B-TYPE AND N-TERMINAL PRO-B-TYPE NATRIURETIC PEPTIDES IN HEART FAILURE

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ABSTRACT

The thesis evaluated the role of the B-type natriuretic peptides and N-terminal pro-B-type natriuretic peptides in the management of heart failure patients. In a population-based retrospective cohort study of electronic medical records, the thesis examined the patterns of natriuretic peptide testing in primary care practices within the United Kingdom. The thesis showed that single brain natriuretic peptides and N-terminal pro brain natriuretic peptides were independent predictors of all-cause mortality and cardiovascular mortality in the primary care setting. In a small subgroup of patients with two natriuretic peptides measured, changes in brain natriuretic peptides did not provide additional prognostic information beyond a single point-in-time peptide level. Age adversely impacted the mortality especially in elderly. In a clinical trial setting, the thesis also investigated the within-subject variability of B-type natriuretic peptides, vital signs and serum creatinine over a three-month period in stable chronic heart failure patients with New York Heart Association class II-III. Correlations between B-type natriuretic peptides and cardiac structure factors, weight, heart rate, blood pressure and serum creatinine were estimated. The B-type natriuretic peptide within-subject variability was 46%, much higher than the heart rate or blood pressure within-subject variability of 10%, serum creatinine within-subject variability of approximately 8% and weigh within-subject variability of 1%. The three-month monitoring interval of B-type natriuretic peptides in stable chronic heart failure was too short. Extended monitoring intervals should be further investigated. Monitoring natriuretic peptides in primary care provides an opportunity to positively impact the burden of heart failure by better directing management for patients with poorer prognoses and better utilizing health care resources.

Abbreviations

ACE-I	Angiontensin-converting enzyme inhibitor
ARB	Angiotensin-receptor blockers
bmi	Body mass index
BNP	B-type natriuretic peptide
CABG	Coronary artery bypass graft
CHF	Chronic heart failure
COPD	Chronic obstructive pulmonary disease
CPRD	Clinical Practice Research Datalink
CRP	C-reactive protein
CV	Cardiovascular
CV_b	Between-subject coefficient of variation
CV_w	Within-subject coefficient of variation
hf	Heart failure
HFrEF	Heart failure with reduced ejection fraction
HFpEF	Heart failure with preserved ejection fraction
ICD	International Classification of Diseases
IMD	Index of multiple deprivation
IL-6	Interleukin-6
ISAC	Independent Scientific Advisory Committee
IQR	Interquartile range
MAGGIC	Meta-analysis Global Group in Chronic Heart Failure
MHRA	Medicines & Healthcare products Regulatory Agency
Min	Minimum
Max	Maximum
NICE	National Institute for Health and Clinical Excellence
NHS	National Health Service
NT-proBNP	The N-terminal fragment of unprocessed proBNP
NYHA	New York Heart Association
ONS	Office of National Statistics
OXMIS	Oxford Medical Information System
PCI	Percutaneous coronary intervention
PICO	Participants, Interventions, Comparisons, Outcomes
RCV	Reference Change Value
Q1	Lower quartile
Q3	Upper quartile
UK	United Kingdom
USA, US	United States of America

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Chapter 1 - Introduction

The overall aim of the thesis was to investigate the prognostic and monitoring roles of B-type natriuretic peptides in chronic heart failure patients. The overall hypothesis was that the B-type natriuretic peptides are predictors of mortality in primary care and their variability impacts the monitoring time interval when treating chronic heart failure patients. The thesis is directed at primary care physicians, cardiologists, heart failure specialist nurses, as well as health care professionals involved with treating chronic heart failure patients.

B-type natriuretic peptide levels have been used to guide and intensify the treatment for heart failure, however such a strategy produced inconsistent benefits to patients.

Heart failure is a major chronic disorder of global epidemic prevalence and poor prognosis. This chapter is an overview of heart failure. Current algorithms for diagnosing heart failure, as well as recommendations for monitoring heart failure by the most recent clinical guideline for chronic heart failure are described (Ponikowski et al. 2016). The roles of B-type natriuretic peptides in diagnosing and treating heart failure are introduced, followed by the research questions addressed in this thesis. The B-type natriuretic peptides and their utility when titrating the heart failure therapy are described in Section 1.6.

1.1. Definitions of heart failure

Heart failure is a syndrome characterized by symptoms and clinical signs caused by various cardiac abnormalities (Ponikowski et al. 2016, NICE 2010). Typical symptoms include

breathlessness, orthopnoea, paroxysmal nocturnal dyspnoea, reduced exercise tolerance, fatigue, tiredness, and increased time to recover after exercise. Less than typical symptoms include a nocturnal cough, wheezing, bloated feeling, loss of appetite, confusion, depression, palpitations, dizziness, syncope. More specific signs are elevated jugular venous pressure, hepatojugular reflux, third heart sound and laterally displaced apical impulse (Ponikowski et al. 2016, NICE 2010, Braunwald 2013, McDonagh, 2011, Davie et al. 1997, Mant et al. 2009, Qudejans et al. 2011, Fonseca et al. 2006). Less specific signs include weight gain of more than 2 kilograms per week, weight loss in advanced heart failure, tissue wasting, cardiac murmur, peripheral oedema, pulmonary crepitations, reduced air entry, tachycardia, irregular pulse, tachypnoea, Cheyne Stokes respiration, hepatomegaly, ascites, cold extremities, oliguria and narrow pulse pressure (Ponikowski et al. 2016, NICE 2010). The heart still works, but the structural and/or functional cardiac abnormalities lead to a reduced cardiac output and elevated intracardiac pressures (Ponikowski et al. 2016). Heart failure can be described based on the left ventricular ejection fraction (LVEF) into heart failure with preserved (LVEF $\geq$ 50%), mid-range (LVEF: 40%-49%) and reduced ejection fraction (LVEF<40%) (Ponikowski et al. 2016).

For most of its course, the disease has a chronic form that is interrupted from time to time by an acute, insidious stage caused by the decompensation of the heart. Precipitants of acute heart failure include events leading to rapid deterioration such as: arrhythmia, acute coronary syndrome, acute pulmonary embolism, hypertensive crisis, cardiac tamponade, aortic dissection, surgery, peripartum cardiomyopathy, as well as events leading to less rapid deterioration such as: infection, exacerbation of COPD/asthma, anaemia, kidney dysfunction, non-adherence to diet or drug therapy, drug interactions, arrhythmias, uncontrolled hypertension, thyroid problems, alcohol and drug abuse (McMurray John 2012).

Etiologies of heart failure are diseased myocardium, abnormal loading conditions and arrhythmias (Ponikowski et al. 2016, McDonagh 2011). The damage to the myocardium could be caused by ischaemic heart disease, toxic damage due to substance abuse, heavy metals, medications, radiation; immune-mediated and inflammatory damage, that may be related to infection; infiltration, whether or not related to malignancy; hormonal and nutritional metabolic abnormalities, as well as genetic abnormalities (Ponikowski et al. 2016, NICE 2010, Braunwald 2013, (McDonagh 2011). Abnormal loading conditions occur due to hypertension, valve and myocardium structural defects, pericardial and endomyocardial pathologies, high output states and volume overload, which is often the case in renal failure (Ponikowski et al. 2016, McDonagh 2011, Braunwald 2013).

1.2. The epidemiology of heart failure

It is estimated that more than 37.7 million people have heart failure around the world (Vos et al. 2013). The British Heart Foundation estimated that there were 493,000 people living with heart failure in 2014 in the UK (Townsend et al. 2015). In the United States, there were 5.7 million (2.2%) adults with heart failure (Mozaffarian et al. 2016), and the prevalence is expected to increase to 8 million (2.97%) people by 2030 (Heidenreich et al. 2013).

The global heart failure estimated incidence varies from 100 to 900 cases per 100,000 person-years according to how heart failure were defined and the population included in the study (Roger 2013, Ziaeian and Fonarow 2016). In 2012, there were an estimated 915,000 new cases of heart failure in the United States (Mozaffarian et al. 2016).

According to the British Heart Foundation, there are over 25,000 new cases of heart failure in the United Kingdom every year (Townsend et al. 2012). The incidence rates of heart failure increase with age, with the highest rates seen in adults over 75 years old. Overall, among all age groups, the incidence rates of heart failure were about 61% higher for men than women, with the incidence rates being twice as high for men in the age group of 55 to 64 years old. Northern Ireland had the most new cases of heart failure followed closely by Scotland, Wales and England (Townsend et al. 2012). These data describe diagnosed conditions, and cannot be used to estimate the hidden burden of heart failure which comes from an undiagnosed disease.

Additionally, there is a high prevalence of undetected heart failure in the long-term care settings where patients are much older, live with several comorbidities and take multiple medications, suffer a cognitive deficit, immobility and poor access to services. The Heart Failure in Care Homes (HFinCH), a prospective study that followed 399 elderly patients living in 33 long-term UK care facilities for 14 months showed high levels of misdiagnosis, missed diagnosis and that the point prevalence of heart failure was 22.8%, much greater than in community populations. The study participants, mostly females, white British, aged 85 or older were registered with 23 general practices, including 93 primary care physicians (Hancock 2013).

Trends in the incidence and prevalence of heart failure suggest that while the incidence has stayed approximately the same over the past decade, there is a marked increase in prevalence (Roger 2013, Ziaeian and Fonarow 2016, McDonagh 2011). This can be explained by a larger elderly population with an increased life expectancy, technological advances in cardiology, better medication to treat heart failure and improved clinical guidelines.

When describing the epidemiology of heart failure, it is important to realize that different clinical and research definitions of heart failure have been applied over time, in various studies

and settings causing the incidence and prevalence estimates to vary widely. Furthermore, most of the heart failure epidemiology has been obtained from developed countries (Ziaeian and Fonarow 2016, Roger 2013).

Despite the technological advances in the diagnosis and management of heart failure, the mortality due to heart failure remains high. A systematic review (Askoxylakis 2010) consisting of large population-based and hospital-based studies from developed countries reported the 5-year relative survival for heart failure being 62%, much worse than the 5-year relative survival for breast cancer, which ranged from 73% to 89% or for prostate cancer, which ranged from 50% to 99%.

A national heart failure audit for England and Wales (Cleland 2011), developed by the British Society for Heart Failure, the Health Care Commission and the National Health Service reported the overall heart failure mortality during the index admission of 34% and 12% during the index hospitalization, while the predicted 1-year mortality rate was higher than 40%, ranging from 26% in those younger than 75 years to 56% in those over 75 years old. The mortality estimates were based on data from 6170 patients admitted to 86 hospitals from April 2008 to March 2009 with a follow-up period of up to 1 year. Heart failure patients with reduced ejection fraction have higher mortality than those with preserved ejection fraction (Pocock 2013).

With respect to the temporal trends in mortality attributed to heart failure, there has been a 40% reduction in the age-standardized death rates from 1987 to 2008 in seven European countries such as England and Wales, Germany, Greece, Spain, France, Finland and Sweden, while mean age at death increased from 80 to 82. 7 years (Laribi 2012). The calculations were based on information collected from the death certificates, also showing that half of the deaths occurred in hospitals. In a large retrospective primary care cohort, the one-year, five-year, and

10-year survival rates of 80%, 50%, and 28%, respectively did not significantly change between 1998 and 2012.

1.3. The economic burden of heart failure on health care systems

Heart failure is an expensive disease with high rates of hospitalization caused by recurrent acute decompensation events. It is estimated that the annual hospitalization costs due to heart failure amount to £563 million in the UK and this is only a partial cost without accounting for medications, specialist care and primary care (Cleland 2011). In 2014, there were 70 million prescriptions for the treatment of hypertension and heart failure in the United Kingdom (Townsend et al. 2015). Globally, there were an estimated US$108 billion spent on heart failure in 2012, primarily in developed countries (Cook et al. 2014). By 2030, the total medical costs to treat heart failure are expected to increase to $53.1 billion in the United States (Heidenreich et al. 2013).

1.4. Diagnosing heart failure

As per the most recent European clinical guidelines, a diagnosis of heart failure of non-acute onset starts with an assessment of heart failure probability based on clinical history, physical examination and an electrocardiogram (Ponikowski et al. 2016). If everything is normal, then heart failure is unlikely. If the patient has at least one of the following: a history of coronary artery disease or arterial hypertension, exposure to cardiotoxic drugs or radiation, difficulty breathing, an abnormal ECG or presents any of the following signs during the physical examination such as: rales, bilateral ankle oedema, heart murmur, jugular venous dilatation, or

laterally displaced apical beat then an echocardiogram is recommended (Ponikowski et al. 2016). However, if prior to echocardiogram, the natriuretic peptides were measured and the levels were less than 35 pg/ml for B-type natriuretic peptides and lower than 125 pg/ml for N-terminal proBNP fragment then the heart failure could be ruled out (Ponikowski et al. 2016).

Diagnosing heart failure with preserved ejection fraction is more challenging due to the normal ejection fraction. Nevertheless, any of the aforementioned symptoms or signs, elevated natriuretic peptides (BNP > 35 pg/ml, NT-proBNP>125 pg/ml) or abnormal functional or structural cardiac parameters such as deceleration time of the mitral valve flow, early versus late mitral valve diastolic inflow, isovolumetric relaxation time are indicative of heart failure (Ponikowski et al. 2016). Heart failure with preserved ejection fraction occurs more often in older women with a history of hypertension, however other comorbidities are: atrial fibrillation, kidney dysfunction, metabolic syndrome, obesity, pulmonary disease and sleep apnea (Ponikowski et al. 2016, Ziaeian and Fonarow 2016, Andersson and Vasan 2014).

The most recent clinical guidelines for heart failure in the United Kingdom (NICE 2010) were issued in 2010, and an updated version is expected this year.

1.5. Treating and monitoring heart failure

Managing heart failure patients is challenging. A timely diagnosis and an accurate determination of the cause and length of heart failure are essential for determining prognosis, developing an optimal treatment plan, and effective monitoring strategies.

Various evidence-based pharmacological treatment options are available for heart failure patients with reduced ejection fraction such as angiotensin-converting enzyme inhibitors, beta-

blockers, mineralocorticoid receptor antagonists, angiotensin receptor blocker, and lately an angiotensin receptor neprilysin inhibitor in patients who cannot tolerate ACE inhibitors or ARBs. Major clinical trials such as CONSENSUS and SOLVD-TREATMENT- Enalapril, ATLAS-Lisinopril, COPERNICUS – Carvedilol, CIBIS-II – Bisoprolol, MERIT-HF – Metoprolol, SENIORS – Nebivolol, RAES – Spironolactone, EMPHASIS-HF – Eplerenone, PARADIGM-HF – Sacubritil/Valsartan, SHIFT – Ivabradine, CHARM-Added and CHARM-Alternative – Candesartan, Val-HeFT – Valsartan showed improvement in all-cause mortality, combined cardiovascular mortality and reduced cardiovascular hospitalizations (CONSENSUS 1987, Glick et al. 1995, Packer et al. 1999, CIBIS-II 1999, Merit-HF 1999, Flather et al. 2005, Pitt et al. 1999, Zannad et al. 2011, McMurray et al. 2013, Swedberg et al. 2010, McMurray et al. 2003, Granger et al. 2003, Cohn and Tognoni 2001).

For each of the evidence-based disease-modifying heart failure drugs, the 2016 ESC clinical guidelines include recommendations on how to pharmacologically treat patients with symptomatic heart failure with reduced ejection fraction (Ponikowski et al. 2016). For each class of heart failure drugs, detailed clinical recommendations are given on the starting dose, target dose, titration steps, time of titration steps, when to stop the particular medicine, how to combine medication, how to evaluate the renal function, electrolytes, and blood chemistry in the context of dosing (Ponikowski et al. 2016). Clinical guideline recommendations are also given on how to address worsening symptoms or signs, low heart rates, low blood pressure, volume overload or volume depletion in the context of dosing, as well as advice to patients (Ponikowski et al. 2016).

Despite the numerous pharmacotherapy options available for heart failure with reduced ejection fraction, they do not work in heart failure patients with preserved ejection fraction due to differing pathophysiology and etiology (Nabeebaccus 2016). Current options in heart failure

patients with preserved ejection fraction include only symptomatic relief and treatment of the existing comorbidities.

The goals of the treatment are different for chronic versus acute heart failure patients. For example, during the acute stages of heart failure, the focus is on: treating symptoms, restoring oxygenation, improving hemodynamics and organ perfusion, limiting cardiac and renal damage, preventing thrombo-embolism, minimizing the length of stay in the intensive care unit, stabilizing patient and optimizing treatment strategy, initiating and up-titrating disease-modifying pharmacological therapy, considering device therapy in severe patients (McMurray John 2012).

Monitoring chronic heart failure patients is essential in order to prevent acute episodes, adverse events, and worse prognosis. The National Institute for Health and Clinical Excellence guidelines (NICE 2010) for chronic heart failure recommend a multidisciplinary team approach to the heart failure management after discharge that involves the primary care physician, carers, local cardiac support networks, home telemonitoring, and referral to cardiologist for the most severe cases or for heart failure cases that do not respond to treatment or cannot longer be managed effectively in the home setting with the assistance of nurses. A recent Cochrane review showed that heart failure patients benefitted from non-invasive remote telemonitoring such as structured telephone support by having reduced mortality and hospitalizations (Inglis, Clark, and Dierckx 2015, Dierckx 2017, Inglis et al. 2017).

1.6. The current role of B-type natriuretic peptides and NT-proBNP in heart failure

B-type natriuretic peptide (BNP) and N-terminal fragment (NT-proBNP) are cardiac biomarkers of myocardial stretch (Lin et al. 2014, Levin, Gardner, and Samson 1998). They are primarily secreted from the ventricles into the circulation in response to volume overload, changes in pressure, wall stress, and ventricular stretching that occur when heart failure develops and worsens (Braunwald 2008). In heart failure patients, the heart has to work harder in an attempt to meet the body needs. Compensatory mechanisms such as cardiorenal hemodynamic and neurohormonal are activated. However, they ultimately lead to a progressive deterioration of the heart due to increasing remodeling and overall work of the heart. (Levin, Gardner, and Samson 1998). BNP and NT-proBNP also have diuretic effects by increasing the glomerular filtration rate, inhibiting the sodium re-absorption by the kidneys, thus allowing for more water and sodium to be eliminated from the body (Braunwald 2008, 2013, Richards, Januzzi, and Troughton 2014). The blood concentrations of these peptides are raised in heart failure patients, with higher levels reflecting higher disease severity and worse symptoms. However, their levels decrease when the heart failure condition is stable (Ponikowski et al. 2016).

BNP is a 32-amino acid biologically active molecule with a half-life of 20 minutes. NT-proBNP is a 76-amino acid biologically inactive molecule with a half-life of 120 minutes and is excreted by the kidneys, which explains why the NT-proBNP values are higher than BNP levels (Braunwald 2008). BNP degrades in the kidneys, liver and vascular endothelium (Nakano et al. 2016, Martinez-Rumayor et al. 2008). There are no conversion factors between BNP and NT-proBNP. Furthermore, there is variability in the type of assays used and availability of testing across the country (Saenger 2017).

BNP and NT-proBNP can potentially aid in diagnosing, treating and monitoring heart failure patients. The most recent 2016 ESC heart failure guidelines recommend using BNP and NT-proBNP part of the diagnosis process to rule out heart failure when BNP< 35 pg/ml or NT-proBNP < 125 pg/ml (Ponikowski et al. 2016). The diagnostic cut-off values may depend on age, gender, existing comorbidities such as renal disease and diabetes, previous exposure to diuretics and ACE inhibitors, therefore BNP and NT-proBNP should be interpreted by assessing the totality of patient's demographics, available clinical and imaging information. An arbitrary cut-off value means little without the clinical context of the patient (Miller and Jaffe 2016).

A recent Cochrane review showed uncertainty in BNP and NT-proBNP guided treatment in heart failure patients concerning all-cause mortality, heart failure mortality, improved quality of life, reduction in heart failure admission and its relation to changes in medication (McLellan et al. 2016). The results of the GUIDE-IT trial showed that the NT-pro-B-type-guided therapy did not improve time to the first heart failure hospitalization or cardiovascular mortality, nor all-cause mortality in heart failure, high-risk patients with a reduced ejection fraction, when compared to usual care (Felker et al. 2017).

The monitoring values of BNP and NT-proBNP are under scientific investigation. The most recent ESC guidelines identify remote monitoring strategies in heart failure as an area that needs further research (Ponikowski et al. 2016).

1.7. Research questions addressed in the thesis

My thesis investigates the role of natriuretic peptides in managing chronic heart failure patients in the primary care setting. The thesis is not about diagnosing heart failure.

In the United Kingdom, primary care physicians are often the first to investigate, diagnose and treat heart failure patients. To date, little is known about the patterns of natriuretic peptide testing in chronic heart failure patients managed by primary care practices throughout the United Kingdom. In England, the estimated annual rate of natriuretic peptide tests ordered by the general practitioners, used mainly for diagnostic purposes ranged from 0.05 to 14.4 per 1000 practice population, a 297-fold variation (NHS 2013). The thesis investigates whether there is any variation in the BNP/NT-proBNP testing in the primary care setting across the United Kingdom among heart failure patients. It also identifies patient-level and practice-level predictors of the likelihood of natriuretic peptide serial testing. The time interval between repeat natriuretic peptide measurements is also estimated for various regions in order to provide additional information on the testing landscape from a population perspective.

Regarding the prognostic value of these peptides, most of the past research involved heart failure patients that were either hospitalized or candidates for transplants and cardiac surgery. Community studies conducted often included people who were not necessarily diagnosed with heart failure. This thesis provides a unique prognostic view of the natriuretic peptides in heart failure patients from a primary care perspective and seeks to present evidence on whether the NT-proBNP and BNP levels are associated with all-cause mortality in the UK primary care chronic heart failure population, in addition to investigating the association to mortality due to cardiovascular diseases.

When monitoring the natriuretic peptides over time, it is important to understand what degree of biomarker change represents clinical and statistical significance. This interplays with the biologic variation of these peptides, greatly affecting the proper interpretation of serial test results by primary care physicians, cardiologists, and specialized nurses. Compared to BNP, NT-proBNP

may be a more sensitive marker for detecting statistically significant changes in serial determinations due to NT-proBNP's longer half-life, clearance by the kidney, and absence of neuropeptides that metabolize BNP, but not NT-proBNP (Wu 2006). However, most of the small size studies were conducted in healthy volunteers rather than heart failure patients and also without considering the clinical contexts and confounding factors affecting natriuretic peptides such as age, gender, and renal function. Given the limited number of studies addressing the biological variability of natriuretic peptides, my thesis provides further insight by determining the variability of NT-proBNP at week 1, week 6 and week 12 in stable heart failure patients with New York Heart Association of class II and III taking placebo. This will directly impact the interpretation of serial natriuretic peptide values so that the clinical resources are effectively used when they are really needed. Another important aspect addressed in this thesis is the comparison of the biologic variability of the natriuretic peptides to other key markers recommended to be monitored such as blood pressure, heart rate, weight, and serum creatinine (Bashi et al. 2017). This will help to identify key clinical parameters with a low biological variability that do not hinder the interpretation of serial test results when monitoring a chronic heart failure patient's response to pharmacotherapy or in the assessment of patient's clinical status, especially considering important risk factors.

Current medical guidelines stress the importance of monitoring weight in heart failure patients, which could be regarded as a marker of fluid retention or accumulation in the body, indicating the further clinical deterioration of the heart. Body weight increases before the cardiac filling pressures arise, preceding hospitalization due to an acute stage of the disease caused either by an acute decompensated cardiac failure described by a deterioration of cardiac performance during a period of days, even weeks prior to the acute event or by an acute vascular failure,

characterized by acute hypertension and increased vascular stiffness (Cotter et al. 2008). Although some (Metra, Cas, and Bristow 2008) may perceive body weight as an insensitive index of fluid accumulation due to new implanted devices monitoring intra-thoracic impedance and pulmonary artery pressure to detect lung fluid accumulation, monitoring natriuretic peptides and body weight in a home-based setting may be effective in preventing heart failure hospitalization due to an acute heart decompensation. Using clinical trial data, my thesis explores the correlations between natriuretic peptides and body weight, blood pressure, heart rate, and serum creatinine for up to three months following the critical period after the hospital discharge due to a cardiovascular event. Understanding these correlations could aid the personalized management of the heart failure patients so that hospital readmissions can be prevented and clinical outcomes improved.

The primary research questions addressed in this thesis are listed in Table 1.1:

Table 1. 1 - Research questions addressed in the thesis

Chapter	Research questions
3	What are the predictors of the likelihood of repeat BNP/NT-proBNP testing in chronic heart failure patients attending primary care practices?
4	Are NT-proBNP levels associated with the all-cause mortality in heart failure patients managed in routine clinical practice? Are NTproBNP levels associated with cardiovascular mortality? Are BNP levels associated with all-cause mortality and cardiovascular mortality in heart failure patients managed in routine clinical practice?
5	Are the changes in BNP and NT-proBNP associated with the all-cause mortality and cardiovascular mortality in heart failure patients managed in the primary care setting?
6	What is the within-subject short-term variability of BNP in stable heart failure subjects (NYHA class II - III)? What is the impact of age, gender, body mass index, heart rate, systolic and diastolic pressure, renal dysfunction, left ventricular ejection fraction, cardiac output, left ventricular left end-diastolic and end-systolic volumes on the BNP variability? What is the correlation between BNP and cardiac structure factors, weight, heart rate, blood pressure, serum creatinine?

Chapter 1 of this thesis introduces the burden of heart failure on patients and healthcare systems, describes the epidemiology of heart failure, current medical and cardiovascular guidelines for treating it and the promising role of natriuretic peptides in managing chronic heart failure patients. The questions of research are presented and the organization of the thesis is described.

Chapter 2 is a Literature Review that summarizes existing systematic reviews and meta analysis for B-type natriuretic peptides with respect to their role in prognosis and monitoring heart failure.

Chapter 3 investigates patient-level and practice-level predictors of the likelihood of repeat natriuretic peptide testing over time in heart failure patients within the United Kingdom.

By taking a population approach, Chapter 4 examines the prognostic utility of natriuretic peptides in primary care setting by determining whether NT-proBNP and BNP levels are associated with all-cause and cardiovascular mortality in heart failure patients managed by general practitioners in the United Kingdom. The statistical analyses presented are both unadjusted, as well as adjusted for important risk factors and clinical characteristics such as age, gender, body mass index, smoking status, including diabetes, hypertension, renal abnormalities and clinical pharmacology treatment.

Chapter 5 examines the associations between changes in NT-proBNP and BNP and all-cause, mortality and cardiovascular mortality in a subset of patients who had repeat natriuretic peptides tests.

Based on a clinical trial data, Chapter 6 estimates the within-subject variability of BNP, blood pressure, heart rate, serum creatinine over a three-month period in stable chronic heart failure patients as well as their serial correlation. It also investigates the impact of age, gender,

body mass index, renal function and cardiac factors on the BNP variability. The cardiac structure factors include: left ventricular ejection fraction, cardiac efficiency indices such as cardiac output and cardiac index, left ventricular end-diastolic and end-systolic volumes.

Chapter 7 represents the conclusion of the thesis, reviewing the most interesting findings in my results and elaborating on the main implications of my research in managing chronic heart failure patients.

Chapter 2 – Literature Review

2.1. Introduction

Previous studies have mainly looked at the prognostic value of natriuretic peptides in more severe patient populations such as hospitalized patients or in need of cardiac surgery. There are also numerous studies about the screening and diagnostic value of these peptides, however much less information is available in already diagnosed chronic heart failure patients. The clinical settings often vary, as some heart failure patients are managed in a hospital setting during an acute event of heart decompensation or are treated by cardiologists, specialist heart failure nurses, primary care or ambulatory setting.

Changes in BNP and NT-proBNP in chronic heart failure could inform treatment options and carry prognostic information. The prognostic utility of changes in BNP or NT-proBNP over time when predicting all-cause mortality or cardiac mortality is not known. The chronic heart failure population is heterogeneous, and this challenges the evaluation of serial B-type and N-terminal pro-B-type natriuretic peptide measurements and their changes over time. There is still an ongoing controversy about the real benefit of repeatedly measuring BNP or NT-proBNP over varying time intervals and how this information contributes to the management of chronic heart failure patients. The optimal time intervals, frequency of testing in various categories of chronic heart failure patients defined by clinical characteristics and stage in the disease progression remains to be determined.

A literature search of systematic reviews in chronic heart failure patients was conducted. The objective of this literature search was to summarize the existing systematic reviews with respect to the prognostic, monitoring and guiding treatment value of the natriuretic peptides (BNP and NT-proBNP) in chronic heart failure patients. The search was not about diagnosing heart failure.

2.2. Methods

2.2.1. Types of studies

Systematic reviews and meta-analysis were included in this summary of systematic reviews.

2.2.2. Types of participants

The participants were chronic heart failure adults.

2.2.3. Types of interventions

The focus of the literature review was the utility of BNP and NT-proBNP in the prognosis, management and monitoring of heart failure patients. The following interventions were considered:

- BNP
- NT-proBNP
- Natriuretic peptide guided treatment (BNP or NT-proBNP)

2.2.4. Types of comparators

No restrictions on the comparators were imposed. All comparators were considered, as well as non-comparators.

In the context of using the natriuretic peptides for guiding the treatment of chronic heart failure, common comparators include usual care and other treatments guided by symptoms, guidelines and clinical experience.

2.2.5. Types of outcome measures

The following types of outcomes were considered:

- All-cause mortality
- Cardiovascular mortality
- Changes in BNP or NT-proBNP
- Variability of BNP or NT-proBNP

There were no restrictions on the duration of follow-up.

2.2.6. Data Sources and Searches

I searched PUBMED, EMBASE, the Cochrane library of Cochrane Database of Systematic Reviews, Cochrane Central Register of Controlled Trials, Cochrane Methodology Register, Database of Abstracts of Reviews of Effects, Health Technology Assessment Database, and NHS Economic Evaluation Database until July 1st, 2018. Combinations of the following search terms were considered: “natriuretic peptide”, “brain natriuretic peptide”, “N-terminal pro B type natriuretic peptide”, “BNP” or “NT proBNP”, “B-type natriuretic peptide”, “heart failure”, “chronic heart failure”, “systematic review”, “meta-analysis”, “prognosis”, “management”, “monitoring”, “changes”, “variability”. The exact search terms and corresponding results can be found in the Appendix A.

2.2.7. Data extraction and quality assessments

The following data was extracted from the eligible studies: number of the randomized clinical trials included in the systematic review, patient characteristics, follow-up duration, clinical setting, size of the systematic review regarding the number of patients included, type of B-type natriuretic peptide and outcomes of interest. The data extracted for each systematic review was tabulated in a descriptive table.

The methodological quality of the systematic reviews was assessed using the framework recommended by the British Medical Journal (e.g., http://bestpractice.bmj.com/info/us/toolkit/learn-ebm/appraising-systematic-reviews/). This tool evaluates the focus of the research questions, reproducibility of results, the inclusion and exclusion criteria of the studies, combination of the randomized clinical trials, reporting, including any variation or heterogeneity between individual studies, as well as the clinical importance of the results.

Given the context of the thesis, there was no second, independent reviewer to select, extract, or assess the methodological information reported in each of the systematic review.

2.3. Results

Upon reviewing 236 records based on titles and abstracts and eliminating duplicates, twenty full-text articles were reviewed for the eligibility. Ten full-text articles were excluded for the following reasons: general, narrative review (Oremus 2014), diagnosis of heart failure (Schaub 2015), mortality estimates reported for composite or combined outcomes or other outcomes or comparisons (e.g., Aimo 2018, Zhong 2017, De Vecchis 2014, Balion 2014), other interventions

(e.g., Gamino-Arroyo 2017, Zhang 2016, Balion 2008), and insufficient information reported such as an estimate of any outcomes of interest (e.g., Balion 2014).

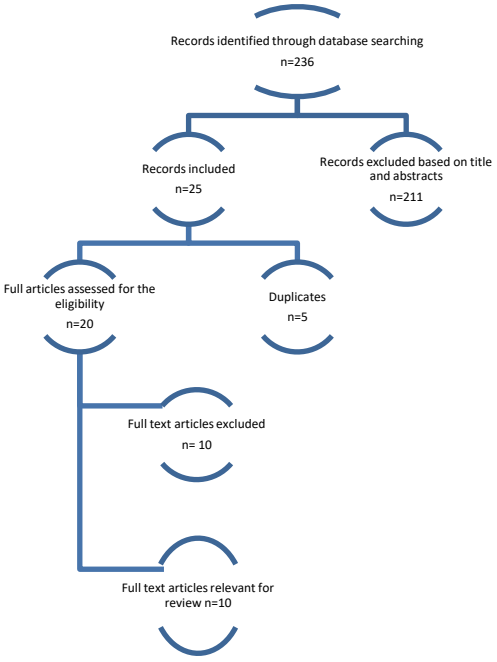
There were 10 systematic reviews that met the inclusion criteria. One review, which was an HTA evidence report investigated the prognostic value of BNP and NTproBNP. Nine systematic reviews (e.g., 7 scientific articles, 1 Cochrane review, 1 HTA evidence report) addressed the monitoring value of the natriuretic peptide when guiding the heart failure treatment. Limited information was found about changes in natriuretic peptides. There was no systematic review that evaluated the variability of BNP or NTproBNP. The search results are presented in the following table.

Table 2. 1 - Broad objectives of systematic reviews

Broad objective of the systematic review	***# of Systematic reviews (n)***	***Systematic reviews by the first author***
Prognostic value of BNP or NT-proBNP	1	Balion 2006
Natriuretic peptide-guided heart failure treatment	9	Khan et al. 2018, Pufulete et al. 2017, McLellan et al. 2016, Xin et al. 2015, Troughton et al. 2014, Li et al. 2013, Savarese et al. 2013, Porapakkham et al. 2010, Felker et al. 2009
Changes in BNP or NT-proBNP	1	Xin et al. 2015
Variability of BNP or NT-proBNP	0	None

The flow chart of databases searches is presented in the figure below.

Figure 2. 1- Flow chart of database searches



2.3.1. Systematic reviews investigating the prognostic value of BNP and NTproBNP

There was one systematic review that investigated the prognostic value of BNP and NT-proBNP (Balion et al. 2006). There was no meta-analysis conducted mainly due to the high heterogeneity of the studies included. Instead, Balion et al. summarized the all-cause mortality and cardiovascular mortality estimates found in 21 BNP studies and 18 NT-proBNP studies. Six studies even reported both BNP and NT-proBNP. The baseline prognostic value of BNP and NT-proBNP was evaluated in prospective cohort studies and clinical trials. The patient population was extremely heterogeneous. Patients were recruited from hospitals, emergency departments, outpatient clinics, primary care, and general populations. The duration of the follow-up ranged from 30 days to 92 months for BNP and 6 months to 92 months for NT-proBNP. The median study size of the studies was 102 (range: 33-4,300) for BNP and 121 for NT-proBNP (range: 48

– 2,320). The average age was around 65 years old. The study populations included a NYHA I-IV and a LVSD lower than 50%, when reported. Several studies did not report any mortality measures, only the number of deaths. Among those who reported them, the mortality estimates varied greatly, had wide confidence intervals, making them difficult to extrapolate. For example, for BNP, the hazard ratios for the all-cause mortality varied from 1 to 4.5 and were as high as 7.2 for the cardiovascular mortality (van der Meer et al. 2004, Kyuma et al. 2004). For NT-proBNP, the unadjusted odds ratio for all-cause mortality was 5 in a study (Gardner et al. 2003), while Hartman et al reported a risk ratio of 1 for the same outcome (Hartman et al. 2015). No study reported an estimate for the cardiovascular mortality for NT-proBNP. Several studies included the systematic review concluded that BNP and NT-proBNP were independent predictors of mortality. However, the systematic review by Balion et al. did not combine any mortality estimates (Balion et al. 2006). Therefore, it is difficult to understand the extent of the prognostic value for BNP and NT-proBNP in a specific clinical setting for chronic heart failure patients, such as primary care.

2.3.2. Systematic reviews investigating the role of BNP and NT-proBNP in guiding the heart failure therapy

There were 9 systematic reviews that investigated the natriuretic peptide-guided treatment and were published between 2009 and 2018. All of them consisted of randomized controlled trials, ranging from 6 to 15 trials per systematic review, with the more recent ones including more patients.

There was a lot of overlap among the studies included in the differing systematic reviews. The following randomized clinical trials were included in all systematic reviews: BATTLESCARRED (Lainchbury et al. 2009), PRIMA (Eurlings et al. 2010), TIME-CHF (Pfisterer et al. 2009), STARS-BNP (Jourdain et al. 2007) and CHRISTCHURCH Pilot (Troughton et al.2000). Randomized clinical trials such as: NorthStar (Schou et al. 2013), UPSTEP (Karlström et al. 2011), SIGNAL-HF (Persson et al. 2010), STARBRITE (Shah et al. 2011) and Berger et al (Berger et al. 2010) were included in all subsequent systematic reviews, once they had been published.

There were some isolated studies included in some of the reviews. For example, the study by Estenban et al. was included only in the systematic review by Porapakkham (Esteban et al. 2006, Porapakkham et al. 2010). The studies by Skvortsov, Li and Krupicka were included only in the systematic review by McLellan et al (Koshkina et al. 2015, Li et al. 2013, McLellan et al. 2016. The most recent systematic review by Khan included data from 18 clinical trials from patients treated for acute and chronic heart failure (Khan et al. 2018). However, in the current chapter only data related to chronic heart failure were included. Therefore, the estimates all-cause estimates for all-cause mortality were based for 14 trials rather than 18. The most recent study included in this review was a large clinical trial in 894 high-risk heart failure patients. This study also demonstrated that guiding the heart failure therapy based on natriuretic peptide levels does not improve the all-cause mortality or cardiovascular mortality. The studies included in each of the systematic reviews are specified in Table 2.2.

Table 2. 2 - Studies included in the systematic reviews

Studies included in the systematic reviews			Systematic Reviews by the first author								
Year	First Author	RCT acronym	Khan 2018 N=14	Pufulete 2017 N=13	McLellan 2016 N=15	Xin 2015 N=14	Troughton 2014 N=10	Li 2013 N=11	Savarese 2013 N=12	Porapakkham 2010 N=8	Felker 2009 N=6
2017	Felker et al.	GUIDE-IT	Yes	No	N/A	N/A	N/A	N/A	N/A	N/A	N/A
2015	Li et al.	-	No	No	Yes	N/A	N/A	N/A	N/A	N/A	N/A
2015	Skvortov et al	-	No	No	Yes	N/A	N/A	N/A	N/A	N/A	N/A
2013	Maeder et al.	TIME-CHF: HFpEF	Yes	Yes	Yes	Yes	No	No	No	N/A	N/A
2013	Schou et al.	NORTHSTAR	Yes	Yes	Yes	Yes	No	No	No	N/A	N/A
2012	Shochat et al	-	No	Yes	Yes	No	No	No	No	N/A	N/A
2011	Gaggin et al.	-	Yes	No	No	No	No	No	No	N/A	N/A
2011	Januzzi et al.	PROTECT	No	Yes	Yes	Yes	Yes	Yes	Yes	N/A	N/A
2011	Karlstrom et	UPSTEP	Yes	Yes	Yes	Yes	Yes	Yes	Yes	N/A	N/A
2011	Shah et al.	STARBRITE	No	Yes	Yes	Yes	Yes	Yes	Yes	N/A	Yes
2010	Anguita et al.	-	Yes	Yes	Yes	Yes	Yes	No	Yes	N/A	N/A
2010	Berger et al.	-	Yes	Yes	Yes	Yes	Yes	Yes	Yes	N/A	N/A
2010	Eurlings et al.	PRIMA	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
2010	Krupicka et al	-	No	No	Yes	No	No	No	No	N/A	N/A
2010	Lainchbury	BATTLESCARRED	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
2010	Persson et al.	SIGNAL-HF	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	N/A
2009	Pfiesterer et al	TIME-CHF: HFrEF	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
2007	Jourdain et al.	STARS-BNP	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
2006	Esteban et al.	-	No	No	No	No	No	No	No	Yes	No
2005	Beck-da-Silva	-	Yes	No	Yes	Yes	No	Yes	Yes	Yes	No
2000	Troughton et al.	CHRISCHURCH Pilot	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

N/A=Not Applicable means that the results of the study were not published in a medical journal at a time

The clinical setting was often not reported in the systematic reviews. Khan et al. included patients evaluated by heart failure specialists. Pufulete et al. examined patients seen at heart failure clinics. McLellan et al. focused on the in-hospital and out-of-hospital settings, while Porapakkham et al. evaluated outpatients (McLellan 2016, Porapakkham 2010).

Characterizing patients was challenging because most of the reviews combined aggregate data. More recent reviews started to incorporate more individual patient data, but even so the average clinical characteristics could not be summarized for the entire systematic review, only for the subset of studies that provided individual patient data. However, ranges for the mean values from individual studies, were presented, when reported in the systematic review.

The patients' characteristics described in the systematic reviews reflect the heterogeneity of heart failure. Most of the patients were males. Some reviews reported the mean age of the participants, while others enumerated the age in each of the studies included. For example, McLellan et al. reported the mean age of the participants as between 57 and 80 years old, while Khan et al., Li et al., Savarese et al. focused on older patients with a mean age above 70 years old. When reported, the heart failure was due to the left ventricular systolic dysfunction and was described by a low ventricular ejection fraction.

The left ventricular ejection fraction was not reported in the most recent systematic review by Khan, but the ejection fraction was ≤ 40% in many of the studies included, such as GUIDE-IT, Beck-da-Silva et al, Berger et al., PROTECT, STARBRITE, UPSTEP. The left ventricular was less than 50% in SIGNAL-HF and ≤ 45% in STARS-BNP.

The patients' characteristics are presented in the following table:

Table 2. 3 - Summary of systematic reviews of RCTs in heart failure patients with mortality outcomes

Year	Author	No of trials	Setting	Patients' characteristics	Size	Follow-up duration	Type of peptide	Comparator group	Mortality Estimate (95% CI)
2018	Khan et al.	14	HF specialists	Chronic heart failure, NYHA II-IV Median age=72 yrs	3,859	Mean follow up was 16 months	BNP or NTproBNP	Guideline-guided	All-cause mortality RR=0.92 [0.81, 1.04] CV mortality RR=0.90 [0.72, 1.12]
2017	Pufulete et al. Evidence report	13	HF clinics	Heart failure;	3,074	1 – 24 months; some NR	BNP or NTproBNP	Symptom-guided therapy	All-cause mortality HR=0.87 (0.73, 1.04)
2016	McLellan et al. Cochrane Review	15 6	In-hospital and out-of-hospital	Adults treated for heart failure; Mean age: 57 to 80 years;	3,169 853	3 – 54 months 6-24 mo	BNP or NTproBNP	No BNP nor NTproBNP/ usual care	All-cause mortality RR=0.87 (0.76, 1.01) HF mortality RR=0.84 (0.54, 1.30)
2015	Xin et al.	14	NR	Chronic heart failure; NYHA I-IV;Mean age:61-80	3,004	3 – 36 months	BNP or NTproBNP	Clinical group	All-cause mortality RR=0.83 [0.81, 1.08]
2014	Troughton et al.	10	NR	HF due to LVSD mostly; Mean age= 72 yrs; 2/3 males	2,431	3 – 24 months	BNP or NTproBNP	Clinically guided	Overall mortality HR=0.82 (0.67, 1)
2013	Li et al.	11	NR	Chronic heart failure; Mean age= 70 yrs;63.7% men	2,414	3 -36 months	BNP or NTproBNP	Guideline-guided drug therapy	All-cause mortality RR=0.83 (0.69, 0.99)
2013	Savarese et al.	12	NR	Chronic heart failure due to systolic dysfunction; Mean age=70; 67% men	2,686	Mean follow-up=1.2 ± 0.7 years	BNP or NTproBNP BNP: (n=5) NTproBNP (n=7)	Clinically guided/ usual care	All-cause mortality OR=0.74 (0.60, 0.91) OR_{BNP}= 0.81 (0.52, 1.28) $OR_{NTproBNP}$=0.72(0.56,0.91
2010	Porapakkham	8	Outpatient	Chronic hf,NYHA II-IV, LVEF<50%, mostly men	1,726	Mean=17 months	BNP or NTproBNP	Usual care	All-cause mortality RR=0.76 (0.63-0.91)
2009	Felker	6	NR	Chronic heart failure, mostly men, Mean age 61-77yrs, LVEF < 37%	1,627	3 – 18 months	BNP or NTproBNP	Usual care	All-cause mortality HR=0.69 (0.55, 0.86)

The duration of follow up varied by systematic review and studies included. It ranged from 1 month to 54 months. All systematic reviews considered both BNP and NT proBNP in the B-type natriuretic peptide guided treatment of heart failure. The comparators were usual care, symptom-guided, or clinically-guided or guideline-guided groups.

All systematic reviews reported estimates for all-cause mortality using various measures of associations such as odds ratios, hazard ratios and risk ratios. Khan et al. also reported the cardiovascular mortality, while McLellan et al. estimated the heart failure mortality too.

Quality Assessment

The quality criteria recommended by the British Medical Journal for assessing systematic reviews were met most of the time. All systematic reviews performed a comprehensive and reproducible literature search with clear focused questions and eligibility criteria while appropriately assessing, reporting and combining data from primary randomized controlled trials. While Khan et al. and Troughton et al. assessed the quality of the randomized clinical trials, it was not clear how this information was accounted for in the analyses (Khan 2018, Troughton 2014). Khan et al. concluded that the heart failure treatment guided by the B-type natriuretic peptide did not improve the all-cause mortality compared to the guideline-guided group, but it did not elaborate on the clinical relevance of such results. Additionally, each systematic review assessed the quality of the randomized clinical trials included in the specific review based on randomization, risk of bias, precision, consistency. The systematic review quality criteria recommended by the British Medical Journal are shown in the following table.

Table 2. 4 - Assessing the quality of systematic reviews

Author	Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8	Q9	Q10
Khan et al.	Yes	Yes	Yes	Yes	Can't tell	Yes	Yes	Yes	Yes	Can't tell
Pufulete et al.	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
McLellan et al.	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Xin et al.	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Troughton et al.	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes
Li et al.	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Savarese et al.	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Porapakkham et al	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Felker et al.	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

Q1 - Does the SR explicitly report and perform a comprehensive and reproducible literature search?
Q2 - Does the SR formulate a clearly focused question?
Q3 - Does the SR's methods section explicitly state the basis for inclusion or exclusion of primary RCTs?
Q4 - Does the SR report data from primary RCTs (e.g. size, interventions used, results from individual RCTs)
Q5 – Does the SR assess the methodological quality of primary studies, and take these into account where necessary?
Q6 – Meta-analysis: does the SR combine primary studies appropriately?
Q7 – Meta-analysis: does the SR state how results are combined statistically?
Q8 – Meta-analysis: does the SR report absolute numbers as well as appropriate statistics?
Q9 – Does the SR discuss the reason for any variations/heterogeneity between individual RCTs/overall results?
Q10 – Does the SR report on the clinical relevance/importance of the results?
Reference: http://bestpractice.bmj.com/info/us/toolkit/learn-ebm/appraising-systematic-reviews/

All-cause mortality

All systematic reviews reported estimates for all-cause mortality using various measures of associations such as odds ratios, hazard ratios and risk ratios.

The systematic reviews by Khan et al., Pufulete et al., McLellan et al., Xin et al., and Troughton et al., all concluded that the heart failure treatment guided by BNP or NTproBNP does not significantly reduce the all-cause mortality (Khan et al. 2018, Pufulete et al. 2017, McLellan et al. 2016, Xin et al. 2015, Troughton et al. 2014]. These systematic reviews were published between 2014 and 2018.

The systematic reviews from 2009 to 2013 by Felker et al., Porapakkham et al., Savarese et al., Li et al. showed that increasing the dose based on serial BNP or NT-proBNP values was significantly associated with a reduced all-cause mortality.

There was only one review by Savarese et al. that separately analyzed BNP and NT proBNP with respect to the all-cause mortality (Savarese et al. 2013]). While the heart failure treatment guided by BNP or NT proBNP significantly reduced the all-cause mortality, the separate analyses indicated that it was the NT proBNP – guided treatment and not the BNP-guided treatment that reduced the all-cause mortality when compared to usual care. Possible explanations include better properties of NT-proBNP such as higher concentrations in blood, longer half-life, and reduced in vitro degradation (Steiner et al. 2008). Patients who followed the BNP-guided treatment had lower ejection fractions, suggesting a more severe heart failure population with possibly smaller room for increasing the dosage. Additionally, there were fewer patients in the BNP trials than NT-proBNP trials, and no single study performed a head-to-head comparison of BNP versus NT proBNP. The all-cause mortality estimates resulted from combing the data from various randomized controlled trials (Savarese et al. 2013).

Cardiovascular mortality

There was only one systematic review by Khan et al. that reported the cardiovascular mortality based on six trials in 2,069 patients. The B-type natriuretic peptide-guided treatment for chronic heart failure had no effect on the cardiovascular mortality when compared to the guideline-guided group. The risk ratio was 0.90 (95% CI: (0.72, 1.12), p=0.33, I^2=0%). In subgroup analyses, the risk ratio for the cardiovascular mortality was not statistically significant for those younger than 72 years old (e.g., RR=0.80 (95% CI: (0.52, 1.22)) or patients with LVEF ≤ 30% (e.g. RR=0.92 (95% CI: (0.65, 1.30)) (Khan et al. 2018).

Heart failure mortality

The Cochrane review by McLellan et al. was the only review that reported the heart failure mortality (McLellan et al. 2016). Low-quality evidence from six studies in 853 patients showed uncertainty for heart failure mortality following serial BNP or NTproBNP-guided treatment. The risk ratio was 0.84 (95% CI: (0.54, 1.30)).

2.3.3. Systematic reviews investigating changes in B-type natriuretic peptides

Only the systematic review by Xin et al. investigated the impact of changes in BNP on clinical outcomes (Xin et al. 2015). Xin et al. reported that the between-group BNP changes ranged from -47 to 6%. Overall, the between group BNP changes did not impact the all-cause mortality. However, stratified analyses defined by cut-offs based on quartiles of BNP changes suggested a lower risk of all-cause mortality when changes of BNP were at least 5%. For example, when BNP decreased by at least 5%, the relative risk of all-cause mortality was 0.76 (95% CI: 0.58, 0.99) compared to the clinical group. When BNP decreased by at least 15%, the relative risk was 0.53 (95%CI: 0.27, 1.02), suggesting a trend of decreased all-cause mortality with higher changes in BNP.

2.3.4. Systematic reviews investigating the variability of B-type natriuretic peptides

No systematic review reported any information on the variability of B-type natriuretic peptides.

2.4. Discussion

Although there were differences among the studies included in the systematic reviews, it appears that the natriuretic peptide-guided treatment for heart failure did not improve the all-cause mortality, cardiovascular mortality and heart failure mortality, when compared to usual care. Usual medical care refers to standard-of-care or symptom-based care, or guideline-directed, or clinical experience, or a control care. GUIDE-IT (Felker et al. 2017) was the most recent study added to the most recent systematic review by Khan et al. This was a large multi-center study in severe heart failure patients, characterized by a reduced ejection fraction (e.g., median LVEF of 25%) and a history of prior hospitalization or emergency visit due to heart failure (Felker et al. 2017). Although the study intended to enroll 1,100 patients, a safety data monitoring committee recommended stopping the trial earlier with 894 heart failure patients due to the futility of the NT-proBNP guided therapy. These patients with a median age of 68 years were followed-up for a median of 15 months. Approximately a third of them were women.

The optimal BNP or NT-proBNP levels based on which the pharmacotherapy should be guided by remains unknown. Clinicians set the same natriuretic levels for the entire study, but natriuretic peptide levels are higher in elderly, females and lower in obese chronic patients (Wang et al. 2002, Redfield et al. 2002, Ponikowski et al. 2016). These confounders should be accounted for when escalating the medication doses. A too aggressive titration of heart failure medication to reduce the natriuretic peptide levels may be detrimental to some heart failure patients, especially those elderly with a renal dysfunction. Increasing heart failure medication such as spironolactone, ACE inhibitors, and Beta-blockers are known to reduce mortality (Hjalmarson et al. 2000, Pitt et al. 1999, Polinowski et al. 2016). Chronic heart failure patients

randomized to the natriuretic peptide-guided treatment were prescribed higher doses of heart failure medication than the clinically-controlled, symptom-guided, usual care groups.

Overall, the follow-up period ranged from 1 month to 54 months. Many studies included in the systematic reviews had a too short follow-up to be able to observe death.

The systematic review by Xin et al. was the only review that investigated changes in B-type natriuretic peptides (Xin et al. 2015). Changes depend on how the dose is safely escalated in chronic heart failure patients. Xin et al. reported that the between-group BNP changes ranged from -47 to 6%. When change in BNP decreased by at least 5%, the all-cause mortality was reduced for the natriuretic peptide-guided treatment group compared to the clinical group (Xin et al. 2015). Changes in B-type natriuretic peptide were more clinically important than absolute values.

The effect of age on the treatment effect was investigated by several systematic reviews. While the systematic review by Xin et al., Saverese et al. suggested that younger chronic heart failure patients (e.g., <75 years) may benefit from the natriuretic peptide-guided treatment, Pufulete et al. and McLellan et al. showed uncertainty in all-cause mortality for subgroups of patients younger or older than 75 years (Xie et al. 2015, Savarese et al. 2013, McLellan et al. 2016, Pufulete et al. 2017). Older patients tend to be sicker, with a more severe renal dysfunction that may preclude dose titration to control the adverse events.

The debate over the usefulness of the B-type natriuretic peptides in treating chronic heart failure patients hasn't been settled. More recent evidence tends to suggest that the serial BNP and NT-proBNP measurements do not improve the mortality. However, the quality of evidence is affected by several limitations.

