected.

Test modality (NP cut off values in pM)	Scans to detect 1 case of LVSD	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	+LR	-LR	Cost per case (€)	% cases missed
Echo alone	48.6	100	100	NA				7285	0
N-ANP (175.79)	44.1	100	9.5	2.3	99.2	1.1	NA	7686	0
BNP (18.84)	27.9	100	44.7	3.7	99.8	1.8	NA	5121	0
N-ANP (966.05)	39.9	96.4	18.5	2.5	99.6	1.18	0.19		
BNP (35.89)	8.6	96.4	85.3	12.3	99.9	6.56	0.04	2244	3.6
N-BNP (26)	29.5	96.4	40.4	3.4	99.8	1.62	0.09	5636	3.6
Major ECG abnormalities	8.7	67.9	85.0	8.8	99.2	4.51	0.38	2886	22.1
Major and Minor ECG abnormalities	19.1	92.9	60.9	4.8	99.8	2.37	0.18	3961	7.1
Sequential – ECG, then BNP	12.3	92.9	74.7	7.3	99.8	3.67	0.10	3250	7.1

Table 8.2. Different performance characteristics of various screening modalities in diagnosing LVSD in my trial. (PPV:Positive predictive vale, NPV:Negative predictive value, LR+: Positive likelihood ratio, LR- :Negative likelihood ratio)

8. 10 Study Limitations

- The cohort involved only Caucasian subjects. The aetiology of LVSD in different ethnic groups has been investigated. The Harrow Heart Failure watch [210] has found that the prevalence of IHD is significantly higher in the non-white population (largely South Asian) with LVSD. This may have implications for a screening program. No data exist regarding the effect of ethnicity on natriuretic peptide levels.

- The study did not formally asses diastolic function. An accurate echocardiographic analysis would have involved further measurements including tissue Doppler studies. Some subjects would have had diastolic dysfunction in the presence of normal systolic function. These are likely to have had higher levels of peptides but, in this study, would have been classed as having "normal" left ventricular function. Because the study aims were to identify cases of systolic dysfunction, it would be correct to confine the primary investigation to LVSD.

- The assay used for BNP was an in-house immunoluminetric assay. Commercial assays have since become widely available and therefore standard in many centers. It would be wrong to assume that the measurements correlate precisely and furthermore, direct comparisons using cut-off values would not be exact.

8.11 Final Conclusions:

- The incidence of previously unidentified cases of LVSD in this unselected sample of the general population was 2.1%. Approximately half did not have symptoms of dyspnoea or reduced exercise tolerance.
- The presence of ECG abnormalities, a previous history of IHD, elevated peptide levels and QRS duration were independent predictors of LVSD. N-ANP, BNP and N-BNP levels were significantly higher in the group with LVSD.
- In normal subjects, gender, age and heart rate were independent predictors of peptide levels. Age and gender specific cut-off values may lead to improved diagnostic utility of cardiac peptides.
- Using ECG or peptides as a pre-screening test prior to echocardiography to detect LVSD was cost effective. However, BNP was superior to the ECG as well as N-ANP and N-BNP. Using BNP as a pre-screening test reduced to overall cost of detecting a single case of LVSD by 30%. Although a logistic model of ECG and peptides improved diagnostic utility, a serial application of ECG and BNP did not result in significant cost saving over that of BNP used alone.
- Screening for previously undetected LVSD in the general population is feasible. Although the 12 lead ECG has significant utility, pre-screening with BNP before formal echocardiography is most cost-effective.

8.12 Suggested future research

1. A large scale, multi-center, prospective screening trial with long-term follow-up would be required to assess the impact of screening for heart failure. Ideally a randomized control trial comparing the effects of screening versus no screening should be performed. Long-term data about cost effectiveness and improvement in quality of life would be required to assess the true value and long-term impact of screening for LVSD. A further question would be the selection of the target group, be it the general population or high-risk groups. Ultimately, the issue should be less of which modality we use to screen and should focus on the actual long-term health impact of such a screening program.

2. A long-term follow-up of my study population would be very illuminating. In particular, I would want to assess the longer term outcome of subjects with high NP levels despite having relative preserved LV function with no other obvious cause of high NP levels.

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