2.5. Limitations

Not all systematic reviews included individual patient data in their analyses. Older systematic reviews used aggregate data, while the more recent ones made considerable efforts to use individual patient data when combining the studies (Khan et al. 2018, Pufulete et al. 2017, McLellan et al. 2016, Throughton et al. 2014).

Compliance to the prescribed heart failure medication was not monitored nor accounted for in the analyses of the systematic reviews. It is possible that the chronic heart failure patients took less of the medication prescribed.

Some heterogeneity was noticed. The results are subject to biases when combining studies of differing follow-up periods, comparators, target doses, inconsistent target natriuretic peptide levels, patient groups and clinical settings.

The risk of publication bias could not be excluded.

Based on quality assessments of the randomized clinical trials, McLellan et al. rated the quality of their evidence as low, while Pufulete et al. rated their evidence between low and moderate (McLellan et al. 2016, Pufulete et al. 2017). This was mainly due to the uncertainty in the results, risk of bias, potential for publication bias, differing study designs and inconsistencies of the data for the clinical trials included.

No systematic review focused solely on data from primary care. The impact of changes in natriuretic peptides on mortality within the primary care setting is not known.

2.6. Implications for research

There are an ongoing number of randomized clinical trials focusing on the serial BNT or NT-proBNP-guided treatment. It is desirable to obtain individual patient data from such new trials and conduct an updated review on the role of the natriuretic peptide in treating chronic heart failure. More data on cardiovascular mortality or heart failure mortality is needed, as most of the reviews focused on the all-cause mortality. Subgroup analyses based on subsets of randomized clinical trials with similar designs, similar clinical setting and comparators should be considered to alleviate some of the expected heterogeneity. Future studies should enroll many more heart failure patients, matched on essential confounders such as age, sex and weight to elucidate the real impact of the natriuretic peptide-guided therapy.

Other views should be explored. For example, while BNP or NT-proBNP may not affect the mortality, changes in natriuretic peptides over time may impact the adverse events or quality of life of chronic heart failure patients. Important outcomes such as cardiac function, NYHA class, quality of life and adverse events would need to be investigated.

Since in the United Kingdom, chronic heart failure patients are mainly treated by their primary care physician, it would be very helpful to investigate the role of natriuretic peptides in the primary care setting. The B-type natriuretic peptides may be better suited in the primary care setting.

2.7. Implications for practice

Recent systematic reviews did not show an association between the serial BNP or NT-proBNP-guided treatment and all-cause mortality, cardiovascular mortality or heart failure mortality. Should new data prove such an association, algorithms on how to titrate the natriuretic peptides in various clinical scenarios based on the patient's age, gender, length of heart failure, cause of heart failure, other comorbidities, tolerance to medication should be developed.

Therapy should be optimized at baseline before titrating based on natriuretic peptide levels. The target BNP or NTproBNP levels should take into the account the patients' characteristics as well as the expected levels in a stable heart failure population. One target dose or one target natriuretic peptide levels for the entire cohort of patients should be avoided. One size does not fit all in medicine. The length of follow-up should be at least two years. If change in therapy is evaluated, the reference change values should be used to facilitate the clinical interpretation.

More data on the use of BNP or NT-proBNP in primary care is needed.

2.8. Conclusion

The most recent systematic reviews suggested that guiding the heart failure medication based on serial B-type natriuretic peptide levels was not associated with a reduction in all-cause mortality, cardiovascular mortality or heart failure mortality.

No systematic review reported the variability of B-type natriuretic peptides over any time period. Based on limited data, changes in natriuretic peptides can positively affect mortality, if such changes are big. The degree of change remains to be further investigated.

Chapter 3 - A primary care perspective of the of B-type natriuretic peptide testing in heart failure patients

3.1. Introduction

In the United Kingdom, primary care physicians are often the first who diagnose heart failure, but they also manage and treat chronic heart failure patients. Current cardiovascular and clinical guidelines in the United Kingdom (NICE 2010, McMurray John 2012, Ponikowski et al. 2016) recommend the use of natriuretic peptides for diagnosis purposes only, but more evidence is needed for assessing the monitoring values of these peptides. The most recent European guidelines for heart failure do not recommend monitoring of natriuretic peptides due to inconsistent results from various studies (Ponikowski et al. 2016).

While the screening value of the B-type natriuretic peptides (e.g., BNP and NTproBNP) for diagnosing chronic heart failure had been previously studied in communities (Taylor et al. 2014)), to date little is known about the patterns of natriuretic peptide testing in chronic heart failure patients managed by primary care practices throughout the United Kingdom. In England, the estimated annual rate of natriuretic peptide tests ordered by the general practitioners, used mainly for diagnostic purposes ranged from 0.05 to 14.4 per 1000 practice population, a 297-fold variation (NHS 2013). Understanding the patterns of natriuretic peptide

testing may provide an opportunity for improving the management of chronic heart failure patients.

This chapter describes the natriuretic peptide testing in the primary care setting across the United Kingdom among heart failure patients and identifies patient-level and practice-level predictors of the likelihood of natriuretic peptide serial testing. In a subset of patients with repeat natriuretic peptide measurements, the time interval between repeat natriuretic peptide measurements is also estimated. Having multiple B-type natriuretic peptide tests provides an opportunity to investigate the potential use of changes as prognostic markers.

3.2. Objectives

The following primary research questions is addressed in this chapter:

Primary research question: What are the predictors of the likelihood of repeat natriuretic peptide (e.g., BNP and NTproBNP) testing in chronic heart failure patients attending primary care practices?

3.3. Methods

3.3.1. Study design

A retrospective cohort study was conducted in established heart failure patients using primary care electronic medical records. The study period was from January 1st, 2005 until August 1st, 2012. The entry into the cohort or the index date was the date of the first BNP or NT-proBNP measurement. The exit from the cohort was either the event of death or the end of the study period. There were no restrictions on the duration of the follow-up period. Thus all data

available within the study period was used. The heart failure cases were identified based on the following diagnostic codes in the CPRD data source:

Table 3. 1 - List of READ/OXMIS codes for identifying heart failure patients

READ/OXMIS code	Medcode	Description
G580.00	398	Congestive heart failure
G58..00	2062	Heart failure
G58z.00	4024	Heart failure NOS (not otherwise specified)
1O1..00	9913	Heart failure confirmed
G580.12	10079	Right heart failure
662T.00	12366	Congestive heart failure monitoring
14A6.00	15058	H/O: heart failure
8HBE.00	17851	Heart failure follow-up
9Or0.00	19380	Heart failure review complete
G580000	23707	Acute congestive heart failure
G582.00	27964	Acute heart failure
662W.00	30779	Heart failure annual review
G580100	32671	Chronic congestive heart failure
9Or..00	32911	Heart failure monitoring administration

The READ codes for the exclusion criteria can be found in the Appendix B.

3.3.2. Setting

The research presented in this chapter used fully anonymized, existent patient data from the Clinical Practice Research Datalink (CPRD). It is the world's largest computerized database of de-identified longitudinal medical records from primary care. The patient population captured in the database is broadly representative of the demographic breakdown of the population in the United Kingdom (www.cprd.com, last accessed on 22-Sep-2017).

CPRD is an excellent source for conducting medical and epidemiological research, especially for long-term follow-up studies, as there are patients followed for over 20 years. CPRD has medical records for about 11 million research standard patients drawn from almost 700 general

practices throughout the UK, covering about 17% of the UK population (www.cprd.com, last accessed on 22-Sep-2017). It includes individual patient data such as demographic information, lifestyle information such as body mass index, smoking, and alcohol consumption, clinical information (diagnoses, symptoms, procedures and medical history), referrals to secondary care including hospital specialty and emergency; prescriptions issued along with dosage and some adverse drug reactions; lab tests including qualitative and quantitative test results along with the normal ranges. Data quality is monitored continuously by the UK Medicine and Healthcare products Regulatory Agency (MHRA).

Given the retrospective nature of the study design, no contact was made with patients at any time and no patient information including UK identifying number, names, address was used nor obtained. The research protocol was reviewed and approved by the Independent Scientific Advisory Committee for Medicines and Healthcare Products Regulatory Agency database research.

3.3.3. Study population

The eligibility criteria applied to the study population are described below.

The inclusion criteria were: age ≥ 18; males & females; heart failure patients with a six-month pre-enrolment period to minimize a potential immortal time bias.

The exclusion criteria were: severe pulmonary disease (i.e., pulmonary embolism, COPD, lung cancer) or severe renal disease. NT-proBNP and BNP levels are much higher in renal insufficiency (Eggers et al. 2013, Scrutinio et al. 2015), characterized on the basis of glomerular filtration rate. Chronic kidney disease is identified by plasma creatinine (Perrone, Madias, and Levey 1992). When the kidney has an impaired capacity to excrete waste products, the levels of

the serum creatinine increase, and the glomerular filtration rate decreases (www.mayoclinica.com). Severe pulmonary diseases such as pulmonary embolism, primary pulmonary hypertension, COPD and lung cancer are associated with increased brain natriuretic peptides. Therefore, in such cases, the NT-proBNP and BNP levels should not be interpreted without the corresponding clinical scenario.

3.3.4. Variables

The following variables were considered for patients: age, gender, body mass index and smoking. Age was treated as a continuous variable in statistical analyses, and as a categorical variable in summary tables using the following classification: 0-44, 45-54, 55-64, 65-74 and 75+ years. Similarly, the body mass index was treated as a continuous variable when conducting statistical tests, and using the classification described in the Table 4.2. (e.g., underweight, normal weight, overweight, obese) when summarizing it.

The average time between two consecutive B-type natriuretic peptide measurements was computed. The time interval calculations included patients with up to three BNP or NT-proBNP measurements. Three time intervals were calculated: the time between the first two natriuretic peptide tests for 22% of the patients who had two measurements taken, the time between the second and third measurements for 5% of patients who had three measurements taken, as well as an arithmetic average between these two time intervals.

The following variables were considered at the practice level: number of chronic heart failure patients, index of multiple deprivation for the practice's postal code, and practice region.

3.3.5. Study size

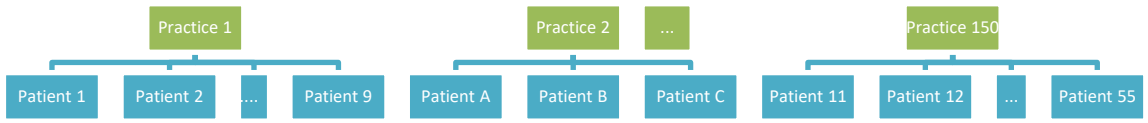
The chapter included 4,252 heart failure patients in whom B-type natriuretic peptides were tested. Overall, there were 87,672 heart failure patients in CPRD during the study period. Therefore, B-type natriuretic peptides were tested in approximately 5% of all heart failure patients.

3.3.6. Statistical Methods

Patients' characteristics were summarized using counts and percentages. The two groups of patients with single and repeat natriuretic peptide tests were statistically compared using t-tests for age and body mass index, the chi-square test for smoking and Fisher Exact test for gender. The number of chronic heart failure patients managed by primary care practices was summarize using medians and interquartile range. The index of multiple deprivation and practice regions were summarized using counts and percentages.

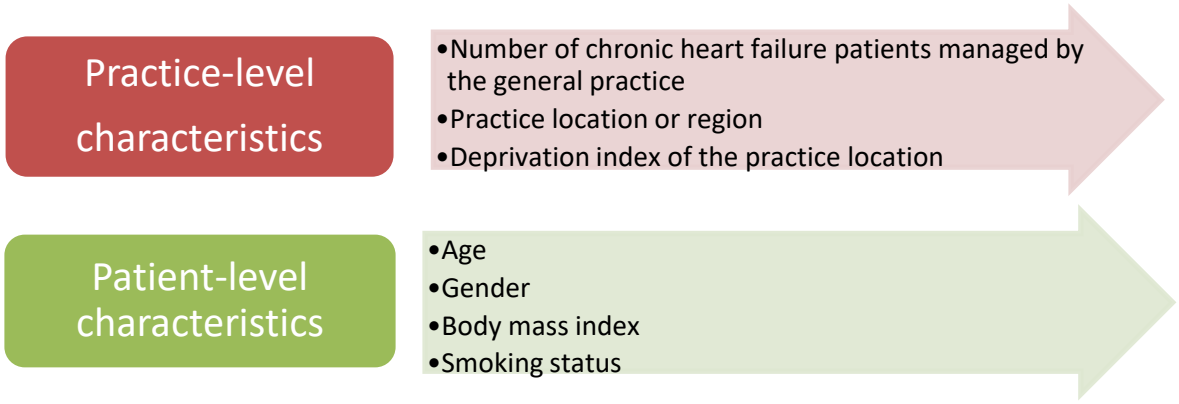
The data had a two-level hierarchical structure, as patients were clustered within general practices. This translated into a complex pattern of variability since there was variability among general practices, as well as variability among patients who were clustered within a general practice. The data structure is visualized in the figure below:

Figure 3. 1 - The two-level hierarchical structure of the data set



The following practice-level and patient-level characteristics were investigated in a statistical model aimed at identifying factors that may increase the likelihood of repeat brain natriuretic peptide testing in the primary care setting.

Figure 3. 2 - Practice-level and patient-level characteristics.



To appropriately account for the two sources of variability, both at the practice and patient levels, the hierarchical logistic regression model was employed. The form of the model is specified below:

$$\text{logit}(\pi_{ij}) = \alpha_j + \beta x_{ij}$$

$$\alpha_j = \alpha + \gamma z_j + u_j \quad ,$$

where π_{ij} is the probability of repeat testing for patient i attending the general practice j. At level 1 of the hierarchy, the outcome was expressed as the sum of an intercept and the patient's risk factor, while at the level 2, the general practice intercepts were specified. In this model, the general practice or primary care effects were measured by the random intercepts (j=1, ..., J), which was a linear combination of a grand mean α, a general practice fixed effect (γ), and general practice random effects (u_j)). The differences between primary care practices were explained by the observed general practice characteristic z, in addition to the random effects. The equations for the two levels above could be combined into one equation as:

$$logit(\pi_{ij}) = \alpha + \gamma z_j + u_j + \beta x_{ij}$$

The two-level hierarchical model was fit in SAS (Statistical Analysis System) version 9.2 (SAS Institute, North Carolina, USA) using the GLIMMIX procedure.

3.4. Results

There were 4,252 chronic heart failure patients meeting the eligibility criteria. As expected, most of the patients were older, aged 75 years or more, overweight or obese. There were approximately a similar number of males and females in the cohort. The patients' characteristics were statistically summarized by the type of BNP/NT-proBNP testing (e.g., single or repeat) in Table 3.3.

There were no differences in age and gender between patients that were tested once versus those tested repeatedly. Also, the rates of single and repeat testing were very similar across all body mass index groups, including underweight, normal, overweight and obese patients. A statistically significant difference was observed for smoking, although the difference did not seem to be clinically significant. Furthermore, the statistical difference was more likely due to the slightly higher percentages of single BNP/NT-proBNP testing of smokers and ex-smokers, as well as repeat testing of non-smokers. The null hypothesis of equal proportions of the chi-square test was rejected at the significance level of 5% and a p-value of 0.045.

Table 3. 2 - Patient characteristics

Characteristic	Single testing (n=3157)	Repeat testing (n=1095)	p-value
Age (years)			0.058
18-44	18 (0.6%)	10 (0.9%)	
45-54	79 (2.5%)	24 (2.2%)	
55-64	191 (6%)	83 (7.6%)	
65-74	608 (19.3%)	238 (21.7%)	
75+	2261 (71.6%)	740 (67.6%)	
Gender			0.194
Male	1572 (49.8%)	520 (47.5%)	
Female	1585 (50.2%)	575 (52.5%)	
Body mass index (kg/m2)			0.045
Underweight (<18.5)	90 (2.9%)	35 (3.2%)	
Normal weight (18.5-25)	930 (29.5%)	324 (29.6%)	
Overweight (25-30)	1042 (33%)	327 (29.9%)	
Obese (>30)	906 (28.7%)	364 (33.2%)	
Unknown	189 (5.9%)	45 (4.1%)	
Smoking			0.003
Yes	249 (7.9%)	73 (6.5%)	
No	1491 (47.2%)	584 (53.4%)	
Ex-smoker	1403 (44.5%)	437 (40%)	
Unknown	14 (0.4%)	1 (0.1%)	

Data presented as frequencies and percentages;
The p-values were derived from t-tests for age and body mass index; Fisher's exact test for gender; Chi-square test for smoking

The cohort was attended by 281 primary care practices. The general practices managed an average of 14 chronic heart failure patients (e.g., median=8, interquartile range=15, standard deviation=18). A proxy measure of the socioeconomic status, the index of multiple deprivations is derived based on the general practice postcode. The index of multiple deprivation indicated that the primary care practices were fairly distributed among the five quantiles, with slightly more practices belonging to the second most deprived quintile 4. Most general practices were located in London (e.g. 16%), closely followed by South East Coast (15%) and North West and South Central, each with 11.4%. The least number of practices were located in Wales, North East and Scotland. The general practice characteristics are summarized in Table 3.3.

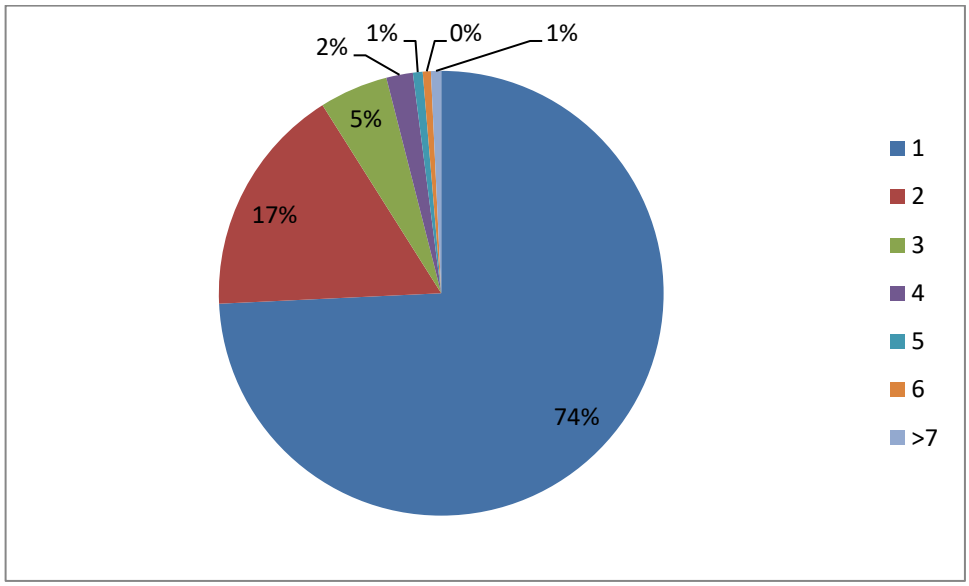
Table 3. 3 - General practice characteristics

Practice characteristic	Summary statistics (N=281)
No of chf[1] patients per practice	8 (IQR=15)[2]
Index of multiple deprivation	
Quintile 1 (least deprived)	47(19.1%)
Quintile 2	43 (17.5%)
Quintile 3	46 (18.7%)
Quintile 4	59 (24%)
Quintile 5 (most deprived)	51 (20.7%)
Practice region	
East Midlands	11 (3.9%)
East of England	24 (8.5%)
London	45 (16%)
North East	7 (2.5%)
North West	32 (11.4%)
Northern Ireland	22 (7.8%)
Scotland	8 (2.8%)
South Central	32 (11.4%)
South East Coast	42 (15%)
South West	17 (6.1%)
Wales	4 (1.4%)
West Midlands	26 (9.3%)
Yorkshire and The Humber	11 (3.9%)
[1]chronic heart failure patients;[2]Median=8 and interquartile range=15	

The general practices from London managed the highest number of the patients, followed by practices in South Central, South East Coast and Northern Ireland. Primary care practices in Scotland and Wales had the least number of chronic heart failure patients.

The total number of brain natriuretic peptide tests on a patient ranged from 1 to a maximum of 18 during the study period. Almost three quarters of the cohort had only one BNP or NT-proBNP test, while one quarter had a repeat test. 17% of the patients studied had two BNP/NT-proBNP tests, 5% had three such tests, and less than 5% of the cohort had 4 tests or more. The distribution of the total number of BNP and NT-proBNP tests is visualized next:

Figure 3. 3 - Number of BNP and NT-proBNP tests per patient



No of tests	1	2	3	4	5	6	7	8	9	10	11	12	13	16	18
Count	3157	714	212	82	30	25	12	6	6	2	1	1	1	2	1
%	74.25	16.79	4.99	1.93	1.71	0.59	0.28	0.14	0.14	0.05	0.02	0.02	0.02	0.05	0.02

Overall, almost three-quarters of the natriuretic peptide tests were NT-proBNP, while little over a quarter were BNPs. This reflects the test availability, rather than physicians' preferences.

The results of the hierarchical logistic regression are summarized in Table 3.4. Due to small numbers, Wales, and Scotland were excluded from the analysis in order to obtain estimable functions for the other regions.

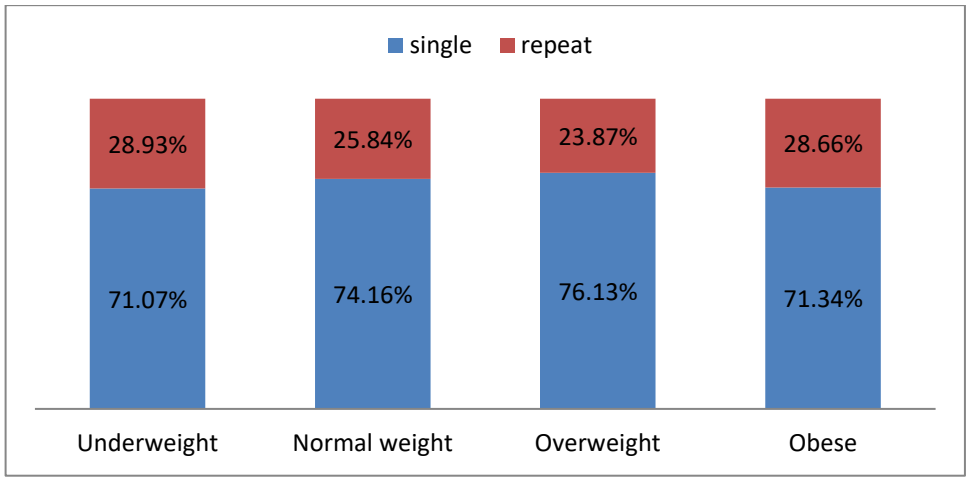
Table 3. 4 - Hierarchical logistic regression modelling the probability of repeat natriuretic peptide testing

Effect	Estimate	Standard error	p-value
Intercept	-1.81	0.52	0.004
Patient's age	-0.01	0.004	0.024
Patient's gender			0.106
Male	-0.09	0.08	
Female (ref)	-	-	
Patient's smoking status			0.123
Smoker	-0.19	0.16	
Non-smoker	0.12	0.09	
Ex-smoker (ref)	-	-	
Patient's bmi	0.02	0.006	0.013
Deprivation index (practice)	0.01	0.05	0.812
No of chronic heart failure patients per practice	0.02	0.003	<0.0001
Practice region			<0.0001
East Midlands	-0.23	0.01	
East of England	-0.51	0.55	
London	1.01	0.51	
North East	0.05	0.57	
North West	-0.40	0.52	
Northern Ireland	0.58	0.049	
South Central	0.24	0.48	
South East Coast	0.07	0.47	
South West	0.39	0.52	
West Midlands	0.27	0.49	
Yorkshire & The Humber (ref)			
Ref=reference category; bmi=body mass index			

The results suggested that the patient's age and body mass index, as well as the average number of the chronic heart failure patients per practice and practice location statistically, impacted the probability of repeat natriuretic peptide testing. Despite the statistical significance at the 5% level, the estimates were very close to zero to have a significant impact on repeat natriuretic peptide testing. The estimated variance of the practice random intercepts was 0.5478 with a standard error of 0.1094. This parameter measured the variability among the primary care practices.

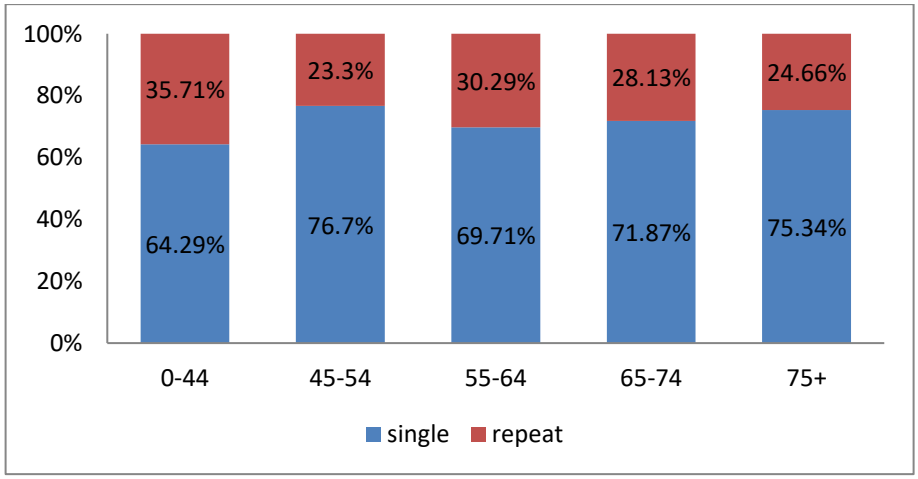
The NT-proBNP and BNP natriuretic peptide testing by body mass index are displayed below, which shows a higher proportion of repeat testing was higher in the underweight and obese groups.

Figure 3. 4 - Natriuretic peptide testing by the body mass index



As illustrated in Figure 3.5, the rates of repeat NT-proBNP and BNP tests were highest in the patients less than 45 years old. The rate of repeat natriuretic peptide testing was 23.3% in patients between 45 and 54 years old. This was the lowest percentage among all groups.

Figure 3. 5 - Natriuretic peptide testing by age groups



Overall, there were approximately 11 months between the first two consecutive natriutretic peptide tests, but the median time between the second and third tests was 7.2 months. The high inter-patient variation in the time intervals is illustrated in the table below.

Table 3. 5 - Time interval between consecutive natriuretic peptide tests

Time (months)	n	Median (min – max)
Time between tests 1 and 2	926	10.9 (0-92)
Time between tests 2 and 3	212	7.2 (0.1 - 63.4)
Average time between two consecutive tests	926	11.3 (0-92)

Heart failure men appeared to have BNP or NT-proBNP levels tested slightly earlier than women. As presented in Table 3.6, the median period of time between the first and second natriuretic peptide tests were 10.1 months for males and 11.6 for females. The median time intervals between the second and third natriuretic peptide tests were shorter: 6 months in men and 7.6 months in females. High inter-patient variation was observed, especially in the time interval between the first and second natriuretic peptide measurements, as indicated by the high ranges in the time intervals.

Table 3. 6 - Time interval between consecutive natriuretic peptide tests by gender

Time (months)	Median (min-max	
	Males	**Females**
Time between tests 1 and 2	10.1 (0-92)	11.6 (0-89.1)
Time between tests 2 and 3	6 (0.1-50.4)	7.6 (0.2-63.4)
Average time between two consecutive tests	10.7 (0-92)	11.5 (0-89.1)

The median and range of the time intervals between the first and second natriuretic peptide tests and between the second and third consecutive tests for various age groups are presented in the following table.

Table 3. 7 - Time interval between consecutive natriuretic peptide tests by age

Time (months)	Median (min-max)				
	18-44 yrs	**45-54 yrs**	**55-64 yrs**	**65-74 yrs**	**75+ yrs**
Time between tests 1 and 2	7.6 (0.2-37.9)	11.2 (0.1-71)	7.7 (0.2-78.8)	11.9 (0-92)	10.5 (0-89.3)
Time between tests 2 and 3	5.8 (1.5-15.9)	3 (0.9- 49.2)	3 (0.1-20.9)	11.8 (0.1-50.4)	6.6 (0.3-63.4)
Average time between two consecutive tests	6.1 (0.2-33.3)	11.2 (0.1-71.1)	8.1 (0.2-78.8)	12.7 (0-92)	10.8 (0-89.3)

Overweight and obese patients had about one year between their first two consecutive natriuretic peptide tests, followed by approximately eight months between their second and third consecutive tests, as illustrated in Table 3.8. The average time between two consecutive tests was much shorter in underweight and normal patients. A high variation in the data was observed.

Table 3. 8 - Time interval between consecutive natriuretic peptide tests by weight

Time (months)	Median (range)			
	Under-weight	**Normal weight**	**Over-weight**	**Obese**
Time between tests 1 and 2	4.9 (0.4-66.5)	9.6 (0-91.9)	11.7 (0-79.9)	12 (0-89.3)
Time between tests 2 and 3	1.3 (0.4-3.7)	7.3 (0.1-63.4)	8.4 (0.2-44.8)	7.5 (0.1-50.4)
Average time between two consecutive tests	4.9 (0.4-66.5)	10.7 (0-91.9)	11.7 (0-79.9)	12.4 (0-89.3)

The median time between the first two consecutive natriuretic peptide tests was 12.5 months y for smokers, 10 months for non-smokers and 11.5 months for ex-smokers. The time interval between the second and the third consecutive natriuretic peptide tests was shorter for smokers than non-smokers or ex-smokers.

Table 3. 9 - Time interval between consecutive natriuretic peptides by smoking status

Time (months)	Median (range)		
	Smoker	**Non-smoker**	**Ex-smoker**
Time between tests 1 and 2	12.5 (0-78.8)	9.9 (0-92)	11.5 (0.1-89.3)
Time between tests 2 and 3	3.2 (0.9-15.7)	7.4 (0.2-51.9)	7.6 (0.1-63.4)
Average time between two consecutive tests	11.9 (0-78.8)	10.4 (0-92)	11.9 (0.1-89.3)

3.5. Discussion

This descriptive study provided population-based evidence on the patterns of the B-type natriuretic peptide laboratory testing in heart failure patients managed by primary care physicians in the UK. The study showed that the B-type natriuretic peptides are heavily underused in the primary care setting. Only 5% of the chronic heart failure managed in primary care had their natriuretic peptides tested.

During the study period, the NT-proBNP and BNP repeat testing varied greatly within the UK. The British Heart Foundation reported in 2010 that the prevalence of heart failure in men was 1% in Scotland (e.g. number of cases 6,166) or Wales (number of cases 6,697), compared to 0.9% in the United Kingdom (number of cases 82,781). In women, the prevalence of heart failure was 0.8% in Scotland (number of cases 6,676) and 0.7% in Wales (number of cases 7,803), compared to 0.7% overall in women in the United Kingdom (number of cases 94,598). The incidence of heart failure in men was 43.4 per 100,000 in Scotland (number of cases 202) and 44.6 per 100,000 in Wales (number of cases 233) compared to the overall rate of 39.1 per 100,000 across the United Kingdom (number of cases 2,659). The incidence of heart failure in women was lower, 32.1 per 100,000 in Scotland (number of cases 206) and 24.9

per 100,000 in Wales (number of cases 197) compared to the overall incidence in women of 24 per 100,000 across the United Kingdom (number of cases 2,334).

No patients' characteristics such as age, gender, body mass index, smoking status clinically impacted the likelihood of serial natriuretic-peptide testing. No practice characteristics such as the deprivation index of the practice location or number of chronic heart failure patients treated at the current practice clinically impacted the likelihood of repeat natriuretic testing. Patient's age and body mass index, as well as the average number of the chronic heart failure patients per practice and practice location reached the statistical significance, but considering the estimates were very close to zero, they did not reach clinical significance.

There were approximately 11 months between consecutive natriuretic peptide tests, while age, sex, and body mass index had little effect on the time intervals between the tests. It was unclear whether the repeat natriuretic peptide testing was done for monitoring purposes, or it was part of a serial diagnosis, which is often the case with heart failure. The electronical medical records didn't allow the investigation of the scope of repeat testing.

3.5.1. Limitations

The study population represents a small sample of the entire heart failure population managed in the primary care setting within the United Kingdom. This study shows how underused natriuretic peptides are in the primary care setting. Only 5% of all heart failure patients had their natriuretic peptide levels measured and only 1.2% had repeat natriuretic peptide tests. The point of care BNP testing was not represented in this study.

The systematic review by Herrett (Herrett 2010) showed high validity of the diagnoses for the circulatory system in the general practice research database. The median proportion of cases

confirmed was 85.3%. In this study, approximately 92% of the heart failure patients were also exposed to hypertension and/or heart failure medication. The medication is listed in the Appendix D.

This study is descriptive in nature and in the absence of data collected by electronical medical records, it is impossible to state whether the repeat natriuretic peptide testing was done for diagnosis or monitoring purposes. However, considering that most of the heart failure patients had a single natriuretic peptide test, this suggests that it was mostly used to support diagnosis.

3.5.2. Implications for research

Natriuretic peptides are highly underused in the primary care setting.

One way to investigate the purpose of natriuretic peptide testing is to contact the CPRD team and suggest adding such a field in the database for future research. Another way to elucidate the scope of testing, as well as physician adherence to the NICE guidelines with respect to the use of natriuretic peptide in primary care is to conduct a representative, prospective cohort study in primary care where this information is being collected.

3.5.3. Implications for practice

No patients' characteristics studied such as age, sex, body mass index and smoking were predictors of repeat natriuretic peptide testing in the primary care setting. However, these factors should be considered by the multidisciplinary team when ordering brain natriuretic tests in heart failure patients, as they help with risk stratification and targeting therapies (Ponikowski et al. 2016).

To actively and effectively manage heart failure patients in the primary care setting, regular natriuretic peptide testing could be an invaluable tool to be used throughout the United Kingdom especially in high-risk patients who are elderly and frequently admitted. High natriuretic peptide levels reflect high disease severity and worse symptoms (Ponikowski et al. 2016). Regular natriuretic peptide testing provides an opportunity in primary care to monitor chronic heart failure patient's response to pharmacology and continually assess the disease progression. This is expected to reduce requirements for more expensive echocardiography and waiting times, as well as inform policy makers about the clinical needs of the heart failure patients. Furthermore, given the dire prognosis of heart failure patients, B-type natriuretic peptide tests may improve the management of heart failure patients (Ibrahim and Januzzi 2017, Savarese 2016, Faggiano et al. 2010, Miller et al. 2007).

3.6. Conclusion

B-type natriuretic peptides are heavily underused in the primary care setting within the United Kingdom. While the electronical medical records do not capture the scope of testing, the existing data suggests that these natriuretic peptides are mainly used for diagnosis purposes, since most of the patients had just one test.

Chapter 4 - The prognostic value of B-type and N-terminal pro-B-type natriuretic peptides in heart failure patients – A primary care perspective

4.1. Introduction

Previous studies showed that B-type natriuretic peptides are independent predictors of all-cause mortality. However, the magnitude of the risk varied considerably in individual studies and the literature search led to no systematic review and meta-analysis that combined any mortality estimates. The HTA evidence report by Balion et al. summarized the mortality estimates obtained from various studies, but the differences in the study designs, study sizes, duration of follow-up, inconsistency in study reporting, heterogeneity of the study population recruited from various clinical settings such as hospitals, emergency departments, outpatient clinics, and general population precluded the estimation of a combined mortality risk (e.g., all-cause mortality, cardiovascular mortality) for BNP and NT-proBNP (Balion et al. 2006). Therefore, while BNP and NT-proBNP are expected to be independent predictors of mortality, the extent of the prognostic values of these biomarkers is not known in primary care.

This chapter addresses the prognostic value of both BNP and NT-proBNP peptides levels in chronic heart failure patients managed by primary care physicians in the UK.
The following research questions are addressed:

Primary research question: Are NT-proBNP levels associated with the all-cause mortality in heart failure patients managed by primary care physicians?

Secondary research question: Are BNP levels associated with the all-cause mortality in heart failure patients managed by primary care physicians?

Secondary research question: Are NT-proBNP levels associated with the all-cause mortality and cardiovascular mortality in heart failure patients managed by primary care physicians?

4.2. Methods

4.2.1. Study design, study setting and study participants

The study design and study participants were described in detail in Chapter 3. However, key design elements are summarized here for ease of reference. The study was a retrospective cohort conducted in heart failure patients managed by primary care physicians. The study setting was the Clinical Research Data Link, the largest database of de-identified electronic medical records in the United Kingdom. Each patient had a variable entry into the cohort, defined as the date of the first natriuretic peptide measurement, regardless its type (e.g. BNP or NT-proBNP). The exit from the cohort was either the event of death or end of study, which was set as September 30th, 2012. The study participants were heart failure adult patients with a least one B-type natriuretic peptide measurement and without any severe pulmonary or renal diseases. While a little over 95% of the entire cohort had their baseline BNP or NT-proBNP test measured between January 1st, 2005 and September 30th, 2012, the analysis included all BNP and NT-proBNP data available. Therefore, the 199 patients or 4.65% with baseline B-type natriuretic peptides measured in 2003 and 2004 were also included in the statistical analysis. The B-type natriuretic peptides were first mentioned in the heart failure NICE clinical guidelines in 2003 (Chronic Heart Failure, (NICE clinical guidelines 5 (2003)), which was later updated and replaced in 2010 (NICE 2010).

4.2.2. Study endpoints

The primary study endpoint was all-cause mortality. This was ascertained based on the death information available in the primary records, which included the date of death for the deceased patients.

The secondary study endpoint was cardiovascular mortality. This was obtained from the Office for National Statistics through the CPRD linkage for patients belonging to English practices that had consented to this linkage scheme. The causes of death were coded according to the International Classification of Diseases, tenth revision (ICD-10), listed by the World Health Organization. The advantage of adopting such a classification of diseases into broad groups or chapters is the ability of comparing frequencies and causes of deaths across countries and different periods of time. The ICD-10 group of diseases of the heart and circulatory system are described in table below. For a complete description of all codes, refer to Appendix C.

Table 4. 1- ICD10 codes for groups of cardiovascular diseases

ICD10 code	Group of disease
I00-I09	Acute rheumatic fever and chronic rheumatic heart diseases
I10-I13	Hypertensive diseases
I20–I25	Ischemic heart diseases
I26-I51	Other heart diseases*
I60-I69	Cerebrovascular diseases
I70-I79	Diseases of arteries, arterioles and capillaries
I80-I99	Other disorders of the circulatory system
*includes heart failure	

In some patients, especially those with multiple comorbidities, establishing the primary cause of death could present a challenge. The mortality data from the Office of National Statistics included up to 15 different fields for recording causes of death. The cardiovascular mortality

occurred if at least one the recorded causes of death was attributed to a disease of the heart and circulatory system with an ICD10 code listed in Table 4.1, even if it was not the primary cause of death, for patients with multiple causes of death.

4.2.3. Sample size calculations

Sample size calculations were performed for the primary endpoint, all-cause mortality and included several scenarios using the two-sided hypothesis testing for a univariate Cox regression that meets the proportional hazards assumption. The calculations assumed the probability of rejecting a true null hypothesis or an alpha significance level of 5% with 80% and 90% power, as the probabilities of rejecting a false null hypothesis. In this context, the null hypothesis corresponds to a hazard ratio of 1, that is no association between the outcome and the independent factor studied.

The required sample size varies according to the event rate or the proportion of subjects in which the event of interest (all-cause mortality) occurs during the study. This is the proportion of non-censored subjects.

Sample size curves are displayed in Figures 4.1 by the regression coefficient from the Cox model, the predicted change in log (base e) hazards at one unit change in the risk factor when the other covariates are held constant. For example, log hazard ratios of 0.2, 0.3, 0.4, 0.5, 0.6, 0.7 and 0.8 correspond to hazard ratio of 1.22, 1.35, 1.5, 1.65, 1.82, 2, and 2.2 respectively.

Figure 4. 1 - Sample size curves for 90% power and 80% power

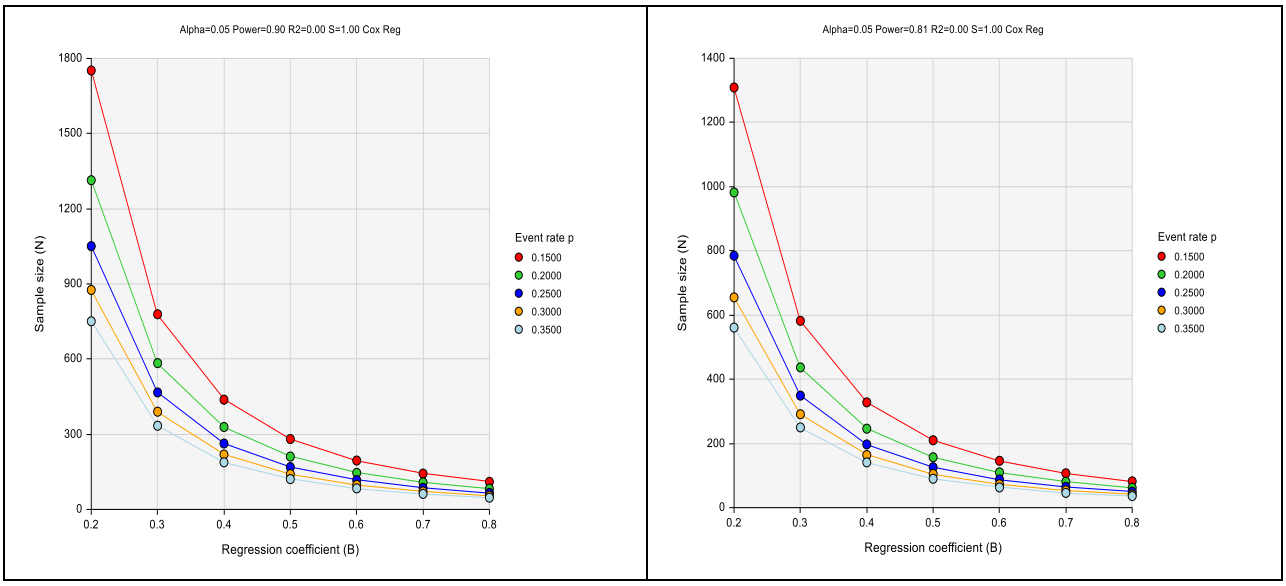


Figure 4.1 shows that a Cox regression of the log hazard ratio on a covariate with a standard deviation of 1 based on a sample of 876 patients achieves 90% power at a 5% significance level to detect a regression coefficient equal to 0.2. The sample size was adjusted for an anticipated event rate of 0.3 or 30%. For a power of 80% under the same other assumptions, the corresponding sample size would be 655 patients. As the study cohort had over 4,000 patients, the sample and power analyses demonstrated enough data to support the primary and secondary objectives addressed in this chapter.

About half of the clinical practices consented to have their data linked to the CPRD Data Linkage Scheme (ONS Mortality Data) and causes of death available. Concerning the secondary endpoint, cardiovascular mortality, there will be at least 582 records of cardiovascular death which will ensure 80% power at a 5% significance level to detect a hazard ratio of 1.5, assuming an anticipated event rate of 15%.

4.2.4. Study variables or covariates

The study included the following variables: natriuretic peptides, age at study entry, gender, body mass index, smoking status, geographical region, serum creatinine, diabetes, hypertension and heart failure medication. All covariates were assessed during the pre-enrollment period, defined as six months before the index date, the date of the patient's first BNP or NT-proBNP test. Gender, smoking and geographical region were readily available in the CPRD. However, the remainder variables were derived based on the information available.

Higher levels of natriuretic peptides indicate a worse condition of the heart (Braunwald 2013, Mentz and Felker 2011). In CPRD, the natriuretic peptides were available in various units. Conversions were applied so that all B-type natriuretic peptide levels were expressed in pg/ml. To convert plasma concentrations from pmol/L to pg/ml, I used the conversion factor of 3.46 for BNP and 8.457 for NT-proBNP (Balion et al. 2008). BNP and/or NT-proBNP were treated both as continuous, as well as categorical variables. The four categories were separately defined based on the quartile values for the baseline BNP and NT-proBNP measurements, respectively.

Heart failure is a disease of the aging, and the mortality is higher in older people (NICE 2010, Atherton et al. 2016). For each patient, the date of birth was available; thus the age at study entry was calculated as the date of the first B-type natriuretic peptide minus the date of birth.

Chronic heart failure men are expected to die at younger age than women (Martínez-Sellés et al. 2012, Metra 2016, Ziaeian and Fonarow 2016).

Although an increased body mass index is a risk factor for heart failure, it is paradoxical, inversely associated with survival. A meta-analysis by Oreopoulos (Oreopoulos et

al. 2008) confirmed the protective effect of overweight and obesity on all-cause mortality and cardiovascular mortality in chronic heart failure patients. This has been termed as the obesity paradox or reverse epidemiology in the literature. Clark et al argued that the serum lipoproteins are increased in overweight and obese patients and they may down-regulate inflammatory cytokines, which are associated with increased mortality (Oreopoulos et al. 2008, Rauchhaus et al. 2003). Also, it is possible that overweight or obese patients are diagnosed and treated earlier than normal or underweight people. The following formula was applied when calculating the body mass index: weight (kg)/height^2 (m^2). In statistical analyses, the body mass index was treated as a continuous variable, however, in summary tables, it was categorized using the following classification:

Table 4. 2 - Body mass index categories

BMI category	BMI range (kg/m^2)
Underweight	<18.5
Normal weight	18.5 – 24.9
Overweight	25 – 30
Obese	>30

A relatively recent systematic review and meta-analysis comprising of 17 studies from 7 countries showed that smoking is associated with all-cause mortality in people at least 60 years old (Gellert, Schottker, and Brenner 2012).

Elevated levels of serum creatinine increase the risk of all-cause mortality and cardiovascular mortality (Ford et al. 2015). As a measure of kidney function, I included the serum creatinine in the statistical analyses rather than estimating the glomerular filtration rate using the Cockcroft-Gault (CG) equation, because age, gender, and weight were already accounted for in the model, thus avoiding the potential of multi-collinearity.

Whether or not a patient is diabetic may have little impact on the all-cause or cardiovascular mortality (Ford et al. 2015). The diabetes status was ascertained based on the diagnosis codes related to diabetes mellitus. The complete list of READ/OXIMS codes for identifying diabetic patients is available in Appendix C. A patient was considered diabetic as long as the patient had any of the diagnosis codes listed in Appendix C. The diabetes status was established at baseline, defined as the 6-month pre-enrollment period prior to the index date.

The exposure to heart failure and/or hypertension medication was ascertained based on the medicines' codes available in the CPRD according to the British National Formulary. The hypertension and heart failure chapter of the British National Formulary includes the following classes of such drugs: vasodilators antihypertensive drugs, centrally acting antihypertensive drugs, adrenergic neuron blocking drugs, alpha-adrenoceptor blocking drugs and drugs affecting the renin-angiotensin system (BMJ 2012).

The hypertension medication is prescribed according to the patient's age, gender, ethnic origin, medical problems, medical history, as well as the patient's response to the first line of therapy (NICE 2010). The main drugs for treating elevated blood pressure are ACE inhibitors, ARBs, Calcium channel blocker, diuretics, beta-blockers and combination of such drugs (NICE 2010). ACE inhibitors inhibit or block the production of the Angiontensin II hormone, ARBs block the effects of this hormone in the body, while Calcium-channel blockers block the effect of Calcium on the blood vessels (www.bloodpressureuk.org, last accessed on 22-Sep-2017). Diuretics, also known as "water pills" help eliminate the excess of water and salt by the direct stimulation of kidney (NICE 2010). In addition to blocking Angiontensin II, beta-blockers also block the effect of the adrenaline and noradrenaline hormones by slowing down the heart rate and the force with which the blood is pumped throughout the body (www.bloodpressureuk.org,

last accessed on 22-Sep-2017). ACE inhibitors, ARBs, Calcium-channel blockers and beta-blockers make the blood vessels relax and widen so that the blood could flow smoothly throughout the body (www.bloodpressureuk.org, last accessed on 22-Sep-2017).

As the majority of heart failure patients take hypertension medication, and some of them are prescribed combinations of drugs such as ACE inhibitor and Calcium channel blocker or diuretics, the drug exposure to either of heart failure and/or hypertension medication was considered altogether rather than separately. The British National Formulary chapters used in the derivation of this variable are listed in Appendix D. A patient was considered exposed to hypertension and/or heart failure medication as long as the patient took any of the medicines listed in Appendix D, regardless the dose or duration.

4.2.5. Statistical methods

SAS version 9.2 (SAS Institute Inc, Cary, NC) was used for the statistical analyses. The PASS software (PASS v12, NCSS, LLC, Kaysville, Utah, USA) was used for the sample size calculations.

Summary statistics were generated by the mortality status (e.g., dead or alive) for both all-cause mortality and cardiovascular mortality. Continuous variables were summarized using mean and standard deviations, while categorical variables were summarized using count, frequencies, medians, and ranges. Data were visualized with distributional graphs, histograms, stack bars and line plots.

The baseline characteristics of the two groups (e.g., dead or alive) were statistically compared using t-tests for normally distributed variables and non-parametric tests such as the Wilcoxon rank sum sign tests, Fisher's exact tests or chi-square tests for variables that did not meet the normality assumptions. To meet the normality assumptions, the variables had to be continuous,

normally distributed with the same variance or standard deviation in the two groups, and have independent observations.

Time to death was statistically analyzed with Cox proportional hazards models. As a particular, semiparametric class of survival analyses, these models make no assumption about the shape of the underlying hazard function, although hazards should be proportional over time. This means that, for example, if men are twice as likely as women to die at age 50, they will be twice as likely to die at 65 years old or any other age for that matter. Another key assumption in survival analyses is random censoring, which ensures that subjects censored at one point in time are representative of all subjects still alive at that time.

Unlike other regression models such as linear regression, logistic regression or Poisson regression, Cox regression models do not have an intercept, rather a baseline hazards, which is the hazard function when all covariates equal to 0. The general form of the Cox regression model is given below:

$h(t;X)=h_0(t) * \exp(\beta_1 x_1 + \beta_2 x_2 + ... + \beta_k x_k)$,

where $h(t;X)$ represented the hazard function at time t for a subject with a vector of covariates $X=(x_1,\ldots,x_k)$ such as age, gender, body mass index, smoking status, diabetes, serum creatinine, exposure to heart failure and/or hypertension medication and geographic region, $h_0(t)$ was the baseline hazard, while β_i was the regression coefficient for the covariate x_i ($i=1, \ldots, k$). Regarding interpretation, a positive beta parameter indicated that the mortality risk increased with time, while a negative regression coefficient meant that the hazard function decreased over time.

Univariate and multivariable Cox regression models were developed. The univariate Cox regression model had one covariate, BNP or NT-proBNP, respectively. Although the initial

multivariable models included all available variables, the final model employed was based on the stepwise selection method. For a variable to enter or stay in the model, it had to be significant at the 5% level.

The model fitting was performed by checking for outliers, functional form of continuous covariates, as well as the proportional hazards assumption. I tested for outliers by visually inspecting the deviance residual plots versus the observation number. I checked for the functional form of continuous covariates by plotting martingale residuals against the continuous covariates. Martingale residuals estimate a difference between the observed number of events and the expected number of events, while the deviance residuals are a transformation of Martingale residuals. A non-pattern in these plots indicated proper fit. I checked the proportional hazards assumption by plotting the log of the negative log of the survival function versus time, computing and plotting the Schoenfeld residuals versus the number of observation and by testing the interactions between time and covariates, as well as conducting a Kolmogorov-type supremum test using 1000 simulated patterns.

Table 4.3 summarizes the statistical models performed to investigate the prognostic role of the baseline BNP and NT-proBNP on the all-cause mortality and cardiovascular mortality. Model fitting and diagnostic details can be found in Appendix E.

Table 4. 3 - Statistical models performed

Model No[b]	Outcome	B-type natriuretic peptide	Scale	Cox regression statistical model
1-2	All-cause mortality and CV mortality	BNP	log_e	Unadjusted
3-4			log_e	Adjusted for age, gender, body mass index, smoking, geographic region, serum creatinine, hypertension and/or heart failure medication and diabetes status at baseline
5-6			log_e	Sensitivity analysis - stepwise selection from all baseline characteristics aforementioned while removing the 5% highest BNP values
7-8			categorical quartile[d]	Unadjusted – quartile as a categorical variable
9-10			categorical quartile[d]	Adjusted for age, gender, body mass index, smoking, geographic region, serum creatinine, hypertension and/or heart failure medication and diabetes status at baseline
11-12			categorical[d] quartile	Sensitivity analysis – stepwise selection from all baseline characteristics aforementioned
13-14			continuous[e] quartile	Unadjusted - quartile as a continuous variable
15-16			continuous[e] quartile	Adjusted for age, gender, body mass index, smoking, geographic region, serum creatinine, hypertension and/or heart failure medication and diabetes status at baseline
17-18			continuous[e] quartile	Sensitivity analysis – stepwise selection from all baseline characteristics aforementioned
19-20		NT-proBNP	log_e	Unadjusted
21 -22			log_e	Adjusted for age, gender, body mass index, smoking, geographic region, serum creatinine, hypertension and/or heart failure medication and diabetes status at baseline
23-24			loge	Sensitivity analysis - stepwise selection from all baseline characteristics aforementioned while removing the 5% highest NT-proBNP levels
25-26			categorical[d] quartile	Unadjusted – quartile as a categorical variable
27-28			categorical[d] quartile	Adjusted for age, gender, body mass index, smoking, geographic region, serum creatinine, hypertension and/or heart failure medication and diabetes status at baseline
29-30			categorical[d] quartile	Sensitivity analysis – stepwise selection from all baseline characteristics aforementioned

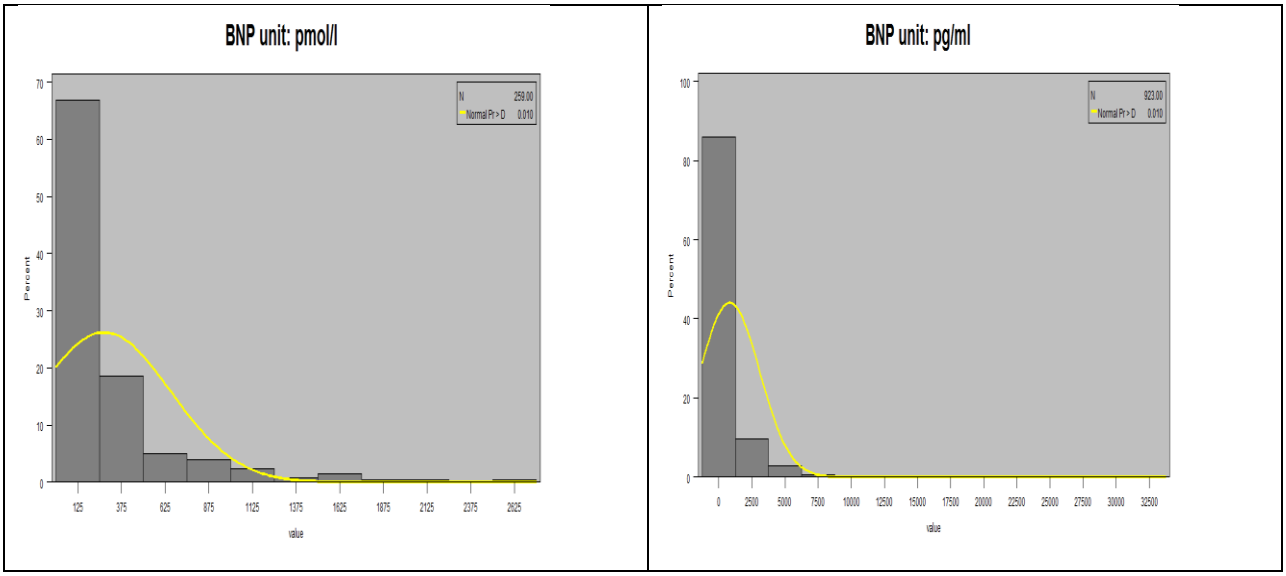
31-32			continuous[e] quartile	Unadjusted - quartile as a continuous variable
33-34			continuous[e] quartile	Adjusted for age, gender, body mass index, smoking, geographic region, serum creatinine, hypertension and/or heart failure medication and diabetes status at baseline
35-36			continuous[e] quartile	Sensitivity analysis – stepwise selection from all baseline characteristics aforementioned
37-38	All-cause mortality and CV mortality	Combined[c] BNP and NT-proBNP	categorical[d] quartile	Unadjusted – quartile as a categorical variable
39-40			categorical[d] quartile	Adjusted for age, gender, body mass index, smoking, geographic region, serum creatinine, hypertension and/or heart failure medication and diabetes status at baseline
41-42			categorical[d] quartile	Sensitivity analysis – stepwise selection from all baseline characteristics aforementioned
43-44			continuous[e] quartile	Unadjusted - quartile as a continuous variable
45-46			continuous[e] quartile	Adjusted for age, gender, body mass index, smoking, geographic region, serum creatinine, hypertension and/or heart failure medication and diabetes status at baseline
47-48			continuous[e] quartile	Sensitivity analysis – stepwise selection from all baseline characteristics aforementioned

a. Separate statistical analyses were conducted for BNP and NT-proBNP.
b. Odd model numbers denote the statistical models performed for assessing the all-cause mortality. Even model numbers denote the statistical models performed for assessing the cardiovascular mortality.
c. In the combined analyses, the BNP and NT-proBNP quartiles were combined according to the respective quartiles at baseline. The first combined quartile consisted of patients in the first BNP quartile and first NT-proBNP quartile. The second combined quartile consisted of patients in the second BNP quartile and NT-proBNP quartile. The third combined quartile consisted of patients in the third BNP quartile and NT-proBNP quartile. The fourth combined quartile consisted of patients in the fourth BNP quartile and NT-proBNP quartile. The higher the quartile, the more severe the patient.
d. The categorical quartiles estimate the effect of each quartile on the mortality, as compared to the first quartile.
e. The continuous quartiles estimate the effect of each subsequent quartile on the mortality

4.2.6. Missing data

There were 15 subjects (1.25%) with BNP levels available at baseline. However, the units were missing. In order to apply the proper conversion factor, the statistical distributions for BNP levels were separately graphed for each unit, as displayed in Figure 4.3. Summary statistics were also produced by each of the units in Table 4.4. The following simple rule was applied when imputing the missing units: if the BNP level was less than 200, then the unit of pmol/l was assigned and the conversion factor of 3.46 was applied. When the BNP level was greater than 200, then the preferred unit of reporting of pg/ml was assigned, and no conversion was needed.

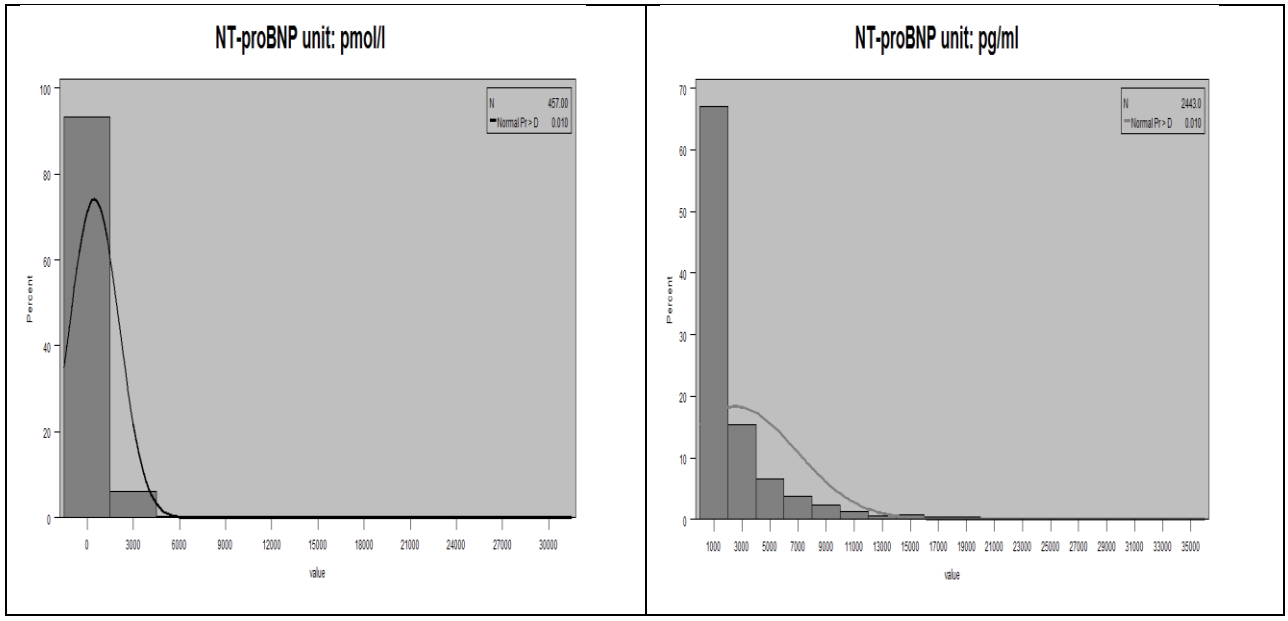
Figure 4. 2 - BNP distributions by units of measurements



There were 155 subjects (5%) with available NT-proBNP levels at baseline, but missing units of measurements. In order to apply the proper conversion factor, the statistical distributions for NT-proBNP levels were separately graphed for each unit, as displayed in Figure 4.4, as well as summarized in Table 4.4. The following simple rule was applied when imputing the missing

units for NT-proBNP: if the peptide level was less than 350, than the unit of pmol/l was assigned and the conversion factor of 8.457 was applied. If NT-proBNP was greater than 350, then the preferred unit of reporting of pg/ml was assigned and no conversion was needed.

Figure 4. 3 - NT-proBNP distributions by units of measurements



Mean, median, lower, and upper quartile are presented in Table 4.4 for each of the measurement unit for BNP and NT-proBNP.

Table 4. 4 - Summary statistics for BNP and NT-proBNP by measurement unit

Analyte (unit)	Mean	Lower quartile	Median	Upper quartile
BNP (missing unit)	700.3	39	165.4	1763
BNP (pmol/l)	800.1	118	274.7	706
BNP (pg/ml)	276.5	57	133	314
NT-proBNP (no units)	1392.9	174.7	573	1619
NT-proBNP (pmol/l)	474.9	55	160	395
NT-proBNP (pg/ml)	2547.9	322	1052	2657

The impact of missing data for covariates was evaluated and was considered to be minimal. Data was not imputed for the covariates. Table 4.5 shows the percentage of missing data for each of the covariates.

Table 4. 5 - Missing data for covariates

Covariate	% missing
Age	0
Gender	0
Body mass index	5.5%
Smoking status	0.3%
Diabetes status at baseline	0.1%
Serum creatinine	5.3%
Hypertension/heart failure medication	0.8%
Geographic region	1.6%

The denominator was the entire cohort of 4250 patients

Table 4.6 shows the percentage of missing data for each of the covariates by the first B-type natriuretic peptide test (e.g., BNP, NT-proBNP). Missing data was similar for both BNP and NT-proBNP.

Table 4. 6 - Missing data for covariates by the baseline BNP or NT-proBNP test

Covariate	BNP test* n=1,196	NT-proBNP test* n=3,053
Age	0	0
Gender	0	0
Body mass index	4.8%	5.8%
Smoking status	0.2%	0.1%
Diabetes status at baseline	0	0.1%
Serum creatinine	5.6%	5.3%
Hypertension/heart failure medication	1.3%	0.6%
Geographic region	1.7%	1.5%
*The denominator was 1,196 for patients with a baseline BNP test and 3,053 for those with a baseline NT-proBNP test.		

4.3. Results

There were 4249 patients included in the survival analysis of the present chapter. While the previous chapter included 4252 patients, two of these patients had missing B-type natriuretic peptides levels, although the dates of the tests, as well as tests' measurement units were recorded. These two patients were a 68-year old male patient and an 87-year old female patient. One additional patient had a negative study period, as the recorded death date of 19/10/2006 before the date of the first B-type natriuretic peptide test, which was 08/05/2007. Given the observational nature of the study, this was deemed as a data entry error. None of these three patients died during the study. Consequently, there were 4249 patients included in the survival analysis of Chapter 4.

The primary outcome of all-cause mortality occurred in 1260 patients or 29.6%. The secondary outcome of cardiovascular mortality took place in 628 patients or 14.8%.

Information about the outcomes was not available in less than 7% of the patients, as they had left the existing clinical practice. The following reasons for transferring out of the clinical practice were listed in the CPRD database: "Removal to new TP/HB/CSA" (1.9%), internal transfer (1.8%), "embarkation" (0.02%), "Deduction at GP's request" (0.05%), other reason (0.7%), "returned undelivered" (0.09%), "internal transfer – address change" (2.2%), and "Practice advise outside" (0.05%).

The study population is summarized by the mortality status in Table 4.7. As expected, patients who died during the study period were older. There was a fairly similar distribution of gender between the two groups of patients with women representing 51.4% of those dead and 50.6% of those alive, respectively. Patients who survived were significantly heavier at baseline than those who died during the study period. Obesity was more prevalent among those alive (35.2%)

than those who died (22.6%). Most participants were either non-smokers or ex-smokers and were seen at medical practices located in London, South Central, and South East Coast. Overall, the serum creatinine levels at baseline were significantly higher for those who died during the study period. The average serum creatinine measurements were 102.5 umol/l (95%CI: (100.9, 104.1)) for those alive and 114.8 umol/l (95%CI: (112.2, 117.5)) for those who died during the study.

Regarding smoking and diabetes status, there was no statistically significant difference between those who died or were alive. Regardless the mortality status, approximately 92% of the study participants had been previously exposed to hypertension and/or heart failure medication before the study entry.

About 72% of the study participants had NT-proBNP levels, and 28% had BNP levels measured at baseline. Patients who died belonged to the more severe third (28.8%) and fourth (30.4%) BNP quartiles and fourth NT-proBNP quartile (34.2%) than those who were still alive by the end of the study period in the respective quartiles.

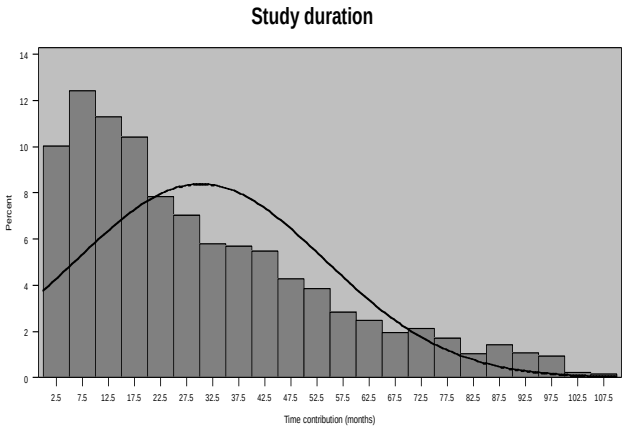
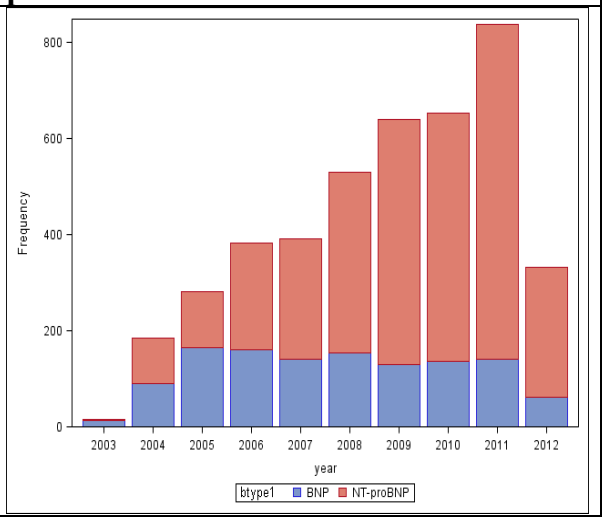
Table 4. 7 - Patients characteristics

Patient characteristic	Alive (n=3010)	Dead (n=1239)	p-value
Age (years)	77.2 ± 10.4	81.1 ± 9.3	<0.0001
Gender			0.636
Males	1488 (49.4%)	602 (48.6%)	
Females	1522 (50.6%)	637 (51.4%)	
Body mass index (kg/m^2)	28.7 ± 7.1	26.2 ± 6.0	<0.001
Underweight (<18.5)	55 (1.9%)	66 (5.8%)	
Normal weight (18.5-24.9)	814 (28.%)	439 (38.5%)	
Overweight (25-30)	992 (34.5%)	377 (33.1%)	
Obese (>30)	1011 (35.2%)	258 (22.6%)	
Smoking			0.521
Yes	222 (7.4%)	100 (8.1%)	
No	1485 (49.5%)	588 (47.7%)	
Ex-smoker	1296 (43.1%)	543 (44.1%)	
Region			<0.0001
East Midlands	229 (7.8%)	127 (10.3%)	

West Midlands	270 (9.2%)	93 (7.5%)	
London	523 (17.8%)	232 (18.7%)	
East of England	237 (8.1%)	102 (8.2%)	
North East	150 (5.1%)	78 (6.3%)	
North West	203 (6.9%)	42 (3.4%)	
South West	143 (4.8%)	46 (3.7%)	
Northern Ireland	336 (11.4%)	145 (11.7%)	
Scotland	17 (0.6%)	8 (0.6%)	
South Central	411 (13.9%)	211 (17%)	
South East Coast	360 (12.2%)	130 (10.6%)	
Yorkshire and the Humber	55 (1.9%)	23 (1.9%)	
Wales	9 (0.3%)	2 (0.1%)	
Serum creatinine (umol/L)	102.5 ± 43.4	114.8 ± 46.6	<0.0001
First BNP value (pg/ml)	n=795	n=401	<0.0001
≤ Q1 (≤131)	228 (28.7%)	72 (17.9%)	
Q1 to Q2 (131 to 304)	209 (26.3%)	92 (22.9%)	
Q2 to Q3 (304 to 800)	181 (22.7%)	115 (28.8%)	
>Q3 (>800)	177 (22.3%)	122 (30.4%)	
First NT-proBNP value	n=2115	n=838	<0.0001
(pg/ml)	623 (28.1%)	140 (16.7%)	
≤ Q1 (≤350)	558 (25.2%)	205 (24.5%)	
Q1 to Q2 (350 to 1100)	558 (25.2%)	206 (24.6%)	
Q2 to Q3 (1100 to 2738)	476 (21.5%)	287 (34.2%)	
>Q3 (> 2738)			
Diabetes at baseline	246 (8.2%)	108 (8.7%)	0.542
Hypertension and/or heart failure medication	2747 (92.1%)	1135 (92.2%)	0.949
Quartile cut off value rounded to the nearest integer.			

The patients were followed up to nine years. The average follow-up was 30.2 months or 2 ½ years, while the median follow-up was 23.7 months or almost two years. The study duration and the year when the B-type measurement was taken are displayed in the Figure 4.4.

Figure 4. 4 - Study duration and the year of baseline BNP or NT-proBNP measurements

Study duration	The year of the baseline BNP and NT-proBNP measurements
	

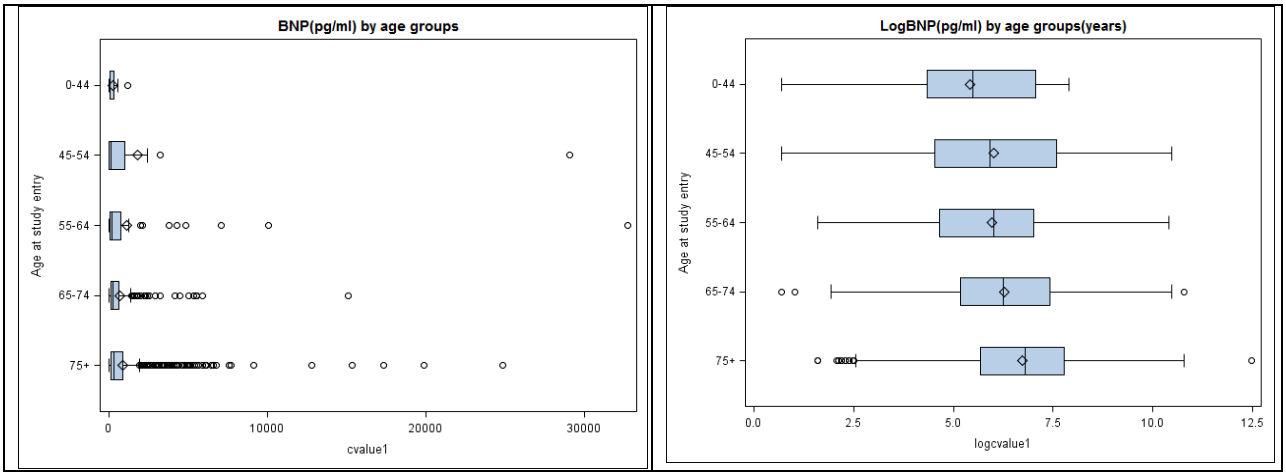
Consistent with the NICE clinical guidelines for heart failure published in 2003, the B-type natriuretic peptides testing started in 2003, recommended for diagnosing heart failure at that time. The study period ended on September 1st, 2012, which explains a lower number of B-type natriuretic peptides tests for this year in the Figure 4.4.

4.3.1. B-type natriuretic peptides in subgroups of patients

BNP and age

Most of the patients with baseline BNP levels were 75 years old or older. In this age group, the mean BNP levels were 870.4 pg/ml (95% CI: (747.7, 993.1)) based on 828 patients. Figure 4.5 displays the BNP baseline values by age groups.

Figure 4. 5 - Baseline BNP levels by age groups



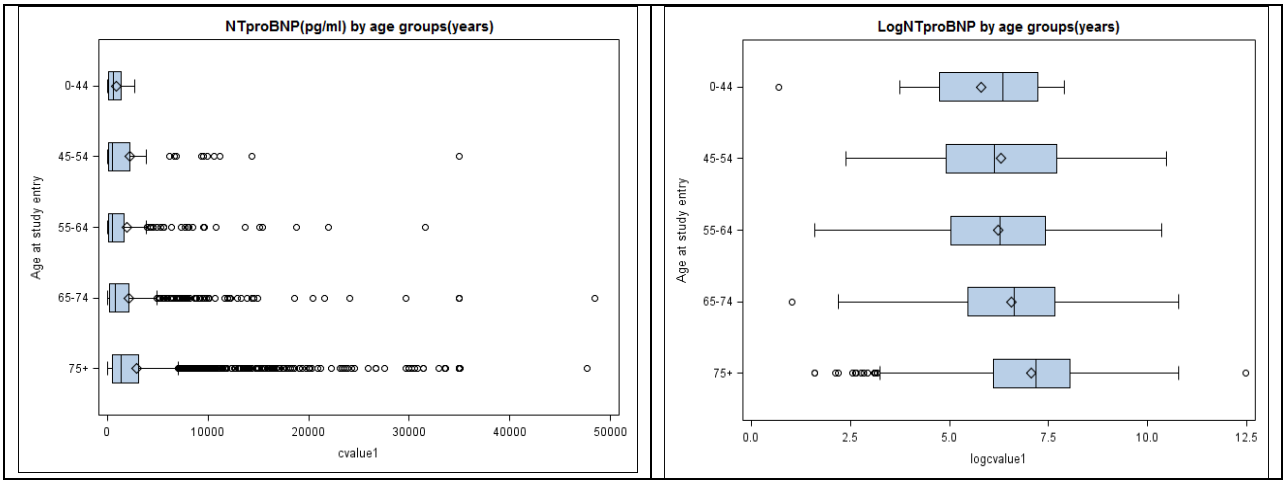
There was no linear correlation between baseline BNP levels and age. The Pearson correlation coefficient was 0.01 (95%CI: (-0.04, 0.06), p-value=0.782). However, there was a weak, non-linear association between age at the study entry and first BNP measurements. The Spearman correlation coefficient was 0.16 (95%CI: (0.10, 0.21, p-value < 0.0001).

NT-proBNP levels and age

Among the 3,053 patients with NT-proBNP measurements taken at the baseline, the highest NT-proBNP levels were observed in those 75 years old or older. In this age group, the mean NT-proBNP was 2,990.3 pg/ml (95% CI: (1796.2, 2510.8)). The mean NT-proBNP baseline levels for the 80 patients with ages between 45 and 54 years old was: 2,255 pg/ml (95% CI: (1195.6, 3314.4)). The average NT-proBNP baseline value for the 592 patients in the age group of 65-74.9 years old was 2,153. 5 pg/ml (95%CI: (1,796.2, 2,510.8)).

There was a weak correlation between age and baseline NT-proBNP levels. The Pearson correlation coefficient was 0.10 (95%CI: (0.06, 0.14), p-value <0.0001). The non-linear Spearman correlation coefficient was 0.22 (95%CI: (0.19, 0.25, p-value < 0.0001).

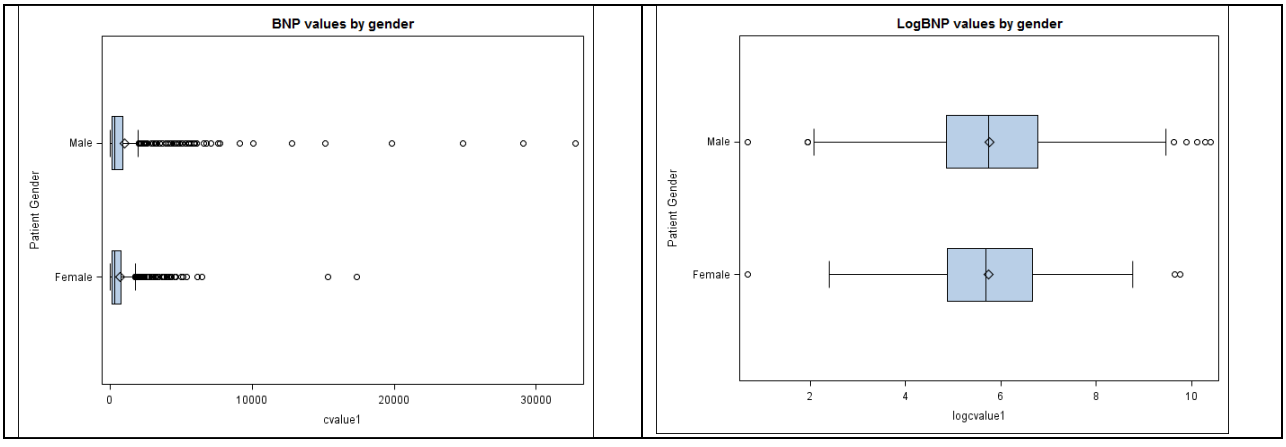
Figure 4. 6 - Baseline NT-proBNP levels by age groups



BNP and gender

The BNP levels, as well as BNP variation appeared higher for males compared to women. The mean BNP levels were 1025.5 pg/ml (95% CI: (801.7, 1249.3)) for males and 704.4 pg/ml (95%CI: (604.6, 804.1)) for females. The BNP boxplots are displayed by gender in Figure 4.7 in both original and logarithmically (base e) transformed scales.

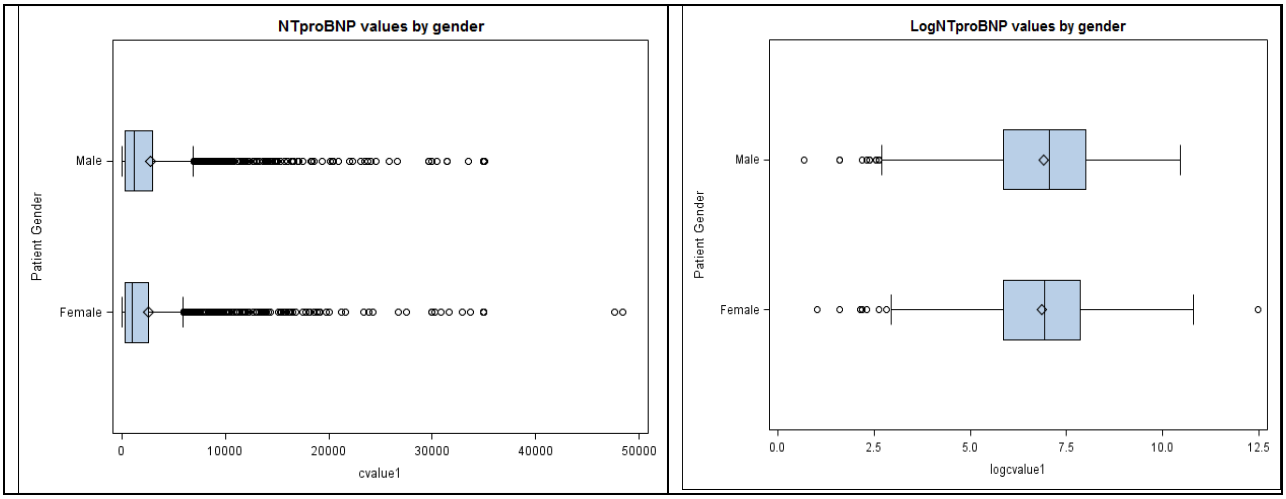
Figure 4. 7 – Baseline BNP values by gender



NT-proBNP and gender

The average NT-proBNP values were very similar for males and females.. The average NT-proBNP levels were 2,766.7 pg/ml (95% CI: (2534.6, 2998.7)) in 1526 males and 2,690.6 pg/ml (95%CI: (2,283.6, 3,097.6)).

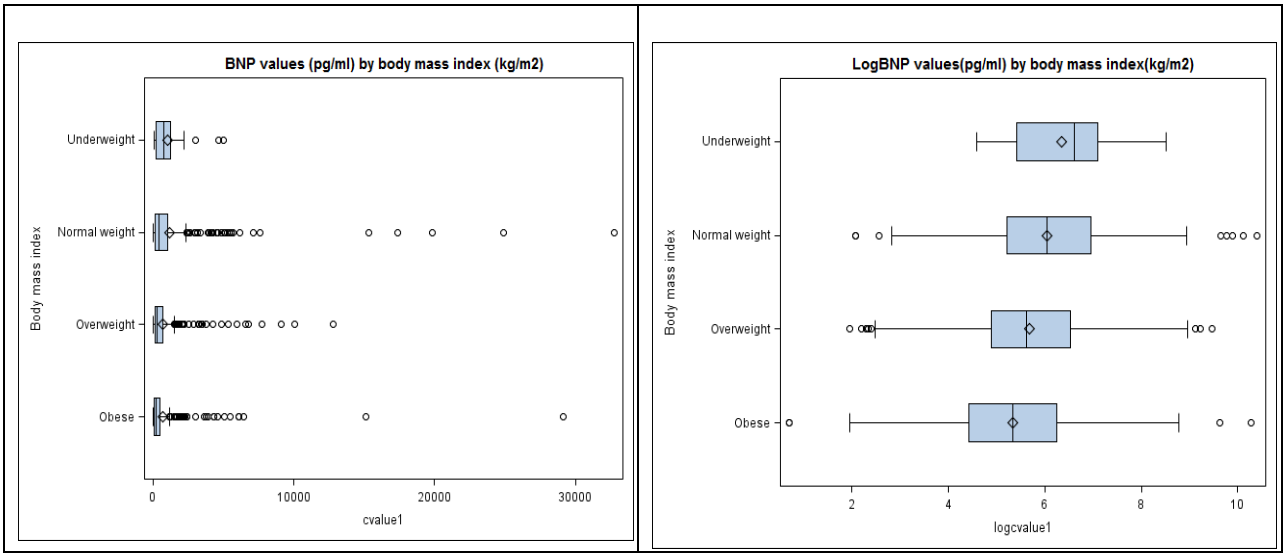
Figure 4. 8 - Baseline NT-proBNP values by gender



BNP and body mass index

Baseline BNP levels indicated a protective effect of the weight and body mass index. On average, the heavier the patients, the lower their BNP values. For the 319 obese patients, the average BNP baseline levels at baseline were 654.9 pg/ml (95%CI: (431.3, 878.5)). The mean BNP values at baseline were 680.8 pg/ml (95%CI: (550.1, 811.6)) in 387 overweight patients and 1,153.8 pg/ml (95%CI: (877.8, 1,429.8)) in 391 patients with normal weight. Figure 4.9 displays boxplots of the first BNP measurement grouped by body mass index categories.

Figure 4. 9 - Baseline BNP levels by body mass index

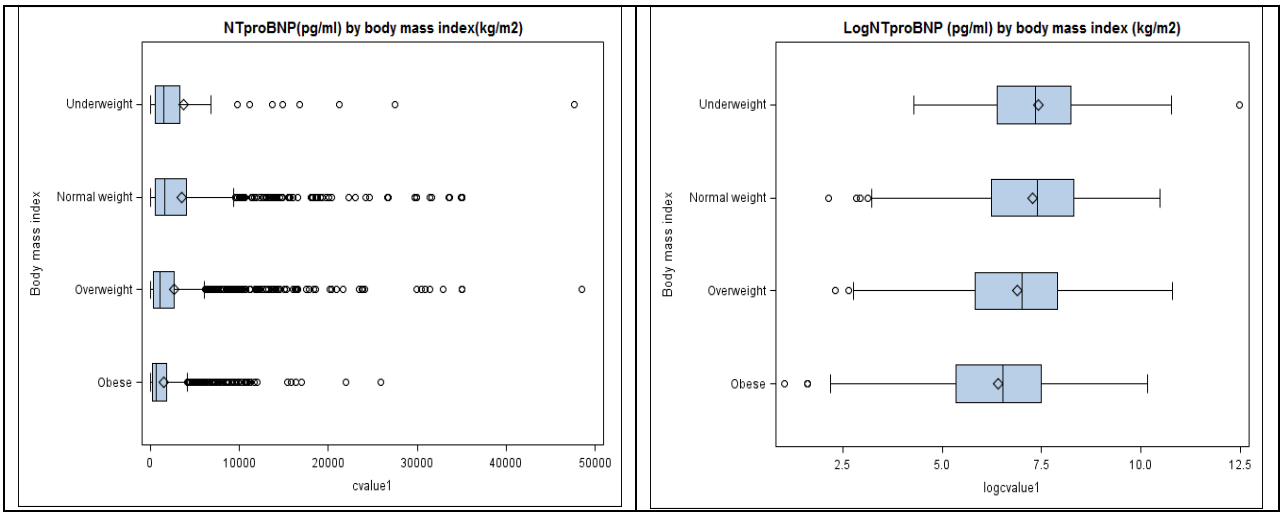


There was a mild, negative correlation between the baseline BNP levels and body mass index or weight. The Pearson correlation coefficient was -0.10 (95%CI: (-0.16, -0.05), p-value =0.0002) for the body mass index and slightly lower, -0.07 (95%CI: (-0.13, -0.01), p-value=0.013) for the weight. The non-linear Spearman correlation coefficient was -0.23 (95%CI: (-0.29, -0.18), p-value < 0.0001) for the body mass index and -0.19 (95%CI: (-0.24, -0.13), p-value<0.0001) for weight.

NT-proBNP and body mass index

A similar trend was noticed for NT-proBNP levels. On average, the heavier the patients, the lower the NT-proBNP values. For example, the average NT-proBNP levels were 1550.4 pg/ml (95%CI: (1392.3, 1708.4)) in 947 obese patients, 2699.9 pg/ml (95%CI: (2402.6, 2997.2)) in 984 overweight patients and 3494.7 pg/ml (95% CI: 3133.7, 3855.6) in 863 normal weight patients, respectively.

Figure 4. 10 - Baseline NT-proBNP levels by body mass index



The correlations between the baseline NT-proBNP levels and body mass index or weight were weak and negative. The Pearson correlation coefficient was -0.14 (95%CI: (-0.17, -0.10), p-value <0.0001) for both the body mass index and weight. The non-linear Spearman correlation coefficient was -0.24 (95%CI: (-0.28, -0.21), p-value < 0.0001) for the body mass index and -0.20 (95%CI: (-0.24, -0.17), p-value<0.0001) for weight.

B-type natriuretic peptides in diabetics

On average, both BNP and NT-proBNP levels were lower in the diabetic patients compared to the non-diabetic patients. The average BNP values at baseline were: 504.7 pg/ml (95%CI: (319.4, 690.1)) in 96 diabetic patients compared to 886.5 pg/ml (95%CI: (759.1, 1013.8)) in the 1100 non-diabetic patients at baseline. The mean NT-proBNP levels at baseline were: 2024.7 (95%CI: (1634.6, 2414.8)) in 258 diabetic patients compared to 2793.2 pg/ml (95%CI: (2539.8, 3046.6)) in 2791 non-diabetic patients at baseline. The variation of both BNP and NT-proBNP was higher in non-diabetic versus diabetic patients.

B-type natriuretic peptides and exposure to heart failure and/or hypertension medication

Those who had been exposed to heart failure and/or hypertension medication prior to the baseline had lower average BNP levels. For example, the mean BNP levels were 835.5 pg/ml (95%CI: 720.1, 950.8)) in 1073 patients exposed to heart failure and/or hypertension compared to 1069.2 pg/ml (95%CI: (432.8, 1705.5)) for 107 patients who had not been exposed to such medication by the baseline. On the other hand, the average NT-proBNP measurements were slightly higher in those who had been previously exposed to heart failure and/or hypertension medication. The average NT-proBNP values were 2,739.8 pg/ml (95%CI: (2490.8, 2988.7)) in 2809 patients who had been taking heart failure and/or hypertension medication compared to 2349.3 pg/ml (95%CI; (1834.9, 2863.6)) in 224 patients who had not been exposed to such medication.

4.3.2. B-type natriuretic peptides and all-cause mortality

The patients in the higher BNP or NT-proBNP quartiles were followed less than those in milder quartiles. Given their more severe heart failure condition, these patients died sooner. For example, those in the BNP first quartile were followed for approximately 48 months, while those in the fourth BNP quartile were in the study for about 34 months, on average. Among those with NT-proBNP data, patients in the first NT-proBNP quartile were followed for approximately 36 months, while patients in the fourth NT-proBNP quartile were followed for approximately 26 months. The median time contribution to the analysis according to the BNP, NT-proBNP and overall B-type natriuretic quarterlies is summarized in the Table 4.8.

Table 4. 8 - Time contribution (months) by the B-type natriuretic peptides

Quartile[a]	BNP[b] n Median (lower, higher quartile)	NT-proBNP[c] n Median (lower, higher quartile)	Combined[d] BNP and NT-proBNP, n Median (lower, higher quartile)
1st quartile	n=228 49.7 (23.8, 72.3)	n=623 28.8 (15.8, 49.5)	n=851 34.7 (16.8, 55.7)
2nd quartile	n=209 42.0 (18.7, 71.5)	n=558 23.4 (12.5, 40.3)	n=767 27.6 (13.3, 47.9)
3rd quartile	n=181 34.4 (14.6,)	n=558 24.5 (12.5, 41.1)	n=739 26.1 (12.6, 45.4)
4th quartile	n=177 27.8 (13.7, 47.1)	n=475 20.5 (10.6, 37.7)	n=652 22.1 (11.4, 40.0)

a. Quartiles defined based on BNP values, NT-proBNP values and overall B-type natriuretic peptides according to the column being displayed.

b. BNP quartiles were defined based on the following cutoff BNP values: Q1 (≥131 pg/ml), Q1 to Q2 (131 to 304 pg/ml), Q2 to Q3 (304 to 800 pg/ml), ≤Q3 (≤800 pg/ml)

c. NT-proBNP quartiles were defined based on the following NT-proBNP values: ≥Q1 (≥350 pg/ml), Q1 to Q2 (350 to 1100 pg/ml), Q2 to Q3 (1100 to 2738 pg/ml), >Q3 (> 2738 pg/ml)

d. First combined quartile if patient was either in the 1st BNP or 1st NT-proBNP, regardless the type of natriuretic peptide measurement. Similar definition for the second, third and fourth quartiles.

e. Time contribution was expressed in months since the study start

When treated as a continuous variable, the prognostic impact of a single peptide on the all-cause mortality is shown in Table 4.9. For both BNP and NT-proBNP, a unit increase in both log(base-e)-BNP and log (base e)-NT-proBNP led to an increased all-cause mortality.

Table 4. 9 – Prognostic analysis of B-type natriuretic peptides (log scale) and all-cause mortality

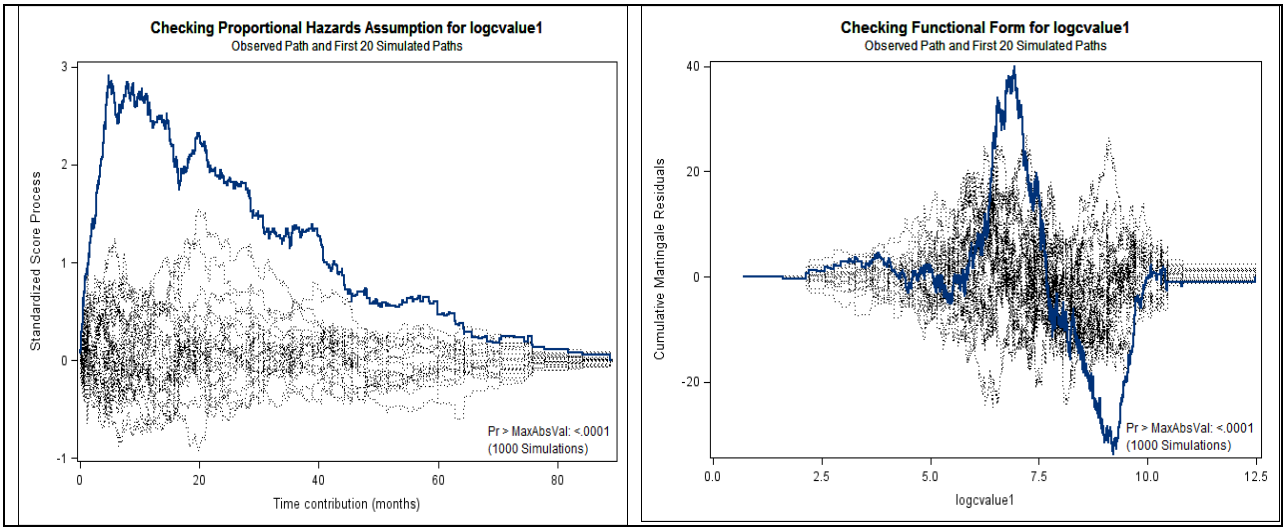
Natriuretic Peptide	Model	N	All cause-mortality HR (95% CI)
Log-BNP	Unadjusted	1196	1.32 (1.23,1.41)
	Adjusted Model[a]	1048	1.23 (1.13, 1.34)
Log-NT-proBNP	Unadjusted	3053	1.37 (1.30, 1.43)
	Adjusted Model [a]	2671	1.24 (1.17, 1.32)

1. Model adjusted for age, gender, BMI, smoking, geographic region, serum creatinine, hypertension and/or heart failure medication and diabetes at baseline

The all-cause mortality hazard ratio estimates for $\log_e$-BNP and $\log_e$-NT-proBNP were very similar. The unadjusted all-cause mortality hazard ratio estimate for $\log_e$-BNP was 1.32 (95%CI: (1.23, 1.41)) and 1.37 (95%CI: (1.30, 1.43)) for $\log_e$-NT-proBNP. This means that for every unit increase in the $\log_e$-BNP, there is a 1.32 fold increase in the risk of death. Similarly, for every unit increase in the $\log_e$-NT-proBNP there is a 1.37 fold increase in the risk of death. Both BNP and NT-proBNP were independent predictors of all-cause mortality. The unadjusted all-cause mortality survival model for $\log_e$BNP indicated a proper fit.

The Figure 4.11 shows the lack of proportional hazards and linearity for $\log_e$NTproBNP, however the form was not clear. Following a Brownian process, a cloud of random paths was generated under the proportional hazards or linearity and then the actual data was compared to the randomly generated paths under the proportional hazards or linearity assumptions, respectively. Since the actual data paths were not within the cloud of random paths, it indicated non-proportional hazards, as well as non-linearity for $\log_e$NT-proBNP, but the form was not clear. Model fitting details can be found in the Appendix E.

Figure 4. 11 - Checking proportional hazards and linearity for logNT-proBNP



Given the shortcomings of this model, the prognostic effect of the NT-proBNP on the all-cause mortality was also analyzed when NT-proBNP was treated as a categorical variable based on its quartiles.

Compared to the unadjusted results, the hazard ratio estimates were slightly lower when adjusting for baseline patient characteristics such as: age at the study entry, gender, body mass index, smoking and diabetes status, geographic region, serum creatinine, and exposure to hypertension and/or heart failure medication. The adjusted all-cause mortality hazard ratio estimates were 1.23 (95% CI: (1.13, 1.34)) for $\log_e$-BNP and 1.24 (95%CI: (1.17, 1.23)) for log-NT-proBNP.

In addition to the baseline BNP measurements, age and creatinine appeared to be statistically significant at the 5% significance level, however the hazard ratio estimates were close to unity. For example, the hazard ratios for creatinine was 1.005 (95%CI: (1.003, 1.007))

respectively. The hazards ratio estimates for age are presented below. No other baseline characteristics reached statistical significance.

Table 4. 10 - The effect of age on all-cause mortality from the adjusted Cox regression model with logBNP

Parameter	Hazard ratio	95% Confidence interval
Age[1]		
1-unit change	1.05	(1.03, 1.06)
10-unit change	1.64	(1.42, 1.90)
At 40 years[2]	0.95	(0.91, 1.00)
At 50 years[2]	0.98	(0.95, 1.01)
At 60 years[2]	1.01	(0.98, 1.03)
At 70 years[2]	1.03	(1.02, 1.04)
At 80 years[2]	1.06	(1.04, 1.07)
At 90 years[2]	1.09	(1.06, 1.11)

[1] Age at baseline; [2]1-unit change
Model adjusted for logBNP, age, gender, BMI, smoking, geographic region, serum creatinine, hypertension and/or heart failure medication and diabetes at baseline

In addition to the baseline NT-proBNP measurement, age at the study entry, baseline body mass index and serum creatinine had a statistically significant effect on the all-cause mortality, although the hazard ratios were very close to the unity. For example, the hazard ratio for body mass index and creatinine were 0.97 (95% CI: (0.96, 0.99)), and 1.002 (95%CI: (1.001, 1.003)) respectively. The hazard ratios for age are presented below. The table shows how the risk of all-cause mortality changes with every decade starting at 40 years old. Age statistically significantly impacted the all-cause mortality once the patients turned 70 years old/ Thus, for every additional year, there was a 2% increased risk of all-cause mortality. Overall, for each 10-year increase in age, the risk of all-cause mortality increased by 39%. No other baseline characteristics reached statistical significance at the 5% level.

Table 4. 11 - The effect of age on all-cause mortality from the adjusted Cox regression model with logNTproBNP

Parameter	Hazard ratio	95% Confidence interval
Age[1]		
1-unit change	1.03	(1.02, 1.04)
10-unit change	1.39	(1.26, 1.54)
At 40 years[2]	0.95	(0.92, 0.99)
At 50 years[2]	0.97	(0.95, 1.00)
At 60 years[2]	0.99	(0.98, 1.01)
At 70 years[2]	1.02	(1.01, 1.03)
At 80 years[2]	1.04	(1.03, 1.05)
At 90 years[2]	1.06	(1.04, 1.08)

[1] Age at baseline; [2]1-unit change
Model adjusted for logNTproBNP, age, gender, BMI, smoking, geographic region, serum creatinine, hypertension and/or heart failure medication and diabetes at baseline

Additional Cox regression models treated the first B-type natriuretic peptides as categorical variables defined by their respective quartiles. The BNP quartiles were defined based on the following cutoff BNP values: Q1 (≤131 pg/ml), Q1 to Q2 (131 to 304 pg/ml), Q2 to Q3 (304 to 800 pg/ml), ≥Q3 (≥800 pg/ml). The NT-proBNP quartiles were defined based on the following NT-proBNP values: ≤ Q1 (≤350 pg/ml), Q1 to Q2 (350 to 1100 pg/ml), Q2 to Q3 (1100 to 2738 pg/ml), >Q3 (> 2738 pg/ml). The overall quartiles were defined according to the patient quartile classification, irrespective of the type of quartile. For example, if a patient was in the 3rd NT-proBNP quartile, then this patient would also be in the third overall quartile.

The association between the BNP and NT-proBNP quartiles and all-cause mortality is presented in Table 4.12. The risk of all-cause mortality increased with each subsequent BNP quartiles and combined natriuretic peptide quartiles.

Table 4. 12 - Prognostic analysis of B-type natriuretic peptides (categorical quartiles) and all-cause mortality

Natriuretic peptide	Comparison	Unadjusted HR (95% CI)	Adjusted[a] HR (95% CI)
BNP	Quartile 2 vs. Quartile 1	1.43 (1.05, 1.96)	1.29 (0.92, 1.82)
	Quartile 3 vs. Quartile 1	2.27 (1.69, 3.05)	1.98 (1.42, 2.75)
	Quartile 4 vs. Quartile 1	2.79 (2.08, 3.75)	2.23 (1.59, 3.13)
NT-proBNP	Quartile 2 vs. Quartile 1	1.88 (1.52, 2.33)	1.67 (1.31, 2.13)
	Quartile 3 vs. Quartile 1	1.84 (1.48, 2.29)	1.46 (1.14, 1.87)
	Quartile 4 vs. Quartile 1	3.27 (2.66, 4.01)	2.18 (1.71, 2.78)
NT-proBNP and BNP respective quartiles combined	Quartile 2 vs. Quartile 1	1.72 (1.44, 2.06)	1.56 (1.29, 1.90)
	Quartile 3 vs. Quartile 1	1.98 (1.67, 2.36)	1.67 (1.37, 2.03)
	Quartile 4 vs. Quartile 1	3.10 (2.62, 3.67)	2.27 (1.87, 2.78)

a. Model adjusted for age, gender, BMI, smoking, geographic region, serum creatinine, hypertension and/or heart failure medication and diabetes at baseline

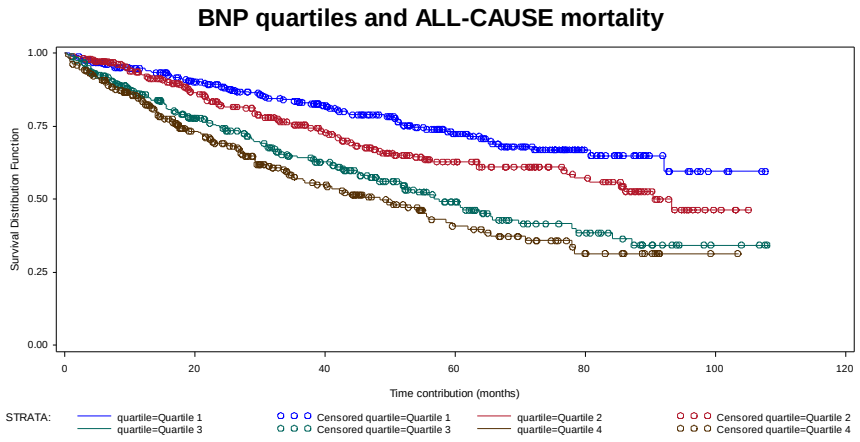
b. BNP quartiles were defined based on the following cutoff BNP values: Q1 (≤131 pg/ml), Q1 to Q2 (131 to 304 pg/ml), Q2 to Q3 (304 to 800 pg/ml), ≥Q3 (≥800 pg/ml)

c. NT-proBNP quartiles were defined based on the following NT-proBNP values: ≤ Q1 (≤350 pg/ml), Q1 to Q2 (350 to 1100 pg/ml), Q2 to Q3 (1100 to 2738 pg/ml), >Q3 (> 2738 pg/ml)

d. First combined quartile if patient was either in the 1st BNP or 1st NT-proBNP, regardless the type of natriuretic peptide measurement. Similar definition for the second, third and fourth quartiles

The risk of the all-cause mortality increased with each subsequent BNP quartile compared to the first quartile. This can also be visualized in Figure 4.12. The four BNP quartiles were statistically different. The chi-square value of the log-rank test of equality over strata was 61.1495 (degrees of freedom=3, p-value < 0.0001). The same conclusion was reached based on the Wilcoxon rank test, which had a statistically significant chi-square test value of 57.2271 (degrees of freedom=3. p-value < 0.0001). The percent censored for the first, second, third and fourth quartiles were: 77%, 69.46%, 58.86% and 57.19%, respectively.

Figure 4. 12- The survival distribution function for BNP quartiles



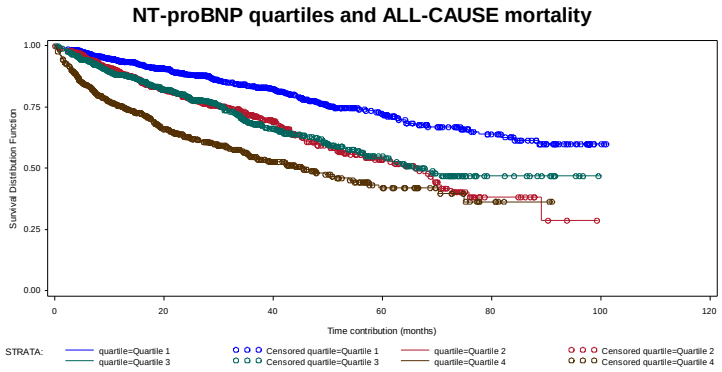
Patients with B-type natriuretic peptide concentrations in the highest quartile had a 2.79 fold unadjusted increase in mortality compared to those in the lowest quartile. When adjusting for age, gender, body mass index, smoking status, geographic region, serum creatinine, hypertension and/or heart failure medication, and diabetes status, the hazard ratio became 2.23 (95%CI: (1.59, 3.13)). Patients in the second and third BNP quartiles had a 1.43 and 2.27 fold unadjusted increase in the all-cause mortality compared to those in the lowest quartile. When adjusting for the patients' demographics and baseline characteristics, the hazard ratios decreased to 1.29 (95%CI: (0.92, 1.82)) and 1.98 (95%CI: (1.42, 2.75)) for the second and third BNP quartiles respectively.

Adjusting for age, gender, body mass index, smoking status, geographic region, serum creatinine, hypertension and/or heart failure medication, diabetes status at the baseline resulted in a lower all-cause mortality risk. BNP quartiles, age, serum creatinine and smoking status hypertension and/or heart failure medication at baseline significantly impacted the all-cause mortality at the 5% significance level.

The second and third NT-proBNP quartiles led to a similar all-cause mortality risk in the first five years, however this risk gets higher for the second rather than third quartile beyond 70

months from the study start. The fourth quartile was significantly higher when compared to the first NT-proBNP quartile. This can also be visualized in the Figure 4.13. The four NT-proBNP quartiles were statistically different. The chi-square value of the log-rank test of equality over strata was 145.5787 (degrees of freedom=3, p-value < 0.0001). The same conclusion was reached based on the Wilcoxon rank test, which had a statistically significant chi-square test value of 171.3652 (degrees of freedom=3. p-value < 0.0001). The percent censored for the first, second, third and fourth quartiles were: 81.83%, 72.05%, 72.74% and 62.12%, respectively.

Figure 4. 13 - The survival distribution function for NT-proBNP quartiles

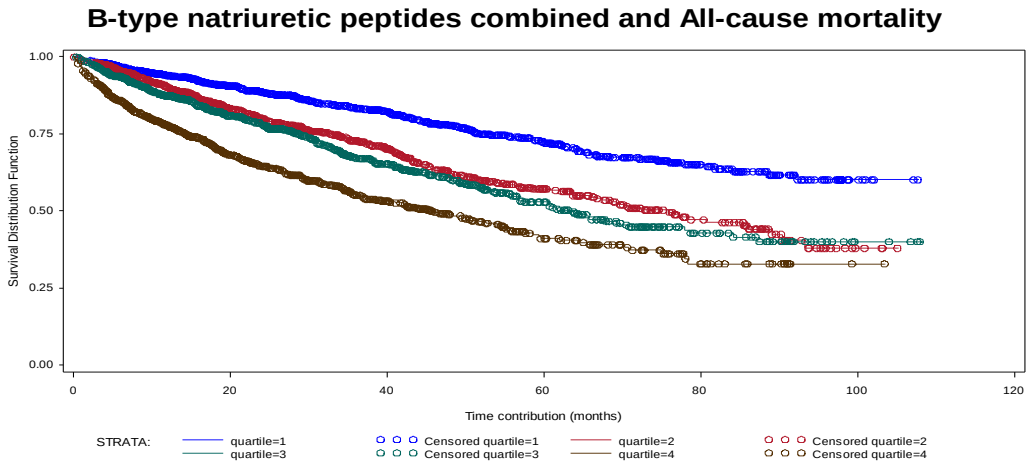


Patients with NT-proBNP natriuretic peptide concentrations in the highest quartile had a 3.27-fold unadjusted increase in mortality compared to those in the lowest quartile. When adjusting for age, gender, body mass index, smoking status, geographic region, serum creatinine, hypertension and/or heart failure medication, and diabetes status, the hazard ratio decreased to 2.18 (95%CI: (1.70, 2.78)). Patients in the second and third NT-proBNP quartile had a 1.8-fold unadjusted increase in the all-cause mortality compared to those in the lowest quartile. When adjusting for the patients' demographics and baseline characteristics, the hazard

ratios slightly decreased to 1.67 (95%CI: (1.31, 2.13)) and 1.46 (95%CI: (1.14, 1.87)) for the second and third NT-proBNP quartiles respectively.

\The risk of the all-cause mortality increased with each subsequent quartile, when combining all B-type natriuretic peptides according to their respective quartiles at baseline. This can also be visualized in the Figure 4.14. The four combined quartiles were statistically different. The chi-square value of the log-rank test of equality over strata was 196.5675 (degrees of freedom=3, p-value < 0.0001). The same conclusion was reached based on the Wilcoxon rank test, which had a statistically significant chi-square test value of 214.6362 (degrees of freedom=3. p-value < 0.0001). The percent censored for the first, second, third and fourth quartiles were: 80.47%, 71.32%, 68.83% and 60.73%, respectively.

Figure 4. 14 - The survival distribution function for BNP quartiles



Combining all quartiles led to a 1.72, 1.98 and 3.10 fold unadjusted increase in the all-cause mortality for the second, third and fourth quartile when compared to the first combined quartile. When adjusting for age, gender, body mass index, smoking status, geographic region, serum creatinine, hypertension and/or heart failure medication, and diabetes status, apatients in the

second, third and fourth combined quartiles had a 1.56 1.67, and 2.27-fold unadjusted increase in the all-cause mortality compared to those in the lowest quartile, respectively.

Additional survival analyses were conducted where the B-type natriuretic peptides quartiles were also treated as a continuous variable taking the following values: 1, 2, 3, and 4. The results are summarized below.

Table 4. 13 - Prognostic analysis of B-type natriuretic peptides (continuous quartile) and all-cause mortality

Natriuretic peptide (quartile continuous)	N	Unadjusted HR (95% CI)	Adjusted[a] HR (95% CI)
BNP	1,196	1.41 (1.29, 1.54)	1.32 (1.19, 1.47)
NT-proBNP	3,053	1.41 (1.33, 1.51)	1.23 (1.14, 1.33)
NT-proBNP and BNP respective quartiles combined	4,249	1.41 (1.34, 1.49)	1.26 (1.19, 1.34)
a. Model adjusted for age, gender, BMI, smoking, geographic region, serum creatinine, hypertension and/or heart failure medication and diabetes at baseline			

With every B-type natriuretic peptide quartile, there is an increased risk of death by a factor of 1.41. The 95% confidence interval for the hazard ratio does not include 1, indicating the statistical significance of the BNP quartiles.

Adjusting for all demographics and baseline characteristics led to a slightly lower risk of death of a factor of 1.32 for every increasing BNP quartile. Factors significantly impacting the all-cause mortality at the 5% significance level were: quartiles, age, smoking status, and serum creatinine. For example, for every additional year, the risk of all-cause mortality is increased by approximately 5%, all other factors staying the same (hazard ratio of 1.058 (95% CI: (1.04, 1.07)). As expected, ex-smokers and non-smokers had a lower mortality risk than smokers. The hazard ratios were 0.44 (95%CI: (0.30, 0.64)) for ex-smokers and 0.36 (95%CI:

(0.30, 0.64)) when compared to non-smokers. The serum creatinine hazard ratio estimates were very close to unity (1.006 (95% CI: (1.003, 1.008)).

Similar to the results observed for the BNP quartiles, with every NT-proBNP quartile, there is an increased risk of death by a factor of 1.41. The 95% confidence interval for the hazard ratio was (1.33, 1.51), indicating the statistical significance of the NT-proBNP quartiles.

Adjusting for age, gender, body mass index, smoking status, geographical region, heart failure and/or hypertension medication and diabetes status at baseline led to a slightly lower hazards ratio of 1.23 (95%CI: (1.14, 1.33)) for all-cause mortality. The following baseline characteristics had a statistically significant impact on the all-cause mortality: NT-proBNP quartiles, age, body mass index, smoking status and serum creatinine. For example, a patient 1 year older than another patient had an increased risk of dying by a factor of 1.03 (95%CI: (1.02, 1.04)), assuming that all other characteristics are the same. The body mass index seemed to have a protective effect on survival, since the hazard ratio for the all-cause mortality was 0.97 (95% CI: (0. 95, 0.98)). Ex-smokers and non-smokers were less likely to die than smokers. The all-cause mortality hazard ratio was 0.69 (95%CI: (0.50, 0.93)) for ex-smokers and 0.66 (95% CI: (0.49, 0.89)) for non-smokers as compared to smokers. The effect of the serum creatinine was minimal, as the hazard ratio was very close to1 (HR=1.003 (95%CI: (1.002, 1.004)).

Combining all the B-type natriuretic peptides according to the baseline quartiles resulted in similar results. The unadjusted hazard ratio for the continuous quartile was 1.41 (95%CI: (1.34, 1.49)). When adjusting for the demographics and baseline characteristics, the all-cause mortality hazard ratio was slightly lower (HR=1.26, 95%CI: (1.19, 1.34)). The combined B-type natriuretic peptide quartiles, age, body mass index, smoking, serum creatinine

and diabetes status at baseline significantly impacted the all-cause mortality. The hazard ratio estimates were very similar to previous results: 1.04 (95%CI: (1.03, 1.05)) for age, 0.97 (95%CI: (0.96, 0.98)) for the body mass index, 0.59 (0.46, 0.74) and 0.54 (0.42, 0.68) for ex-smokers and non-smokers as compared to smokers, 1.003 (95%CI: (1.002, 1.004)) for serum creatinine, and 1.26 (1.02, 1.55) for diabetics at baseline.

4.3.3. B-type natriuretic peptides and cardiovascular mortality

The entire study cohort consisted of 4,249 patients, of whom 1239 or 29.2% died of any cause. There were 628 patients or 14.8% who died of cardiovascular diseases. Half of them were males and half were females. These patients were old and slightly overweight. The median age was 82 years old, while the median body mass index was 25.7 kg/m2. Approximately 36% were of normal weight, 35% were overweight and only 22% were obese. Eighty-two percent of these patients who died of cardiovascular diseases were 75 years old or older at the study entry. On average, they were followed for 22 months, ranging from three days to 7.5 years. Almost all (92.2%) of this sub-set of patients were taking hypertension and/or heart failure medication at baseline. Although approximately 9% of the patients who died of cardiovascular diseases had diabetes at the study start, 27% became diabetic at one point during the study. When treated as a continuous variable, the prognostic impact of a single B-type natriuretic peptide on the cardiovascular mortality is shown in the table below. For both BNP and NT-proBNP, a unit increase in both log(base-e)-BNP and log (base e)-NT-proBNP led to an increased risk of cardiovascular mortality.

Table 4. 14 - Prognostic analyses of B-type natriuretic peptides (log scale) and cardiovascular mortality

Natriuretic Peptide	Model	All cause-mortality HR (95% CI)
Log-BNP	Unadjusted	1.41 (1.29,1.55)
	Adjusted Model[a]	1.32 (1.18, 1.46)
Log-NT-proBNP	Unadjusted	1.39 (1.29, 1.49)
	Adjusted Model [a]	1.33 (1.22, 1.44)

1. Model adjusted for age, gender, BMI, smoking, geographic region, serum creatinine, hypertension and/or heart failure medication and diabetes at baseline

The cardiovascular mortality hazard ratio estimates for $\log_e$-BNP and $\log_e$-NT-proBNP were very similar. On the naturally logarithmic scale, each unit increase in the B-type natriuretic peptides was associated with a 1.4 fold increase in the cardiovascular mortality. The unadjusted cardiovascular mortality hazard ratio estimate for $\log_e$-BNP was 1.41 (95%CI: (1.29, 1.55)) and 1.39 (95%CI: (1.29, 1.49)) for $\log_e$-NT-proBNP. Both BNP and NT-proBNP were independent predictors of cardiovascular mortality.

The cardiovascular mortality risk slightly decreased upon adjusting for all variables. Each unit increase in the logarithmic value of BNP or NT-proBNP was associated with a 1.3 fold increase in the cardiovascular mortality. The adjusted cardiovascular mortality hazard ratio estimate for $\log_e$-BNP was 1.32 (95% CI: (1.18, 1.46)) and 1.33 (95% CI: (1.22, 1.44) for $\log_e$-NT-proBNP.

In addition to the logBNP, age, serum creatinine and smoking status at the baseline had significantly impacted the cardiovascular mortality. While being an ex-smoker or non-smoker had a protective effect, every additional year in age increased the risk of cardiovascular

mortality by 7% in chronic heart failure patients. Although serum creatinine had a statistically significant impact, the result was not clinically significant. These results are presented below.

Table 4. 15 - Adjusted Cox regression model for cardiovascular mortality with logBNP

Parameter	Hazard ratio	95% Confidence interval
$\log_e$BNP	1.32	(1.18, 1.46)
Age[1]		
1-unit change	1.07	(1.05, 1.09)
10-unit change	1.85	(1.53, 2.23)
At 40 years[2]	0.96	(0.90, 1.02)
At 50 years[2]	0.98	(0.94, 1.03)
At 60 years[2]	1.01	(0.98, 1.04)
At 70 years[2]	1.04	(1.02, 1.06)
At 80 years[2]	1.07	(1.05, 1.09)
At 90 years[2]	1.10	(1.06, 1.13)
Serum creatinine[1]	1.005	(1.002, 1.008)
Smoking[1]		
Ex-smoker	0.39	(0.24, 0.65)
No	0.39	(0.23, 0.65)
Yes	reference	reference

[1] Age, serum creatinine and smoking were assessed at baseline; [2]1-unit change
Model adjusted for logBNP, age, gender, BMI, smoking, geographic region, serum creatinine, hypertension and/or heart failure medication and diabetes at baseline

In the adjusted Cox regression model for logNT-proBNP, the following factors reached statistical significance at the 5% level: logNT-proBNP, age, serum creatinine, body mass index, and diabetes status at baseline. Higher body mass index seemed to have a slight protective effect on the cardiovascular mortality. For every additional year in the age of chronic heart failure patients there is a 3% increased the risk of cardiovascular mortality. No clinical impact on the cardiovascular mortality was noted for the serum creatinine. Chronic heart failure patients who were diabetic at the start of the study were 1.45 more likely to die due to a cardiovascular cause than the non-diabetics. These results are presented in the Table 4.16:

Table 4. 16 - Adjusted Cox regression model for cardiovascular mortality with logNT-proBNP

Parameter	Hazard ratio	95% Confidence interval
logNT-proBNP	1.33	(1.22, 1.44)
Age[1]		
1-unit change	1.03	(1.01, 1.04)
10-unit change	1.38	(1.19, 1.60)
At 40 years[2]	0.95	(0.90, 1.00)
At 50 years[2]	0.97	(0.93, 1.01)
At 60 years[2]	0.99	(0.97, 1.02)
At 70 years[2]	1.02	(1.01, 1.03)
At 80 years[2]	1.04	(1.02, 1.06)
At 90 years[2]	1.06	(1.04, 1.09)
Serum creatinine[1]	1.002	(1.001, 1.004)
Body mass index	0.96	(0.94, 0.98)
Diabetes		
Yes	1.45	(1.02, 2.06)
No	reference	reference

[1] Age, serum creatinine, smoking, body mass index, diabetes at baseline; [2]1-unit change
Model adjusted for logNTproBNP, age, gender, BMI, smoking, geographic region, serum creatinine, hypertension and/or heart failure medication and diabetes at baseline

The associations between the B-type natriuretic peptide quartiles and cardiovascular mortality are presented below. The risk of cardiovascular mortality increased with each subsequent BNP or NT-proBNP quartiles, as well as combined quartiles.

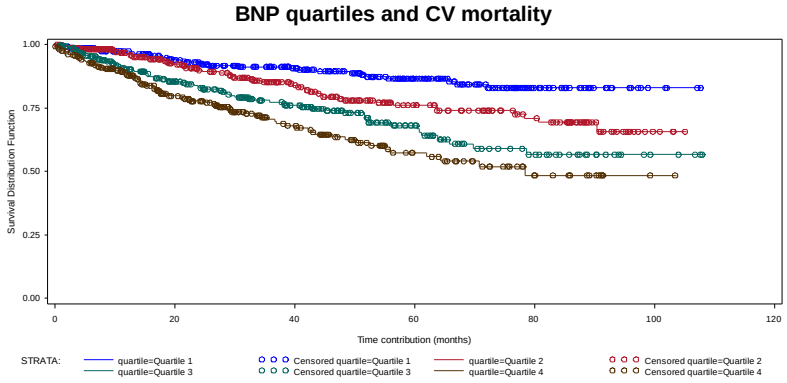
Table 4. 17 - Prognostic analyses of BNP, NT-proBNP quartiles and CV mortality

Natriuretic peptide	Comparison	Unadjusted HR (95% CI)	Adjusted* HR (95% CI)
BNP	Quartile 2 vs. Quartile 1	1.81 (1.16, 2.81)	1.67 (1.06, 2.77)
	Quartile 3 vs. Quartile 1	2.78 (1.83, 4.23)	2.26 (1.61, 4.18)
(n=1196)	Quartile 4 vs. Quartile 1	3.88 (2.58, 5.85)	3.20 (1.99, 5.13)
NT-proBNP	Quartile 2 vs. Quartile 1	1.59 (1.16, 2.18)	1.72 (1.21, 2.44)
	Quartile 3 vs. Quartile 1	1.50 (1.09, 2.06)	1.60 (1.12, 2.29)
(n=3053)	Quartile 4 vs. Quartile 1	3.35 (2.51, 4.48)	2.98 (2.12, 4.20)
NT-proBNP and BNP respective quartiles combined (n=4249)	Quartile 2 vs. Quartile 1	1.65 (1.28, 2.12)	1.64 (1.24, 2.17)
	Quartile 3 vs. Quartile 1	1.90 (1.48, 2.44)	1.89 (1.43, 2.51)
	Quartile 4 vs. Quartile 1	3.51 (2.78, 4.44)	3.01 (2.29, 3.96)

a. Model adjusted for age, gender, BMI, smoking, geographic region, serum creatinine, hypertension and/or heart failure medication and diabetes at baseline
b. BNP quartiles were defined based on the following cutoff BNP values: Q1 (≤131 pg/ml), Q1 to Q2 (131 to 304 pg/ml), Q2 to Q3 (304 to 800 pg/ml), ≥Q3 (≥800 pg/ml)
c. NT-proBNP quartiles were defined based on the following NT-proBNP values: ≤ Q1 (≤350 pg/ml), Q1 to Q2 (350 to 1100 pg/ml), Q2 to Q3 (1100 to 2738 pg/ml), >Q3 (> 2738 pg/ml)
d. First overall quartile if patient was either in the 1st BNP or 1st NT-proBNP, regardless the type of natriuretic peptide measurement. Similar definition for the second, third and fourth quartiles
e. Natriuretic peptides treated as categorical variables

Figure 4.15 shows that the risk of the cardiovascular mortality increased with each subsequent BNP quartile compared to the first BNP quartile. The four BNP quartiles were statistically different. The chi-square value of the log-rank test of equality over strata was 53.5685 (degrees of freedom=3, p-value < 0.0001). The same conclusion was reached based on the Wilcoxon rank test, which had a statistically significant chi-square test value of 49.5532 (degrees of freedom=3. p-value < 0.0001). The percent censored for the first, second, third and fourth quartiles were: 89.3%, 82.2%, 76.6% and 71.9%, respectively.

Figure 4. 15 - Cardiovascular mortality and the survival distribution for BNP quartiles



Patients with B-type natriuretic peptide concentrations in the highest quartile were almost 4 times most likely to die from a cardiovascular disease than those in the first, lowest BNP quartile (HR=3.88 (95%CI: (2.58, 5.85)). When adjusting for age, gender, body mass index, smoking status, geographic region, serum creatinine, hypertension and/or heart failure medication, and diabetes status, this hazard ratio was slightly lowered to 3.2 (95% CI: (1.99, 5.13). Patients in the second and third BNP quartiles had a 1.81 and 2.78 fold unadjusted increase in the cardiovascular-cause mortality compared to those in the lowest quartile. When adjusting for the patients' demographics and baseline characteristics, the hazard ratios slightly decreased to 1.67 (95%CI: (1.06, 2.77)) and 2.26 (95%CI: (1.61, 4.18)) for the second and third BNP quartiles respectively.

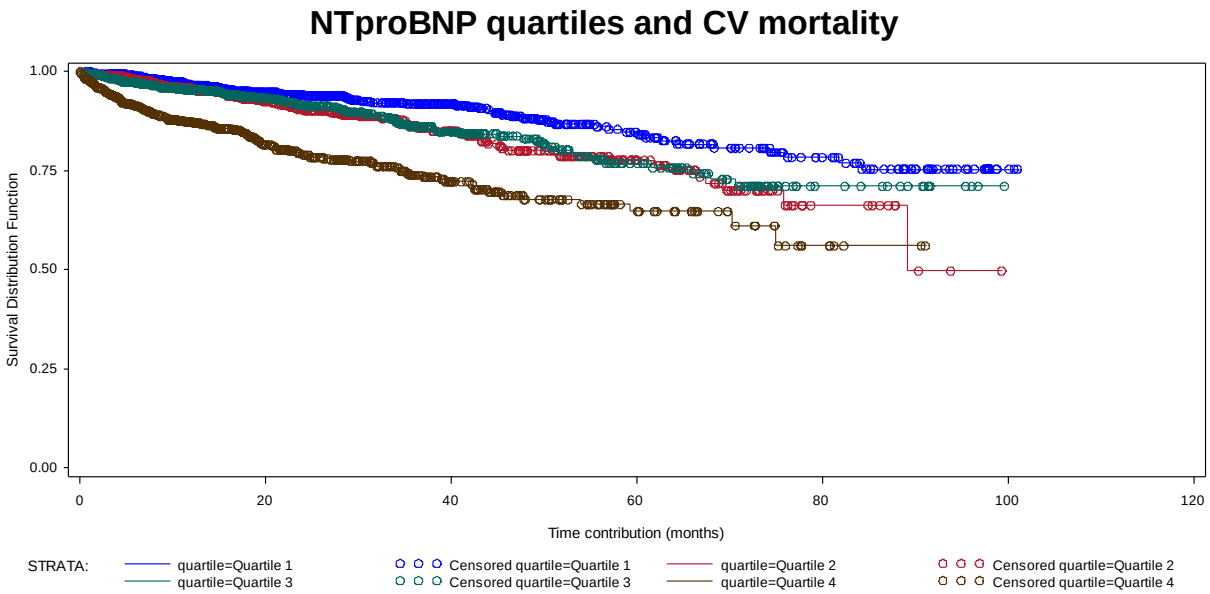
When BNP quartiles were treated as a continuous variable taking the values of 1, 2, 3, 4, for every BNP quartile, there was an increased risk of death due to cardiovascular diseases by a factor of 1.54 (95%CI: (1.36, 1.73)). The result was statistically significant at the 5% level (p-value < 0.0001). Model diagnostics indicated a proper statistical fit.

When adjusting for the baseline characteristics such as age, gender, body mass index, smoking, geographic region, serum creatinine, hypertension and/or heart failure medication, and diabetes status, the risk of cardiovascular death slightly decreased. For every BNP continuous quartile, there was a risk of cardiovascular death by a factor of 1.44 (95% CI: (1.25, 1.65)). The following factors impacted the cardiovascular mortality at the 5% level: BNP continuous quartiles, age, smoking status and serum creatinine at the baseline. For every year getting older, there was a 6,9% increased risk of cardiovascular death in chronic heart failure patients (HR=1.069 (95% CI: (1.048, 1.090), p-value<0.0001). Not smoking or being an ex-smoker decreased the risk of cardiovascular death. The hazard ratio for ex-smokers was 0.41 (95%CI:

(0.24, 0.67)) and 0.40 (95%CI: (0.24, 067)) for non-smokers, when compared to smokers. Although, the serum creatinine reached statistical significance, the hazard ratio estimates were very close to the unity: 1.006 (95%CI: (1.003, 1.009)). The diagnostic modelling showed mixed results, which are detailed in the Appendix E.

As depicted in the Figure 4.16, the risk of cardiovascular mortality is similar for the second and third NT-proBNP quartiles for the first six years into the study. Overall, the four NT-proBNP quartiles had statistically different cardiovascular risks. The chi-square value of the log-rank test of equality over strata was 88.2427 (degrees of freedom=3, p-value < 0.0001). The same conclusion was reached based on the Wilcoxon rank test, which had a statistically significant chi-square test value of 105.1266 (degrees of freedom=3. p-value < 0.0001). The percent censored for the first, second, third and fourth NT-proBNP quartiles were: 90.85%, 88.32%, 88.99% and 80.87%, respectively.

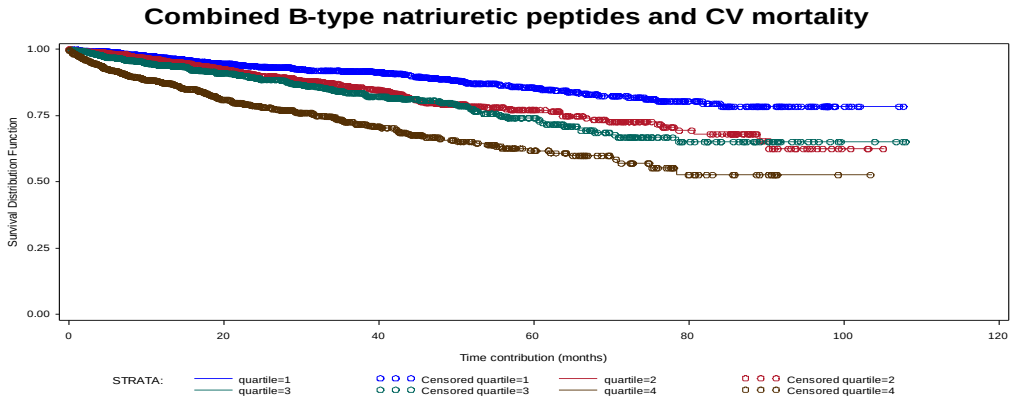
Figure 4. 16 - Cardiovascular mortality and survival distribution for NT-proBNP quartiles



Chronic heart failure patients in the second and third NT-proBNP quartile had an approximately 1.5 fold increased risk in the cardiovascular mortality when compared to the lowest NT-proBNP quartile. Patients in the highest quartile had a 3.35 fold unadjusted increased risk in mortality due cardiovascular diseases. When adjusting for the baseline patient characteristics, the cardiovascular mortality risk slightly increased for the second and third quartile, while it decreased for the fourth quartile when compared to the lowest NT-proBNP quartile. Surprisingly, the adjusted cardiovascular mortality risk was higher for the second NT-proBNP quartile (HR=1.72 (95%CI: (1.21, 2.44))) than the third quartile (HR=1.60 (95%CI: (1.12, 2.29))).

Combining all quartiles, regardless of the type of BNP or NT-proBNP tests showed that higher quartiles led to a higher risk of cardiovascular death. This can be visualized in the Figure 4.17. Chronic heart failure patients in the most severe combined BNP and NT-proBNP quartile had three times higher risk of cardiovascular death than patients in the lowest combined quartile. The unadjusted hazard ratio for the second, third and fourth combined quartiles were 1.6, 1.9 and 3.5, respectively when compared to the lowest quartile. Upon adjusting for all baseline characteristics such as: age, gender, body mass index, smoking status, geographic region, serum creatinine, hypertension and/or heart failure medication, and diabetes status, the unadjusted and adjusted cardiovascular death risks were almost identical for the second and third quartile. In the fourth combined quartile, taking into account the baseline patient characteristics, led to a slight decrease in the cardiovascular risk, as the hazard ratio estimate decreased from 3.51 (95% CI: (2.78, 4.44)) to 3.01 (95% CI: (2.29, 3.96)).

Figure 4. 17 - The survival distribution for the combined natriuretic peptide quartiles



In addition to each of the combined quartiles, age, body mass index, serum creatinine and smoking and diabetes status at the baseline had significantly impacted the cardiovascular mortality. While being an ex-smoker or non-smoker had a protective effect, every additional year in age increased the risk of cardiovascular mortality by 4% in chronic heart failure patients. The serum creatinine did not have a clinical impact on the cardiovascular mortality. Chronic heart failure patients who were diabetic at the start of the study were 1.35 more likely to die due to a cardiovascular cause than the non-diabetics. These results are presented below.

Table 4. 18 - Adjusted Cox regression model for cardiovascular mortality and logBNP

Parameter	Hazard ratio	95% Confidence interval
Combined quartiles		
2 vs. 1	1.64	(1.24, 2.17)
3 vs. 1	1.89	(1.42, 2.50)
4 vs. 1	2.97	(2.25, 3.90)
Age[1]	1.04	(1.03, 1.05)
Serum creatinine[1]	1.003	(1.002, 1.004)
Diabetes[1]	1.35	(1.01, 1.80)
Smoking[1]		
Ex-smoker	0.63	(0.45, 0.88)
No	0.64	(0.46, 0.90)
Yes	reference	reference
[1] Age, serum creatinine and smoking were assessed at baseline		

The table below presents the results when B-type natriuretic peptide quartiles were treated as a continuous.

Table 4. 19 - Prognostic analyses of the combined natriuretic peptides and CV mortality

Natriuretic peptide (quartile continuous)	N	Unadjusted HR (95% CI)	Adjusted[a] HR (95% CI)
BNP	1,196	1.54 (1.36, 1.73)	1.44 (1.25, 1.65)
NT-proBNP	3,053	1.45 (1.32, 1.59)	1.38 (1.24, 1.54)
NT-proBNP and BNP respective quartiles combined	4,249	1.49 (1.38, 1.60)	1.40 (1.28, 1.52)

a. Model adjusted for age, gender, BMI, smoking, geographic region, serum creatinine, hypertension and/or heart failure medication and diabetes at baseline
b. BNP quartiles were defined based on the following cutoff BNP values: Q1 (≤131 pg/ml), Q1 to Q2 (131 to 304 pg/ml), Q2 to Q3 (304 to 800 pg/ml), ≥Q3 (≥800 pg/ml)
c. NT-proBNP quartiles were defined based on the following NT-proBNP values: ≤ Q1 (≤350 pg/ml), Q1 to Q2 (350 to 1100 pg/ml), Q2 to Q3 (1100 to 2738 pg/ml), >Q3 (> 2738 pg/ml)
d. Combined quartiles treated as a continuous variable

With each severe B-type natriuretic peptide quartile, the risk of cardiovascular death increases approximately by a factor of 1.5. Upon adjustment for the baseline patient characteristics such as: age, gender body mass index, smoking, geographic region, serum creatinine, diabetes, and exposure to hypertension and/or heart failure medication, the cardiovascular death risk slightly decreased to approximately 1.4.

Specifically, with every BNP quartile, there is an increased risk of cardiovascular death by a factor of 1.54 (95%CI: (1.36, 1.73)). The 95% confidence interval for the hazard ratio did not include 1, indicating the statistical significance of the BNP quartiles.

Adjusting for all demographics and baseline characteristics led to a slightly lower risk of cardiovascular death of a factor of 1.44 (95%CI: (1.25, 1.65)) for every increasing BNP

quartile. Factors significantly impacting the cardiovascular mortality at the 5% significance level were: BNP quartile, age, smoking status, and serum creatinine. For example, for every additional year, the risk of the cardiovascular mortality was increased by approximately 7%, all other factors staying the same (hazard ratio of 1.069 (95% CI: (1.04, 1.09)). As expected, ex-smokers and non-smokers had a lower cardiovascular mortality risk than smokers. They are approximately 0.4 times more likely to die of cardiovascular causes than the smokers. The hazard ratios were 0.41 (95%CI: (0.24, 0.67)) for ex-smokers and 0.40 (95%CI: (0.24, 0.67)) when compared to smokers. The serum creatinine hazard ratio estimates were very close to unity (1.006 (95% CI: (1.003, 1.009)).

With every NT-proBNP quartile, there is an increased risk of cardiovascular death by a factor of 1.45. The 95% confidence interval for the hazard ratio was (1.32, 1.59), indicating the statistical significance of the NT-proBNP quartiles.

The adjusted hazard ratio was 1.38 (95%CI: (1.24, 1.54)) after adjusting for all variables. The following baseline characteristics had a statistically significant impact on the cardiovascular mortality: NT-proBNP quartiles, age, body mass index, serum creatinine and diabetes status at baseline. For example, a patient one year older than another patient had an increased risk of dying due to cardiovascular diseases by a factor of 1.03 (95%CI: (1.02, 1.05)), assuming that all other characteristics are the same. The body mass index seemed to have a slight protective effect on survival, as the hazard ratio for the cardiovascular mortality was 0.95 (95% CI: (0.93, 0.97)). The effect of the serum creatinine was minimal, as the hazard ratio was very close to 1 (HR=1.003 (95%CI: (1.002, 1.004)). The diabetic patients are 1.43 times most

likely to die due to cardiovascular diseases than the non-diabetics (HR=1.43 (95%CI: (1.01, 2.04)).

Combining all quartiles into a continuous variable taking on values from 1 to 4 led to similar results. With every increasing combined quartile, the unadjusted risk of cardiovascular mortality significantly increased by a factor of 1.49. Taking into account the baseline patient characteristics slightly decreased the cardiovascular mortality risk to 1.40. The proportional hazards assumption was not met for the combined quartiles.

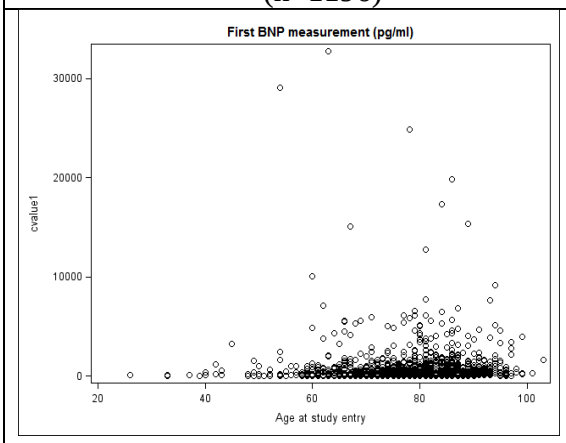
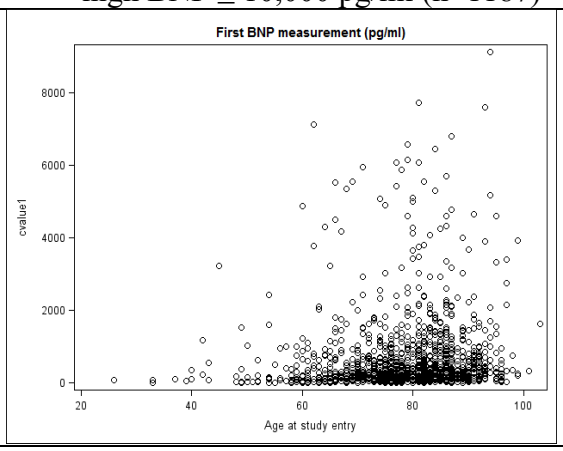
4.3.4. Sensitivity Analyses

There were several high B-type natriuretic peptide values present that required closer attention. Given the observational nature of the electronical medical record, it could not be determined whether there were just abnormally high observations or data entry errors. The impact of these high values on the association with other risk factors is described below.

Among the 1,196 patients with BNP measurements, there were 9 patients with BNP levels higher than 10,000 pg/ml (from 10,008 pg/ml to 32,765 pg/ml). Seven of these patients were males and two were females. Their age varied from 54 to 89 years old. All of them, but a 63-year old male of normal weight had been previously exposed to heart failure and/or hypertension medication. None of them were diabetic at the start of the study. When ignoring these 9 subjects with high BNP levels, there was a weak, linear, positive association between the age at the study entry and BNP levels. The Pearson correlation coefficient was 0.09 (95%CI: (0.04, 0.15), p-value=0.001) based on 1187 patients. Nevertheless, there was no impact on the non-linear association between the age at study entry and BNP levels at baseline. For example, the non-parametric Spearman correlation coefficient was 0.16 (95% CI: (0.10,

0.22, p-value < 0.0001)). Figure 4.18 displays the correlation between the age at study entry and BNP levels with and without these nine patients.

Figure 4. 18 - The association between age and BNP at baseline

All patients with first BNP measurements (n=1196)	After removing 9 patients with abnormally high BNP ≥ 10,000 pg/ml (n=1187)
	

Following the removal of these nine, high BNP values, the correlation between the baseline BNP levels and body mass index or weight became slightly stronger, though remained in the same negative direction. For example, the Pearson correlation coefficient was -0.15 (95%CI: (-0.21, -0.09), p-value <0.0001) for the body mass index and slightly lower, -0.11 (95%CI: (-0.17, -0.06), p-value<0.0001) for the weight. There was no impact on the non-linear Spearman correlation.

Removing the highest NT-proBNP value of 263,063.4 pg/ml resulted into a smaller NT-proBNP variation in females. The standard deviation was reduced from 8,107.7 pg/ml to 4,614 pg/ml in females. The linear correlation between the baseline NT-proBNP and age at the study start was slightly stronger. The Pearson correlation coefficient increased from 0.10 (95%CI: (0.06, 0.14)) to 0.11 (95%CI: (0.07, 0.15)), while the Spearman correlation coefficient did not change. There was a minimal effect on the linear correlation between the NT-proBNP levels and mass index or weight and no effect on the non-linear association when the highest NT-

proBNP value. For example, the Pearson correlation coefficient was slightly stronger in the negative direction: -0.17 (95% CI: (-0.21, -0.14), p-value <0.0001) for body mass index and -0.16 (95% CI: (-0.19,-0.12), p-value < 0.0001).

Additional sensitivity analyses were conducted upon removing the highest 5% BNP and NT-proBNP levels, while applying the stepwise variable selection in the respective Cox regression models. Consequently, 57 subjects with baseline BNP values higher than 3,500 pg/ml and 148 patients with NT-proBNP values higher than 10,500 pg/ml were removed from the Cox regression models. The demographics and clinical characteristics of these subjects with extremely high B-type natriuretic peptides are tabulated in the Appendix F.

The Table 4.20 shows the results of the sensitivity analyses when the B-type natriuretic peptides were treated as continuous variables and statistically analyzed on the logarithmic (base e) scale. The magnitude of the impact of BNP on the all-cause mortality and cardiovascular mortality was very similar to the results described in the Sections 4.3.2 and 4.3.3. However, the prognostic impact of NT-proBNP on the all-cause mortality was slightly higher and slightly lower on the cardiovascular mortality, while adjusting only for the significant risk factors in the model, as opposed to all baseline patient characteristics.

Table 4. 20 - Sensitivity analyses for BNP, NT-proBNP and all-cause mortality

Natriuretic Peptide	**N[a]**	**All cause-mortality HR (95% CI)**	**Cardiovascular mortality HR (95% CI)**
Log-BNP	999	1.25 (1.14, 1.38)[b]	1.39 (1.23, 1.57)[d]
Log-NT-proBNP	2546	1.16 (1.09, 1.23)[c]	1.23 (1.13, 1.33)[e]

a. Sample size based on complete patient records upon removing BNP values ≥ 3500 pg/ml and NT-proBNP values ≥10,500 pg/ml
b. AlSamplel-cause mortality Cox regression model adjusted for age, smoking, serum creatinine, hypertension and/or heart failure medication following the stepwise model selection at the 5% significance level

c. All-cause mortality Cox regression model adjusted for age, body mass index, serum creatinine and smoking status following the stepwise model selection at the 5% significance level
d. Cardiovascular mortality Cox regression model adjusted for logBNP, age, smoking and serum creatinine upon applying the stepwise model selection at the 5% significance level
e. Cardiovascular mortality Cox regression model adjusted for logNT-proBNP and age upon applying the stepwise model selection at the 5% significance level

Sensitivity analyses were conducted while treating the B-type natriuretic peptides as categorical quartiles upon removing the highest 5% BNP and NT-proBNP levels and applying the stepwise variable selection in the respective Cox. The prognostic impact of the BNP quartiles, NT-proBNP quartiles and combined B-type natriuretic peptides are shown in the Table 4.21. The results for the all-cause mortality were very similar to the ones presented in the Section 4.3.2. The patients in the most severe NT-proBNP and combined quartiles had slightly higher cardiovascular mortality risk than presented in the Section 4.3.3. For example, patients in the fourth NT-proBNP quartile were 3.5 times more likely to die of cardiovascular diseases compared to the first NT-proBNP quartile. In the Section 4.3.3 this hazard ratio was 3.25. Also, the prognostic impact of the BNP quartiles on the cardiovascular mortality was slightly stronger.

Table 4. 21 - Sensitivity analyses for B-type natriuretic peptide quartiles (categorical) and all-cause mortality

Natriuretic peptide	Comparison	All-cause mortality HR (95% CI)	Cardiovascular mortality HR (95% CI)
BNP	Quartile 2 vs. Quartile 1	1.33 (0.95, 1.86)[a]	1.71 (1.08, 2.70)[d]
	Quartile 3 vs. Quartile 1	1.98 (1.43, 2.72)[a]	2.53 (1.63, 3.93)[d]
	Quartile 4 vs. Quartile 1	2.24 (1.62, 3.08)[a]	3.19 (2.07, 4.90)[d]
NT-proBNP	Quartile 2 vs. Quartile 1	1.68 (1.33, 2.13)[b]	1.67 (1.18, 2.36)[e]
	Quartile 3 vs. Quartile 1	1.51 (1.19, 1.91)[b]	1.61 (1.17, 2.28)[e]
	Quartile 4 vs. Quartile 1	2.26 (1.79, 2.85)[b]	3.50 (2.55, 4.81)[e]
NT-proBNP and BNP respective quartiles combined	Quartile 2 vs. Quartile 1	1.55 (1.27, 1.88)[c]	1.57 (1.19, 2.07)[f]
	Quartile 3 vs. Quartile 1	1.66 (1.36, 2.02)[c]	1.79 (1.36, 2.35)[f]
	Quartile 4 vs. Quartile 1	2.22 (1.83, 2.71)[c]	3.10 (2.39, 4.02)[f]

a. All-cause mortality Cox regression model adjusted for the BNP quartiles, age, smoking and serum creatinine following the stepwise model selection at 5% significance level;

b. All-cause mortality Cox regression model adjusted for the NT-proBNP quartiles, age, serum creatinine and body mass index following the stepwise model selection at 5% significance level

c. All-cause mortality model Cox regression adjusted for the combined quartiles, age, serum creatinine, smoking status, body mass index, geographic region and diabetes status at baseline following the stepwise model selection at the 5% significance level

d. Cardiovascular mortality Cox regression model adjusted for the BNP quartiles, age, smoking and serum creatinine following the stepwise model selection at the 5% significance level

e. Cardiovascular mortality Cox regression model adjusted for the NT-proBNP quartiles following the stepwise model selection at the 5% significance level

f. Cardiovascular mortality Cox regression model adjusted for the combined quartiles and age following the stepwise model selection at the 5% significance level

g. BNP quartiles were defined based on the following cutoff BNP values: Q1 (≤131 pg/ml), Q1 to Q2 (131 to 304 pg/ml), Q2 to Q3 (304 to 800 pg/ml), ≥Q3 (≥800 pg/ml)

h. NT-proBNP quartiles were defined based on the following NT-proBNP values: ≤ Q1 (≤350 pg/ml), Q1 to Q2 (350 to 1100 pg/ml), Q2 to Q3 (1100 to 2738 pg/ml), >Q3 (> 2738 pg/ml)

i. First combined quartile if patient was either in the 1st BNP or 1st NT-proBNP, regardless the type of natriuretic peptide measurement. Similar definition for the second, third and fourth combined quartiles.

j. Sample size based on complete patient records upon removing BNP values ≥ 3500 pg/ml and NT-proBNP values ≥10,500 pg/ml

k. B-type natriuretic peptide quartiles treated as categorical quartiles

Sensitivity analyses were performed with the B-type natriuretic peptides as continuous quartiles. The results are presented below and were very similar to the ones presented in Sections 4.3.2 and 4.3.3. With each severe B-type natriuretic peptide quartile, the risk of all-cause death increased approximately by a factor of 1.3, upon adjustment for the baseline patient characteristics. The adjusted risk of cardiovascular death was close to 1.5.

Table 4. 22 - Sensitivity analyses for B-type natriuretic peptides (continuous quartiles) and all-cause mortality

Natriuretic peptide (quartile continuous)	N[a]	All-cause mortality HR (95% CI)	Cardiovascular mortality HR (95% CI)
BNP	1,048	1.30 (1.19, 1.43)[b]	1.45 (1.27, 1.65)[b]
NT-proBNP	2,671	1.25 (1.16, 1.34)[c]	1.47 (1.33, 1.63)[e]
NT-proBNP and BNP respective quartiles combined	3,719	1.27 (1.19, 1.34)[d]	1.41 (1.30, 1.51)[f]

a. Sample size based on complete patient records upon removing BNP values ≥ 3500 pg/ml and NT-proBNP values ≥10,500 pg/ml
b. Cox regression model adjusted for BNP continuous quartiles, age, smoking and serum creatinine following the stepwise model selection at the 5% significance level
c. All-cause mortality Cox regression model adjusted for NT-proBNP continuous quartiles, age, serum creatinine and body mass index following the stepwise model selection at the 5% significance level
d. All-cause mortality Cox regression model adjusted for the combined, continuous quartiles, age, body mass index, smoking status, geographic region, serum creatinine and diabetes at baseline following the stepwise model selection at the 5% significance level
e. Cardiovascular mortality Cox regression model adjusted for the NT-proBNP continuous quartiles following the stepwise model selection at the 5% significance level
f. Cardiovascular mortality Cox regression model adjusted for the combined, continuous quartiles and age following the stepwise model selection at the 5% significance level
g. BNP quartiles were defined based on the following cutoff BNP values: Q1 (≤131 pg/ml), Q1 to Q2 (131 to 304 pg/ml), Q2 to Q3 (304 to 800 pg/ml), ≥Q3 (≥800 pg/ml)

h. NT-proBNP quartiles were defined based on the following NT-proBNP values: ≤ Q1 (≤350 pg/ml), Q1 to Q2 (350 to 1100 pg/ml), Q2 to Q3 (1100 to 2738 pg/ml), >Q3 (> 2738 pg/ml)

i. First combined quartile if patient was either in the 1st BNP or 1st NT-proBNP, regardless the type of natriuretic peptide measurement. Similar definition for the second, third and fourth combined quartile.

As described in Section 4.2.6, there was a small percentage of missing units for the B-type natriuretic peptide tests in the database. Sensitivity analyses were conducted with and without these tests with missing units, but there was no impact on the statistical inference.

4.4. Discussion

The patient population of this study consisted of 4,249 chronic heart failure patients managed by primary care physicians. The average age of the patients was 78.3 years at the study entry. Sample size calculations indicated that there was sufficient power to obtain valid statistical results. Given the real-word observational nature of the database, the current 4,249 patients had a variable entry into the cohort. The overall length of follow-up was 9 years after the chronic heart failure diagnosis.

The current study showed that the $\log_e$-NT-proBNP was associated with the all-cause mortality in the primary care setting. The unadjusted all-cause mortality hazard ratio was 1.37 (95% CI: (1.30, 1.43)). The hazard ratio estimate became slightly lower 1.24 (95% CI: (1.17, 1.32)) when adjusting for the patients' age, gender, body mass index, smoking, geographic region, serum creatinine, hypertension and/or heart failure medication and diabetes status at baseline. In the sensitivity analyses that excluded the 5% highest NT-proBNP observations, the hazard ratio was lowered to 1.16 (95%CI: (1.09, 1.23)) when adjusting for the patients' age, body mass index, serum creatinine, and smoking, which were the only baseline factors that significantly impacted the all-cause mortality at the 5% significance level. Patients in the third

and fourth NT-proBNP quartiles were 1.46 and 2.18, respectively more likely to die than the patients in the first, less severe NT-proBNP quartile. The study showed that the $\log_e$-NT-proBNP was also associated with the cardiovascular mortality in the primary care setting. The unadjusted cardiovascular mortality hazard ratio was 1.39 (95%CI: (1.29, 1.49)), which was very similar to the hazard ratio of 1.33 (95%CI: (1.22, 1.44)) when adjusting for all baseline patients' characteristics. In the sensitivity analyses, hazard ratio was slightly reduced to 1.23 (95%CI: (1.13, 1.33)), when adjusting for age.

As for the secondary objectives, the current study showed that the $\log_e$-BNP was associated with the all-cause mortality and cardiovascular mortality in the primary care setting. The unadjusted all-cause mortality hazard ratio was 1.32 (95%CI: (1.23, 1.41)) and 1.23 (95% CI: (1.13, 1.34)) when adjusting for all baseline patients' characteristics such as: age, gender, body mass index, smoking, geographic region, serum creatinine, hypertension and/or heart failure medication and diabetes status at baseline. The adjusted all-cause mortality hazard ratio was almost identical to the results of the sensitivity analyses, which excluded the 5% highest BNP observations.

When evaluating the impact of $\log_e$-BNP on the cardiovascular mortality, the unadjusted hazard ratio was slightly higher,1.41 (95% CI: (1.29, 1.55)). The adjusted hazard ratio for all patients' baseline characteristics was 1.32 (95% CI: (1.18. 1.46)), which was very similar to the hazard ratio of 1.39 (95% CI: (1.23, 1.58)) obtained in the sensitivity analyses upon removing the highest 5% BNP observations and adjusting for age, smoking and serum creatinine. None of the individual studies mentioned at the beginning of this section reported the prognostic value of the BNP on the cardiovascular mortality.

Both BNP and NT-proBNP were inversely related to the body mass index and weight. The non-linear associations were stronger than the linear ones. The non-linear Spearman correlation coefficient between the BNP and body mass index was -0.23 (95%CI: (-0.29, -0.18), p-value < 0.0001) and -0.19 (95%CI: (-0.24, -0.13), p-value<0.0001) between the baseline BNP and weight. Similarly, the non-linear Spearman correlation coefficient between the NT-proBNP and body mass index was -0.24 (95%CI: (-0.28, -0.21), p-value < 0.0001) and -0.20 (95%CI: (-0.24, -0.17), p-value<0.0001) between the NT-proBNP and weight. This paradox of inverse or reverse epidemiology had been previously observed in the medical literature (Nadruz et al. 2016, Kube et al. 2016, Oreopoulos et al. 2008, Wannamethee et al. 2014).

The risk of mortality increased with age. Upon adjusting for BNP or NTproBNP and other baseline characteristics, age significantly impacted the all-cause mortality and cardiovascular mortality in patients 70-years old or older. At 70-year old, for each additional year, there was approximately 2% to 4% increase in the risk of mortality. At 80-years old, the yearly adjusted mortality risk ranged from 4.4% to 7.3%. At 90-years old, the yearly adjusted mortality risk ranged from 6.7% to 10.3%. Overall, regardless the age, for every decade older, the adjusted risk of all-cause mortality and cardiovascular mortality increased by 64% and 85%, respectively, upon adjusting for BNP, age, gender, body mass index, smoking, geographic region, serum creatinine, hypertension and/or heart failure medication and diabetes status at baseline. In the logNTproBNP models, for each 10-year increase in age, the adjusted risk of all-cause mortality and cardiovascular mortality were 39% and 38%, respectively.

The study also provided a detailed summary of the B-type natriuretic peptide levels in subgroups of patients defined by age, gender, body mass index categories which can be used to inform the design of future studies in such subgroups of patients.

4.4.1 Limitations

The study had several limitations.

The study was a retrospective cohort of electronical medical records.

The residual confounding due to unrecorded variables pertaining to the etiology of heart failure, disease severity, type of heart failure, left ventricular ejection fraction could not be ruled out. There was no information recorded about the B-type natriuretic peptide assays tested on the patients to provide further insight of the peptides' abnormal levels, analytic ranges and coefficient of variation. Nevertheless, the amount of missing data for the existing variables was low. The data records met the high quality standards needed for research.

The NT-proBNP diagnostic modeling consistently revealed that the proportional hazard assumption was not met. Other studies were not reported in such detail, thus it was impossible to assess the extent of this problem outside the current study. Nevertheless, the hazard ratio for BNP and NT-proBNP were very similar, which alleviated some of the concerns.

Another limitation is that the Clinical Practice Research Datalink does not collect patient's race. However, the data source is representative of the UK population which consists of 86% Whites, according to the 2011 Census (ONS 2012)

Not all medical practices consented to linkage to the CPRD Data Linkage Scheme or ONS Mortality Data in order to have the cause of death evaluated. It is possible that more patients died of cardiovascular diseases, and therefore the cardiovascular hazard ratios

presented in this chapter may underestimate the true association between the baseline B-type natriuretic peptides and mortality due to cardiovascular diseases. Nevertheless, a systematic review covering the period between 1987 and April 2008 demonstrated the high validity of diagnoses for the circulatory system, as well as causes of mortality in the data source (Herrett et al. 2010). It is also possible that the estimates for the cardiovascular mortality were over-estimated because some heart failure patients may have died of other primary cause, not necessarily a cardiovascular disease. The ONS mortality data often reports multiple causes of death. This study defined cardiovascular mortality if one of the reported causes of death was a cardiovascular disease.

A small degree of measurement bias may have been introduced because the smoking status was self-reported, which in turn would underestimate the association between the B-type natriuretic peptides and mortality. However, research suggests that self-reported smoking is highly reliable, thus the impact of smoking status on the results was considered minimal (Patrick et al. 1994, Blank et al. 2016).

The results are not generalizable to patients of non-White race residing in the United Kingdom. The results are not generalizable outside the United Kingdom.

4.4.2. Implications for research

The limitations encountered in the current study could be overcome in a long-term prospective study conducted in heart failure patients within the primary care setting. A representative sample of primary care practices could be selected. These primary care practices should include similar information about patients such as: age, gender, body mass index, smoking, geographic region, etiology of heart failure, type of heart failure, disease severity, comorbidities,

symptomatic assessment such as NYHA, treatment information and type of assay to be used for measuring BNP and NT-proBNP. The pharmacotherapy, symptomatic heart failure assessment, and clinical variables should be regularly assessed in all patients so that the impact of changes could be evaluated. A follow-up period of five years with clinical assessments at every 3 months is suggested. The primary cause of death should be carefully evaluated to ensure the appropriate estimation of the true association between BNP or NT-proBNP and cardiovascular mortality.

4.4.3. Implications for practice

Baseline BNP and NT-proBNP are independent predictors of all-cause mortality and cardiovascular mortality in the primary care setting. On a logarithmically transformed scale, for every unit increase in BNP or NT-proBNP, there was a 1.3-fold increase in the unadjusted risk of death. The impact of the natriuretic peptides on the cardiovascular mortality was slightly higher. Adjusting for the baseline patients' characteristics such as age, gender, body mass index, smoking, geographic region, serum creatinine, hypertension and/or heart failure medication and diabetes at baseline led to slightly lower mortality estimates.

When treating heart failure patients, physicians should consider not only the baseline BNP or NT-proBNP levels, but also the age of the patients, since age significantly impacts mortality, especially in elderly. For example, at 70-year old, for each additional year, there was approximately 2% to 4% increase in the risk of mortality. This risk increased to 4.4% to 7.4% in 80-year old patients.

4.5. Conclusion

This large population-based primary care cohort of 4,249 chronic heart failure patients managed by general practitioners within the United Kingdom confirmed that the baseline BNP and NT-proBNP are predictors of all-cause mortality and cardiovascular mortality in the primary care setting too.

The risk of mortality increased with age, especially in patients 70-years old or older.

These results are not generalizable outside to the United Kingdom.

Chapter 5 – The prognostic value of changes in B-type and N-terminal pro-B-type natriuretic peptides in heart failure patients – A primary care perspective

5.1. Introduction

This chapter is an extension of Chapter 4, and it examines the prognostic impact of changes in B-type natriuretic peptides on the all-cause mortality and cardiovascular mortality.

The clinical relevance of changes in B-type natriuretic peptides in chronic heart failure patients remains little understood. It is not clear how much of a change in these peptides is clinically relevant and under what clinical scenarios. Additionally, the time horizon over which the changes in B-type natriuretic peptides should be measured is highly inconsistent.
There is limited information in the literature about the changes of BNP or NT-BNP over time and how they impact mortality in chronic heart failure. The literature search from Chapter 2 found only one systematic review that evaluated changes in BNP when comparing the natriuretic peptide-guided treatment to usual clinical care (Xin et al. 2015). While the between-group BNP changes ranged from -47 to 6%, overall the between group BNP changes did not impact the all-cause mortality. However, stratified analyses showed a trend of decreased all-cause mortality with higher changes in BNP, of at least 5%.

While the primary research question of direct association with mortality, was addressed in Chapter 4, the following secondary research question about changes in B-type and

N-terminal pro-B-type natriuretic peptides and their association with mortality is discussed in this chapter:

Secondary research question: Are changes in B-type natriuretic peptides (e.g. BNP and NTproBNP0) associated with all-cause mortality and cardiovascular mortality in the primary care setting?

5.2. Methods

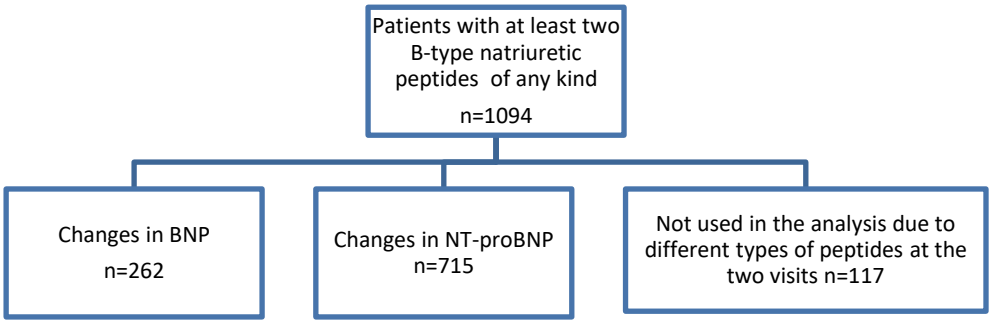
5.2.1. Study design, study setting, study participants and endpoints

The study design and study participants were described in detail in Chapter 3 and summarized in Chapter 4. Briefly, this was a retrospective cohort in heart failure patients, managed by primary care practitioners in the United Kingdom. The data source was de-identified electronic medical records from the Clinical Research Data Link. Patients entered the cohort on the day of their first B-type natriuretic peptide test. 95% of the patients had their index date after January 1, 2005, while 5% patients had their index date in 2003 or 2004. The exit from the cohort was either the event of death or the end of the study, which was September 30th, 2012. The study participants of this chapter were heart failure adult patients without any severe pulmonary or renal diseases with two or more B-type natriuretic measurement tests. The study endpoints were the all-cause mortality and cardiovascular mortality. See Section 4.2.2 for how these endpoints were ascertained.

5.2.2. Analysis populations

The analysis population of this chapter included chronic heart failure patients with at least two B-type natriuretic peptide measurements. Approximately 25% of the chronic heart failure patients had at least two B-type natriuretic peptide tests on separate medical office visits. However, 117 (10%) had different types of B-type natriuretic peptide tests taken during the two visits such as: BNP first and then NT-proBNP or NT-proBNP first and BNP on the following visit. Since there is no conversion between BNP and NT-proBNP, these 117 patients were excluded from the analysis. A flow chart of the analysis population included in this chapter is provided in Figure 5.1:

Figure 5. 1 - Flow chart of the patient population included in Chapter 5



5.2.3. Statistical methods

SAS version 9.2 (SAS Institute Inc, Cary, NC) was used for the statistical analyses.

No sample size calculations were performed for the secondary objectives concerning changes in the B-type and N-terminal pro-B-type natriuretic peptides. Therefore, the work presented in this chapter takes an explorative approach. Changes in BNP and NT-proBNP were calculated

as the difference between the test value on the second visit minus the test value on the first visit.

Continuous variables were summarized using mean and standard deviations, while categorical variables were summarized using count, frequencies, medians, and ranges. Data were visualized with distributional graphs, histograms, stack bars and line plots. The baseline characteristics of groups of patients were statistically compared using t-tests for the normally distributed variables as well as non-parametric tests such as Wilcoxon rank sum tests, Fisher's exact tests and chi-square tests when the normality assumptions were not met.

Pearson and Spearman's correlations were computed to assess the linear and non-parametric associations between changes in B-type natriuretic peptides and baseline characteristics.

Time to death was statistically analyzed with multivariable Cox proportional hazards models. Survival analyses for the changes in natriuretic peptides were conducted on a logarithmic (base 2) scale to reflect the impact of a doubling in the BNP or NT-proBNP change. For the statistical analyses of change in natriuretic peptides, time zero was considered the time of the second B-type natriuretic peptide visit. The objective was to investigate whether changes in B-type natriuretic peptides provide additional information on mortality beyond a single point-in-time peptide level. Separate analyses were conducted for BNP and NT-proBNP. The model diagnostics were performed by examining outliers, the functional form of the continuous variables as well as testing the proportional hazards assumption using a Kolmogorov supremum test.

The following covariates were included in the multivariable Cox regression analysis: age, gender, body mass index, smoking, geographical region, serum creatinine, diabetes, hypertension and heart failure medication. The covariates were assessed at baseline.

The overall, missing data in the entire sub-cohort, as well as by the type of B-type natriuretic peptide tests are displayed in Table 5.1. Considering the small percentage, missing data were not imputed.

Table 5. 1 - Missing data for covariates by the baseline BNP and NT-proBNP tests

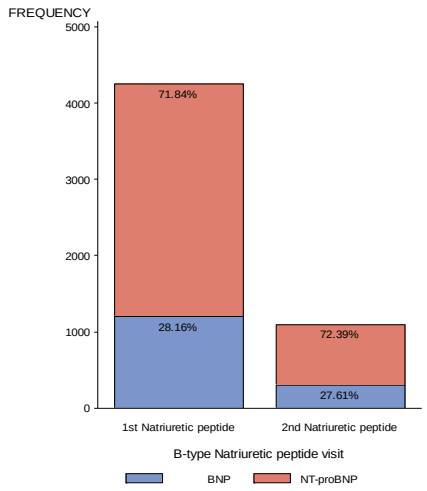
Covariate	All sub-cohort n=1094	Patients with 2 BNP tests (n=262)	Patients with 2 NT-proBNP tests (n=715)
Age	0	0	0
Gender	0	0	0
Body mass index	4.1%	1.9%	5.2%
Smoking status	0.01%	0	0.1%
Diabetes status at baseline	0	0	0
Serum creatinine	4.8%	5.7%	4.4%
Hypertension/heart failure medication	0.6%	1.5%	0.3%
Geographic region	0.9%	1.5%	0.6%
Missing overall (any missing)			
The denominator was 1,094 for all sub-cohort, 262 for patients with two consecutive BNP tests and 715 for patients with two consecutive NT-proBNP tests.			

5.3. Results

There were 977 patients included in the survival analysis of the present chapter. Overall, there were 1,094 patients who had at least two B-type natriuretic peptide measurements of any kind, of whom 262 patients had two consecutive BNP tests, and 715 patients had two successive NT-proBNP tests. Although the remainder 117 patients had two tests, they were different; either BNP followed by NT-proBNP or vice versa and therefore they were excluded from the analyses. Figure 5.2 shows the type of B-type natriuretic peptide

measurements taken during the first two visits. Approximately, three times more patients had NT-proBNP tests than BNP tests.

Figure 5. 2 - First two measurements of B-type natriuretic peptides



The patients' baseline characteristics of the entire sub-cohort of patients with at least two B-type natriuretic peptides of any kind, as well as for those with two consecutive BNP and NT-proBNP tests respectively are summarized in the Table 5.2. The groups of patients were very similar regarding gender, age, body mass index, creatinine, smoking and diabetes status, exposure to heart failure and hypertension medication. The average patients' age was 77 years old. There were slightly more women than men. Approximately 65% of the patients were either overweight or obese. A little over half of the patients were non-smokers. Less than 8% of the patients had diabetes at baseline. Most of the patients had been exposed to hypertension and/or heart failure medication.

Table 5. 2 - Patients' baseline characteristics

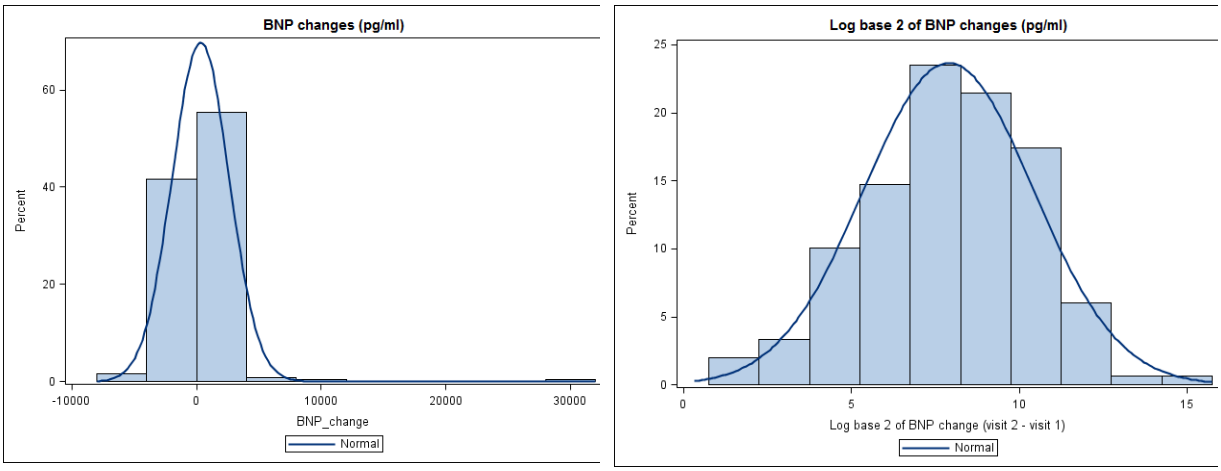
Patient characteristic	All sub-cohort n=1094	Patients with 2 BNP tests (n=262)	Patients with 2 NT-proBNP tests (n=715)	p-value[a]
Age (years)	77.2 ± 10.3	76.8 ± 10.2	77.5 ± 10.3	0.207
Gender				0.563
Males	519 (47.4%)	120 (45.8%)	343 (47.9%)	
Females	575 (52.6%)	142 (54.2%)	372 (52.1%)	
Body mass index (kg/m^2)	28.2 ± 6.8	27.3 ± 5.7	28.4 ± 7.2	0.097
Underweight (<18.5)	35 (3.3%)	8 (3.1%)	23 (3.4%)	
Normal weight (18.5-24.9)	324 (30.9%)	91 (35.4%)	209 (30.8%)	
Overweight (25-30)	326 (31.1%)	79 (30.7%)	204 (30.1%)	
Obese (>30)	364 (34.7%)	79 (30.7%)	242 (35.7%)	
Smoking				0.986
Yes	73 (6.7%)	18 (6.9%)	49 (6.9%)	
No	583 (53.3%)	142 (54.2%)	383 (53.6%)	
Ex-smoker	437 (40%)	102 (38.9%)	282 (39.5%)	
Geographical region				<0.001
East Midlands	94 (8.7%)	64 (24.4%)	27 (3.8%)	
West Midlands	78 (7.2%)	0	77 (10.8%)	
London	314 (28.9%)	96 (36.6%)	169 (23.7%)	
East of England	36 (3.3%)	0	16 (2.2%)	
North East	73 (6.7%)	2 (0.7%)	56 (7.8%)	
North West	33 (3.1%)	3 (1.1%)	28 (3.9%)	
South West	41 (3.8%)	0	40 (5.6%)	
Northern Ireland	168 (15.5%)	14 (5.3%)	149 (20.9%)	
Scotland	2 (0.2%)	0	2 (0.2%)	
South Central	152 (14%)	16 (6.1%)	108 (15.2%)	
South East Coast	81 (7.5%)	45 (17.1%)	27 (3.8%)	
Yorkshire and the Humber	21 (1.1%)	0	12 (1.7%)	
Serum creatinine (umol/L)	104.2 ± 33.4	105.8 ± 31.9	104.1 ± 34.8	0.259
Diabetes at baseline	81 (7.4%)	17 (6.5%)	56 (7.8%)	0.582
Hypertension and/or heart failure medication	998 (91.8%)	228 (87.1%)	660 (92.6%)	0.051

[a.] P-value from statistically comparing the patients with two consecutive BNP tests versus those with two consecutive NT-proBNP tests

Among the patients included in this chapter, the primary outcome of all-cause mortality occurred in 285 patients or 26%. The secondary outcome of cardiovascular mortality occurred in 170 patients or 15.5%. The median follow-up for the patients included in this chapter was 37.6 months (Q1=20.8, Q3=56.8).

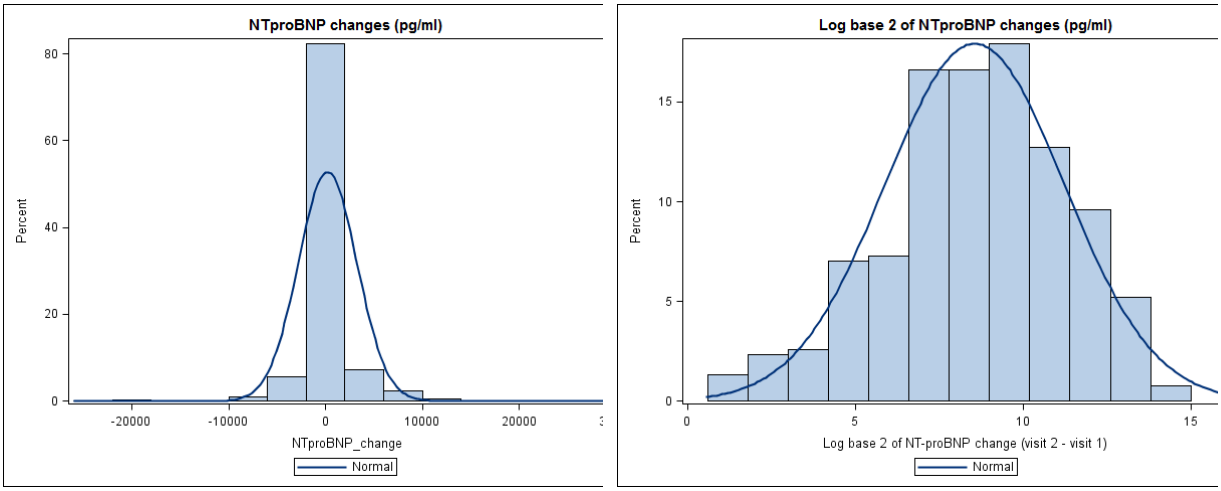
The average time between two consecutive BNP tests was 32 months or 2.6 years, ranging from 2 days to 8.9 years. The distribution of the time between two successive BNP tests and NT-proBNP tests can be found in the Appendix G. Figure 5.3 shows the skewed distribution of the BNP changes on the original scale, as well as the suitability of the logarithmic base 2 transformation in preparation for the statistical analyses.

Figure 5. 3 - The distribution of BNP changes from baseline



The average time between two NT-proBNP tests was very similar to the average time between two BNP tests. Figure 5.4 shows the distribution of the N-terminal pro BNP changes on the original scale, as well as following the logarithmic base 2 transformations in preparation for the statistical analyses.

Figure 5. 4 - The distribution of NT-proBNP changes from baseline



On average, BNP increased from visit 1 to visit 2 by 323.61 pg/ml, although the median change was only 28.2 pg/ml. While the mean N-terminal proBNP increase was 205.7 pg/ml between two visits, half of the patients hardly experienced any increases in their NT-proBNP levels. The median NT-proBNP change from visit 1 to visit 2 was only 20 pg/ml. Table 5.3 summarizes changes in B-type natriuretic peptides.

Table 5. 3 - Changes in BNP and NT-proBNP (pg/ml)

Change (2^{nd} measurement – 1^{st} measurement)	BNP change (n=262)	NT-proBNP change (n=715)
Lower quartile (Q1)	-87	-316
Median (Q2)	28.2	20
Upper quartile (Q3)	354	510
Interquartile range (Q3-Q1)	414	862
Mean	323.6	205.7
Standard deviation	2,287.5	3,022.8

Among patients with two consecutive BNP tests, almost half of them did not experience an increase in their BNP levels. Approximately 12% of these patients had an increase in BNP of up to 30%. Almost 10% or 26 patients had BNP increases of over 400% from the first visit

to the next one. This subset of patients was an average of 75 years old and slightly overweight, as reflected by the mean body mass index of 25.7 kg/m^2.

A similar trend was noticed in the patients with two consecutive NT-proBNP tests. Almost half of these patients either had their NT-proBNP levels decreased or unchanged from visit 1 to visit 2. Approximately 15% of these patients had their NT-proBNP levels increased by up to 30% and a little less than 7% or 49 patients had their NT-proBNP measurements increased to levels four times higher. Count and frequencies for percent changes in BNP and NT-proBNP are presented in Table 5.4.

Table 5. 4 - Percent changes in BNP and NT-proBNP from baseline

Percent change[a] category	BNP n (%)	NT-proBNP n (%)
Decrease	113 (43.1%)	308 (43.1%)
No change[b]	0 (0%)	24 (3.4%)
Increase 1-29.9%	31 (11.8%)	112 (15.7%)
Increase 30-49.9%	14 (5.3%)	51 (7.1%)
Increase 50-99.9%	29 (11.1%)	77 (10.8%)
Increase 100-199.9%	20 (7.6%)	57 (7.9%)
Increase 200-399.9%	29 (11.1%)	37 (5.2%)
Increase ≥400%	26 (9.9%)	49 (6.8%)
Total	262 (100%)	715 (100%)

[a] Change was defined as visit 2 – visit 1
[b] Percent changes between 0 and 0.99 were considered as "No change"

The 49 patients with NT-proBNP increases of over 400% had an average age of 79.5 years old at study entry. Approximately 45% of this subset of patients were obese, while the average body mass index was 30.5 kg/m2. Almost 60% were women.

The patients' characteristics for those who experienced an increase in the B-type natriuretic peptides between the two consecutive visits are summarized in Table 5.5.

Table 5. 5 - Characteristics of patients with BNP and NT-proBNP increases from baseline

Patient characteristic	All sub-cohort n=1094	Patients with BNP increases[a] (n=149)	Patients with NT-proBNP increases[a] (n=383)	P-value[b]
Age (years)	77.2 ± 10.3	77.5 ± 10.1	77.5 ± 10.1	0.862
Gender				0.772
Males	519 (47.4%)	73 (48.9%)	194 (50.6%)	
Females	575 (52.6%)	76 (51.1%)	189 (49.4%)	
Body mass index (kg/m^2)	28.2 ± 6.8	26.5 ± 5.2	28.4 ± 7.5	0.022
Underweight (<18.5)	35 (3.3%)	3 (2.1%)	17 (4.7%)	
Normal weight (18.5-24.9)	324 (30.9%)	62 (42.1%)	111 (30.4%)	
Overweight (25-30)	326 (31.1%)	50 (34.1%)	109 (29.8%)	
Obese (>30)	364 (34.7%)	32 (21.7%)	128 (35.1%)	
Smoking				0.978
Yes	73 (6.7%)	12 (8.1%)	29 (7.6%)	
No	583 (53.3%)	78 (52.3%)	200 (52.2%)	
Ex-smoker	437 (40%)	59 (39.6%)	154 (40.2%)	
Region				<0.0001
East Midlands	94 (8.7%)	41 (27.5%)	10 (2.6%)	
West Midlands	78 (7.2%)	0	42 (11.1%)	
London	314 (28.9%)	59 (39.6%)	103 (27.1%)	
East of England	36 (3.3%)	9 (6.1%)	9 (2.4%)	
North East	73 (6.7%)	1 (0.6%)	28 (7.3%)	
North West	33 (3.1%)	0	15 (3.9%)	
South West	41 (3.8%)	0	17 (4.5%)	
Northern Ireland	168 (15.5%)	4 (2.7%)	0	
Scotland	2 (0.2%)	0	0	
South Central	152 (14%)	8 (5.4%)	0	
South East Coast	81 (7.5%)	27 (18.1%)	15 (3.9%)	
Yorkshire and the Humber	21 (1.1%)	0	5 (1.3%)	
Serum creatinine (umol/L)	104.2 ± 33.4	102.7 ± 29.6	103.2 ± 35.2	0.865
Diabetes at baseline	81 (7.4%)	7 (4.7%)	32 (8.3%)	0.194
Hypertension and/or heart failure medication	998 (91.8%)	127 (86.9%)	354 (92.4%)	0.058

[a]Increases ≥ 1% from the baseline
[b] P-value from statistically comparing the patients with increases in BNP versus those with increases in NT-proBNP

Patients who experienced BNP increases were very similar to those who suffered NT-proBNP increases from the first visit to the next, regarding most baseline characteristics. The average age was approximately 77 years old in both groups. The proportions of men and women were

relatively similar between the two groups. More patients with two consecutive NT-proBNP tests were obese. The serum creatinine levels were very similar. There were a slightly higher percentage of patients with diabetes at baseline and exposed to heart failure and/or hypertension medication among those with two consecutive NT-proBNP tests than those with two BNP successive tests.

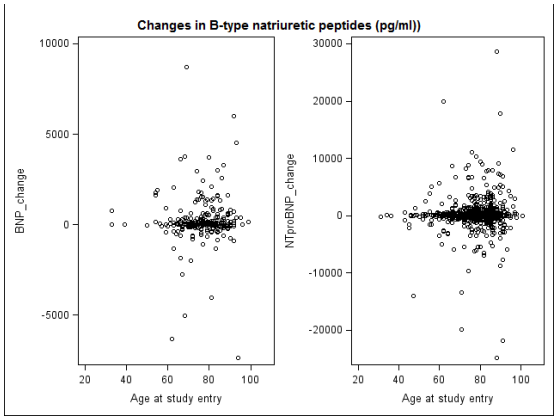
5.3.1. Changes in B-type natriuretic peptides in subgroups of patients

Changes in B-type natriuretic peptides and age

There was no linear relationship between the B-type natriuretic peptide changes and age at the study entry, as depicted in Figure 5.5. The linear Pearson correlation coefficient between the change in BNP and age at study entry was -0.03 (95% CI: [-0.15, 0.08], p-value=0.609, alpha=0.05). The non-parametric Spearman correlation coefficient between the change in BNP and age at study entry was 0.05 (95% CI: (-0.07, 0.17), p-value=0.409, alpha=0.05).

There was no statistical association between the change in NT-proBNP and age at the study entry. The linear Pearson correlation coefficient was 0.04 (95% CI: (-0.02, 0.11), p-value=0.228, alpha=0.05). The non-parametric Spearman correlation coefficient between the NT-proBNP change and age was 0.02 (95%CI: (-0.04, 0.10), p-value=0.426, alpha=0.05).

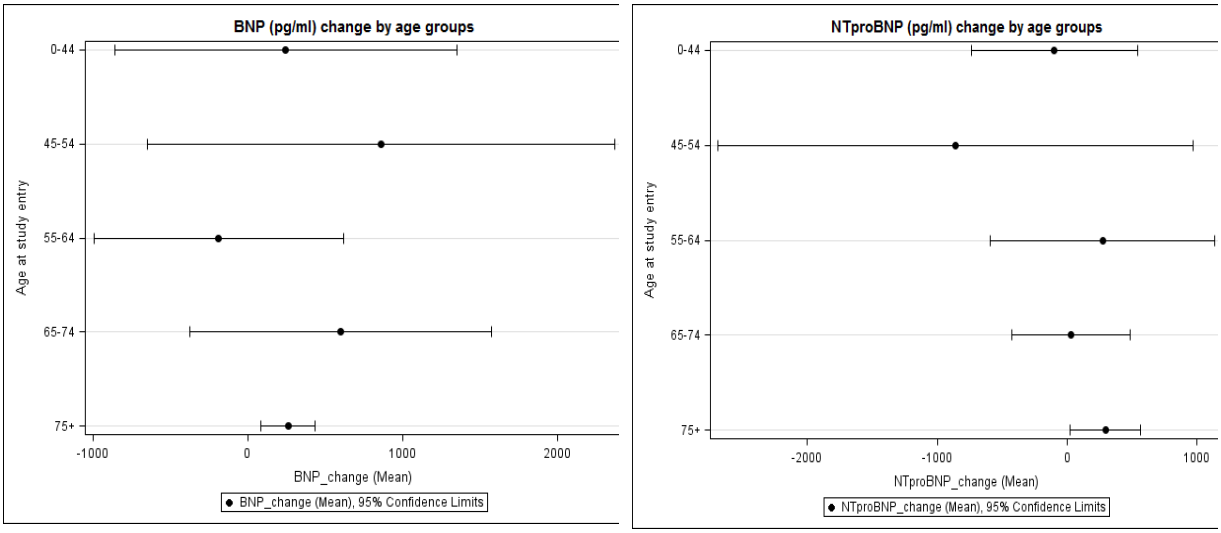
Figure 5. 5 - Changes in B-type natriuretic peptides and age



Age subgroups were also examined. Except for the age group of 55 to 64 years old, all other age subgroups had their BNP levels increased from one test to the next. On average, the highest increases in BNP changes from visit 1 to visit 2 were seen in the age group of 45-54 years old, followed by the group with ages between 65 and 74 years old. However, there were only four patients with ages between 45 and 54 years old. The standard deviation of BNP changes in these four patients was 948.74 pg/ml, which was higher than the average BNP changes of 860.59 pg/ml (median=868.75 pg/ml and IQR=1,636.36 pg/ml), reflecting the high variation in BNP changes. Surprisingly, patients 75 years old or older had the smallest increases in BNP. The average BNP change from visit 1 to visit 2 was 258.9 pg/ml (95%CI: (85.2, 432.5) pg/ml, median (IQR) of 43.49 (481.67) pg/ml).

The results were slightly different for NT-proBNP changes. On average, groups of patients over 55 years old experienced increases in NT-proBNP, while younger subgroups had decreases in NT-proBNP from visit 1 to visit 2. On average, the highest increase in NT-proBNP was observed in 488 patients over 75 years old. In this subgroup of patients, the mean NT-proBNP change was 292.7 pg/ml (95% CI: (20.2, 565.2), median (IQR) of 25.29 (1,017.58) pg/ml). Patients aged between 65 and 74.9 years old had minimal increases in NT-proBNP levels. The average NT-proBNP increase was 271.8 pg/ml (95% CI: (-590.1, 1133.9), median (IQR) of 23 (477.6) pg.ml) in 55 patients with ages between 55 and 64.9 years old. The changes in BNP and NT-proBNP from visit 1 to visit 2 are displayed by age groups in Figure 5.6.

Figure 5. 6 - Changes in BNP and NT-proBNP by age groups

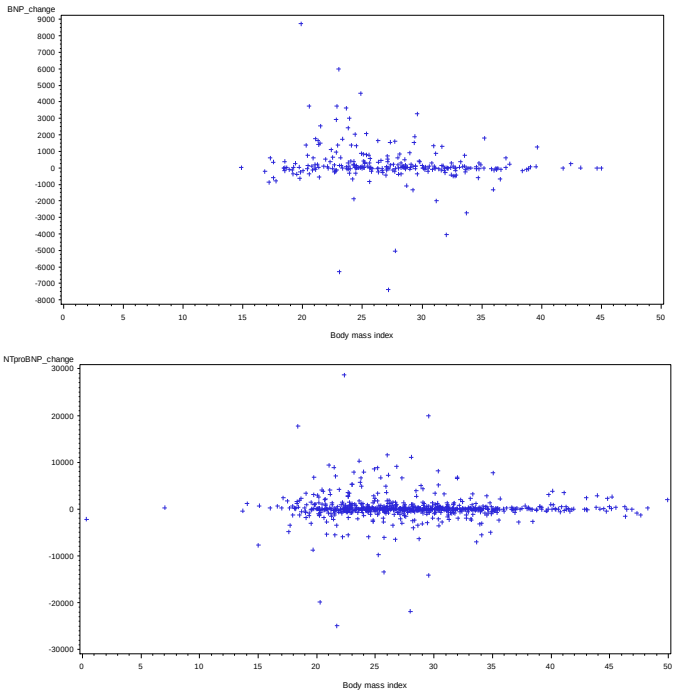


Changes in B-type natriuretic peptide and body mass index

The correlations between the changes in B-type natriuretic peptides and body mass index were slightly negative, as displayed in Figure 5.7. The Pearson correlation coefficient between the BNP changes and body mass index was -0.13 (95%CI: (-0.25, -0.01), p-value=0.028, alpha=0.5), while the non-parametric Spearman correlation was -0.14 (95%CI: -0.26, -0.02, p-value=0.017, alpha=0.5).

There was no correlation between the changes in NT-proBNP from visit 1 to visit 2 and body mass index. The Pearson correlation coefficient was -0.01 (95%CI: (-0.08, 0.06), p-value=0.765, alpha=0.05), while the non-parametric Spearman correlation coefficient was 0.02 (95%CI: (-0.12, 0.02), p-value=0.155, alpha=0.05).

Figure 5. 7 - Scatterplot betweeen BNP, NT-proBNP and body mass index



The changes in BNP and NT-proBNP from visit 1 to visit 2 were also assessed by the body mass index groups. On average, the highest increase in BNP levels was noticed in normal-weight patients, while the obese experienced decreases in BNP measures. The average BNP change was 926.3 pg/ml (95%CI: (182.1, 1670.6), median (IQR) of 109.8 (1,011) pg/ml in 91 normal weight patients and -59.8 pg/ml (95%CI: (-224.5, 104.8), median (IQR) of 55.36 (208.95) pg/ml) in 79 obese patients. On average, all body mass index subgroups experienced increases in NT-proBNP levels. In 209 normal weight patients, the average NT-proBNP change was 403.1 pg/ml (95%CI: (-121.3, 927.4), median (IQR) of 20 (1,313) pg/ml). In 242 obese patients, the mean NT-proBNP change was 130.9 pg/ml (95%CI: (-71.4, 333.4), median (IQR) of 7 (590) pg/ml). The obese patients had the lowest NT-proBNP variability. Figure 5.8 displays changes in BNP and NT-proBNP by body mass index subgroups.

Figure 5. 8 - Changes in BNP and NT-proBNP by body mass index groups

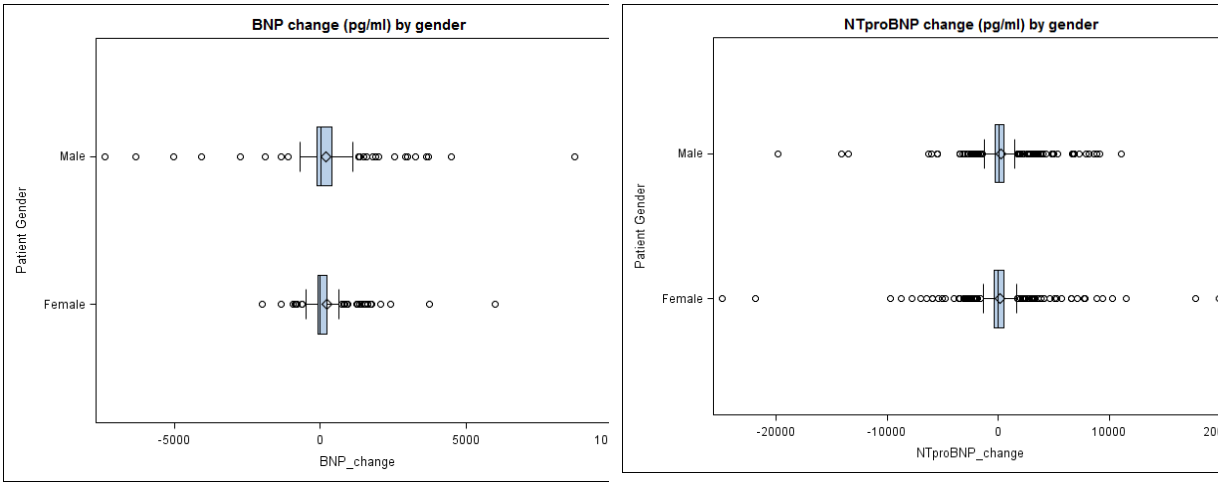


Similar results were obtained when examining the association between the B-type natriuretic peptides and weight.

Changes in B-type natriuretic peptides and gender

Males experienced higher increases in both BNP and NT-proBNP from visit 1 to visit 2, although the increase was more pronounced in BNP. The average BNP increase in males was 446. 2 pg/ml (95%CI: (-143.1, 1035.6), median (IQR) of 47.8 (499.5) pg/ml), while the mean BNP increase in females was 219.9 pg/ml (95%CI: (82.26, 357.6), median (IQR) of 12.9 (317.1) pg/ml). Compared to women, the variability of the BNP change was higher in males, as evidenced by the standard deviation of 3260.6 pg/ml in 120males and 829.9 pg/ml in 142 females. The average NT-proBNP increase was 211.7 pg/ml (95% CI: (-56.7, 480.2), median (IQR) of 35 (774) pg/ml) in 343 males and 200.1 pg/ml (95%CI: (-148.6, 548.7), median (IQR) of 7 (830)) in 372 females. The BNP and NT-proBNP changes from visit 1 to visit 2 by gender are displayed in Figure 5.9.

Figure 5. 9 - Changes in BNP and NT-proBNP by gender

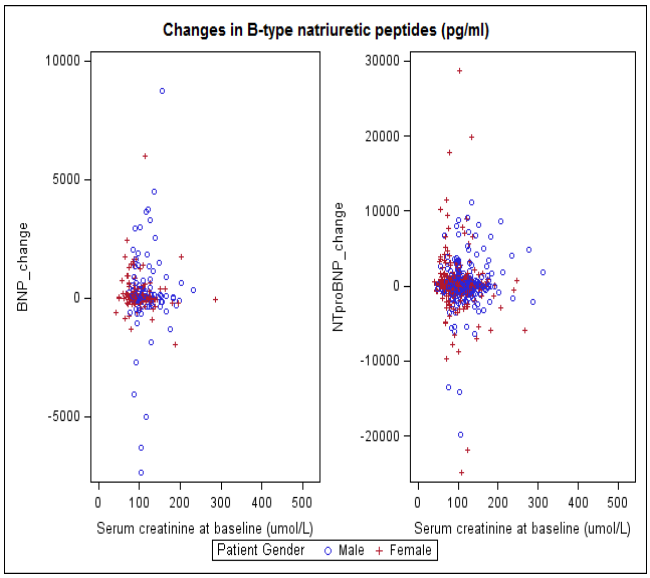


Changes in B-type natriuretic peptides and serum creatinine

It is well known that the serum creatinine levels depend on the patient's gender with women having lower levels than men because of a smaller muscle mass. The association between the B-type natriuretic peptide changes and serum creatinine is visualized in the Figure 5.10 overall, as well as by gender. Creatinine levels could temporarily be increased due to the toxicity associated with heart failure medication. (Ponikowski et al. 2016, Moe 2016) For males, there was no association between the BNP changes and serum creatinine levels at baseline. The linear Pearson correlation coefficient was 0.06 (95%CI: (-0.12, 0.24), p-value=0.509, alpha=0.05)), while the non-parametric Spearman correlation coefficient was 0.04 (95%CI: (-0.14, 0.22), p-value=0.645, alpha=0.05). A very mild positive linear association was noticed between the NT-proBNP changes from baseline and serum creatinine levels at baseline in males. The linear Pearson correlation coefficient was 0.11 (95%CI: (0.01, 0.22), p-value=0.033, alpha=0.05), while the non-parametric Spearman correlation was 0.02 (95%CI: (-0.08, 0.13), p-value=0.639, alpha=0.05).

In females, no linear association was observed between changes in any of the B-type natriuretic peptides and serum creatinine. The Pearson correlation coefficient between the BNP changes and serum creatinine was -0.08 (95% CI: (-0.24, 0.08), p-value=0.335, alpha=0.05). The Pearson correlation coefficient between the NT-proBNP changes and serum creatinine was -0.09 (95%CI: (-0.19, 0.01), p-value=0.081, alpha=0.053). However, the non-linear correlations showed that both BNP changes and NT-proBNP changes from baseline were mildly associated with the serum creatinine at baseline in females. For example, the Spearman correlation coefficient between the BNP changes and serum creatinine was -0.21 (95% CI: (-0.36, -0.03), p-value=0.016, alpha=0.05). The Spearman correlation coefficient between NT-proBNP changes and serum creatinine was -0.11 (95%CI: (-0.21, -0.01), p-value=0.042, alpha=0.05).

Figure 5. 10 - Changes in BNP. NT-proBNP and serum creatinine

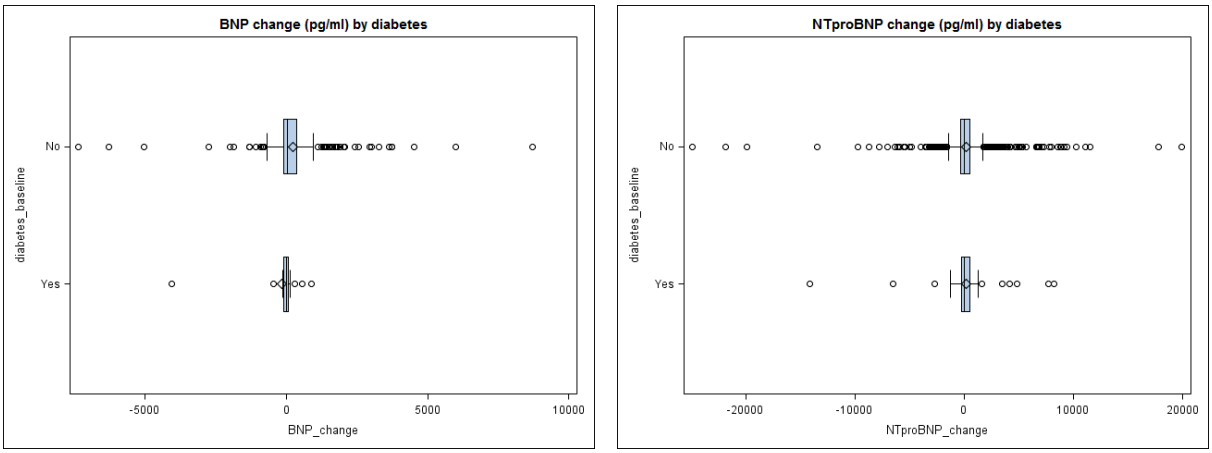


Changes in B-type natriuretic peptides and diabetes status at baseline

Focusing on changes in B-type natriuretic peptides, in the current study there were only 17 patients who had diabetes at the study entry and had at least two BNP measurements taken during the study. On average, their BNP levels decreased from visit 1 to visit 2. The mean

BNP change for diabetics at baseline was -179.2 pg/ml (95%CI: (-715.2, 356.7), median (IQR) of -13.8 (144) pg/ml). The non-diabetics experienced an increase in the mean BNP from visit 1 to visit 2. Based on 245 non-diabetic patients, the average BNP change was 358.4 pg/ml (95%CI: (63.2, 653.8), median (IQR) of 33 (455) pg/ml). On the other hand, on average NT-proBNP levels raised from visit 1 to visit 2 for both diabetics and non-diabetics. The diabetic patients experienced higher increases. The average NT-proBNP change from visit 1 to visit 2 in the 56 diabetic patients at baseline was 687.1 pg/ml (95%CI: (-586.7, 1960.8), median (IQR) of 18.5 (914.4) pg/ml). The changes in BNP and NT-proBNP can be visualized in Figure 5.11.

Figure 5. 11 - Changes in BNP and NT-proBNP by diabetes status at baseline

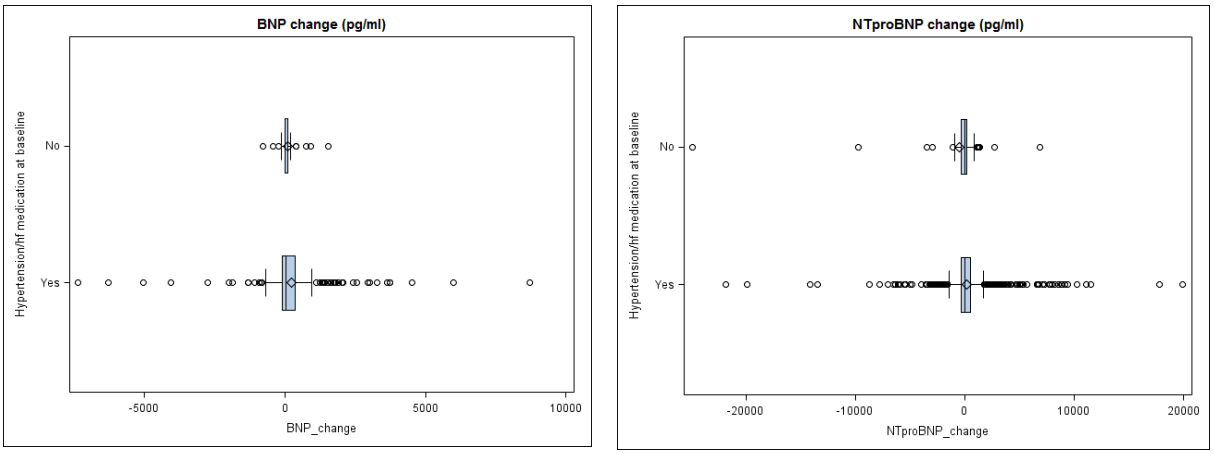


Changes in B-type natriuretic peptides and exposure or heart failure and/or hypertension medication

The BNP and NT-proBNP levels of those who had been exposed to heart failure and hypertension medication increased from visit 1 to visit 2. For example, the mean BNP increase was 350.9 pg/ml (95%CI: (31.9, 670.1), median (IQR) of 34 (476.3)), while the average NT-proBNP increase was 265.5 pg/ml (95%CI: (40.7, 490.3), median (IQR) of 20 (825.5). The

BNP changes and NT-proBNP changes from visit 1 to visit 2 are displayed by the status of hypertension and/or heart failure medication at baseline.

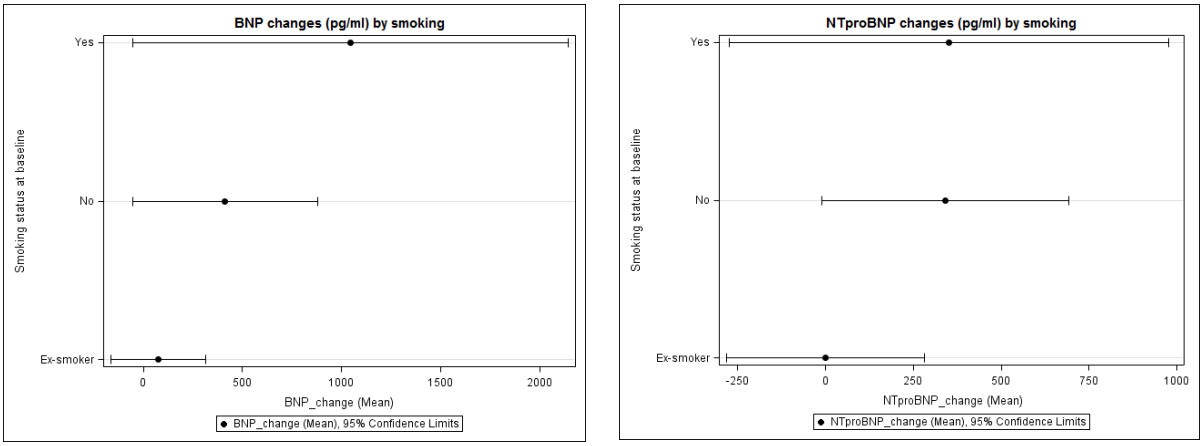
Figure 5. 12 - Changes in BNP, NT-proBNP and baseline exposure to hypertension/hf medication



Changes in B-type natriuretic peptides and smoking status

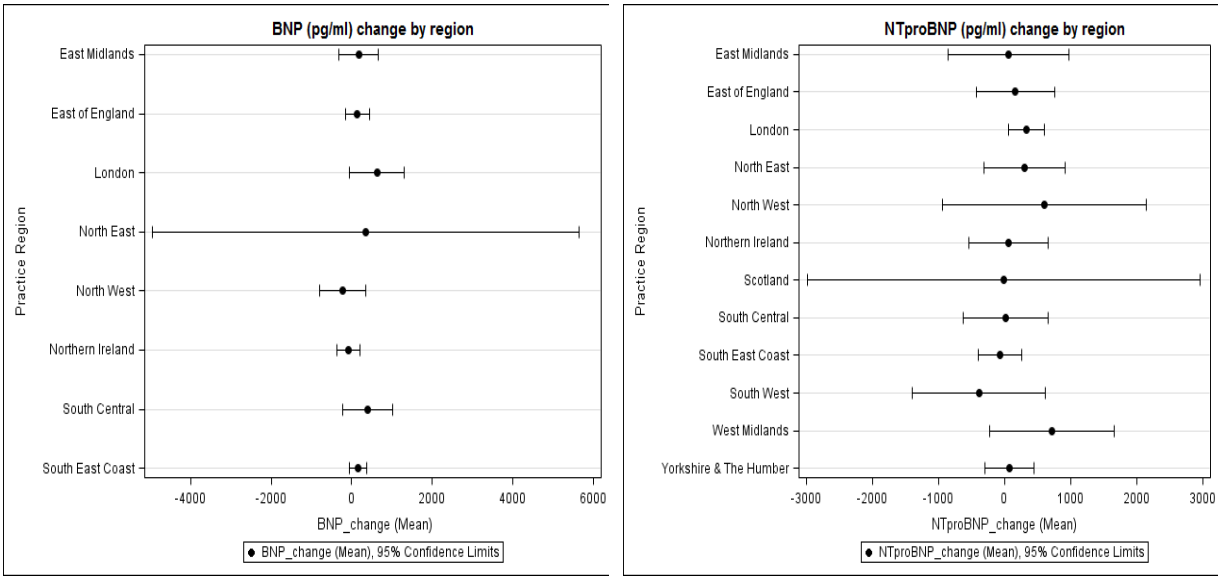
Figure 5.13 shows the changes in the B-type natriuretic peptides by the smoking status at baseline. Regardless the self-reported smoking status, on average, all subgroups experienced increases in the BNP or NT-proBNP levels from visit 1 to visit 2. The magnitude of the BNP change was higher in smokers compared to non-smokers or ex-smokers. Similarly, average NT-proBNP concentrations were higher for smokers than ex-smokers, although somewhat similar to non-smokers.

Figure 5. 13 - Changes in BNP and NT-proBNP by smoking



While no relationship was expected between changes in B-type natriuretic peptides and the region where the patient received care, for consistency purposes, Figure 5.14 shows the changes in BNP and NT-proBNP from visit 1 to visit 2.

Figure 5. 14 - Changes in BNP and NT-proBNP by practice region

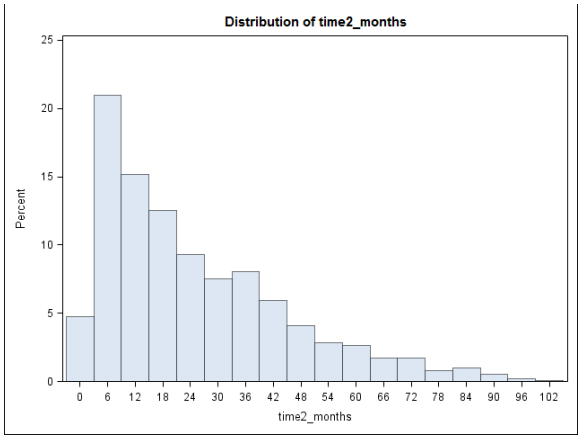


5.3.2. Changes in B-type natriuretic peptides and all-cause mortality

The primary outcome of all-cause mortality occurred in 285 patients or 26%. These patients were on average 79.6 years old and body mass index of 26.5 kg/m^2 at the study entry. There were slightly more females than males (51.6% versus 48.4%) who died. Approximately half of these patients were non-smokers, and most of them (e.g., 92.2%) had been exposed to heart failure and/or hypertension medication. Although at the baseline only 25 or 8.7% were diabetic, there were 81 diabetics or 28.4% by the end of the study.

To investigate whether the changes in B-type natriuretic peptides provided additional information on the mortality beyond a single point-in-time peptide level, the time zero was considered the time of the second visit. Overall, this time interval varied from 10 days to 83.5 months or almost seven years. The median follow-up period since the second B-type natriuretic peptide visit was a little over a year or 14.8 months. The distribution of the time since the second visit is displayed in Figure 5.15.

Figure 5. 15 - The distribution of the time interval since the second B-type natriuretic peptide visit



Considering the type of B-type natriuretic peptide tests, the median follow-up period after the second visit was 21.7 months for those who experienced increases in BNP from the first visit to the next visit, and 15.8 months for those with NT-proBNP increases.

For both BNP change and NT-proBNP change, the logarithmic (base 2) scale transformations were applied before conducting the statistical analyses. As such, the statistical models included only patients with increasing B-type natriuretic peptide levels from visit 1 to visit 2 for the change to be positive and its logarithmic value to be mathematically defined.

The association of the change in BNP and NT-proBNP from visit 1 to visit 2 and all-cause mortality is presented in Table 5.6. The "unadjusted" model was based on the log base 2 of change and the B-type natriuretic peptide value at visit 2. In addition to these variables, the "adjusted" model also included patients' characteristics such as age, gender, body mass index, smoking, geographic region, serum creatinine, diabetes, and hypertension and/or heart failure medication.

Table 5. 6 - The association between changes in BNP, NT-proBNP and all-cause mortality

Natriuretic Peptide	Model	n	All cause-mortality HR (95% CI)	p-value
Change[a] in BNP (visit 2 – visit 1)	Unadjusted[b]	149	1.01 (0.80, 1.26)	0.927
	Adjusted Model[c]	131	0.97 (0.73, 1.29)	0.855
Change[a] in NT-proBNP (visit 2- visit 1)	Unadjusted[b]	385	1.12 (0.96, 1.30)	0.143
	Adjusted Model[c]	344	1.16 (0.97, 1.38)	0.091

a. Change was log-base 2 transformed to reflect the doubling effect
b. Model adjusted for the log base 2 of change and the visit 2 peptide
c. Model adjusted for the log base 2 of change, visit 2 peptide, age, gender, BMI, smoking, geographic region, serum creatinine, hypertension and/or heart failure medication and diabetes at baseline, as well as the length of time between the first two consecutive tests

The results showed that the change in BNP did not have a significant impact on the all-cause mortality. The hazard ratio estimates and corresponding 95% confidence intervals were very similar between the "unadjusted" and "adjusted" models. The model diagnostics showed a good fit of the data, while the required assumptions, including the proportional hazards assumptions, were met.

Sensitivity analyses were conducted by applying the stepwise selection method. Among all variables considered, it was only the BNP level at time zero and serum creatinine that met the model entry criteria set at the 5% significance level. The BNP change did not meet this threshold.

The change in NT-proBNP initially did not provide additional information on the all-cause mortality beyond a single point-in-time NT-proBNP level. Diagnostic modeling indicated statistical assumptions were met, including the proportional hazards assumption.

Sensitivity analyses were conducted. When the region was removed from the statistical model while keeping all other factors aforementioned, the hazard ratio for the change in NT-proBNP slightly decreased to 1.16 (95% CI: (0.99, 1.37), p-value=0.065). Additionally, when the stepwise selection was applied, only the log base 2 of the NT-proBNP change and serum creatinine reached statistical significance at the 5% level. The hazard ratio for the serum creatinine was very close to the unity, and the result did not appear to have a clinically significant impact on the all-cause mortality. The hazard ratio for the log base 2 of the NT-proBNP change slightly increased to 1.27 (95% CI: (1.16, 1.38), p-value<0.0001).
The modeling diagnostics and verification of the assumptions indicated proper modeling fit.

The hazards ratio estimates for age are presented below. For every decade, the risk of all-cause mortality increased by approximately 20% (e.g. 27% from the change in BNP models,

and 21% from the change in NTproBNP models). Although the impact of age on the all-cause mortality increased with every decade, the impact was not statistically significant in the Cox regression models for changes in BNP or NTproBNP.

Table 5. 7 - The effect of age on the all-cause mortality

Parameter/ AGE	Hazard ratio 95% Confidence Intervals – change in BNP adjusted Cox regression model[a]	Hazard ratio 95% Confidence Intervals – change in NTproBNP adjusted Cox regression model[b]
Age		
1-unit change	1.02 (0.97, 1.07)	1.02 (0.99, 1.05)
10-unit change	1.27 (0.80, 2.03)	1.21 (0.90, 1.63)
At 40 years[c]	0.93 (0.73, 1.18)	0.92 (0.81, 1.04)
At 50 years[c]	0.95 (0.80, 1.13)	0.94 (0.86, 1.03)
At 60 years[c]	0.98 (0.87, 1.09)	0.97 (0.92, 1.03)
At 70 years[c]	1.01 (0.95, 1.06)	1.00 (0.97, 1.03)
At 80 years[c]	1.03 (0.98, 1.09)	1.03 (1.00, 1.06)
At 90 years[c]	1.06 (0.95, 1.18)	1.06 (1.00, 1.13)

[a]Model adjusted for the log base 2 of change in BNP, visit 2 BNP level, age, gender, BMI, smoking, geographic region, serum creatinine, hypertension and/or heart failure medication and diabetes at baseline, as well as the length of time between the first two consecutive BNP tests
[b]Model adjusted for the log base 2 of change in NTproBNP, visit 2 NTproBNP level, age, gender, BMI, smoking, geographic region, serum creatinine, hypertension and/or heart failure medication and diabetes at baseline, as well as the length of time between the first two consecutive NTproBNP tests
[c]1-unit change

5.3.3. Changes in B-type natriuretic peptides and cardiovascular mortality

There were 170 patients or 15.5% who died due to cardiovascular diseases and had at least two B-type natriuretic peptide tests measured. The 170 patients were older and slightly overweight. On average, their age was 81.7 years old, and the body mass index was 25.9 kg/m^2. Approximately half of them were males (51.8% or 88). Over 90% of these patients were taking heart failure medication and were non-diabetics at the baseline. They were followed for almost two years, on average.

The associations between the changes in BNP and NT-proBNP from visit 1 to visit 2 and cardiovascular mortality are presented in Table 5.7. The "unadjusted" model included the log base 2 of change and the B-type natriuretic peptide measure at visit 2. In addition to these variables, the "adjusted" model also included patients' characteristics at the baseline such as age, gender, body mass index, smoking, geographic region, serum creatinine, diabetes, and hypertension and/or heart failure medication, as well as the length of time between the first two successive natriuretic peptide tests. The results showed that the changes in B-type natriuretic peptides did not provide additional information on the cardiovascular mortality beyond a single point-in-time peptide level. The hazard ratio estimates and corresponding 95% confidence intervals for the change in NT-proBNP were very similar between the "unadjusted" and "adjusted" models. As for BNP, accounting for the length of time between the first two successive BNP tests, as well as patients' characteristics led to a higher hazard ratio of 1.25 (95% CI: (0.71, 2.18), p-value=0.424). Model diagnostics indicted proper fit, including the suitability of the proportional hazards assumption, functional forms of the continuous variables, as well as a random pattern of martingale and deviance residuals.

Table 5. 8 - Association between changes in BNP, NT-proBNP and CV mortality

Natriuretic Peptide	Model	n	CV-mortality HR (95% CI)	p-value
Change[a] in BNP (visit 2 – visit 1)	Unadjusted[b]	149	1.01 (0.74, 1.37)	0.923
	Adjusted Model[c]	131	1.25 (0.71, 2.18)	0.424
Change[a] in NT-proBNP (visit 2- visit 1)	Unadjusted[b]	385	1.14 (0.94, 1.37)	0.162
	Adjusted Model[c]	344	1.13 (0.90, 1.40)	0.267

a. Change was log-base 2 transformed to reflect the doubling effect
b. Model adjusted for the log base 2 of change and the visit 2 peptide
c. Model adjusted for the log base 2 of change, visit 2 peptide level, age, gender, BMI, smoking, geographic region, serum creatinine, hypertension and/or heart failure medication and diabetes at baseline, as well as the length of time between the first two successive natriuretic peptide measurements

The hazards ratio estimates for age are presented below. For every decade, the risk of cardiovascular mortality increased by approximately 10% - 15% (e.g. 15% from the change in BNP models, and 10% from the change in NT-proBNP models) but these are not statistically significant. Age adversely impacts the cardiovascular mortality once the patients turn 80-years old.

Table 5. 9 - The effect of age on the cardiovascular mortality

Parameter/ AGE	Hazard ratio 95% Confidence Intervals – change in BNP adjusted Cox regression model[a]	Hazard ratio 95% Confidence Intervals – change in NTproBNP adjusted Cox regression model[b]
Age		
1-unit change	1.01 (0.95, 1.08)	1.01 (0.97, 1.04)
10-unit change	1.15 (0.60, 2.23)	1.10 (0.78, 1.55)
At 40 years[c]	0.68 (0.51, 0.91)	0.86 (0.78, 0.94)
At 50 years[c]	0.76 (0.62, 0.94)	0.90 (0.84, 0.96)
At 60 years[c]	0.86 (0.75, 0.98)	0.95 (0.90, 0.99)
At 70 years[c]	0.96 (0.90, 1.03)	1.00 (0.97, 1.03)
At 80 years[c]	1.08 (1.01, 1.16)	1.04 (1.01, 1.09)
At 90 years[c]	1.21 (1.05, 1.39)	1.10 (1.03, 1.16)

[a]Model adjusted for the log base 2 of change in BNP, visit 2 BNP level, age, gender, BMI, smoking, geographic region, serum creatinine, hypertension and/or heart failure medication and diabetes at baseline, as well as the length of time between the first two consecutive BNP tests
[b]Model adjusted for the log base 2 of change in NTproBNP, visit 2 NTproBNP level, age, gender, BMI, smoking, geographic region, serum creatinine, hypertension and/or heart failure medication and diabetes at baseline, as well as the length of time between the first two consecutive NTproBNP tests
[c]1-unit change

Sensitivity analyses were conducted. When applying the stepwise selection method to the adjusted model for the BNP change, no variables met the 5% level for entry into the model. When applying the stepwise selection method to the adjusted model for the NT-proBNP change, it was only the log base 2 change in NT-proBNP that met the entry criteria at the 5% significance level. In this model, the hazard ratio was 1.13 (95% CI: (1.02, 1.25), p-value=0.017). Each doubling in NT-proBNP change over time was associated with a 13% increased likelihood of dying due to cardiovascular disease.

5.4. Discussion

By taking an exploratory approach, this study examined the association between important risk factors and changes in B-type natriuretic peptides in chronic heart failure patients managed by primary care physicians.

The patients included in this chapter had at least two B-type natriuretic measurements taken at separate visits. The average time between two consecutive B-type natriuretic peptide measurements was approximately 2.5 years. Few patients did not experience changes in their B-type natriuretic peptides, while approximately half of them had their levels increased from one measurement to the next.

The patients' baseline characteristics were very similar between those with two consecutive BNP tests and those with two successive NT-proBNP tests regarding age, gender, smoking, serum creatinine, diabetes status, exposure to hypertension and/or heart failure medication. The 715 patients with two consecutive NT-proBNP tests were slightly heavier than those with two successive BNP tests, as more obese patients had NT-proBNP tests measured. The average body mass index was 28.4 kg/m2 for those with two consecutive NT-proBNP tests and 27.3 kg/m2 for those with two BNP tests.

No association was noted between age and changes in B-type natriuretic peptides. Patients 75 years old or older had the smallest increases in BNP. This may be because older patients are exposed to more heart failure and hypertension medication, which may lead to lower B-type natriuretic peptide levels (Nakano et al. 2016). The risk of mortality increased with age, but the impact on the all-cause mortality was not statistically significant. Age

adversely impacted the cardiovascular mortality once the patients turn 80-years old. Overall, regardless the age, for every decade older, the adjusted risk of all-cause mortality and cardiovascular mortality increased by 27% and 15%, respectively, upon adjusting for the change in BNP, age, gender, body mass index, smoking, geographic region, serum creatinine, hypertension and/or heart failure medication, diabetes status at baseline and length of time between the first two successive BNP tests. Upon adjusting for the change in NTproBNP, patients' characteristics and models, for each 10-year increase in age, the adjusted risk of all-cause mortality and cardiovascular mortality were 21% and 10%, respectively.

There was a mild, negative association between the BNP changes and body mass index. Both the linear and non-parametric results were statistically significant at the 5% significance level. The study was not formally powered to detect such differences. Despite the association observed for BNP changes, there was no correlation between the N-terminal fragment BNP and the body mass index. Both the Pearson and Spearman correlations were zero.

Males experienced higher increases in BNP than females, although the average changes in NT-proBNP were very similar between men and women.

A weak association was noticed between the NT-proBNP changes from baseline and serum creatinine levels at baseline. This mild association was linear and positive in males and non-linear and negative in females. In men, the linear Pearson correlation coefficient was 0.11 (95%CI: (0.01, 0.22). In women, the Spearman correlation coefficient was -0.11 (95%CI: (-0.21, -0.01)). No linear association between BNP changes from baseline and serum creatinine at baseline was observed in males or females. However, in female patients, a non-linear, weak correlation between the BNP changes and serum creatinine was noted. The Spearman

correlation coefficient between the BNP changes and serum creatinine was -0.21 (95% CI: (-0.36, -0.03).

Approximately 7% of patients had diabetes at the study start. Given the small numbers, the results were not consistent between those with BNP and NT-proBNP tests. For example, the average BNP levels decreased, and NT-proBNP levels increased among diabetics. Alonso et al reported higher NT-proBNP levels in diabetic than non-diabetic ambulatory heart failure patients (Alonso et al. 2016). In the Swedish Heart Failure registry, BNP levels were higher in those with type II diabetes mellitus among patients with an ischemic heart disease (Johansson et al. 2016). However, the NT-proBNP levels were very similar between diabetics and non-diabetics with an ischemic heart disease (Johansson et al. 2016). Among heart failure patients without an ischemic heart disease, both BNP and NT-proBNP levels were lower in diabetics than non-diabetics (Johansson et al. 2016).

On average, smokers had the highest increase in B-type natriuretic peptides compared to non-smokers or ex-smokers. While the smoking status was self-reported and thus subject to measurement bias, Patrick reported the accuracy of self-reporting smoking (Patrick et al. 1994).

Since the statistical analysis for the changes in the B-type natriuretic peptides was conducted on the logarithmic (base 2) scale, only patients who experienced increases in these peptide levels from visit 1 to visit 2 were included in these analyses, as the logarithmic transformation can be applied only to positive numbers.

In this study, changes from baseline in BNP did not provide additional information on the all-cause and cardiovascular mortality beyond a single point-in-time peptide level. The interaction between the change and the peptide level was not significant.

Concerning NT-proBNP, each doubling in NT-proBNP change over time was associated with a 16% increased likelihood of dying of any cause upon adjustment for patient's age, gender, body mass index, smoking, diabetes, serum creatinine, geographic region, previous exposure to hypertension and/or heart failure medication, as well as the length of time between the first two successive natriuretic peptide tests. Each doubling in NT-proBNP change over time was associated with a 14% increased likelihood of dying due to cardiovascular disease. However, these changes were not statistically significant.

In the PARADIGM-HF trial, increases in NT-proBNP were associated with an increased risk of cardiovascular death or heart failure hospitalization per doubling of NT-proBNP (Zile et al. 2016). The hazard ratio was 1.24 (95% CI: (1.11, 1.38), p-value < 0.0001) (Maisel et al. 2016, Zile et al. 2016). Although the results appeared to be similar, key differences included different outcomes, different patient population and different treatment. In the PARADIGM-HF, the endpoint was the first occurrence of cardiovascular death or heart failure hospitalization, while in the current study the endpoints were all-cause mortality and cardiovascular mortality (McMurray et al. 2013). In the PARADIGM-HF trial, all chronic heart failure patients had been hospitalized in the previous 12 months, which was not necessarily the case for the patients managed by the primary care practices (McMurray et al. 2013). The chronic heart failure patients in the current study were expected to be less severe. Patients in PARADIGM-HF were exposed to treatment with ARNI (angiotensin receptor-neprilysin inhibitor), which is known to decrease the NT-proBNP levels (Packer et al. 2015). The results for NT-proBNP were similar to the ones observed in the PARADIGM-HF, where increases in NT-proBNP (Zile et al. 2016, McMurray et al. 2014b, McMurray et al. 2013).

In an ambulatory care setting, Nunez showed that a single BNP measurement was better than a categorical BNP change over time (Núñez et al. 2012). Although the current results could not directly be compared due to different reporting methods, it does raise the question of the added value of the serial BNP measurements. Michtalik studied two groups of NT-proBNP changes (e.g., ≥ 50% and < 50%) from admission to discharge in an acute setting, while the current study investigated the actual change on a continuous scale in a primary care setting (Michtalik 2011). Additionally the study outcome was readmission or death within one year from admission to hospital, while the current study looked at the all-cause mortality and cardiovascular mortality over almost seven years since the time zero of the second B-type natriuretic peptide level (Michtalik 2011).

Additional sensitivity analyses in subgroups of patients who experienced BNP and NT-proBNP increases of at least 200% and 400% from baseline were conducted. Given the small subgroup sizes and low event rates, the results were inconclusive, marked by huge confidence intervals and occasionally non-estimable parameters.

5.4.1. Limitations

Most of the study limitations introduced in Chapters 3 and 4 are also present in the current chapter.

The study population represents a very small sample of the entire heart failure population managed in the primary care setting within the United Kingdom. This study shows that B-type natriuretic peptides are repeatedly tested in only approximately 1.2% of such patients. The point of care BNP testing was not represented in this study.

Since about half of the medical practices had consented to have the cause of death evaluated, it is possible that more patients died of cardiovascular diseases. On the other hand, since the ONS Mortality Data sometimes records multiple causes of death, it is possible that the primary cause of death was not a cardiovascular one. The current study defined cardiovascular mortality if one of the reported causes of death was a cardiovascular disease. Therefore, the hazard ratios presented in this chapter may not fully characterize the true association between changes in B-type natriuretic peptides and cardiovascular mortality.

The residual confounding due to unrecorded variables pertaining to the etiology and severity of heart failure or B-type natriuretic peptide assays could not be ruled out. Therefore, the prognostic value of the changes in B-type natriuretic peptides on the all-cause mortality and cardiovascular mortality could not be evaluated in NYHA subgroups of chronic heart failure patients or patients with reduced or preserved ejection fraction or subgroups defined by the ischemic etiology of heart failure. The assays that measured the B-type natriuretic peptides were not recorded by the general practices. Thus no information about the coefficients of variation of the assays was available. The accuracy of the prognostic values of the changes in such peptides depends on the analytic variability of the tests, as well as the biological variability within the chronic heart failure patients over time

It is possible that the relationship between changes from baseline in B-type natriuretic peptides and mortality was influenced by the treatment and management of the chronic heart failure patients throughout the disease. Certain heart failure medications produce hemodynamic and biological benefits that decrease B-type natriuretic peptide levels (Ponikowski et al. 2016, NICE 2010). Most of heart failure patients take hypertension medication, and some of them are prescribed combinations of drugs such as ACE inhibitor and Calcium channel blocker or

diuretics depending on patient's age, gender, comorbidities, medical history, response to previous treatment, tolerance to certain medication. Since electronical medical records don't accurately capture the start and end date of specific heart failure medication and medication compliance is not monitored, the approach taken in this study was to define exposure to heart failure and/or hypertension if the patient took any medicine listed in the Appendix D, regardless the dose or duration or combination of pharmacotherapy.

The statistical models did not incorporate time-varying covariates. Given the observational nature of the study and the variable entry of the patients into the cohort, patients did not have covariates measured at regular intervals of time. This is more likely to occur in a clinical trial setting.

The selection bias was assessed by comparing the baseline characteristics between those with BNP tests and NT-proBNP tests. The patients' baseline characteristics were very similar between the two groups. The percentage of missing data was small. Similar missing patterns were observed for those with BNP tests and those with NT-proBNP tests.
The results are not generalizable outside the United Kingdom.

5.4.2. Implications for research

One option to overcome the limitations encountered in the current study is to conduct a long-term prospective study in heart failure patients managed within the primary care setting. A representative sample of primary care practices could be selected. These primary care practices should collect similar information about patients such as: age, gender, body mass index, smoking, geographic region, etiology of heart failure, type of heart failure, disease severity, comorbidities, symptomatic assessment such as NYHA, treatment information and type of assay to be used for measuring BNP and NTproBNP. The pharmacotherapy, symptomatic heart

failure assessment, and clinical variables should be regularly assessed in all patients so that the impact of changes could be evaluated. A follow-up period of five years with clinical assessments at every 3 months is suggested.

5.4.3. Implication for clinical practice

The study showed the changes in BNP and NTproBNP did not provide additional information on the all-cause mortality and cardiovascular mortality beyond a single point-in-time peptide level. From a monitoring perspective, measuring the next B-type natriuretic peptide after more than two years from the initial test in the primary care setting offers little value in optimizing the management of chronic heart failure patients.

The risk of mortality increased with age, but the impact on the all-cause mortality was not statistically significant. Age adversely impacted the cardiovascular mortality once the patients turn 80-years old.

Novel pharmacotherapy research for treating chronic heart failure patients is undergoing. The clinical interpretation of the changes in BNP and NT-proBNP will have to be assessed in the context of the drugs' mechanisms of action in addition to patient's demographics and clinical characteristics. For example, the angiotensin-neprilysin inhibition leads to decreasing NT-proBNP, but not necessarily BNP (Packer et al. 2015) and is expected to change the interpretation of future studies.

5.5. Conclusion

Conducted in a primary care setting, this observational study showed that changes in BNP and NTproBNP did not provide additional information on the all-cause mortality and cardiovascular mortality beyond a single point-in-time peptide level. Each doubling in NT-proBNP change over time was associated with a 16% increased likelihood of dying of any cause and 13% increased likelihood of dying of cardiovascular diseases upon adjustment for patient's age, gender, body mass index, smoking, diabetes, serum creatinine, geographic region, previous exposure to hypertension and/or heart failure medication and length of time between the natriuretic peptide tests. Each doubling in BNP change over time was associated with a 25% increased likelihood of dying due to cardiovascular disease upon adjustment for the patients' characteristics. These associations were not statistically significant.

More studies are needed to link changes in B-type natriuretic peptides to complex pathophysiological mechanisms and clinical outcomes in a continuous effort to improve and extend the life of chronic heart failure patients.

Chapter 6 - BNP variability and clinical factors influencing it

6. 1. Introduction

B-type natriuretic peptides serve an essential role when diagnosing heart failure patients in the presence of dyspnea, risk stratifying, predicting adverse outcomes, and determining the response to pharmacotherapy. The monitoring value of B-type natriuretic peptides remains to be proven. The most recent medical guidelines (Ponikowski et al. 2016) acknowledge that heart failure patients may benefit from monitoring of biomarkers, although the monitoring regimen may vary from patient to patient. They also identify a gap in research concerning the role of remote monitoring strategies in heart failure (Ponikowski et al. 2016).

Devising an optimal interval between consecutive BNP tests is critical when developing, evaluating and implementing a patient monitoring scheme (Glasziou 2008). It is essential to understand whether clinically relevant change occur during the time interval, as well as the responsiveness of the test (Glasziou 2008). One crucial aspect becomes the variability of BNP when assessing changes in clinical status. The changes may vary according to the patient's heart failure stage and existing comorbidities.

Compared to cardiac imaging, the low cost and broad accessibility of blood tests measuring B-type natriuretic peptides are definite advantages. The high variability of B-type natriuretic peptides may hamper the clinical interpretation of the patient's status, potentially affecting their utility over time, especially considering the large heterogeneity of chronic heart failure patients.

Previous studies have published reference change values for BNP from 66% to 198% (Piper 2016, Schindler 2016, Frankenstein 2011, Meijers 2017). These studies were small with different monitoring intervals, often short and including healthy individuals. More recently, Schindler et al. assessed the BNP variability in 39 stable heart failure patients, reporting a within-subject coefficient of BNP variation of 27.6%, an analytical variability of 4.1% (Abbott Architect assay) and reference change value of 113% (Schindler 2016). The patients were routinely followed-up at a heart failure hospital clinic. The blood draw times were not controlled but rather based on the availability of study participants and hospital staff. Apart from gender and BNP, no other demographics and clinical information were reported about the stable heart failure patients.

Since B-type natriuretic peptides are higher in elderly or female patients, and lower in those obese or exposed to diuretics, smaller BNP values may not necessarily reflect a clinical change in heart failure status (Ibrahim 2017, Latini 2002, Levin 1998, Wang 2004). While echocardiography can assist in the evaluation of the left ventricular structure and function, it is not immediately available in the primary care setting.

It is of interest to investigate how the variability of other clinical variables often measured in the primary care setting such as weight, heart rate, blood pressure and serum creatinine relate to BNP. The serum creatinine levels are elevated in chronic heart failure patients, which may be partly due to the accumulated toxicity associated with the heart failure medication (Dobre 2012). When ACE inhibitors and ARBs are taken concomitantly, the renal function may worsen even more [https://en.wikipedia.org/wiki/Creatinine, last accessed on April 9, 2016]. Clinical guidelines in the United Kingdom recommend monitoring creatinine for signs of renal impairment in chronic heart failure patients taking an ACE inhibitor, ARB or

Aldosterone antagonist (NICE 2010). The monitoring schedule depends on the patient's clinical status; however, the emphasis is on short time intervals of up to two weeks when changing the dose or when the patients' condition worsens, and every six months for stable chronic heart failure patients (NICE 2010).

The objectives of this chapter are:

1. To estimate the within-subject short-term variability of BNP in non-diabetic, stable mild and moderate heart failure subjects (NYHA class II-III)
2. To explore the impact of clinical, cardiac and other factors on the BNP variability

Clinical factors: heart rate, systolic and diastolic blood pressure, renal dysfunction,
Cardiac factors: left ventricular ejection fraction, cardiac output, left ventricular left end-diastolic and end-systolic volumes obtained from an echocardiography
Other factors: age, gender, body mass index

3. To examine the correlation between BNP and cardiac structure factors, weight, heart rate, blood pressure, serum creatinine in stable chronic heart failure subjects

6.2. Methods

The objectives were addressed based on data from a phase 2 clinical trial in stable chronic heart failure patients in a post-hoc analysis. This clinical trial is registered on www.clinicaltrials.gov with the following clinical identifier number: NCT01357850 at https://clinicaltrials.gov/ct2/show/NCT01357850?id=112670&rank=1 (last accessed on 01-07-2018). This includes: study description, condition/disease, study design, study start and completion dates, eligibility criteria, primary and secondary outcomes, participating sites and

the primary publication (Lepore 2016). Access to additional study documents such as statistical analysis plan, individual participant datasets, clinical study report, annotated case report forms, data specification, informed consent, study protocol can be requested through the above link. This webpage is also depicted in the Appendix H. A clinical trial setting provides an exciting opportunity to investigate the BNP variability because patients are assessed similarly and at the same time intervals throughout the study duration.

6.2.1. Description of trial design

The study was a multi-center, randomized, placebo-controlled clinical trial that evaluated the safety of a new therapeutic agent in stable NYHA class II-III heart failure subjects. The investigational drug was in development for improved myocardial efficiency and increased exercise ability in heart failure patients. The clinical researchers and subjects were blinded to the study drug. The study design was a parallel group with four arms: placebo and three increasing dose levels of the new therapeutic agent. The thirty patients included in this chapter were not exposed to any dose of a new investigational medicine. Therefore, the BNP variability studied here cannot be attributed to such potential confounding. The study duration was 13 weeks followed by an additional visit approximately one month after the study was completed. The clinical trial was initiated in September 2010 and ended in August 2012. The B-type natriuretic peptide, cardiac output, left ventricular ejection fraction, left ventricular end-diastolic volume, and left ventricular end-systolic volume was measured every six weeks, while the weight, heart rate, diastolic and systolic blood pressure were collected weekly. The serum creatinine was measured every other week, as described in Table 6.1.

Table 6. 1 - Schedule of clinical measurements

Measurement	Week												
	1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*
BNP	x						x						x
Cardiac output	x						x						x
LVEF	x						x						x
LVEDV	x						x						x
LVESV	x						x						x
Heart rate	x	x	x	x	x	x	x	x	x	x	x	x	x
Blood pressure	x	x	x	x	x	x	x	x	x	x	x	x	x
Weight	x	x	x	x	x	x	x	x	x	x	x	x	x
Serum creatinine	x		x		x		x		x		x		x
"x" indicates that the clinical variable was evaluated during the respective week													

6.2.2. Study participants

The clinical trial included clinically stable chronic heart failure patients on optimal therapies for at least three months before the baseline visit. The etiology of chronic heart failure was ischemic or non-ischemic cardiomyopathy. Patients were in NYHA class II or III for at least six months before enrollment into the study. They had a left ventricular ejection fraction of less than 40% within the past two years from the study start. Both males and females were between 21 and 75 years of age.

Table 6. 2 - New York Heart Association symptom classification for heart failure

NYHA class	Description
I	"No limitation of physical activity"
II	"Slight limitation of physical activity, which may result in fatigue, palpitation or shortness of breath; Comfortable at rest"
III	"Clear limitation of physical activity, which causes fatigue, palpitation or shortness of breath; Comfortable at rest"
IV	"Unable to carry on physical activity without discomfort; Heart failure symptoms at rest"
Source: www.heart.org; Last accessed on 30/5/2017 *Adapted from Dolgin M, Association NYH, Fox AC, Gorlin R, Levin RI, New York Heart Association. Criteria Committee. Nomenclature and criteria for diagnosis of diseases of the heart and great vessels. 9th ed. Boston, MA: Lippincott Williams and Wilkins; March 1, 1994.* *Original source: Criteria Committee, New York Heart Association , Inc. Diseases of the Heart and Blood Vessels. Nomenclature and Criteria for diagnosis, 6th edition Boston, Little, Brown and Co. 1964, p 114.*	

Patients with any of the following current or recent conditions were excluded: active ischemia, diabetes mellitus, thyroid disease, genetic disorders of skeletal muscle, clinically significant pericardial diseases, renal dysfunction, history of coronary revascularization, history of alcohol or drug abuse, history of deep vein thrombosis or pancreatitis and other medical conditions with the life expectancy of less than 1 year. Diabetes mellitus was assessed to be present based on a fasting glucose level greater than 140 mg/dL or HbA1c more than 7%. The renal dysfunction was determined based on an estimated glomerular filtration rate (eGFR) of less than 40 ml/min/1.73m^2 at screening. Patients with a positive Hepatitis B or C or HIV test in the previous three months were excluded. Other reasons for exclusions at screening were: Calcitonin levels greater than 100 pg/ml or triglycerides higher than 850 mg/dl triglycerides or resting low (< 85mmHg) or high (>170 mmHg) systolic blood pressure or high diastolic blood pressure (>110 mmHg). Mentally incapacitated patients and pregnant women were also excluded from the study.

6.2.3. Outcomes

The primary outcome of this analysis was BNP. The unit of reporting was ng/l, which is the same as pg/ml. While the assay type was not collected, all blood tests were processed at a central laboratory. The within-subject BNP variability was estimated over a 3-month period.

The between-subject BNP variability was described in subgroups of patients based on demographics, clinical characteristics, medical history, and previous heart failure medication exposure. The changes in BNP levels up to 3 months were described.

The serial correlations between BNP and cardiac structure factors, weight, heart rate, blood pressure, serum creatinine in stable chronic heart failure subjects were estimated.

6.2.4. Sample size and randomization

The sample size was 30 subjects. The sample size was based on feasibility and no formal power calculations for estimating the variability of BNP were conducted. The patients were randomly assigned to not receiving investigational medication for heart failure. The allocation ratio was the same across all treatment groups.

6.2.5. Blinding

The clinical investigators and patients were blinded to the treatment received.

6.2.6. Statistical methods

No formal statistical hypothesis was tested. The approach was exploratory or hypothesis testing.

Summary statistics (e.g., n, mean, 95% confidence interval for the mean, geometric mean, 95% confidence interval for the geometric mean, standard deviation, standard error, between-subject coefficient of variation, median, range, interquartile range) were generated for BNP, weight, heart rate, serum creatinine, systolic and diastolic blood pressure over time. A similar approach was adopted for the absolute changes from baseline and percent changes from baseline in BNP over time. To explore possible trends, summary statistics were produced by various subgroups defined by gender, weight, age, renal dysfunction, prior medication exposure, history of arrhythmia, hypertension, angina, ischemic cardiomyopathy, non-ischemic cardiomyopathy. They are presented in the Appendix H.

Repeated measures mixed models were employed to estimate the within-subject variability. Unlike the generalized linear models, mixed models incorporate random and fixed effects,

allowing for correlations among measurements as well as heterogeneous variance, while the normality assumption of the observations remains. Random factors were included to account for sources of variation, and their values were assumed to be a random sample from a population of interest whose values are normally and independently distributed. Consequently, some values could be higher or lower than the overall average effect. The model fit was evaluated by examining the marginal residuals, conditional residuals, covariance parameter diagnostics and influence diagnostics.

Using matrix notations, the general form of the mixed models is: $Y=X\beta + Z\gamma + \varepsilon$ (Laird 1982), where X is the known design matrix for the fixed effects, β is the vector of fixed effects, Z is the known design matrix for the random effects, γ is the unknown parameter vector of random effects and ε is the normally distributed random error. When variability is of interest, the focus is on the random part of the mixed models. I fitted random intercept models with the intercept as a random effect and week as a fixed effect, as well as random coefficient models, where the subject was a random effect and week was a fixed effect. The natural logarithm of the BNP baseline value was also modeled as a covariate.

BNP measurements taken over time in the same subject are expected to be correlated. Usually, the further apart the measurements, the lesser the correlation. The following covariance structures were considered: variance components, unstructured, compound symmetry, heterogeneous autoregressive and heterogeneous Toeplitz. The matrix form of these structures could be found in Appendix H.

In the statistical analysis, BNP was logarithmically (base e) transformed due to the right skewness of the distribution. The summary statistics were presented in the original scale. The within-subject coefficient of variation was calculated using the following formula:

$CV_w(\%)=100* \sqrt[2]{exp^{\sigma^2}-1}$, where σ was the within-subject mean square error from the mixed model analysis of the BNP log-transformed levels (Laird 1982).

The reference change values (RCV) were calculated using a lognormal approach based on the following derivations (Fraser 2001, Iglesias 2005, Frankenstein 2011):

$RCV_{positive}=[\exp(1.96 \times 2^{1/2} \times \sigma) - 1] \times 100$

$RCV_{negative}=[\exp(-1.96 \times 2^{1/2} \times \sigma) - 1] \times 100$,

, where σ is the estimated within-subject mean square error mentioned above.

The lognormal distribution has asymmetrical limits for the upward (e.g., positive) or downward (e.g., negative) values.

The statistical analysis of weight, heart rate, serum creatinine, systolic and diastolic blood pressure were conducted on the original scale, without any data transformation. The within-subject coefficient of variation was estimated as the square root of the residual variance from the intercept model divided by the overall mean. Since each subject had several measurements taken, the subject's means were calculated for weight, heart rate, serum creatinine, systolic, and diastolic blood pressure. Additionally, the within-subject coefficient of variation from the subject's mean was computed for each subject. An average within-subject coefficient of variation was obtained for the clinical variable of interest.

The between-subject coefficient of variation CVb(%) was calculated according to the following formula: $CV_b(\%) = 100 *$ standard deviation/mean.

The serial linear correlations between BNP and weight, heart rate, blood pressure, serum creatinine were characterized by the Pearson correlation coefficients at weeks 1, 7, and 13. The correlations based on ranks were described using the Spearman correlation coefficients

at the same time points. 95% confidence intervals were generated for the correlation coefficients.

Patients' characteristics were summarized using means and standard deviations for the continuous variables and counts and percentages for the categorical variables.

The BNP measurement for week 1 was considered as baseline. Changes in BNP over time were calculated as follows:

Change in BNP to week 7= $BNP_{week7} - BNP_{baseline}$

Change in BNP to week 13 = $BNP_{week13} - BNP_{baseline}$

Percent change in BNP to week 7 (%) = 100* $(BNP_{week7} - BNP_{baseline})/BNP_{baseline}$

Percent change in BNP to week 13 (%) = 100* $(BNP_{week13} - BNP_{baseline})/BNP_{baseline}$

BNP, weight, heart rate, blood pressure, and serum creatinine were visualized over time with individual profile plots, boxplots, mean/standard errors, as well as mean plots with double axes.

The body mass index was categorized into the following subgroups:

Table 6. 3 - Body mass index subgroups

BMI subgroups	Body mass index range (kg/m^2)
Underweight	< 18.5
Normal weight	18.5 – 24.99
Overweight	25 – 29.99
Obese	≥ 30
Source: World Health Organization http://apps.who.int/bmi/index.jsp?introPage=intro_3.html Last accessed on 30-May-2017	

The kidney function was categorized based on the glomerular filtration rate into the following subgroups:

Table 6. 4 - Chronic kidney disease stages

Chronic kidney disease stage	Kidney function	GFR (ml/min/1.73m^2)
I	Normal	≥ 90
II	Mildly reduced	60-89
III	Moderately reduced	30-59
IV	Severely reduced	15-29
V	End-stage renal kidney failure	< 15 or on dialysis

GFR= Glomerular filtration rate

Source: The Renal Association; www.renal.org; Last accessed on 30/5/2017 http://www.renal.org/information-resources/the-uk-eckd-guide/ckd-stages#sthash.CnSMKkls.dpbs

All statistical analyses were performed with SAS® v9.3, SAS Institute Inc., Cary, NC, US.

6.3. Results

Thirty subjects were included in this chapter. One subject was withdrawn due to not entering the follow-up period, yielding to twenty-nine placebo subjects completing the trial. One subject had a missing BNP value at week 13. Missing BNP values at week 13 for the two subjects mentioned above were not imputed. They did, however, provide BNP data for weeks 1 and 7. Therefore, there were 30 subjects analyzed for the primary outcome at week 1 and week 7, while 28 subjects were analyzed for the primary outcome at week 13.

The study population was relatively young for a chronic heart failure group with a mean age of 55.6 years old. Most of the participants were males (70%) and of white race (86.7%). Almost half of the cohort was obese (46.6%). The mean serum cholesterol was 4.8 mmol/l, of which the average LDL cholesterol was 2.7 mmol/l. The triglyceride levels were mostly in the normal upper range with a mean of 1.7 mmol/l. Almost half (46.7%) of the cohort had a mildly reduced kidney function, while approximately third (30%) had a normal kidney function. The mean

creatinine levels were 89.6 umol/l, while urea levels were slightly higher on average. The study excluded diabetes patients and the average blood glucose levels were slightly higher than normal. The platelets, calcium, potassium, and sodium levels were normal. The patients' characteristics of the cohort are presented next.

Table 6. 5 - Demographics and clinical characteristics at baseline

Patient characteristics	Summary statistics*
Age (years)	55.6 ± 9.6
0-44	4 (13.3%)
45-54	9 (30%)
55-64	13 (43.4%)
65-74	4 (13.3%)
Female	9 (30%)
Male	21 (70%)
Body mass index (kg/m2)	31.3 ± 6.4
Normal weight	5 (16.7%)
Overweight	11 (36.7%)
Obese	14 (46.6%)
Race	
African/American	3 (10%)
Native Hawaiian/Pacific Islander	1 (33%)
White	26 (86.7%)
Cholesterol (mmol/l)	4.8 ± 1.1
LDL Cholesterol (mmol/l)	2.7 ± 1
Triglycerides (mmol/l)	1.7 ± 0.8
Glomerular filtration rate (ml/m/1.73m^2)	77.3 ± 21.8
CKD Stage 1	9 (30%)
CKD Stage 2	14 (46.7%)
CKD Stage 3	7 (23.3%)
Creatinine (umol/l)	89.6 ± 22.8
Urea (mmol/l)	7.2 ± 2.3
Hemoglobin A1C	5.8 ± 0.4
Platelet (GI/L)	221.7 ± 50.2
Calcium (mmol/l)	2.4 ± 0.1
Potassium (mmol/l)	4.5 ± 0.4
Sodium (mmol/l)	139.1 ± 2.5
*Mean ± standard deviation for continuous variables; Frequency(percentage) for categorical variables	

Approximately half (53.3%) of the participants reported hypertension as part of their medical history; however, a little over a third (36.7%) of the cohort were hypertensive at baseline. The etiology of the heart failure was mostly non-ischemic. Eighty percent of these chronic heart failure patients had left ventricular systolic dysfunction, while 56.7% had a history of arrhythmia. As expected, most of the patients had taken beta-blockers, ACE inhibitors, diuretics and Aldosterone antagonists. The cardiovascular medical history and past exposure to the cardiovascular medication are summarized below.

Table 6. 6 - Cardiovascular medical history and medication exposure

CV medical history	Count (%)
Angina	4 (13.7%)
Arrhythmia	17 (56.7%)
Hypertension	16 (53.3%)
Ischaemic cardiomyopathy	11 (36.7%)
Non-ischaemic cardiomyopathy	19 (63.3%)
Left ventricular systolic dysfunction	24 (80%)
Left ventricular diastolic dysfunction	8 (26.7%)
Myocardial infarction	5 (16.7%)
Valvular heart disease	7 (23.3%)
CV medication exposure history	
ACE inhibitor	22 (73.3%)
Aldosterone antagonist	20 (66.7%)
Angiotensin 2 antagonist	8 (26.7%)
Beta-blocker	27 (90%)
Diuretics	21 (70%)

Consistent with a stable, relatively young, chronic heart failure population, there were relatively few heart failure medication changes over the course of the clinical trial. One patient was prescribed Losartan 100 mg once daily on the 10th day into the trial and then added Amlodipine 5 mg on the 29th day into the clinical trial to control his hypertension. Another patient had a dose increase in Carvedilol (e.g., from 3.125 mg BID on Day 76 to 6.25 mg BID on Day 77). One patient had a dose reduction in Lisinopril (e.g., from 30 mg once daily

starting on Day 6 to 20 mg once daily beginning on Day 29) while taking Furosemide 30 mg BID. Another patient was given an ace inhibitor starting on Day 45 (e.g., Lisinopril 2.5 mg once daily), adding a diuretic (e.g., Furosemide 40 mg once daily) and a potassium replacement on Day 49th. Another patient had a decrease in diuretics during the trial (e.g., Furosemide 40 mg BID from Day 49 to Furosemide 40 mg once daily starting with Day 52).

The baseline BNP levels ranged from 5 ng/l to 605 ng/l at baseline. The B-type natriuretic peptides were lower than 100 ng/l for 60% of the patients (n=18) and between 100 and 199.9 ng/l for eight subjects. There were only four placebo subjects with a baseline BNP value higher than 200 ng/l, and for two of them, such values were more than 400 ng/l at baseline. Counts and frequency of patients based on their BNP levels at baseline are presented below.

Table 6. 7 - Baseline BNP categories

BNP category	Counts	Percent of patients
<100 ng/l	18	60%
100-199.9 ng/l	8	26.6%
200-400 ng/l	2	6.7%
>400 ng/l	2	6.7%
Total	30	100%

Means and standard deviations over time for weight, heart rate, systolic and diastolic blood pressure, serum creatinine and B-type natriuretic peptides are presented in Table 6.8.

Table 6. 8 - Means and standard deviations for weight, vital signs and creatinine by week

Week	Weight (kg)	Heart rate (bpm)	Systolic blood pressure (mmHg)	Diastolic blood pressure (mmHg)	Creatinine* (umol/l)	BNP** (ng/l)
Week 1	93.4 ± 23.2	71.4 ± 12.7	114.8 ± 14.6	69 ± 8.4	93.9 ± 22.8	114.8±136.8
Week 2	93.6 ± 23	68.9 ± 10.1	116.2 ± 21.9	66.9 ± 10.6	-	-
Week 3	94.7 ± 23.4	67.9 ± 10.8	115.6 ± 17.2	68.5 ± 8.1	90.2 ± 23.8	-
Week 4	95.1 ± 22.8	69.1 ± 12.8	115.6 ± 16.7	69.7 ± 8.4	-	-
Week 5	94 ± 23.1	71.9 ± 11.4	115.6 ± 17.1	69.9 ± 8.8	94.1 ± 23.5	-
Week 6	94.3 ± 23.6	69.7 ± 13.6	112.5 ± 17.3	66.7 ± 7.6	-	-
Week 7	94 ± 23	66.8 ± 11.2	115.2 ± 15.6	67.7 ± 9	91.1 ± 23.4	112.9±121.6
Week 8	93.7 ± 23.8	66.8 ± 10.2	112.7 ± 17	67.9 ± 10.3	-	-

Week 9	95.5 ± 22.9	68.9 ± 11.2	114.6 ± 18.4	68.2 ± 10.9	90.1 ± 20.9	-
Week 10	94.8 ±24	69.2 ± 10.7	114.2 ± 16.5	68.6 ± 9.1	-	-
Week 11	94.5 ± 23.7	68.2 ± 11.8	111 ± 18.7	68.5 ± 11.6	92.7 ± 22.8	-
Week 12	94.3 ± 23.8	69.4 ± 10.6	113.7 ± 17.1	68.3 ± 9.1	-	-
Week 13	93.8 ± 24	69.6 ± 11.7	115.6 ± 16.7	70.7 ± 10.8	92.9 ± 21.6	103.5±141.6
*Serum creatinine was measured at weeks 1, 3, 5, 7, 9, 11 and 13; ** BNP was measured at weeks 1, 7, 13						

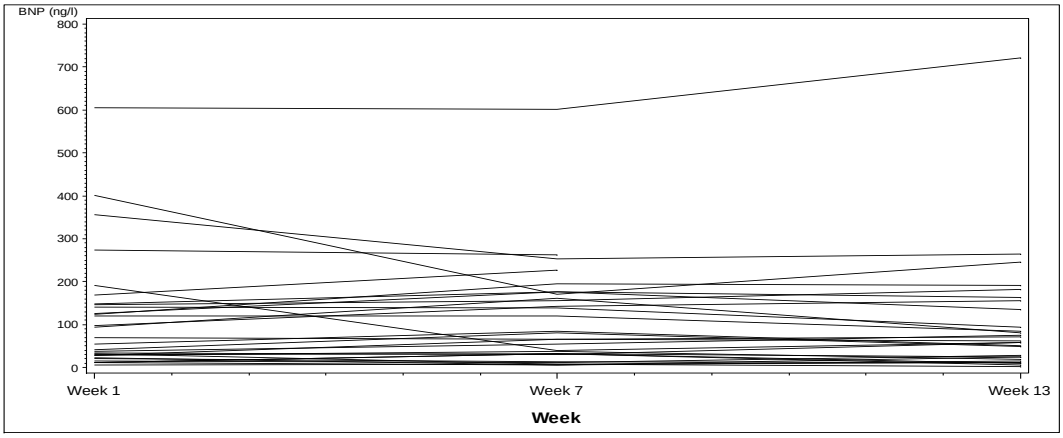
The average weight was close to 95 kg. On average, the mean heart rate was normal, centered around 70 beats per minute. Although a little over half of the cohort had reported a diagnosis of hypertension in the past, most of the subjects were exposed to blood pressure lowering medication before entering the study. During the study period, the blood pressure was normal on average. The mean diastolic blood pressure for the cohort was less than 70 mmHg during most of the weeks. On average, the serum creatinine was in the low 90s umol/l with a standard deviation varying from 21.6 umol/l (week 13) to 23.9 umol/l (week 3).

6.3.1. Describing the between-subject BNP variability

The individual BNP profiles in Figure 6.1 reveal no trends of BNP over time. One subject had BNP values over 600 ng/l at all time points: 605 ng/ml at week 1, 601 ng/ml at week 7 and 721 ng/l at week 13 from the study start. This subject was a 50-year old white male, weighing 84.7 kg. His medical history included: left ventricular systolic dysfunction, dilated cardiomyopathy, as well as pulmonary embolic disease, which explains the high BNP values. Prior medication exposure included Ramipril (5 mg once daily), Bisoprolol (1.25 mg once daily), Spironolactone (25mg once daily), Furosemide (80 mg once daily) and Warfarin (7 mg once daily). Current medication during the study period included Furosemide (40 mg once daily) and Erythromicyn (500 mg) taken during the seventh week in the trial due to an upper tract respiratory infection.

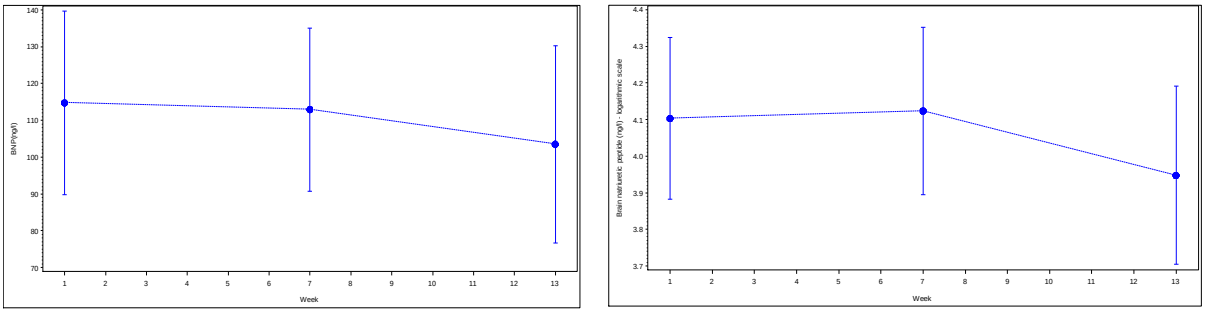
The ranges of BNP values were 600 ng/l, 595 ng/l, and 718 ng/l at week 1, week 7 and week 13 respectively. Two subjects did not have BNP measurements at week 13 either due to withdrawal or missing values.

Figure 6. 1 - BNP individual profiles throughout the clinical trial duration



As illustrated in Figure 6.2, BNP decreases over time, especially between week 7 and week 13. The mean BNP levels were 114.8 ng/l at week 1, 112.9 ng/l at week 7 and 103.5 ng/l at week 13. The BNP variation decreases to week 7, and then it peaks at week 13. The between-subject coefficient of variation was 119.2%, 107.7% and 136.8% at week 1, week 7, and week 13 respectively. This shows the extent of BNP variability about the mean of the population.

Figure 6. 2 - Mean/standard error plot for BNP over time (original and logarithmic scales)



The geometric means were identical in week 1 and week 7, decreasing to 49.4 ng/l at week 13.

Table 6. 9 - Measures of location and variability for BNP (ng/l) values over time

Summary measure	Week 1	Week 7	Week 13
n	30	30	28
Arithmetic mean (95% CI)	114.8 (63.7, 165.8)	112.9 (67.5, 158.3)	103.5 (48.6, 158.4)
Standard deviation	136.8	121.6	141.6
Standard error	25	22.2	26.8
Geometric mean (95% CI)	60.3 (40.4, 99.5)	60.3 (40.4, 99.5)	49.4 (30, 81.5)
Standard deviation on natural logarithmic scale	1.2	1.3	1.3
Between-subject coefficient of variation (CV_b%)	119.2%	107.7%	136.8%
Median (range)	62 (600)	73 (595)	61 (718)
Minimum, maximum	5, 605	6, 601	3, 721
Lower, upper quartile (Q1, Q3)	28, 146	32, 170	21.5, 145.5
Inter-quartile range (Q3-Q1)	118	138	124
Unless otherwise noted (standard deviation on natural logarithmic scale), the data was reported in the original scale.			

On average, although the week 7 and week 13 BNP levels were slightly lower than baseline BNP, there were no significant changes in BNP from baseline to week 7 or week 13. The 95% confidence interval for the mean change included zero. The standard deviation, standard error and between-subject coefficient of variation for the changes in BNP appeared to be higher at week 7 than week 13. The inter-quartile range for the change from baseline in BNP to week 7 and 13 were 35 ng/l and 42.5 ng/l, respectively. All summary statistics generated for the change from baseline in BNP are presented below.

Table 6. 10 - Changes from baseline over time in BNP (ng/l)

Summary measure	Change from baseline to week 7	Change from baseline to week 13
n	30	28
Arithmetic mean (95% CI)	-1.8 (-25, 21.3)	-3.7 (-25.1, 17.7)
Standard deviation	62	55.2
Standard error	11.3	10.4
Geometric mean (95% CI)	1 (0.8, 1.3)	0.9 (0.7, 1.1)
Standard deviation on natural logarithmic scale	0.7	0.5
Coefficient of variation (CV_b%)	-3381	-1500
Median (range)	3 (302)	-3(271)
Minimum, maximum	-231, 71	-155, 116
Lower, upper quartile (Q1, Q3)	-5, 30	-12.5, 30
Inter-quartile range (Q3-Q1)	35	42.5
Change from baseline to week 7 = week 7 – baseline (week 1) Change from baseline to week 13 = week 13 – baseline (week 1) Unless otherwise noted (e.g. standard deviation on natural logarithmic scale), the data was reported in the original scale.		

On average, there was a 21.5% change from baseline to week 7 and only a 7% change from baseline to week 13. Half of the subjects had an 8.1% increase in the percent change from baseline to week 7 and 11% lower BNP values at week 13 compared to the baseline. The variation was high again, indicated by the percent range of 270% at week 7 and 196.9% at week 13. Summary statistics for the percent changes from baseline are presented below.

Table 6. 11 - Percent changes from baseline in BNP (ng/l) over time

Summary measure	% change from baseline to week 7	% change from baseline to week 13
n	30	28
Mean	21.5%	7%
Standard deviation	66.8%	56.2%
Median (range)	8.1% (270%)	-11% (196.9%)
Minimum, Maximum	-79.1%, 190.9%	-69.6%, 127%
Data reported in the original scale (ng/l)		

6.3.2. Describing the between-subject BNP variability in subgroups of patients

Heart failure is a heterogeneous disease. The between-subject variability was generated by subgroups defined by age, gender, body mass, race, and clinical characteristics. The BNP between-subject coefficient of variation varied over time and was higher for those younger than 55 years old. The cohort included only five subjects with a normal body mass index. As such, in the present chapter, weight was categorized as normal/overweight and obese to allow meaningful comparisons between subgroups. Nevertheless, Appendix H includes summary statistics for both classifications applied for completeness. For both normal/overweight and obese subgroups, the BNP between-subject coefficients of variation decreased from baseline to week 7 and then peaked at week 13. The between-subject coefficient of variation was lower among the obese than normal or overweight subjects.

Table 6. 12 - The between-subject coefficient of variation of BNP by age subgroups

Parameter	Subgroup	CV_b(%), n		
		Week 1	Week 7	Week 13
Age (years)	<55	149.3% n=13	145% n=13	169.9% n=13
	≥ 55	100% n=17	74.2% n=17	88.4% n=15
Body mass index	Normal/ Overweight	129.7% n=16	112.6% n=16	133.5% n=16
	Obese	104.9% n=14	97.6% n=14	120.4% n=12

The BNP between-subject coefficient of variations for various subgroups defined by a history of hypertension, arrhythmia, ischemic and non-ischemic cardiomyopathy are presented in Table 6.13. The between-subject variability in BNP was also described in subgroups of

subjects defined by prior exposure to medication commonly prescribed to heart failure patients: beta-blockers (e.g. Carvedilol, Carvedilol phosphate, Metoprolol, Metoprolol succinate, Nebivolol hydrochloride, and Bisoprolol), ACE inhibitors (e.g. Captopril, Lisinopril, Enalapril, Ramipril, or Quinapril), Aldosterone antagonists (e.g. Spironolactone, Eplerenone or Aldactone), Angiotensin 2 antagonists (e.g. Losartan, Losartan Potassium, Valsartan, Candesartan or Candesartan Cilexetil), and diuretics (e.g. Torasemide, Furosemide, Metolazone, Hydrochlorothiazide or Bumetanide). As often heart failure patients have multiple comorbidities and are prescribed multiple medications, the table should be read in the context of each line without comparing the subgroups or multiple lines at a time.

As patients may have multiple comorbidities, the table should be read in the context of each line without comparing the subgroups or multiple lines at a time.

Table 6. 13 - The between-subject coefficient of variation of BNP by CV medical history and CV medication exposure

Subgroup	CV_b(%), n		
	Week 1	Week 7	Week 13
History of hypertension	101.9% n=16	83.6% n=16	97.5% n=14
History of arrhythmia	94.1% n=17	63% n=17	73.5% n=16
History of ischaemic cardiomyopathy	102% n=11	71% n=11	75.6% n=10
History of non-ischaemic cardiomyopathy	124.4% n=19	119.7% n=19	154.8% n=19
Prior beta-blocker	122.5% n=27	111.5% n=27	147% n=25
Prior ACE inhibitor	130% n=22	125.9% n=22	169.5% n=20
Prior Aldosterone antagonist	134.8% n=20	126.4% n=20	165.8% n=18
Prior Angiotensin 2 antagonist	92.5% n=8	63.6% n=8	71.5% n=8
Prior Diuretic	121.7% n=21	117.6% n=21	148.4% n=21

The between-subject coefficient of variation was high in those with a history of non-ischaemic cardiomyopathy and those previously exposed to Aldosterone antagonists, ACE inhibitors beta-blockers or diuretics.

6.3.3. A statistical estimation of the within-subject BNP variability using repeated measures mixed models

Two random intercept models were fitted with subject treated as a random effect. One of these statistical models included the BNP baseline, while the other did not. As both models had the same effect on the average increase in BNP per week, the random intercept model with the BNP baseline as a covariate was dropped to avoid model over-parametrization. BNP was naturally logarithmically transformed in the analyses.

The final intercept random model included week as a fixed effect in addition to the random intercept. The model was fitted with the restricted maximum likelihood estimation for variance components. Most of the subjects had three BNP measurements over time, and eighty-eight observations were used in the model. The covariance parameter estimates are displayed below. The BNP within-subject coefficient of variance was approximately 46%.

Table 6. 14 - Covariance parameter estimates

Covariance parameter	Estimate
Intercept	1.3802
Residual	0. 1909
Within-subject CV	45.86%
RCV_+ (upward) RCV_- (downward)	242% -70.7%
RCV=Reference change value based on the lognormal approach	

The solution for the fixed effects is displayed in Table 6.15. The average BNP value at baseline on the naturally logarithmic scale was 4.1267, corresponding to a BNP geometric mean of 62 ng/l. There was no average increase in BNP per week. The estimate for the week was -0.0055 and the null hypothesis failed to be rejected, as evidenced by the p-value of 0.5704.

Table 6. 15 - Solution for the fixed effects

Effect	Estimate	Standard error	DF	t value	Pr > |t|
Intercept	4.1267	0.2291	29	18.01	0.0001
Week	-0.0055	0.0096	57	-0.57	0.5704
DF=Degrees of freedom					

As hinted by the BNP individual profiles over time (Figure 6.1), the results of the random intercept model showed that there was little evidence of any real progression or deterioration over time in BNP. Since the within-subject coefficient of variation was reasonably large, the results suggested that monitoring over three months was unlikely to detect true BNP change against background noise in this small chronically stable heart failure population. From a monitoring respective, the lognormal reference change values were high. A change in BNP is statistically significant if there is an increase in BNP of at least 235% or a decrease of at least 70.2% between two consecutive visits.

Sensitivity analyses were conducted when excluding the two subjects with missing BNP values at week 13. The results were very similar. For example, the within-subject coefficient of variation was 46.6%, while the lognormal positive and negative reference changes were 242% and -70.7%, respectively.

The within-subject variability for BNP was much higher than the variability for other clinical variables often measured in primary care practice such as: weight, heart rate, blood pressure or serum creatinine. The estimated within-subject coefficients of variation are displayed below:

Table 6. 16 - The within-subject coefficient of variation for weight, heart rate, blood pressure and serum creatinine

Group of patients	n	Estimated CV_w(%) from mixed models*	Average CV_w(%)**
Weight	30	1.2%	1.1%
Heart rate	30	10.3%	9.6%
Systolic blood pressure	30	9.7%	8.7%
Diastolic blood pressure	30	10.7%	10.3%
Serum creatinine			
All	30	8.9%	7.3%
Females	9	10.7%	8.2%
Males	21	8.1%	6.9%

*Estimated from the random intercept mixed model
**Derived as the average of CVw(%)=100*within-subject standard deviation/subject's mean

6.3.4. The short-term correlation between BNP and cardiac imaging factors, weight, heart rate, blood pressure, serum creatinine

Cardiac imaging techniques are often used to detect heart abnormalities, identify disease patterns, manage and monitor heart failure patients (McDonagh 2011). Regularly assessing a heart condition with a simple BNP blood test may be more cost-effective than repeat echocardiographic assessments in chronic heart failure patients. In this clinical trial, Doppler echocardiography was performed at baseline and week 13. As such the following cardiac imaging endpoints were available: cardiac output, left ventricular ejection fraction, left ventricular ejection fraction index, left ventricular end-diastolic volume, left ventricular end-diastolic volume index, left ventricular end-systolic volume and left ventricular end-systolic volume index. The volume indexes were normalized for the body surface areas.

The cardiac imaging parameters were indicative of a moderate to severe heart failure population (Lang 2015). On average, the left ventricular ejection fraction values at week 1 and

week 13 were 32.5% and 37%, respectively. The left ventricular end-diastolic volume index improved throughout the clinical trial from an average of 91.3 mL/m^2 at week 1 to 82.5 mL/m^2 at week 13. Although the left ventricular end-systolic volume index mildly improved during the study period, the levels were still severe, suggesting pressure or volume overload.

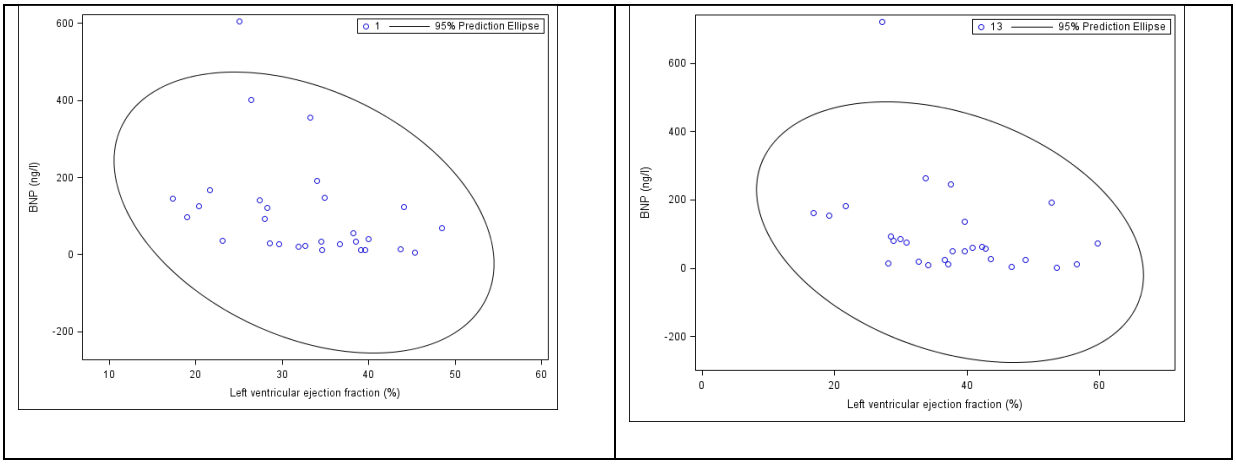
The Pearson and Spearman correlation coefficients in Table 6.18 showed weak to moderate, positive correlations between BNP and left ventricular volumes, with the Pearson coefficients varying from 0.25 to 0.46. A negative, moderate correlation was observed between BNP and left ventricular ejection fraction. While both the linear and rank correlations yielded to the same inference, the Spearman correlation coefficients were slightly stronger.

Table 6. 17 - The Pearson and Spearman correlations between BNP and cardiac factors

BNP (ng/l)	Pearson correlation coefficients (p-value)		Spearman correlation coefficients (p-value)	
	Week 1	Week 13	Week 1	Week 13
Cardiac output (L/min)	-0.09 p-val=0.634 n=28	-0.20 p-val=0.299 n=28	-0.12 p-val=0.538 n=28	-0.18 p-val=0.337 n=28
Left ventricular ejection fraction (%)	-0.363 p-val=0.052 n=29	-0.33 p-val=0.086 n=28	-0.51 p-val=0.004 n=29	-0.432 p-val=0.021 n=28
Left ventricular ejection fraction index (%/m^2)	0.26 p-val=0.169 n=29	-0.21 p-val=0.268 n=28	-0.35 p-val=0.061 n=29	-0.28 p-val=0.144 n=28
Left ventricular end diastolic volume (mL)	0.25 p-val=0.185 n=29	0.36 p-val=0.056 n=28	0.28 p-val=0.139 n=29	0.30 p-val=0.118 n=28
Left ventricular end diastolic volume index (mL/m^2)	0.35 p-val=0.060 n=29	0.48 p-val=0.008 n=28	0.28 p-val=0.127 n=29	0.43 p-val=0.019 n=28
Left ventricular end systolic volume (mL)	0.26 p-val=0.158 n=29	0.36 p-val=0.057 n=28	0.30 p-val=0.113 n=29	0.36 p-val=0.054 n=28
Left ventricular end systolic volume index (mL/m^2)	0.35 p-val=0.058 n=29	0.46 p-val=0.013 n=28	0.41 p-val=0.027 n=29	0.44 p-val=0.016 n=28

The amount of the blood that is pumped out from the left ventricle throughout the body is measured by the left ventricular ejection fraction (McDonagh 2011). The negative association between the BNP and left ventricular ejection fraction is depicted in Figure 6.3. The left ventricular ejection fraction ranged from 17.4% to 48.5% at week 1 and from 16.9% to 59.7% at week 13. Half of the patients had a left ventricular ejection fraction lower than 33% at baseline and lower than 37% at week 13. The correlation between BNP and left ventricular ejection fraction was very similar at baseline and the end of the trial.

Figure 6. 3 - BNP and left ventricular ejection fraction at week 1 and 13



The left ventricular end-diastolic volume index helps to understand the extent of the ventricular remodeling (McDonagh 2011). In heart failure patients, remodeling worsens the condition of the heart (McDonagh 2011). The left ventricular end-diastolic volume index ranged from 43.3 mL/m^2 to 202.6 mL/m^2 at week 1 and from 41.5 mL/m^2 to 202.1 mL/m^2 at week 13. A weak to moderate, positive correlation between BNP and the left ventricular end-diastolic volume index was noted. The Pearson correlation coefficient was 0.353 in week 1 and 0.489 at week 13. Gender appeared to modify this relationship, since the correlation was much stronger in females than males, even attaining statistical significance at week 13. For example, in nine

females the Pearson correlation coefficient was 0.551 in week 1 and 0.819 (p-value=0.0069) at week 13. The relationship between the BNP levels and left ventricular end-diastolic volume index is visualized in the Appendix H.

The left ventricular end-systolic volume index characterizes the left ventricular function relative to the body surface area (McDonagh 2011). Increases in the left ventricular end-systolic volume index predict adverse cardiovascular events, including hospitalizations (McManus 2009). On average, the left ventricular end-systolic volume index was slightly higher at baseline than at the end of the trial. Half of the placebo cohort had a left ventricular end-systolic volume index higher than 51.2 mL/m^2 at week 1 and higher than 46.8 mL/m^2 at week 13, respectively. Overall, there was a weak to moderate, positive correlation between BNP and the left ventricular end-systolic volume index. The Pearson correlation coefficient was 0.355 at week 1 and 0.462 at week 13. The association was stronger in females.

Weak to moderate correlations were noticed between BNP and cardiac imaging parameters reflecting the left ventricular size and function. Gender appeared to be a confounder; however, there were only nine women included in the analysis. Changes in BNP were weakly correlated with changes in the cardiac structure and function factors from baseline to week 13. The correlation coefficients are presented in the Appendix H.

Although weight, heart and blood pressure were collected weekly and serum creatinine biweekly, their serial correlation to BNP was possible to be investigated only every six weeks, when BNP was measured. The linear and rank correlations are presented next..

Table 6. 18 - The Pearson and Spearman correlations between BNP and weight, heart rate, blood pressure, serum creatinine

BNP (ng/l)	Pearson correlation coefficient, p-value			Spearman correlation coefficient, p-value		
	Week 1	Week 7	Week 13	Week 1	Week 7	Week 13
Weight (kg)	-0.134 p=0.474	-0.169 p=0.367	-0.141 p=0.469	-0.136 p=0.468	-0.150 p=0.421	-0.247 p=0.197
Heart rate (beat/min)	0.018 p=0.920	-0.108 p=0.563	-0.125 p=0.518	0.004 p=0.168	0.029 p=0.875	-0.147 p=0.449
Systolic blood pressure (mmHg)	0.066 p=0.724	-0.264 p=0.152	-0.09 p=0.643	0.049 p=0.795	-0.276 p=0.132	-0.005 p=0.977
Diastolic blood pressure (mmHg)	0.017 p=0.925	-0.346 p=0.056	0.011 p=0.954	0.149 p=0.426	-0.328 p=0.071	0.016 p=0.931
Serum creatinine (umol/l)	0.088 p=0.640	0.001 p=0.993	0.068 p=0.725	0.145 p=0.438	-0.026 p=0.889	0.009 p=0.959
Sample size: n=30 at weeks 1 and 7, n=28 at week 13						

No linear correlation was observed between BNP and heart rate, blood pressure and serum creatinine. At week 7, the Pearson correlation between BNP and blood pressure appeared weak; however, this was due to noise rather than a signal. A similar conclusion was drawn based on the rank correlations. At week 1, the Spearman correlation coefficient was 0.25 for the heart rate and 0.14 for serum creatinine.

A weak negative linear relationship between BNP and weight was observed at all points. The Pearson correlation coefficient was relatively constant over time: -0.13 at week 1, -0.14 at week 7 and -0.14 at week 13.

6. 4. Discussion

The clinical trial provided rich laboratory data and cardiac imaging parameters that were repeatedly measured during the study duration. The quality of the data was high, and there were few missing values. Numerous measurements taken over time allowed the evaluation of the within-subject variability for BNP, weight, heart rate, blood pressure, and serum creatinine. Both clinical investigators and patients were blinded to the treatment given. Thus, the investigator bias and misclassification were minimized.

The study participants were stable chronic heart failure patients of NYHA class II-III. Ninety percent of the cohort had been exposed to cardiovascular drugs before the baseline, therefore their natriuretic peptide levels were expected to be similarly affected. Only four subjects had BNP greater than 200 ng/ml at baseline. There were just two subjects with a baseline B-type natriuretic peptide of at least 400 ng/l. Almost half of the chronic heart failure patients were obese, and 36.7% were overweight.

All participants were randomized and clinically stable on optimal therapies for three months before the study start. Despite some severe cardiac imaging values and high BNP levels above 600 ng/l, the clinical trial included a mild to moderate heart failure population. This could be one of the reasons as to why BNP was stable, and few medications were changed over time. Nevertheless, the study provides a detailed insight into the BNP variability in stable heart failure patients. More severe heart failure cases need more intensive and aggressive pharmacotherapy, which is expected to impact by increasing the BNP variability.

A chronic heart failure patient takes several drugs daily to treat their heart failure and various existing comorbidities. Drug-drug interactions, medication errors, poor adherence, adverse events could pose serious risks. It is expected that in a real-world population such

volume of medication would impact the serial correlation results and the within-subject variability in weight, heart rate, blood pressure, and serum creatinine could be higher.

Since BNP was measured only three times for each patient, as opposed to weight, heart rate, blood pressure, and serum creatinine, their serial correlation to BNP could only be evaluated at weeks 1, 7, and 13. Similarly, due to just three dates per patient for the B-type natriuretic peptide, a time series approach to the data could not be applied for exploring serial correlations over time, as such approach would require numerous dates.

In this clinical trial, the serum creatinine was measured biweekly throughout three months. The Glomerular filtration rate was estimated based on the Cockcroft-Gault equation at the baseline only. The serum creatinine underestimates the renal dysfunction in women and older people (Perrone 1992). Although eGFR measurements over time would have been preferred, a minimal impact on the results could be expected, as most of the study participants were relatively young chronic heart failure males.

The BNP within-subject variability over a three-month period was 46%. The reference change values were high. The asymmetrical limits for the upward (positive) value and downward (negative) value for the log-normal RCV were 235% and - 70.2%, respectively. Throughton reported variability of 30% between serial measurements in NT-proBNP (Troughton 2008). Meijers et al. reported lognormal RCV upward and downward values of 104.9 and -40.1 in 83 heart failure patients over a six week-period (Meijers 2017). The Biomarker working study group of the European Society of Cardiology acknowledged the intra-individual biological variability between 30% and 50% for all natriuretic peptides (Thygesen 2012).

Changes in BNP were poorly correlated with changes in cardiac structure factors over a three-month period. No correlations between BNP and heart rate, blood pressure, and creatinine were found over a three-month period. A very weak negative linear relationship was observed between BNP and weight.

Heart rate, blood pressure and serum creatinine had much lower within-subject variability than BNP, making them extremely useful in the primary care setting. Weight had a within-subject variability of approximately 1% over a three-month period in stable chronic heart failure patients. Heart rate and blood pressure had a within-subject variability of roughly 10%. Serum creatinine had a within-subject variability of approximately 8% over a three-month period in stable chronic heart failure patients. The within-subject coefficient was slightly higher in women compared with males.

6.4.1. Limitations

The main limitations were the small sample size and short duration of follow-up. The placebo cohort included only 30 subjects. Therefore, the confidence intervals were wide suggesting high imprecision. Subgroup analyses were affected by the small sample size. The younger age and lower body mass index subgroups included few subjects, but this was not surprising given the heart failure epidemiology. Laboratory and cardiac imaging parameters were summarized on a continuous scale, thus avoiding subgrouping whenever possible. Although thirty percent of the subjects were females, given the small sample size this corresponded to only nine women. The three-month study duration was relatively short. About 30-40% of chronic heart failure patients die within the first year of being diagnosed with heart failure (Cowie 2000, Hobbs 2007).

The results are not generalizable to people with diabetes since the study did not include patients with the Type II Diabetes mellitus. BNP values are higher in diabetic, chronic heart failure patients (Peng 2013). The results are not generalizable to patients with a history of renal failure, characterized by a glomerular filtration rate of less than 40 ml/min.

The BNP reference ranges and analytical performance depend on the assay being used to measure them (Wu 2006). The analytical imprecision of commercially available BNP assays varies from 3.1 to 16.7 (Wu 2006). The NT-proBNP intra-assay precision was 5.4% for the Immulite 2000 assay (Piper 2016). The thirty subjects included in this chapter were enrolled at eleven different sites. Unfortunately, the BNP assay was not collected, and therefore the assay imprecision couldn't be evaluated. The extent of this measurement bias is unknown in this study.

The etiology of the chronic heart failure for all participants was cardiomyopathy due to an ischemic or non-ischemic origin. However, the chronic heart failure duration was not captured to provide additional insight into the clinical course of the disease.

The study included chronic heart failure patients with NYHA class II or III at screening, however, this symptomatic classification was not collected throughout the clinical trial. Thus, a potential change in NYHA could not be assessed.

6.4.2. Implications for research

The study took a descriptive or hypothesis generating approach. Due to the small sample size of only 30 research subjects, the results of this study will need to be replicated in larger cohorts of chronic heart failure patients, potentially of varying degrees of severity. Clinical trial data in chronic heart failure patients being monitored for longer than three months may provide additional insight.

6.4.3. Implications for clinical practice

The high within-subject variability of the B-type natriuretic peptide poses severe limitations when monitoring chronic heart failure patients in the primary care setting. In stable chronic, relatively young, heart failure patients, monitoring BNP over a three-month period is of limited value. Although heart rate, weight, blood pressure and serum creatinine have a much lower within-subject variability, potentially making them useful in monitoring chronic heart failure patients, they were not correlated with BPN over a three-month period. Changes in BNP were poorly correlated with changes in cardiac structure factors over a three-month period. The weight profiles were mostly flat during the study period. The within-subject coefficient of variation of weight was 1.2%. The slight weekly fluctuations in weight during the study period did not appear to be associated with high BNP values.

6.5. Conclusion

The BNP within-subject variability over a three-month period was 46%. The BNP upward and downward values for the log-normal reference change values were 235% and - 70.2%, respectively. There was a weak correlation between changes in BNP and changes in cardiac output, left ventricular ejection fraction, left ventricular end systolic and diastolic volumes.

The within-subject variability for weight, heart rate, blood pressure and serum creatinine were 1%, 10%, 10%, and 8% respectively. While a very weak negative linear correlation was observed between BNP and weight, BNP was not correlated with heart rate, blood pressure, and serum creatinine. Testing in more patients is needed to investigate the reproducibility of these results, as well as increase confidence in the results. An extended duration of follow-up should be investigated.

CHAPTER 7 - CONCLUSION

7.1. Introduction

This thesis evaluated the role of the B-type natriuretic peptides (BNP and NT-proBNP) in heart failure patients. B-type natriuretic peptides add to the clinical judgement when managing chronic heart failure patients. Primary care practices play a central role in treating chronic heart failure patients. Monitoring these peptides can help clinicians identify patients in need of more intensive therapy in order to avoid heart decompensation and improve survival rates. Although the clinical importance of changes in B-type natriuretic peptide is not clear, Miller and Jaffe advocated for the serial B-type natriuretic peptides measurements rather than single values for the effective management of the chronic heart failure patients, especially considering that the disease activity changes over time not only within the same patient but also between patients (Miller 2016, Miller 2007, Miller 2009). Understanding the variability of B-type natriuretic peptides becomes essential to the clinical interpretation of the serial values. A natural question in the primary care setting is whether the B-type natriuretic peptides correlate with other clinical factors often measured such as: weight, heart rate, blood pressure, serum creatinine and even cardiac structure factors, if available.

The following research questions were addressed:

Use of BNP and NTproBNP in primary care:

- What were the predictors of the likelihood of repeat BNP/NT-proBNP testing in chronic heart failure outpatients attending primary care practices?

The prognostic role of BNP and NTproBNP on the all-cause mortality and cardiovascular mortality in primary care

- Were NT-proBNP levels associated to the all-cause mortality and cardiovascular mortality in heart failure outpatients managed by primary care physicians?
- Were BNP levels associated to the all-cause mortality and cardiovascular mortality in heart failure outpatients managed by primary care physicians?

Changes in BNP and NTproBNP over time and the prognostic roles of these changes in primary care

- Were the changes in BNP and NT-proBNP associated with the all-cause mortality and cardiovascular mortality in heart failure patients managed in the primary care setting?

Monitoring BNP over a three-month period

- What was the within-subject short-term variability of BNP in stable heart failure subjects (NYHA class II - III)? How does it compare to the within-subject short-term variability for weight, blood pressure, heart rate and serum creatinine?

Correlation between BNP and other clinical factors over a three-month period

- What was the correlation between BNP and cardiac structure factors such as: left ventricular ejection fraction, cardiac output, left ventricular left end-diastolic and end-systolic volumes
- What was the correlation between BNP and weight, heart rate, blood pressure and serum creatinine in stable heart failure subjects (NYHA class II-III)?

This chapter summarizes the conclusion of the main research objectives, the thesis's contribution to knowledge, while acknowledging its limitations. My findings were compared to

the work of other researchers in the previous chapters. This chapter ends with recommendations linked to my conclusions as well as suggestions for future research.

7.2. Research Objectives: Summary of findings and Conclusions

Taking a population approach, the thesis investigated patterns of B-type natriuretic peptide testing in the primary care setting across the United Kingdom between 2005 and 2012. The results showed that none of patients' characteristics investigated such as age, sex, body mass index and smoking clinically impacted the likelihood of repeat natriuretic peptide testing in the primary care setting.

Based on a population-based primary care cohort of 4,249 chronic heart failure outpatients managed by general practitioners within the United Kingdom, the study confirmed that the baseline B-type natriuretic peptides (BNP and NT-proBNP) were independent predictors of all-cause mortality and cardiovascular mortality in the primary care setting too. On a logarithmically transformed scale, for every unit increase in BNP or NTproBNP, there was a 1.3 fold increase in the risk of death and a 1.4 fold increase in the risk of cardiovascular death. When adjusting for the patient characteristics such as: age, gender, body mass index, smoking, geographic region, serum creatinine, hypertension and/or heart failure medication and diabetes at baseline, there was a1.2-fold increase in the risk of death and 1.3-fold increase in the risk of cardiovascular death. Age significantly impacted the mortality. When treating heart failure patients, physicians should consider not only the baseline BNP or NT-proBNP levels, but also

the age of the patients, since age significantly impacts mortality, especially in elderly. For example, at 70-year old, for each additional year, there was approximately 2% to 4% increase in the risk of mortality. This risk increased to 4.4% to 7.4% in 80-year old patients.

The changes from baseline in BNP or NT-proBNP did not provide additional information on the all-cause and cardiovascular mortality beyond a single point-in-time peptide level.

In a clinical trial setting of 30 chronic heart failure stable patients, the BNP within-subject variability over a 3-month period was 46%, much higher than the heart rate or blood pressure within-subject variability of approximately 10%, the serum creatinine within-subject variability of approximately 8%, and the weight within-subject variability of 1%. The BNP lognormal reference change values were high. A change in BNP would be considered statistically significant if there is an increase in BNP of at least 235% or a decrease of at least 70.2% between two consecutive visits.

The BNP between-subject coefficient of variation was higher in patients younger than 55 years old, of obese or normal weight, and with a history of non-ischaemic cardiomyopathy. While there was no linear correlation between BNP and heart rate, blood pressure orserum creatinine, a weak negative relationship was observed between BNP and weight. Weak to moderate correlations were noticed between BNP and the following cardiac imaging parameters: left ventricular ejection fraction, cardiac output, left ventricular left end-diastolic, and end-systolic volumes reflecting the left ventricular size and function.

7.3. Contribution to Knowledge

While most of the previous studies investigated the impact of either BNP or NT-proBNP on the all-cause mortality, the current work evaluated the prognostic role of both cardiac peptides on both all-cause mortality and cardiovascular mortality in the primary care setting.

The CPRD study also confirmed that baseline B-type natriuretic peptides were independent predictors of death in the primary care setting too. The risk magnitude of these prognostic factors in this study was quantified in the primary care setting. Age significantly impacted the mortality, especially in the elderly. Few prior studies investigated the impact of the serial B-type natriuretic peptide measurements taken over time. The current thesis evaluated both the impact of BNP and NTproBNP changes over time not only on the all-cause mortality, but also on the cardiovascular mortality. It was interesting to note that the BNP change did not provide additional information on the mortality, while the NTproBNP change did. This could be due to a longer NTproBNP half-life than BNP (Braunwald 2008).

Although the current NICE guidelines recommend B-type natriuretic peptide measurements for diagnostic purposes, the most recent European guidelines for the diagnosis and treatment of acute and chronic heart failure acknowledge the benefit of monitoring biomedical parameters such as B-type natriuretic peptides (NICE 2010, Ponikowski et al. 2016).

The thesis contributes evidence towards building monitoring strategies in heart failure patients that includes B-type natriuretic peptides. One important aspect of monitoring evolves around the variability of the biomarkers, which may impact the monitoring intervals (Glasziou 2008). The BNP within-subject variability over a 3-month period of 46% was higher than the

30% variability reported by Throughton and within the individual biological variability range acknowledged by the Biomarker working group of the European Society of Cardiology (Troughton 2008, Thygesen 2012). Therefore, monitoring BNP in stable chronic heart failure patients over a three-month period may provide little value due to the relatively high background noise observed.

7.4. Limitations

Although the limitations were delineated in the previous chapters, they are also summarized here. In the CPRD study, the residual confounding due to unrecorded variables pertaining to the etiology of heart failure, disease severity, type of heart failure, and left ventricular ejection fraction could not be ruled out. The immortal time bias could be present when evaluating the prognostic value of the changes in B-type natriuretic peptides, as heart failure patients had to have at least two tests in order to be included in the analysis. As not all medical practices consented to having the cause of death evaluated, it is possible that more patients have died of cardiovascular death. Other limitations include the self-reported smoking status, the lack of racial diversity and the type of BNP and NTproBNP assays used in the primary care testing. The results are not generalizable outside the United Kingdom.

The main limitation of the clinical trial was the small sample size of 30 heart failure patients, which resulted in wide confidence intervals. The clinical trial included only 9 females. The results were not generalizable to non-diabetics. Information on the BNP analytes was not collected, which precluded the evaluation of the assay imprecision. The results will have to be reproduced in larger clinical trials to increase confidence in results. Monitoring BNP in stable chronic heart failure patients over a three-month period may provide limited value due

to the relatively high background noise observed. The monitoring interval should be further assessed in very elderly, diabetic chronic heart failure patients with renal impairment. Extended B-type natriuretic peptide monitoring intervals could be considered for stable chronic heart failure patients.

7.5. Implications for research

In the United Kingdom, chronic heart failure patients are managed in primary care. A systematic review and meta-analysis of prospective cohort studies and randomized clinical trials should be conducted in the primary care setting to estimate the prognostic value of BNP and NT-proBNP on mortality. Changes in natriuretic peptides should be evaluated along with their impact on mortality, association with cardiac function, NYHA class, quality of life and adverse events.

Natriuretic peptides are heavily underused in the heart failure patients within the United Kingdom. A prospective study within primary care would address the current scope of testing, whether it is diagnosis or monitoring. The physicians' adherence to the current NICE guidelines with respect to the use of B-type natriuretic peptides chronic heart failure patients could also be investigated. Such a long-term prospective study conducted in a representative sample of primary care practices that treat chronic heart failure patients within the United Kingdom would increase confidence in the results. To minimize the unmeasured confounders, the primary care practices should collect relevant information about heart failure patients such as: age, gender, body mass index, smoking, geographic region, etiology of heart failure, type of heart failure, disease severity, comorbidities, symptomatic assessment such as NYHA, treatment information and type of assay to be used for measuring BNP and NT-proBNP. The

pharmacotherapy, symptomatic heart failure assessment, and clinical variables should be regularly assessed in all patients so that the impact of changes could be evaluated. The primary cause of death should be carefully evaluated to ensure the appropriate estimation of the true association between BNP or NT-proBNP and cardiovascular mortality. A follow-up period of five years with clinical assessments at every 3 months is suggested.

The high within subject variability of BNP will need to be replicated in larger cohorts of chronic heart failure patients, potentially of varying degrees of severity. Clinical trial data in chronic heart failure patients being monitored for longer than three months may provide additional insight.

The pharmacology is ever changing. The classes of heart failure medicines developed over the past decades such as beta-blockers, ace inhibitors, angiotensin receptor blockers, aldosterone antagonists and lately, the combined angiotensin receptor and neprilysin inhibitor target heart failure patients with reduced ejection fraction (Ponikowski et al. 2016, Yancy et al. 2016). However, they do not work in heart failure patients with preserved ejection fraction and such patients need new pharmacotherapy options.

The introduction of new classes of heart failure medication will affect the interpretation of future studies in the context of B-type natriuretic peptides. Combining an angiotensin receptor blocker and neprilysin inhibitor inhibits the breakdown of BNP, resulting in high BNP levels (McMurray 2014, Januzzi 2016, Ibrahim, 2017). However, NTproBNP does not appear to be affected, suggesting that NTproBNP could be used for monitoring purposes in patients exposed to such classes of medication (Januzzi 2016).

Although, the B-type natriuretic peptides have been mostly validated among cardiac biomarkers, they represent only one pathophysiologic pathway of the myocardial stretch

(Braunwald 2013, Nabeebaccus 2016). A multi-biomarker approach may be appealing, considering the various pathophysiologic pathways for heart failure biomarkers such as: myocardial stretch, matrix remodeling, myocyte injury, oxidative stress, inflammation, neurohumoral activation, and renal dysfunction (Braunwald 2013, Braunwald 2008). More research is needed to determine whether a multi-marker approach will yield superior results to the B-type natriuretic peptides.

7.6. Implications for practice

Regular natriuretic peptide testing provides an opportunity in primary care to monitor chronic heart failure patient's response to pharmacology and continually assess the disease progression. This is expected to reduce requirements for more expensive echocardiography and waiting times, inform policy makers about the clinical needs of the heart failure patients and improve the management of heart failure patients (Ibrahim and Januzzi 2017, Savarese 2016, Faggiano et al. 2010, Miller et al. 2007). Although, age, sex, body mass index and smoking were not predictors of repeat natriuretic peptide testing in the primary care setting based on CPRD data, these factors should be considered when ordering brain natriuretic tests in heart failure patients, as they help with risk stratification and targeting therapies (Ponikowski et al. 2016).

Baseline BNP and NT-proBNP are independent predictors of all-cause mortality and cardiovascular mortality in the primary care setting. When treating heart failure patients, physicians should consider not only the baseline BNP or NT-proBNP levels, but also the age of the patients, since age significantly impacts mortality, especially in elderly. For example, at 70-year old, for each additional year, there was approximately 2% to 4% increase in the risk of

mortality. This risk increased to 4.4% to 7.4% in 80-year old patients. Changes in BNP and NTproBNP did not provide additional information on the all-cause mortality and cardiovascular mortality beyond a single point-in-time peptide level. Novel pharmacotherapy research for treating chronic heart failure patients is undergoing. The clinical interpretation of the changes in BNP and NT-proBNP will have to be assessed in the context of the drugs' mechanisms of action in addition to patient's demographics and clinical characteristics. For example, the angiotensin-neprilysin inhibition leads to decreasing NT-proBNP, but not necessarily BNP (Packer et al. 2015) and is expected to change the interpretation of future studies.

Serial measurements of B-type natriuretic peptides provide a valuable tool in managing heart failure patients (Miller 2007). Interpreting changes in B-type natriuretic peptides is much more challenging in chronic heart failure patients than acute heart failure patients. This is because of the rapid response of the B-type natriuretic peptides in acute heart failure patients due to the acute hemodynamics, whereas chronic heart failure patients deteriorate more slowly and need to be followed for longer periods of time to observe incremental changes (Miller 2009, Miller 2016, Cioffi 2006, Latini 2006). Although at the moment, medical guidelines include the B-type natriuretic peptides in the diagnosis algorithm for heart failure, serial measurements could predict adverse risks prospectively, as the diseases progresses. This provides an opportunity in the primary care setting to optimally adjust the medication and actively monitor the chronic heart failure patients, only when necessary.

The high within-subject variability of the B-type natriuretic peptide would pose severe limitations when monitoring chronic heart failure patients in the primary care setting. Heart rate, weight, blood pressure and serum creatinine have a much lower within-subject variability,

potentially making them useful in monitoring chronic heart failure patients, but they were not correlated with BPN over a three-month period. Changes in BNP were poorly correlated with changes in cardiac structure factors over a three-month period. However, in primary care the chronic heart failure population is more heterogeneous and may be less stable.

The proper categorization of the heart failure based on the ejection fraction into heart failure with reduced, preserved, or mid-range ejection fraction is essential to optimally manage the care of the chronic heart failure patients. In the United Kingdom, chronic heart failure patients with preserved ejection fractions are most likely managed in the primary care setting, while those with a reduced rejection fraction most likely access cardiology services, due to the advanced disease severity. This provides an important opportunity in the primary care to adequately identify chronic heart failure patients with preserved ejection fraction so that these patients could receive appropriate pharmacology treatments that actually work in this category of patients. This will positively impact the treatment of the heart failure and lead to a better utilization of health care resources.

Upon identification, the primary care practices can enter the appropriate heart failure diagnosis codes such as heart failure with preserved/reduced/mid-range ejection fraction in CPRD rather than the general heart failure code. The collection of the test assays used to measure the B-type natriuretic peptides could aid the interpretation of the test results in the presence of the analytical and biological variation. Such enhanced data entry practices will allow a more informative accumulation of heart failure data that can be used for research purposes, ultimately affecting the patients' lives.

How much of a change in B-type natriuretic peptides represents a significant change? There isn't a one change fits all clinical scenarios answer. It rather depends on the clinical

context and how the patient's disease activity changes over time. For example, in most severe patients with already elevated B-type natriuretic peptide levels even a small change could be clinically significant and portend adverse prognosis, whereas the same change in a younger chronic heart failure patient found in the early stage of the disease could be dismissed easily. At this moment, the heart failure guidelines recommend B-type natriuretic peptides only for diagnosis purposes (Ponikowski 2016, NICE 2010). Monitoring heart failure patients would require a determination of a monitoring interval, which would most likely be based on the patient's disease progression. In this context, changes from baseline in the B-type natriuretic peptides may be more relevant than the first natriuretic peptide measured.

From a primary care perspective, patient education about lifestyle choices, symptom recognition, and organization of care by a multidisciplinary team coupled with an optimal clinical pharmacology treatment remain essential to stabilize the condition, improve patients' quality of life and prevent acute episodes as much as possible (Ponikowski 2016).

7.7. Conclusion

More evidence is needed to establish the monitoring value of B-type natriuretic peptides in the primary care setting. Monitoring natriuretic peptides in addition to clinical assessments could be a cost-effective strategy to detect changes in the patient's clinical status and optimize treatment based on patient's tolerance within primary care.

Bibliography

Alonso, N., J. Lupon, J. Barallat, M. de Antonio, M. Domingo, E. Zamora, P. Moliner, A. Galan, J. Santesmases, C. Pastor, D. Mauricio and A. Bayes-Genis (2016). "Impact of diabetes on the predictive value of heart failure biomarkers." Cardiovascular Diabetology **15**(151): 1-9.

Andersson, C. and R. S. Vasan (2014). "Epidemiology of heart failure with preserved ejection fraction." Heart failure clinics **10**(3): 377-388.

Apple Fred, S. F. (2012). "Increased cardiac troponin I as measured by a high-sensitivity assay is associated with high odds of cardiovascular death: the Minnesota Heart Survey." Clinical Chemistry **58**(5): 930-935.

Askoxylakis, V. (2010). "Long-term survival of cancer patients compared to heart failure and stroke: a systematic review." BMC Cancer **10**(1).

Aspromonte, N. (2007). "Prognostic role of Btype natriuretic peptide in patients with diabetes and acute decompensated heart failure." Diabetic Medicine **24**(2).

Balion, C., P. L. Santaguida, S. Hill, A. Worster, M. McQueen, M. Oremus, R. McKelvie, L. Booker, J. Fagbemi and S. Reichert (2006). Testing for BNP and NT-proBNP in the diagnosis and prognosis of heart failure, Agency for Healthcare Research and Quality.

Balion, C. M., R. S. McKelvie, S. Reichert, P. Santaguida, L. Booker, A. Worster, P. Raina, M.

J. McQueen and S. Hill (2008). "Monitoring the response to pharmacologic therapy in patients with stable chronic heart failure: is BNP or NT-proBNP a useful assessment tool?" Clinical biochemistry **41**(4): 266-276.

Bashi, N., M. Karunanithi, F. Fatehi, H. Ding and D. Walters (2017). "Remote Monitoring of Patients With Heart Failure: An Overview of Systematic Reviews." Journal of Medical Internet Research **19**(1).

Bettencourt, P., A. Azevedo, J. Pimenta, F. Friões, S. Ferreira and A. Ferreira (2004). "N-terminal–pro-brain natriuretic peptide predicts outcome after hospital discharge in heart failure patients." Circulation **110**(15): 2168-2174.

Bhardwaj, A. (2010). "Design and methods of the Pro-B Type natriuretic peptide Outpatient Tailored Chronic heart failure Therapy (PROTECT) study." American Heart Journal **159**(4).

Bjurman, J. J., P. M., H. O. and F. M. (2013). "Assessment of a multimarker strategy for prediction of mortality in older heart failure patients: a cohort study." BMJ Open **3**(3).

Blank, M. D., A. B. Breland, P. T. Enlow, C. Duncan, A. Metzger and C. O. Cobb (2016). "Measurement of smoking behavior: Comparison of self-reports, returned cigarette butts, and toxicant levels." Exp Clin Psychopharmacol **24**(5): 348-355.

BMJ (2012). British National Formulary, Pharmaceutical Press.

Braunwald, E. (2008). "Biomarkers in heart failure." New England Journal of Medicine **358**(20): 2148-2159.

Braunwald, E. (2013). "Chronic heart failure: a look through the rear view mirror." European Heart Journal **34**(19).

Braunwald, E. (2013). "Heart failure." JACC: Heart Failure **1**(1): 1-20.

CIBIS-II, I. (1999). "The cardiac insufficiency bisoprolol study II (CIBIS-II): a randomised trial." The Lancet **353**(9146): 9-13.

Cioffi, G., L. Tarantini, C. Stefenelli, G. Azzetti, R. Marco, S. Carlucci and F. Furlanello (2006). "Changes in plasma N-terminal proBNP levels and ventricular filling pressures during intensive unloading therapy in elderly with decompensated congestive heart failure and preserved left ventricular systolic function." Journal of cardiac failure **12**(8): 608-615.

Cleland, J. G. F. (2011). "The national heart failure audit for England and Wales 20082009." Heart **97**(11).

Cohn, J. N. and G. Tognoni (2001). "A randomized trial of the angiotensin-receptor blocker valsartan in chronic heart failure." New England Journal of Medicine **345**(23): 1667-1675.

CONSENSUS, T. S. G. (1987). "Effects of enalapril on mortality in severe congestive heart failure: results of the Cooperative North Scandinavian Enalapril Survival Study (CONSENSUS)." N Engl j Med **316**: 1429-1435.

Cook, C., G. Cole, P. Asaria, R. Jabbour and D. P. Francis (2014). "The annual global economic burden of heart failure." International Journal of Cardiology **171**(3): 368-376.

Cotter, G., G. M. Felker, K. F. Adams, O. Milo-Cotter and C. M. O'Connor (2008). "The pathophysiology of acute heart failure--is it all about fluid accumulation?" Am Heart J **155**(1): 9-18.

Cowie, M., D. Wood, A. Coats, S. Thompson, V. Suresh, P. Poole-Wilson and G. Sutton (2000). "Survival of patients with a new diagnosis of heart failure: a population based study." Heart **83**(5): 505-510.

Dierckx, R. (2017). "Telemedicine in heart failure: new insights from the Cochrane metaanalyses." European Journal of Heart Failure **19**(3).

Dobre, D., P. Rossignol, M. Metra and F. Zannad (2012). "Can we prevent or treat renal dysfunction in chronic heart failure?" Heart failure reviews **17**(2): 283-290.

Faggiano, P., R. Valle, N. Aspromonte, A. D'Aloia, G. Di Tano, S. Barro, P. Giovinazzo, L. Milani, R. Lorusso and L. Dei Cas (2010). "How often we need to measure brain natriuretic

peptide (BNP) blood levels in patients admitted to the hospital for acute severe heart failure?: Role of serial measurements to improve short-term prognostic stratification." International journal of cardiology **140**(1): 88-94.

Felker, G. M. (2011). "Biomarkers as surrogate end points in heart failure trials." Heart Fail Clin **7**(4): 501-507.

Felker, G. M. and T. Ahmad (2013). "Natriuretic peptides and primary prevention: the new world?" J Am Coll Cardiol **62**(15): 1373-1375.

Felker, G. M., M. Fiuzat, V. Thompson, L. K. Shaw, M. L. Neely, K. F. Adams, D. J. Whellan, M. P. Donahue, T. Ahmad and D. W. Kitzman (2013). "Soluble ST2 in Ambulatory Patients With Heart Failure Association With Functional Capacity and Long-Term Outcomes." Circulation: Heart Failure **6**(6): 1172-1179.

Felker, G. M., V. Hasselblad, A. F. Hernandez and C. M. O'Connor (2009). "Biomarker-guided therapy in chronic heart failure: a meta-analysis of randomized controlled trials." Am Heart J **158**(3): 422-430.

Felker, G. Michael, Kevin J. Anstrom, Kirkwood F. Adams, Justin A. Ezekowitz, Mona Fiuzat, Nancy Houston-Miller, James L. Januzzi et al. "Effect of Natriuretic Peptide–Guided Therapy on Hospitalization or Cardiovascular Mortality in High-Risk Patients With Heart Failure and Reduced Ejection Fraction: A Randomized Clinical Trial." Jama 318, no. 8 (2017): 713-720.

Flather, M. D., M. C. Shibata, A. J. Coats, D. J. Van Veldhuisen, A. Parkhomenko, J. Borbola, A. Cohen-Solal, D. Dumitrascu, R. Ferrari and P. Lechat (2005). "Randomized trial to determine the effect of nebivolol on mortality and cardiovascular hospital admission in elderly patients with heart failure (SENIORS)." European heart journal **26**(3): 215-225.

Frankenstein, L., A. H. Wu, K. Hallermayer, F. H. Wians, E. Giannitsis and H. A. Katus (2011). "Biological variation and reference change value of high-sensitivity troponin T in healthy individuals during short and intermediate follow-up periods." Clinical chemistry **57**(7): 1068-1071.

Fraser, C. G. (2001). Biological variation: from principles to practice, Amer. Assoc. for Clinical Chemistry.

Gaggin, H. K., Q. A. Truong, S. U. Rehman, A. A. Mohammed, A. Bhardwaj, K. A. Parks, D. A. Sullivan, A. Chen-Tournoux, S. A. Moore, A. M. Richards, R. W. Troughton, J. G. Lainchbury, R. B. Weiner, A. L. Baggish, M. J. Semigran and J. L. Januzzi (2013). "Characterization and Prediction of Natriuretic Peptide "Nonresponse" During Heart Failure Management: Results From the ProBNP Outpatient Tailored Chronic Heart Failure (PROTECT) and the NT-proBNP-Assisted Treatment to Lessen Serial Cardiac Readmissions and Death (BATTLESCARRED) Study." Congestive Heart Failure **19**(3): 135-142.

Glasziou, P., L. Irwig and J. Aronson (2008). Evidence-based medical monitoring: from

principles to practice, Blackwell Publishing.

Glick, H., J. Cook, B. Kinosian, B. Pitt, M. G. Bourassa, H. Pouleur and W. Gerth (1995). "Costs and effects of enalapril therapy in patients with symptomatic heart failure: an economic analysis of the Studies of Left Ventricular Dysfunction (SOLVD) Treatment Trial." Journal of cardiac failure **1**(5): 371-380.

Granger, C. B., J. J. McMurray, S. Yusuf, P. Held, E. L. Michelson, B. Olofsson, J. Östergren, M. A. Pfeffer, K. Swedberg and C. I. a. Committees (2003). "Effects of candesartan in patients with chronic heart failure and reduced left-ventricular systolic function intolerant to angiotensin-converting-enzyme inhibitors: the CHARM-Alternative trial." The Lancet **362**(9386): 772-776.

Guyatt, G., A. D. Oxman, H. J. Schünemann, P. Tugwell and A. Knottnerus (2011). "GRADE guidelines: a new series of articles in the Journal of Clinical Epidemiology." Journal of clinical epidemiology **64**(4): 380-382.

Hancock, H. C. (2013). "High prevalence of undetected heart failure in long-term care residents: findings from the Heart Failure in Care Homes (HFinCH) study." European Journal of Heart Failure **15**(2).

Heidenreich, P. A., N. M. Albert, L. A. Allen, D. A. Bluemke, J. Butler, G. C. Fonarow, J. S. Ikonomidis, O. Khavjou, M. A. Konstam and T. M. Maddox (2013). "Forecasting the impact of

heart failure in the United States." Circulation: Heart Failure **6**(3): 606-619.

Herrett, E., S. L. Thomas, W. M. Schoonen, L. Smeeth and A. J. Hall (2010). "Validation and validity of diagnoses in the General Practice Research Database: a systematic review." British journal of clinical pharmacology **69**(1): 4-14.

Hobbs, F. R., A. K. Roalfe, R. C. Davis, M. K. Davies and R. Hare (2007). "Prognosis of all-cause heart failure and borderline left ventricular systolic dysfunction: 5 year mortality follow-up of the Echocardiographic Heart of England Screening Study (ECHOES)." European heart journal.

Ibrahim, N. and J. Januzzi (2017). "Beyond Natriuretic Peptides for Diagnosis and Management of Heart Failure." Clinical Chemistry **63**(1): 211-222.

Iglesias, N., P. H. Petersen and C. Ricós (2005). "Power function of the reference change value in relation to cut-off points, reference intervals and index of individuality." Clinical Chemical Laboratory Medicine **43**(4): 441-448.

Inglis, S. C., R. A. Clark, R. Dierckx, D. Prieto-Merino and J. G. Cleland (2017). Structured telephone support or non-invasive telemonitoring for patients with heart failure, BMJ Publishing Group Ltd and British Cardiovascular Society.

Januzzi James, L. J. "B-Type Natriuretic Peptide Testing in the Era of Neprilysin Inhibition:

Are the Winds of Change Blowing?" Clinical Chemistry **62**(5): 663-665.

Johansson, I., U. Dahlström, M. Edner, P. Näsman, L. Rydén and A. Norhammar (2016). "Prognostic implications of type 2 diabetes mellitus in ischemic and nonischemic heart failure." Journal of the American College of Cardiology **68**(13): 1404-1416.

Kubanek, M., K. M. Goode, V. Lánská, A. L. Clark and J. G. Cleland (2009). "The prognostic value of repeated measurement of N-terminal pro-B-type natriuretic peptide in patients with chronic heart failure due to left ventricular systolic dysfunction." European journal of heart failure **11**(4): 367-377.

Lainchbury, J. G. (2006). "N-terminal proB-type natriuretic peptide-guided treatment for chronic heart failure: results from the BATTLESCARRED (NT-proBNPAssisted Treatment To Lessen Serial Cardiac Readmissions and Death) Trial." Journal of the American College of Cardiology **55**(1).

Laird, N. and J. Ware (1982). "Random-effects models for longitudinal data." Biometrics **38**(4): 11.

Lang, R. M., L. P. Badano, V. Mor-Avi, J. Afilalo, A. Armstrong, L. Ernande, F. A. Flachskampf, E. Foster, S. A. Goldstein and T. Kuznetsova (2015). "Recommendations for cardiac chamber quantification by echocardiography in adults: an update from the American Society of Echocardiography and the European Association of Cardiovascular Imaging."

Journal of the American Society of Echocardiography **28**(1): 1-39. e14.

Laribi, S. (2012). "Trends in death attributed to heart failure over the past two decades in Europe." European Journal of Heart Failure **14**(3).

Latini, R. (2002). "Role of brain natriuretic peptide in the diagnosis and management of heart failure: current concepts." Journal of Cardiac Failure **8**(5).

Lee, S.-C., T. L. Stevens, S. M. Sandberg, D. M. Heublein, S. M. Nelson, M. Jougasaki, M. M. Redfield and J. C. Burnett (2002). "The potential of brain natriuretic peptide as a biomarker for New York Heart Association class during the outpatient treatment of heart failure." Journal of cardiac failure **8**(3): 149-154.

Levin, E. R., D. G. Gardner and W. K. Samson (1998). "Natriuretic peptides." N Engl J Med **339**(5): 321-328.

Lin, D. C. C., E. P. Diamandis, J. L. Januzzi Jr, A. Maisel, A. S. Jaffe and A. Clerico (2014). "Natriuretic peptides in heart failure." Clinical Chemistry **60**(8): 1040-1046.

MacIver, D. H., M. J. Dayer and A. J. I. Harrison (2013). "A general theory of acute and chronic heart failure." International Journal of Cardiology **165**: 25-34.

Maisel, A. (2013). "Primary results of the HABIT Trial (heart failure assessment with BNP in

the home)." Journal of the American College of Cardiology **61**(16).

Maisel, A. S., K. S. Shah, D. Barnard, B. Jaski, G. Frivold, J. Marais, M. Azer, M. I. Miyamoto, D. Lombardo, D. Kelsay, N. Iqbal, P. R. Taub, K. Kupfer, E. Lee, P. Clopton, M. Zile and B. Greenberg (2016). "How B-Type Natriuretic Peptide (BNP) and Body Weight Changes Vary in Heart Failure With Preserved Ejection Fraction Compared With Reduced Ejection Fraction: Secondary Results of the HABIT (HF Assessment With BNP in the Home) Trial." Journal of Cardiac Failure **22**(4): 283-293.

Martínez-Sellés, M., R. N. Doughty, K. Poppe, G. A. Whalley, N. Earle, C. Tribouilloy, J. J. McMurray, K. Swedberg, L. Køber and C. Berry (2012). "Gender and survival in patients with heart failure: interactions with diabetes and aetiology. Results from the MAGGIC individual patient meta-analysis†." European journal of heart failure **14**(5): 473-479.

McDonagh, T. A. (2011). Oxford textbook of heart failure. Oxford, Oxford University Press.

McKie, P. M., A. Cataliotti, B. D. Lahr, F. L. Martin, M. M. Redfield, K. R. Bailey, R. J. Rodeheffer and J. C. Burnett Jr (2010). "The Prognostic Value of N-Terminal Pro-B-Type Natriuretic Peptide for Death and Cardiovascular Events in Healthy Normal and Stage A/B Heart Failure Subjects." Journal of the American College of Cardiology **55**(19): 2140-2147.

McLellan, J., C. Heneghan, R. Perera, A. Clements, P. Glasziou, K. Kearley, N. Pidduck, N. Roberts, S. Tyndel, F. Wright and C. Bankhead (2016). B-type natriuretic peptide-guided

treatment for heart failure (Review), Cochrane Database of Systematic Reviews. **12**.

McManus, D. D., S. J. Shah, M. R. Fabi, A. Rosen, M. A. Whooley and N. B. Schiller (2009). "Prognostic value of left ventricular end-systolic volume index as a predictor of heart failure hospitalization in stable coronary artery disease: data from the Heart and Soul Study." Journal of the American Society of Echocardiography **22**(2): 190-197.

McMurray, J. J., S. Adamopoulos, S. D. Anker, A. Auricchio, M. Böhm, K. Dickstein, V. Falk, G. Filippatos, C. Fonseca and M. A. Gomez-Sanchez (2012). "ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure 2012." European journal of heart failure **14**(8): 803-869.

McMurray, J. J., M. Packer, A. S. Desai, J. Gong, M. Lefkowitz, A. R. Rizkala, J. L. Rouleau, V. C. Shi, S. D. Solomon and K. Swedberg (2014). "Baseline characteristics and treatment of patients in Prospective comparison of ARNI with ACEI to Determine Impact on Global Mortality and morbidity in Heart Failure trial (PARADIGM-HF)." European journal of heart failure **16**(7): 817-825.

McMurray, J. J., M. Packer, A. S. Desai, J. Gong, M. P. Lefkowitz, A. R. Rizkala, J. Rouleau, V. C. Shi, S. D. Solomon and K. Swedberg (2013). "Dual angiotensin receptor and neprilysin inhibition as an alternative to angiotensin-converting enzyme inhibition in patients with chronic systolic heart failure: rationale for and design of the Prospective comparison of ARNI with ACEI to Determine Impact on Global Mortality and morbidity in Heart Failure trial

(PARADIGM-HF)." European journal of heart failure **15**(9): 1062-1073.

McMurray, J. J., M. Packer, A. S. Desai, J. Gong, M. P. Lefkowitz, A. R. Rizkala, J. L. Rouleau, V. C. Shi, S. D. Solomon and K. Swedberg (2014). "Angiotensin–neprilysin inhibition versus enalapril in heart failure." New England Journal of Medicine **371**(11): 993-1004.

McMurray John, J. V. J. (2012). "ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure 2012: The Task Force for the Diagnosis and Treatment of Acute and Chronic Heart Failure 2012 of the European Society of Cardiology. Developed in collaboration with the Heart Failure Association (HFA) of the ESC." European Heart Journal **33**(14): 1787-1847.

Meijers, W. C., A. R. van der Velde, A. C. Muller Kobold, J. Dijck-Brouwer, A. H. Wu, A. Jaffe and R. A. de Boer (2017). "Variability of biomarkers in patients with chronic heart failure and healthy controls." European journal of heart failure **19**(3): 357-365.

Merit-HF, S. G. (1999). "Effect of metoprolol CR/XL in chronic heart failure: metoprolol CR/XL randomised intervention trial in-congestive heart failure (MERIT-HF)." The Lancet **353**(9169): 2001-2007.

Metra, M. (2016). "August 2016 at a glance: the new ESC guidelines, and pathophysiology, epidemiology and prognosis of heart failure." European Journal of Heart Failure **18**(8): 889-890.

Michtalik, H. J. (2011). "Acute changes in N-terminal pro-B-type natriuretic peptide during hospitalization and risk of readmission and mortality in patients with heart failure." American Journal of Cardiology, The **107**(8).

Miller, W. (2009). "Only large reductions in concentrations of natriuretic peptides (BNP and NT-proBNP) are associated with improved outcome in ambulatory patients with chronic heart failure." Clinical Chemistry **55**(1): 78-84.

Miller, W., K. Hartman, M. Burritt, D. Grill, R. Rodeheffer, J. Burnett and A. Jaffe (2007). "Serial biomarker measurements in ambulatory patients with chronic heart failure: the importance of change over time." Circulation **116**(3): 249-257.

Miller, W. L. and A. S. Jaffe (2016). "Biomarkers in heart failure: the importance of inconvenient details." ESC Heart Failure **3**(1): 3-10.

Moe, G. (2016). "Heart failure with multiple comorbidities." Current Opinion in Cardiology **31**(2): 209-216.

Mozaffarian, D., E. J. Benjamin, A. S. Go, D. K. Arnett, M. J. Blaha, M. Cushman, S. R. Das, S. de Ferranti, J.-p. Després and H. J. Fullerton (2016). "Executive summary: Heart Disease and Stroke Statistics-2016 update: A report from the American Heart Association." Circulation **133**(4): 447.

Nabeebaccus, A. (2016). "Heart failure-potential new targets for therapy." British Medical Bulletin **119**(1): 99-110.

Nadruz, W., B. L. Claggett, J. J. McMurray, M. Packer, M. R. Zile, J. L. Rouleau, A. S. Desai, K. Swedberg, M. Lefkowitz and V. C. Shi (2016). "Impact of Body Mass Index on the Accuracy of N-Terminal Pro-Brain Natriuretic Peptide and Brain Natriuretic Peptide for Predicting Outcomes in Patients With Chronic Heart Failure and Reduced Ejection Fraction." Circulation **134**(22): 1785-1787.

Naffaa, M., B. F. Makhoul, A. Tobia, M. Jarous, M. Kaplan, D. Aronson, W. Saliba and Z. S. Azzam (2014). "Brain natriuretic peptide at discharge as a predictor of 6-month mortality in acute decompensated heart failure." American Journal of Emergency Medicine **32**(1): 44-49.

Nakano, H., K. Fuse, M. Takahashi, S. Yuasa, T. Koshikawa, M. Sato, M. Okabe, A. Yamashina and Y. Aizawa (2016). "The response of NT-proBNP to intensified medication in advanced chronic heart failure." IJC Metabolic & Endocrine **10**: 24-29.

Neuhold, S., M. Huelsmann, G. Strunk, J. Struck, C. Adlbrecht, G. Gouya, M. Elhenicky and R. Pacher (2010). "Prognostic value of emerging neurohormones in chronic heart failure during optimization of heart failure–specific therapy." Clinical chemistry **56**(1): 121-126.

NHS (2013). The NHS atlas of variation in diagnostic services; Reducing unwarranted variation to increase value and improve quality. Public Health England. NHS.

NICE (2010). NICE clinical guideline 108 - Chronic heart failure: management of chronic heart failure in adults in primary and secondary care. NICE clinical guideline 108. N. I. f. H. a. C. Excellenc, National Institute for Health and Clinical Excellence
National Collaborating Centre for Acute and Chronic Conditions**:** 1-44.

Núñez, J., E. Núñez, J. Sanchis, V. Bodí, G. C. Fonarow, G. Miñana, P. Palau, V. Bertomeu-González, A. Carratalá and L. Mainar (2012). "Antigen carbohydrate 125 and brain natriuretic peptide serial measurements for risk stratification following an episode of acute heart failure." International journal of cardiology **159**(1): 21-28.

Packer, M. (1992). "The Neurohormonal Hypothesis: A theory to explain the mechanism of disease progression in heart failure." Journal of the American College of Cardiology **20**(1): 248-254.

Packer, M., J. J. McMurray, A. S. Desai, J. Gong, M. P. Lefkowitz, A. R. Rizkala, J. L. Rouleau, V. C. Shi, S. D. Solomon and K. Swedberg (2015). "Angiotensin receptor neprilysin inhibition compared with enalapril on the risk of clinical progression in surviving patients with heart failure." Circulation **131**(1): 54-61.

Packer, M., P. A. Poole-Wilson, P. W. Armstrong, J. G. Cleland, J. D. Horowitz, B. M. Massie, L. Rydén, K. Thygesen and B. F. Uretsky (1999). "Comparative effects of low and high doses of the angiotensin-converting enzyme inhibitor, lisinopril, on morbidity and mortality in

chronic heart failure." Circulation **100**(23): 2312-2318.

Patrick, D. L., A. Cheadle, D. C. Thompson, P. Diehr, T. Koepsell and S. Kinne (1994). "The validity of self-reported smoking: a review and meta-analysis." American journal of public health **84**(7): 1086-1093.

Pellicori, P. (2013). "IVC diameter in patients with chronic heart failure: relationships and prognostic significance." JACC: Cardiovascular Imaging **6**(1).

Peng, Q., W. Hu, H. Su, Q. Yang and X. Cheng (2013). "Levels of B-type natriuretic peptide in chronic heart failure patients with and without diabetes mellitus." Exp Ther Med **5**(1): 229-232.

Perrone, R. D., N. E. Madias and A. S. Levey (1992). "Serum creatinine as an index of renal function: new insights into old concepts." Clinical chemistry **38**(10): 1933-1953.

Piper, S. (2016). "Biologic variability of soluble ST2 in patients with stable chronic heart failure and implications for monitoring." American Journal of Cardiology, The **118**(1).

Pitt, B., F. Zannad, W. J. Remme, R. Cody, A. Castaigne, A. Perez, J. Palensky and J. Wittes (1999). "The effect of spironolactone on morbidity and mortality in patients with severe heart failure." New England Journal of Medicine **341**(10): 709-717.

Pocock, S. J. (2013). "Predicting survival in heart failure: a risk score based on 39 372 patients

from 30 studies." European Heart Journal **34**(19).

Poelzl, G., J. Altenberger, R. Pacher, C. Ebner, M. Wieser, A. Winter, F. Fruhwald, C. Dornaus, U. Ehmsen and S. Reiter (2014). "Dose matters! Optimisation of guideline adherence is associated with lower mortality in stable patients with chronic heart failure." International journal of cardiology **175**(1): 83-89.

Ponikowski, P., A. Voors, S. Anker, H. Bueno, J. Cleland, A. Coats, V. Falk, J. González-Juanatey, V. Harjola and E. Jankowska (2016). "Authors/Task Force Members; Document Reviewers. 2016 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure: The Task Force for the diagnosis and treatment of acute and chronic heart failure of the European Society of Cardiology (ESC). Developed with the special contribution of the Heart Failure Association (HFA) of the ESC." Eur J Heart Fail **18**(8): 891-97530.

Richards, A. M., J. L. Januzzi and R. W. Troughton (2014). "Natriuretic Peptides in Heart Failure with Preserved Ejection Fraction." Heart Failure Clinics **10**(3): 453-470.

Roger, V. L. (2013). "Epidemiology of heart failure." Circulation research **113**(6): 646-659.

Saenger, A. K. (2017). "Specificity of B-Type Natriuretic Peptide Assays: Cross-Reactivity with Different BNP, NT-proBNP, and proBNP Peptides." Clinical Chemistry **63**(1).

Savarese, G. (2016). "Reductions in N-Terminal Pro-Brain Natriuretic Peptide Levels Are

Associated With Lower Mortality and Heart Failure Hospitalization Rates in Patients With Heart Failure With Mid-Range and Preserved Ejection Fraction." Circulation: Heart Failure **9**(11).

Sayer, G. (2014). "The Renin-Angiotensin-Aldosterone System and Heart Failure." Cardiology Clinics **32**(1): 21-32.

Schindler, I. E. (2016). "Short- and Long-term Biologic Variability of Galectin-3 and Other Cardiac Biomarkers in Patients with Stable Heart Failure and Healthy Adults." Clinical Chemistry **62**(2): 360-366.

Scrutinio, D., P. Agostoni, L. Gesualdo, U. Corra, A. Mezzani, M. Piepoli, A. Di Lenarda, A. Iorio, C. Passino, D. Magri, D. Masarone, E. Battaia, D. Girola, F. Re, G. Cattadori, G. Parati, G. Sinagra, G. Q. Villani, G. Limongelli, G. Pacileo, M. Guazzi, M. Metra, M. Frigerio, M. Cicoira, C. Mina, G. Malfatto, S. Caravita, M. Bussotti, E. Salvioni, F. Veglia, M. Correale, A. B. Scardovi, M. Emdin, P. Giannuzzi, P. Gargiulo, M. Giovannardi, P. Perrone-Filardi, R. Raimondo, R. Ricci, S. Paolillo, S. Farina, R. Belardinelli, A. Passantino, R. La Gioia, C. Fiorentini, A. Apostolo, P. Palermo, M. Contini, E. Bertella, V. Mantegazza, F. Pietrucci, A. Ferraironi, M. Casenghi, F. Clemenza, T. Roselli, A. Buono, R. Calabro, D. Santoro, S. Campanale, D. Caputo, D. Bertipaglia, R. Vaninetti, M. Confalonieri, E. Zambon, E. Berton, C. Torregiani, L. D. Cas, V. Carubelli, S. Binno, G. Marchese, F. Oliva and L. Pastormerlo (2015). "Renal function and peak exercise oxygen consumption in chronic heart failure with reduced left ventricular ejection fraction." Circulation Journal **79**(3): 583-591.

Stienen, S., K. Salah, C. Dickhoff, V. Carubelli, M. Metra, L. Magrini, S. Di Somma, J. P. Tijssen, Y. M. Pinto and W. E. Kok (2015). "N-Terminal Pro–B-Type Natriuretic Peptide (NT-proBNP) Measurements Until a 30% Reduction Is Attained During Acute Decompensated Heart Failure Admissions and Comparison With Discharge NT-proBNP Levels: Implications for In-Hospital Guidance of Treatment." Journal of cardiac failure **21**(11): 930-934.

Swedberg, K., M. Komajda, M. Böhm, J. S. Borer, I. Ford, A. Dubost-Brama, G. Lerebours, L. Tavazzi and S. Investigators (2010). "Ivabradine and outcomes in chronic heart failure (SHIFT): a randomised placebo-controlled study." The Lancet **376**(9744): 875-885.

Taylor, C., A. Roalfe, R. Iles and F. Hobbs (2014). "The potential role of NT-proBNP in screening for and predicting prognosis in heart failure: a survival analysis." BMJ open **4**(4): e004675.

Taylor, C. J., A. K. Roalfe, R. Iles and F. D. R. Hobbs (2014). "The potential role of NT-proBNP in screening for and predicting prognosis in heart failure: A survival analysis." BMJ Open **4**(4).

Taylor, C. J., A. K. Roalfe, L. Tait, R. C. Davis, R. Iles, M. Derit and F. R. Hobbs (2014). "Observational longitudinal cohort study to determine progression to heart failure in a screened community population: the Echocardiographic Heart of England Screening Extension (ECHOES-X) study." BMJ open **4**(7): e005256.

Taylor, Clare J, Ronan Ryan, Linda Nichols, Nicola Gale, FD Richard Hobbs, Tom Marshall (2017); Survival following a diagnosis of heart failure in primary care, Family Practice, Volume 34, Issue 2, Pages 161–168

Thygesen, K., J. Mair, C. Mueller, K. Huber, M. Weber, M. Plebani, Y. Hasin, L. Biasucci, E. Giannitsis, B. Lindhal, W. Koenig, M. Tubaro, P. Collinson, H. Katus, M. Galvani, P. Venge, J. Alpert, C. Hamm and A. Jaffe (2012). "Recommendations for the use of natriuretic peptides in acute cardiac care
A position statement from the Study Group on Biomarkers in Cardiology of the ESC Working Group on Acute Cardiac Care." European Heart Journal **33**: 2001-2006.

Townsend, N., P. Bhatnagar, E. Wilkins, K. Wickramasinghe and M. Rayner (2015). Cardiovascular disease statistics, 2015.

Townsend, N., K. Wickramasinghe, P. Bhatnagar, K, M. Nichols, J. Leal, R. Luengo-Fernandez and M. Rayner (2012). Coronary heart disease statistics 2012 edition. B. H. Foundation**:** 211.

Troughton, R., G. Michael Felker and J. L. Januzzi (2014). "Natriuretic peptide-guided heart failure management." European Heart Journal **35**(1): 16-24b.

van Veldhuisen, D. J., G. C. Linssen, T. Jaarsma, W. H. van Gilst, A. W. Hoes, J. G. Tijssen,

W. J. Paulus, A. A. Voors and H. L. Hillege (2013). "B-type natriuretic peptide and prognosis in heart failure patients with preserved and reduced ejection fraction." Journal of the American College of Cardiology **61**(14): 1498-1506.

Vos, T., A. D. Flaxman, M. Naghavi, R. Lozano, C. Michaud, M. Ezzati, K. Shibuya, J. A. Salomon, S. Abdalla and V. Aboyans (2013). "Years lived with disability (YLDs) for 1160 sequelae of 289 diseases and injuries 1990–2010: a systematic analysis for the Global Burden of Disease Study 2010." The Lancet **380**(9859): 2163-2196.

Wang, T. J., M. G. Larson, D. Levy, E. J. Benjamin, E. P. Leip, T. Omland, P. A. Wolf and R. S. Vasan (2004). "Plasma natriuretic peptide levels and the risk of cardiovascular events and death." New England Journal of Medicine **350**(7): 655-663.

Wannamethee, S. G., A. G. Shaper, P. H. Whincup, L. Lennon, O. Papacosta and N. Sattar (2014). "The obesity paradox in men with coronary heart disease and heart failure: The role of muscle mass and leptin." International Journal of Cardiology **171**(1): 49-55.

Wu, A. H. (2006). "Serial testing of B-type natriuretic peptide and NTpro-BNP for monitoring therapy of heart failure: the role of biologic variation in the interpretation of results." Am Heart J **152**(5): 828-834.

Yancy, C. W., M. Jessup, B. Bozkurt, J. Butler, D. E. Casey, M. M. Colvin, M. H. Drazner, G. Filippatos, G. C. Fonarow and M. M. Givertz (2016). "2016 ACC/AHA/HFSA focused update

on new pharmacological therapy for heart failure: an update of the 2013 ACCF/AHA guideline for the management of heart failure: a report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines and the Heart Failure Society of America." Circulation **134**(13): e282-e293.

Zannad, F., J. J. McMurray, H. Krum, D. J. van Veldhuisen, K. Swedberg, H. Shi, J. Vincent, S. J. Pocock and B. Pitt (2011). "Eplerenone in patients with systolic heart failure and mild symptoms." New England Journal of Medicine **364**(1): 11-21.

Ziaeian, B. and G. C. Fonarow (2016). "Epidemiology and aetiology of heart failure." Nature Reviews Cardiology.

Zile, M. R., B. L. Claggett, M. F. Prescott, J. J. McMurray, M. Packer, J. L. Rouleau, K. Swedberg, A. S. Desai, J. Gong and V. C. Shi (2016). "Prognostic implications of changes in N-Terminal pro-B-Type natriuretic peptide in patients with heart failure." Journal of the American College of Cardiology **68**(22): 2425-2436.

APPENDIX A

Search terms and results from the COCHRANE search in Chapter 2

Date Run: 01/07/18 13:59:11.820
Description: Cochrane search supporting Chapter 2

ID	Search	Hits
#1	MeSH descriptor: [Heart Failure] explode all trees	8627
#2	chronic heart failure	9143
#3	MeSH descriptor: [Natriuretic Peptide, Brain] explode all trees	1279
#4	systematic review	56973
#5	MeSH descriptor: [Meta-Analysis as Topic] explode all trees	622
#6	#4 and #5	317
#7	meta-analysis or meta-analyses or meta analysis or meta analyses	40899
#8	#4 and #7	31180
#9	#1 and #8	301
#10	#2 and #8	1897
#11	BNP or NTproBNP or NT proBNP or NT-proBNP	2504
#12	natriuretic peptide	4066
#13	brain natriuretic peptide	2850
#14	B-type natriuretic peptide or B type natriuretic peptide	1171
#15	N terminal pro B type natriuretic peptide or N-terminal pro B type natriuretic peptide or amino terminal pro brain natriuretic peptide	983
#16	#11 or #12 or #13 or #14 or #15	4752
#17	#10 and #16	88
#18	prognosis or prognostic or predict or mortality or death or survival	179754
#19	#17 and #18	83
#20	management or monitoring or variability or changes	265685
#21	#17 and #20	73
#22	#19 or #21	84
#23	#18 or #20	402450
#24	prognosis or prognostic or predict or mortality or death or survival or management or monitoring or variability or changes	402450
#25	chronic heart failure and #24	6429
#26	#25 and #8 and #16	84

Search terms and results from the PUBMED search

Search ID	Search terms	Results
#1	Chronic heart failure	224970
#2	Heart failure	224970
#3	#1 and systematic review	40393
#4	Meta analysis	114573
#5	Meta analyses	119662
#6	Meta-analysis	114573
#7	Meta-analyses	119662
#8	B-type natriuretic peptide	18537
#9	B type natriuretic peptide	18537
#10	N terminal pro B type natriuretic peptide	0
#11	N-terminal pro B type natriuretic peptide	0
#12	Brain natriuretic peptide	16648
#13	Natriuretic peptide	35961
#14	bnp	9196
#15	BNP	9196
#16	NT-proBNP	6794
#17	NTproBNP	4806
#18	#8 or #9 or #10 or #11 or #12 or #13 or #14 or #15 or #16 or #217	37791
#19	#4 or #5 or #6 or #7	155859
#20	#1 and #19	3169
#21	#3 and #20	2043
#22	#21 and #18	128
Date Last Run: 1-JUL-2018		

Embase Session Results

No.	Query	Results
#1	'chronic heart failure' AND ('monitoring'/exp OR 'monitoring' OR 'disease management'/exp OR 'disease management' OR 'diseases management' OR 'disorder management' OR 'disorders management' OR 'illness management' OR 'management of disease' OR 'management of disorder' OR 'medical management' OR 'variability'/exp OR 'prognosis'/exp) AND ('natriuretic peptides' OR 'brain natriuretic peptide'/exp OR 'b-type natriuretic peptide' OR 'n terminal pro b type natriuretic' OR bnp OR 'nt probnp') AND ('systematic review'/exp OR 'review, systematic' OR 'systematic review')	24

EMBASE Last Date Run: 1-JUL-2018

APPENDIX B

Cardiovascular disease causes of death codes according to World Health Organization

Disease classification	Code
Major cardiovascular diseases	I00-I78
Diseases of heart	I00-I09,I11,I13,I20-I51
Acute rheumatic fever and chronic rheumatic heart diseases	I00-I09
Hypertensive heart disease	I11
Hypertensive heart and renal disease	I13
Ischemic heart diseases	I20-I25
Acute myocardial infarction	I21-I22
Other acute ischemic heart disease	I24
Other forms of chronic ischemic heart	I20,I25
Atherosclerotic cardiovascular disease, so described	I25.0
All other forms of chronic ischemic heart disease	I20,I25.1-I25.9
Other heart diseases	I26-I51
Acute and subacute endocarditis	I33
Diseases of pericardium and acute myocarditis	I30-I31,I40
Heart failure	I50
All other forms of heart disease	I26-I28,I34-I38,I42-I49,I51
Essential (primary) hypertension and hypertensive renal disease	I10,I12
Cerebrovascular diseases	I60-I69
Atherosclerosis	I70
Other diseases of circulatory system	I71-I78
Aortic aneurysm and dissection	I71
Other diseases of arteries, arterioles and capillaries	I72-I78
Other disorders of circulatory system	I80-I99

Exclusion Criteria

Read Code	GPRD Medical Code	Read Description
14V2.00	20196	H/O: renal dialysis
14V2.11	44422	H/O: kidney dialysis
1Z13.00	12479	Chronic kidney disease stage 4
1Z14.00	12585	Chronic kidney disease stage 5
66YI.00	18501	COPD self-management plan given
66YL.11	18476	COPD follow-up
66Yd.00	19106	COPD accident and emergency attendance since last visit
66Ye.00	19003	Emergency COPD admission since last appointment
66Yf.00	28743	Number of COPD exacerbations in past year
66Yi.00	46036	Multiple COPD emergency hospital admissions
7B00000	55151	Autotransplant of kidney
7B00100	11745	Transplantation of kidney from live donor
7B00200	24361	Transplantation of kidney from cadaver
7B00z00	5504	Transplantation of kidney NOS
7L1A.11	11773	Dialysis for renal failure
7L1A000	20073	Renal dialysis
7L1A500	30756	Continuous ambulatory peritoneal dialysis
14V2.00	20196	H/O: renal dialysis
7L1B000	8037	Insertion of ambulatory peritoneal dialysis catheter
8H2R.00	11019	Admit COPD emergency
B22z.11	2587	Lung cancer
B4A0.00	1599	Malignant neoplasm of kidney parenchyma
B580.00	1952	Secondary malignant neoplasm of kidney
BB5a.00	8101	[M]Renal adenoma and carcinoma
BB5a000	10668	[M]Renal cell carcinoma
G401.00	1266	Pulmonary embolism
K05..12	53852	End stage renal failure
K050.00	6712	End stage renal failure
K0D..00	8330	End-stage renal disease
L43..00	7174	Obstetric pulmonary embolism
L430.00	31313	Obstetric air pulmonary embolism
L431.00	44192	Amniotic fluid pulmonary embolism
L432.00	49269	Obstetric blood-clot pulmonary embolism
L43z400	44404	Obstetric pulmonary embolism NOS with postnatal complication
SP01500	48639	Mechanical complication of dialysis catheter
SP05613	46438	[X] Peritoneal dialysis associated peritonitis
SP08300	11553	Kidney transplant failure and rejection
TB00100	54990	Kidney transplant with complication, without blame
TB00111	18774	Renal transplant with complication, without blame
TB11.00	28158	Kidney dialysis with complication, without blame
ZV12900	16976	[V] Personal history of pulmonary embolism
ZV42000	5911	[V]Kidney transplanted
ZV45100	22252	[V]Renal dialysis status
ZV56100	52088	[V]Preparatory care for dialysis

APPENDIX C

DIABETES

List of READ/OXMIS codes for identifying diabetic patients

READ/OXMIS code	Medcode	Description
C100112	506	Non-insulin dependent diabetes mellitus
C10..00	711	Diabetes mellitus
C10F.00	758	Type 2 diabetes mellitus
C100011	1038	Insulin dependent diabetes mellitus
C10FJ00	1407	Insulin treated Type 2 diabetes mellitus
C10E.00	1549	Type 1 diabetes mellitus
C108.00	1647	Insulin dependent diabetes mellitus
C101.00	1682	Diabetes mellitus with ketoacidosis
K01x100	2471	Nephrotic syndrome in diabetes mellitus
66AJ100	2478	Brittle diabetes
C109.00	4513	Non-insulin dependent diabetes mellitus
C109.11	5884	NIDDM - Non-insulin dependent diabetes mellitus
C108700	6509	Insulin dependent diabetes mellitus with retinopathy
C108800	6791	Insulin dependent diabetes mellitus - poor control
1252.00	6795	FH: Diabetes mellitus
1434.00	6813	H/O: diabetes mellitus
C106.12	7795	Diabetes mellitus with neuropathy
C109700	8403	Non-insulin dependent diabetes mellitus - poor control
66AR.00	8836	Diabetes management plan given
9OL..00	9897	Diabetes monitoring admin.
C10yy00	10098	Other specified diabetes mellitus with other spec comps
C10ED00	10418	Type 1 diabetes mellitus with nephropathy
C10EM00	10692	Type 1 diabetes mellitus with ketoacidosis
C10B.00	11551	Diabetes mellitus induced by steroids
C314.11	11848	Renal diabetes
8BL2.00	12213	Patient on maximal tolerated therapy for diabetes
66AU.00	12307	Diabetes care by hospital only
C10E.11	12455	Type I diabetes mellitus
66AP.00	12506	Diabetes: practice programme
C10FC00	12640	Type 2 diabetes mellitus with nephropathy
66AQ.00	12675	Diabetes: shared care programme
C10F500	12736	Type 2 diabetes mellitus with gangrene
C104y00	13279	Other specified diabetes mellitus with renal complications
C100100	14803	Diabetes mellitus, adult onset, no mention of complication
C103.00	15690	Diabetes mellitus with ketoacidotic coma
C106.00	16230	Diabetes mellitus with neurological manifestation
C106.13	16491	Diabetes mellitus with polyneuropathy
C104.00	16502	Diabetes mellitus with renal manifestation
C109600	17262	Non-insulin-dependent diabetes mellitus with retinopathy

List of READ/OXMIS codes for identifying diabetic patients (cont'd)

READ/OXMIS code	Medcode	Description
C108F11	17545	Type I diabetes mellitus with diabetic cataract
C108.12	17858	Type 1 diabetes mellitus
C109.12	17859	Type 2 diabetes mellitus
C109G11	18143	Type II diabetes mellitus with arthropathy
C109012	18209	Type 2 diabetes mellitus with renal complications
C109.13	18219	Type II diabetes mellitus
C108J12	18230	Type 1 diabetes mellitus with neuropathic arthropathy
C109J12	18264	Insulin treated Type II diabetes mellitus
C109J00	18278	Insulin treated Type 2 diabetes mellitus
C10E700	18387	Type 1 diabetes mellitus with retinopathy
C10FM00	18390	Type 2 diabetes mellitus with persistent microalbuminuria
C10FB00	18425	Type 2 diabetes mellitus with polyneuropathy
C10F600	18496	Type 2 diabetes mellitus with retinopathy
C108.11	18505	IDDM-Insulin dependent diabetes mellitus
C10EH00	18642	Type 1 diabetes mellitus with arthropathy
C10E500	18683	Type 1 diabetes mellitus with ulcer
C10F000	18777	Type 2 diabetes mellitus with renal complications
9OLA.11	20900	Diabetes monitored
C102.00	21482	Diabetes mellitus with hyperosmolar coma
C108012	21983	Type 1 diabetes mellitus with renal complications
C10N.00	22487	Secondary diabetes mellitus
C106z00	22573	Diabetes mellitus NOS with neurological manifestation
C10EP00	22871	Type 1 diabetes mellitus with exudative maculopathy
C10F.11	22884	Type II diabetes mellitus
C108.13	24423	Type I diabetes mellitus
C109711	24458	Type II diabetes mellitus - poor control
C100000	24490	Diabetes mellitus, juvenile type, no mention of complication
C109G00	24693	Non-insulin dependent diabetes mellitus with arthropathy
C108B00	24694	Insulin dependent diabetes mellitus with mononeuropathy
C109C12	24836	Type 2 diabetes mellitus with nephropathy
C10FQ00	25591	Type 2 diabetes mellitus with exudative maculopathy
C10F700	25627	Type 2 diabetes mellitus - poor control
C10FL00	26054	Type 2 diabetes mellitus with persistent proteinuria
C10B000	26108	Steroid induced diabetes mellitus without complication
C108400	26855	Unstable insulin dependent diabetes mellitus
C109900	29979	Non-insulin-dependent diabetes mellitus without complication
C10EL00	30294	Type 1 diabetes mellitus with persistent microalbuminuria
C10EK00	30323	Type 1 diabetes mellitus with persistent proteinuria
9OLZ.00	31241	Diabetes monitoring admin.NOS
C108900	31310	Insulin dependent diabetes maturity onset
F372.00	31790	Polyneuropathy in diabetes

List of READ/OXMIS codes for identifying diabetic patients (cont'd)

READ/OXMIS code	Medcode	Description
C11y000	32193	Steroid induced diabetes
ZRbH.00	32359	Perceived control of insulin-dependent diabetes
C107.11	32403	Diabetes mellitus with gangrene
C107.12	32556	Diabetes with gangrene
C10FN00	32627	Type 2 diabetes mellitus with ketoacidosis
C105.00	33254	Diabetes mellitus with ophthalmic manifestation
C10y.00	33343	Diabetes mellitus with other specified manifestation
C107200	33807	Diabetes mellitus, adult with gangrene
C10A100	33969	Malnutrition-related diabetes mellitus with ketoacidosis
C10F200	34268	Type 2 diabetes mellitus with neurological complications
C105z00	34283	Diabetes mellitus NOS with ophthalmic manifestation
C10FK00	34450	Hyperosmolar non-ketotic state in type 2 diabetes mellitus
C109400	34912	Non-insulin dependent diabetes mellitus with ulcer
C104100	35105	Diabetes mellitus, adult onset, with renal manifestation
C104z00	35107	Diabetes mellitus with nephropathy NOS
C10E800	35288	Type 1 diabetes mellitus - poor control
C10FH00	35385	Type 2 diabetes mellitus with neuropathic arthropathy
C107.00	35399	Diabetes mellitus with peripheral circulatory disorder
C109K00	36633	Hyperosmolar non-ketotic state in type 2 diabetes mellitus
C10D.00	36695	Diabetes mellitus autosomal dominant type 2
C109J11	37648	Insulin treated non-insulin dependent diabetes mellitus
C10FF00	37806	Type 2 diabetes mellitus with peripheral angiopathy
C108711	38161	Type I diabetes mellitus with retinopathy
C101y00	38617	Other specified diabetes mellitus with ketoacidosis
C100.00	38986	Diabetes mellitus with no mention of complication
C10EE00	39070	Type 1 diabetes mellitus with hypoglycaemic coma
C106100	39317	Diabetes mellitus, adult onset, + neurological manifestation
C102000	40023	Diabetes mellitus, juvenile type, with hyperosmolar coma
C109500	40401	Non-insulin dependent diabetes mellitus with gangrene
C10E900	40682	Type 1 diabetes mellitus maturity onset
C10EN00	40837	Type 1 diabetes mellitus with ketoacidotic coma
C108712	41049	Type 1 diabetes mellitus with retinopathy
C105100	41389	Diabetes mellitus, adult onset, + ophthalmic manifestation
Cyu2000	41686	[X]Other specified diabetes mellitus
C108C00	41716	Insulin dependent diabetes mellitus with polyneuropathy
C101z00	42505	Diabetes mellitus NOS with ketoacidosis
C103000	42567	Diabetes mellitus, juvenile type, with ketoacidotic coma
C108E11	42729	Type I diabetes mellitus with hypoglycaemic coma
C109612	42762	Type 2 diabetes mellitus with retinopathy
C10E200	42831	Type 1 diabetes mellitus with neurological complications
C102100	43139	Diabetes mellitus, adult onset, with hyperosmolar coma

List of READ/OXMIS codes for identifying diabetic patients (cont'd)

READ/OXMIS code	Medcode	Description
C10F311	43227	Type II diabetes mellitus with multiple complications
C10C.00	43453	Diabetes mellitus autosomal dominant
C109D00	43785	Non-insulin dependent diabetes mellitus with hypoglyca coma
C10M.00	43857	Lipoatrophic diabetes mellitus
C10E400	43921	Unstable type 1 diabetes mellitus
C108F00	44260	Insulin dependent diabetes mellitus with diabetic cataract
C108E00	44440	Insulin dependent diabetes mellitus with hypoglycaemic coma
C108500	44443	Insulin dependent diabetes mellitus with ulcer
C109E12	44779	Type 2 diabetes mellitus with diabetic cataract
C10FE00	44982	Type 2 diabetes mellitus with diabetic cataract
C10E312	45276	Insulin dependent diabetes mellitus with multiple complicat
C109B00	45467	Non-insulin dependent diabetes mellitus with polyneuropathy
C10z.00	45491	Diabetes mellitus with unspecified complication
C109712	45913	Type 2 diabetes mellitus - poor control
C108812	45914	Type 1 diabetes mellitus - poor control
C109212	45919	Type 2 diabetes mellitus with neurological complications
C109512	46150	Type 2 diabetes mellitus with gangrene
C108y00	46290	Other specified diabetes mellitus with multiple comps
C10EC00	46301	Type 1 diabetes mellitus with polyneuropathy
C108811	46850	Type I diabetes mellitus - poor control
C10FD00	46917	Type 2 diabetes mellitus with hypoglycaemic coma
C108000	46963	Insulin-dependent diabetes mellitus with renal complications
C10F711	47315	Type II diabetes mellitus - poor control
C10F100	47321	Type 2 diabetes mellitus with ophthalmic complications
C105y00	47377	Other specified diabetes mellitus with ophthalmic complicatn
C109B11	47409	Type II diabetes mellitus with polyneuropathy
C10E000	47582	Type 1 diabetes mellitus with renal complications
C10E100	47649	Type 1 diabetes mellitus with ophthalmic complications
C10E300	47650	Type 1 diabetes mellitus with multiple complications
C109H11	47816	Type II diabetes mellitus with neuropathic arthropathy
C10F900	47954	Type 2 diabetes mellitus without complication
C109E11	48192	Type II diabetes mellitus with diabetic cataract
C10F400	49074	Type 2 diabetes mellitus with ulcer
C108211	49146	Type I diabetes mellitus with neurological complications
C108100	49276	Insulin-dependent diabetes mellitus with ophthalmic comps
C10EF00	49554	Type 1 diabetes mellitus with diabetic cataract
C10F611	49655	Type II diabetes mellitus with retinopathy
C109G12	49869	Type 2 diabetes mellitus with arthropathy
C10E411	49949	Unstable type I diabetes mellitus

List of READ/OXMIS codes for identifying diabetic patients (cont'd)

READ/OXMIS code	Medcode	Description
C109011	50225	Type II diabetes mellitus with renal complications
C109100	50429	Non-insulin-dependent diabetes mellitus with ophthalm comps
C10FB11	50527	Type II diabetes mellitus with polyneuropathy
L180600	50609	Pre-existing diabetes mellitus, non-insulin-dependent
C109A11	50813	Type II diabetes mellitus with mononeuropathy
L180500	50960	Pre-existing diabetes mellitus, insulin-dependent
C100z00	50972	Diabetes mellitus NOS with no mention of complication
C10E.12	51261	Insulin dependent diabetes mellitus
C10FP00	51756	Type 2 diabetes mellitus with ketoacidotic coma
C108511	51957	Type I diabetes mellitus with ulcer
C108300	52104	Insulin dependent diabetes mellitus with multiple complicatn
Cyu2.00	52212	[X]Diabetes mellitus
C108200	52283	Insulin-dependent diabetes mellitus with neurological comps
C109000	52303	Non-insulin-dependent diabetes mellitus with renal comps
C101000	53200	Diabetes mellitus, juvenile type, with ketoacidosis
C10F911	53392	Type II diabetes mellitus without complication
C10EJ00	54008	Type 1 diabetes mellitus with neuropathic arthropathy
C10E412	54600	Unstable insulin dependent diabetes mellitus
C101100	54856	Diabetes mellitus, adult onset, with ketoacidosis
C109F11	54899	Type II diabetes mellitus with peripheral angiopathy
C109411	55075	Type II diabetes mellitus with ulcer
C10EQ00	55239	Type 1 diabetes mellitus with gastroparesis
L180X00	55431	Pre-existing diabetes mellitus, unspecified
C109200	55842	Non-insulin-dependent diabetes mellitus with neuro comps
C109D11	56268	Type II diabetes mellitus with hypoglycaemic coma
C108A00	56448	Insulin-dependent diabetes without complication
C10F011	57278	Type II diabetes mellitus with renal complications
C108D00	57621	Insulin dependent diabetes mellitus with nephropathy
C109611	58604	Type II diabetes mellitus with retinopathy
C10FG00	59253	Type 2 diabetes mellitus with arthropathy
C103y00	59288	Other specified diabetes mellitus with coma
C109C00	59365	Non-insulin dependent diabetes mellitus with nephropathy
C109111	59725	Type II diabetes mellitus with ophthalmic complications
C135.12	60046	Diabetes insipidus – pituitary
C108411	60107	Unstable type I diabetes mellitus
C108J11	60208	Type I diabetes mellitus with neuropathic arthropathy
C108600	60499	Insulin dependent diabetes mellitus with gangrene
C109F12	60699	Type 2 diabetes mellitus with peripheral angiopathy
C10FL11	60796	Type II diabetes mellitus with persistent proteinuria

List of READ/OXMIS codes for identifying diabetic patients (cont'd)

READ/OXMIS code	Medcode	Description
C109D12	61071	Type 2 diabetes mellitus with hypoglycaemic coma
C10H.00	61122	Diabetes mellitus induced by non-steroid drugs
C108011	61344	Type I diabetes mellitus with renal complications
C106y00	61523	Other specified diabetes mellitus with neurological comps
C108212	61829	Type 1 diabetes mellitus with neurological complications
C109511	62107	Type II diabetes mellitus with gangrene
C109300	62146	Non-insulin-dependent diabetes mellitus with multiple comps
C10EM11	62209	Type I diabetes mellitus with ketoacidosis
C108H11	62352	Type I diabetes mellitus with arthropathy
C10EA11	62613	Type I diabetes mellitus without complication
C10FA00	62674	Type 2 diabetes mellitus with mononeuropathy
C108911	63017	Type I diabetes mellitus maturity onset
C107100	63357	Diabetes mellitus, adult, + peripheral circulatory disorder
C10y100	63371	Diabetes mellitus, adult, + other specified manifestation
8CR2.00	63412	Diabetes clinical management plan
C10FR00	63690	Type 2 diabetes mellitus with gastroparesis
C10z100	63762	Diabetes mellitus, adult onset, + unspecified complication
C10zy00	64283	Other specified diabetes mellitus with unspecified comps
C10zz00	64357	Diabetes mellitus NOS with unspecified complication
C108z00	64449	Unspecified diabetes mellitus with multiple complications
C109C11	64571	Type II diabetes mellitus with nephropathy
C10FJ11	64668	Insulin treated Type II diabetes mellitus
C107z00	65025	Diabetes mellitus NOS with peripheral circulatory disorder
C103z00	65062	Diabetes mellitus NOS with ketoacidotic coma
C10F300	65267	Type 2 diabetes mellitus with multiple complications

APPENDIX D

Hypertension and/or heart failure medication listing according to the British National Formulary (BNF) chapters

BNF chapter: ACE inhibitors
Thiazides and related diuretics/ACE inhibitors

Product Name[1]
CAPOZIDE tablets [SQUIBB]
COVERSYL tablets 4mg [SERVIER]
INNOZIDE tablets [M S D]
TRITACE tablets 2.5mg [AVENTIS]
ZESTORETIC 10-tablets 10mg + 12.5mg [ASTRAZENEC]
ZESTORETIC 20-tablets 20mg + 12.5mg [ASTRAZENEC]
ZESTRIL tablets 2.5mg [ASTRAZENEC]
ZESTRIL tablets 20mg [ASTRAZENEC]
ZESTRIL tablets 5mg [ASTRAZENEC]
captopril tablets 12.5mg
captopril tablets 25mg
captopril tablets 50mg
cilazapril tablets 1mg
cilazapril tablets 2.5mg
enalapril tablets 10mg
enalapril tablets 2.5mg
enalapril tablets 20mg
enalapril tablets 5mg
fosinopril tablets 10mg
fosinopril tablets 20mg
lisinopril tablets 10mg
lisinopril tablets 2.5mg
lisinopril tablets 20mg
lisinopril tablets 5mg
lisinopril with hydrochlorothiazide tablets 10mg + 12.5mg
lisinopril with hydrochlorothiazide tablets 20mg + 12.5mg
perindopril arginine tablets 2.5mg
perindopril arginine tablets 5mg
perindopril erbumine tablets 2mg
perindopril erbumine tablets 4mg
perindopril erbumine tablets 8mg
perindopril erbumine with indapamide tablets 4mg + 1.25mg
quinapril tablets 10mg
quinapril tablets 20mg
quinapril tablets 40mg
quinapril tablets 5mg
ramipril capsules 1.25mg
ramipril capsules 10mg
ramipril capsules 2.5mg
ramipril capsules 2.5mg + 5mg + 10mg
ramipril capsules 5mg
ramipril tablets 1.25mg
ramipril tablets 10mg
ramipril tablets 2.5mg
ramipril tablets 2.5mg + 5mg + 10mg
ramipril tablets 5mg
ramipril with felodipine modified release tablet 5mg + 5mg
trandolapril capsules 1mg
trandolapril capsules 2mg
trandolapril capsules 4mg
trandolapril capsules 500 micrograms

[1]Product Name as entered in CPRD by the general practice

BNF chapter: Loop diuretics/Potassium-sparing diuretics and aldosterone aantagonists/Potassium-sparing diuretics with other diuretics;

Thiazides and related diuretics/Potassium-sparing diuretics and aldosterone antagonists/Potassium-sparing diuretics with other diuretics

Product Name[1]
ALDACTIDE film coated tablets 50mg + 50mg [PHARMACIA]
DYAZIDE tablets [GOLDSHIELD]
FRU-CO tablets 5mg + 40mg [IVAX]
FRUMIL LS tablets 20mg + 2.5mg [HELIOS]
FRUMIL LS tablets 20mg + 2.5mg [SANOFI/AVE]
FRUMIL tablets 40mg + 5mg [SANOFI/AVE]
MODURET 25 tablets [M S D]
MODURETIC tablets [BMS]
amiloride hydrochloride with bumetanide tablets 5mg + 1mg
co-amilofruse (amiloride and furosemide) tablets 10mg+80mg
co-amilofruse (amiloride and furosemide) tablets 2.5mg+20mg
co-amilofruse (amiloride and furosemide) tablets 5mg+40mg
co-amilozide (amiloride and hydrochlorothiazide) tablets 2.5mg+25mg
co-amilozide (amiloride and hydrochlorothiazide) tablets 5mg+50mg
co-flumactone (hydroflumethiazide and spironolactone) tablets 50mg+50mg
co-triamterzide (triamterene and hydrochlorothiazide) tablets 50mg+25mg
furosemide with amiloride tablets 20mg + 2.5mg
furosemide with amiloride tablets 40mg + 5mg
furosemide with triamterene tablets 40mg + 50mg
triamterene with furosemide tablets 50mg + 40mg

[1]Product Name as entered in CPRD by the general practice

BNF chapter: Thiazides and related diuretics

Thiazides and related diuretics/Posterior pituitary hormones and antagonists

Thiazides and related diuretics/Potassium-sparing diuretics and aldosterone antagonists/Potassium-sparing diuretics with other diuretics

Product Name[1]
BENDROFLUMETHIAZIDE tablets 5mg [HILLCROSS]
NATRILIX SR sustained release tablets 1.5mg [SERVIER]
NAVISPARE tablets [GOLDSHIELD]
bendroflumethiazide tablets 2.5mg
bendroflumethiazide tablets 5mg
chlortalidone tablets 50mg
cyclopenthiazide with amiloride tablets 250micrograms + 2.5mg
indapamide modified release tablet 1.5mg
indapamide tablets 2.5mg
metolazone tablets 5mg

[1]Product Name as entered in CPRD by the general practice

BNF chapter: **Beta-blockers**
Beta-blockers/Calcium channel blockers
Beta-blockers/Migraine prophylaxis
Beta-blockers/Migraine prophylaxis/Essential tremor, chorea, tics and related disorders/Antithyroid drugs
Supraventricular & ventricular arrhythmias/Beta-blockers
Supraventricular arrhythmias/Beta-blockers/Migraine prophylaxis/Essential tremor, chorea,tics and related disorders/Antithyroid drugs
Thiazides and related diuretics/Beta-blockers

Product Name[1]

BETA-ADALAT capsules 50mg + 20mg [BAYER]
BETALOC tablets 50mg [ASTRAZENEC]
CARDICOR tablets 2.5mg [MERCK SER]
CONGESCOR tablets 2.5mg [TILLOMED]
HALF-INDERAL LA capsules 80mg [ASTRAZENEC]
INDERAL LA capsules 160mg [ASTRAZENEC]
METOPROLOL tablets 50mg [HILLCROSS]
NEBILET tablets 5mg [MENARINI]
SLOW TRASICOR sustained release tablets 160mg [AMDIPHARM]
TENORETIC tablets 100mg + 25mg [ASTRAZENEC]
TENORMIN 25 tablets [ASTRAZENEC]
TENORMIN LS tablets 50mg [ASTRAZENEC]
TENORMIN tablets 100mg [ASTRAZENEC]
atenolol sugar free oral solution 25mg/5ml
atenolol tablets 100mg
atenolol tablets 25mg
atenolol tablets 50mg
atenolol with chlortalidone tablets 100mg+25mg
atenolol with chlortalidone tablets 50mg+12.5mg
bisoprolol tablets 1.25mg
bisoprolol tablets 10mg
bisoprolol tablets 2.5mg
bisoprolol tablets 3.75mg
bisoprolol tablets 5mg
bisoprolol tablets 7.5mg
carvedilol tablets 12.5mg
carvedilol tablets 25mg
carvedilol tablets 3.125mg
carvedilol tablets 6.25mg
celiprolol tablets 200mg
celiprolol tablets 400mg
co-tenidone (atenolol and chlortalidone) tablets 100mg+25mg
co-tenidone (atenolol and chlortalidone) tablets 50mg+12.5mg
labetalol hydrochloride tablets 100mg
labetalol hydrochloride tablets 400mg
metoprolol tartrate modified release tablet 200mg
metoprolol tartrate suspension 50mg/5ml
metoprolol tartrate tablets 100mg
metoprolol tartrate tablets 50mg
nebivolol tablets 2.5mg
nebivolol tablets 5mg
oxprenolol modified release tablet 160mg
oxprenolol tablets 80mg
propranolol modified release capsules 160mg
propranolol modified release capsules 80mg

Product Name[1]
propranolol tablets 10mg
propranolol tablets 160mg
propranolol tablets 40mg
propranolol tablets 80mg
sotalol tablets 160mg
sotalol tablets 40mg
sotalol tablets 80mg
timolol maleate tablets 10mg

[1]Product Name as entered in CPRD by the general practice

BNF chapter: Centrally-acting antihypertensives/Clonidine

Product Name[1]
clonidine tablets 25micrograms

[1]Product Name as entered in CPRD by the general practice

BNF chapter: Calcium channel blockers
Supraventricular arrhythmias/Calcium channel blockers

Product Name[1]

ADALAT LA 20 prolonged release tablet [BAYER]
ADALAT LA 30 prolonged release tablet [BAYER]
ADALAT LA 60 prolonged release tablet [BAYER]
ADALAT LA tablets 30mg [BAYER]
ADALAT LA tablets 60mg [BAYER]
ADALAT RETARD tablets 10mg [BAYER]
ADALAT RETARD tablets 20mg [BAYER]
ADIPINE MR 10 modified release tablet 10mg [CHIESI]
ADIPINE MR 20 modified release tablet 20mg [CHIESI]
ADIPINE XL tablets 30mg [CHIESI]
ADIZEM SR capsules 120mg [NAPPPHARM]
ADIZEM SR capsules 180mg [NAPPPHARM]
ADIZEM SR capsules 90mg [NAPPPHARM]
ADIZEM SR tablets 120mg [NAPPPHARM]
ADIZEM XL capsules 120mg [NAPPPHARM]
ADIZEM XL capsules 180mg [NAPPPHARM]
ADIZEM XL capsules 200mg [NAPPPHARM]
ADIZEM XL capsules 240mg [NAPPPHARM]
ADIZEM XL capsules 300mg [NAPPPHARM]
ANGITIL SR capsules 120mg [CHIESI]
ANGITIL SR capsules 180mg [CHIESI]
ANGITIL SR capsules 90mg [CHIESI]
ANGITIL XL capsules 240mg [CHIESI]
ANGITIL XL capsules 300mg [CHIESI]
CALCICARD CR controlled release tablet 90mg [IVAX]
CARDIOPLEN XL tablets 10mg [CHIESI]
CARDIOPLEN XL tablets 2.5mg [CHIESI]
CARDIOPLEN XL tablets 5mg [CHIESI]
CORACTEN SR modified release capsules 10mg [UCB]
CORACTEN SR modified release capsules 20mg [UCB]
CORACTEN XL modified release capsules 30mg [UCB]
CORACTEN XL modified release capsules 60mg [UCB]
DILCARDIA SR modified release capsules 60mg [GEN (UK)]
DILZEM SR capsules 60mg [CEPHALON]
DILZEM SR capsules 90mg [CEPHALON]
DILZEM XL capsules 120mg [CEPHALON]
DILZEM XL capsules 180mg [CEPHALON]
DILZEM XL capsules 240mg [CEPHALON]
FELOTENS XL tablets 10mg [GENUS]
ISTIN tablets 10mg [PFIZER]
ISTIN tablets 5mg [PFIZER]
NEOFEL XL tablets 2.5mg [NEOLAB]
PLENDIL extended release tablets 10mg [ASTRAZENEC]
PLENDIL extended release tablets 5mg [ASTRAZENEC]
SECURON SR tablets 240mg [ABBOTT]
SECURON tablets 120mg [ABBOTT]
SLOZEM capsules 120mg [MERCK SER]
SLOZEM capsules 180mg [MERCK SER]
SLOZEM capsules 240mg [MERCK SER]
SLOZEM capsules 300mg [MERCK SER]
TILDIEM LA capsules 200mg [SANOFI/AVE]
TILDIEM LA capsules 300mg [SANOFI/AVE]
TILDIEM RETARD tablets 120mg [SANOFI/AVE]
TILDIEM RETARD tablets 90mg [SANOFI/AVE]
TILDIEM modified release tablet 60mg [SANOFI/AVE]
UNIVER sustained release capsules 180mg [CEPHALON]
VERTAB SR tablets 240mg [CHIESI]
VIAZEM XL capsules 120mg [GENUS]
VIAZEM XL capsules 180mg [GENUS]

Product Name[1]
VIAZEM XL capsules 240mg [GENUS]
VIAZEM XL capsules 300mg [GENUS]
VIAZEM XL capsules 360mg [GENUS]
ZANIDIP tablets 10mg [RECORDATI]
ZEMTARD XL capsules 300mg [GALEN]
amlodipine (as besilate) tablets 10mg
amlodipine (as besilate) tablets 5mg
amlodipine (as maleate) tablets 10mg
amlodipine (as maleate) tablets 5mg
amlodipine tablets 10mg
amlodipine tablets 5mg
diltiazem 12 hour modified release capsules 120mg
diltiazem 12 hour modified release capsules 180mg
diltiazem 12 hour modified release capsules 60mg
diltiazem 12 hour modified release capsules 90mg
diltiazem 12 hour modified release tablets 120mg
diltiazem 12 hour modified release tablets 90mg
diltiazem 24 hour modified release capsules 120mg
diltiazem 24 hour modified release capsules 180mg
diltiazem 24 hour modified release capsules 200mg
diltiazem 24 hour modified release capsules 240mg
diltiazem 24 hour modified release capsules 300mg
diltiazem 24 hour modified release capsules 360mg
diltiazem 8 hour modified release tablets 60mg
diltiazem tablets 60mg
felodipine modified release tablet 10mg
felodipine modified release tablet 2.5mg
felodipine modified release tablet 5mg
lacidipine tablets 2mg
lacidipine tablets 4mg
lercanidipine hydrochloride tablets 10mg
lercanidipine hydrochloride tablets 20mg
nicardipine hydrochloride capsules 20mg
nicardipine hydrochloride modified release capsules 45mg
nifedipine 12 hour modified release capsules 10mg
nifedipine 12 hour modified release capsules 20mg
nifedipine 12 hour modified release tablets 10mg
nifedipine 12 hour modified release tablets 20mg
nifedipine 24 hour modified release capsules 30mg
nifedipine 24 hour modified release capsules 60mg
nifedipine 24 hour modified release tablets 20mg
nifedipine 24 hour modified release tablets 30mg
nifedipine 24 hour modified release tablets 60mg
nifedipine capsules 10mg
nifedipine capsules 5mg
verapamil hydrochloride modified release capsules 120mg
verapamil hydrochloride modified release capsules 180mg
verapamil hydrochloride modified release capsules 240mg
verapamil hydrochloride modified release tablet 240mg
verapamil hydrochloride tablets 120mg

[1]Product Name as entered in CPRD by the general practice

BNF chapter: Angiotensin-II antagonists
Thiazides and related diuretics/Angiotensin-II antagonists

Product Name[1]

AMIAS tablets 16mg [TAKEDA]
AMIAS tablets 2mg [TAKEDA]
APROVEL tablets 150mg [SANOFI/AVE]
APROVEL tablets 300mg [SANOFI/AVE]
COAPROVEL tablets 300mg + 12.5mg [SANOFI BMS]
COZAAR tablets 100mg [M S D]
COZAAR tablets 25mg [M S D]
COZAAR tablets 50mg [M S D]
DIOVAN capsules 160mg [NOVARTIS]
DIOVAN capsules 80mg [NOVARTIS]
MICARDIS tablets 40mg [BOEH INGL]
OLMETEC tablets 10mg [DAIIC-SANK]
OLMETEC tablets 20mg [DAIIC-SANK]
candesartan tablets 16mg
candesartan tablets 2mg
candesartan tablets 32mg
candesartan tablets 4mg
candesartan tablets 8mg
eprosartan tablets 300mg
eprosartan tablets 600mg
irbesartan tablets 150mg
irbesartan tablets 300mg
irbesartan tablets 75mg
irbesartan with hydrochlorothiazide tablets 150mg + 12.5mg
irbesartan with hydrochlorothiazide tablets 300mg + 12.5mg
losartan tablets 100mg
losartan tablets 12.5mg
losartan tablets 25mg
losartan tablets 50mg
losartan with hydrochlorothiazide tablets 100mg + 25mg
losartan with hydrochlorothiazide tablets 50mg + 12.5mg
olmesartan medoxomil tablets 10mg
olmesartan medoxomil tablets 20mg
olmesartan medoxomil tablets 40mg
telmisartan tablets 20mg
telmisartan tablets 40mg
telmisartan tablets 80mg
telmisartan with hydrochlorothiazide tablets 40mg + 12.5mg
telmisartan with hydrochlorothiazide tablets 80mg + 12.5mg
valsartan capsules 160mg
valsartan capsules 40mg
valsartan capsules 80mg
valsartan tablets 320mg
valsartan with hydrochlorothiazide tablets 160mg + 12.5mg

[1]Product Name as entered in CPRD by the general practice

BNF chapter: Loop Diuretics

Product Name[1]
BURINEX tablets 1mg [LEO]
FUROSEMIDE tablets 40mg [CP PHARM]
FUROSEMIDE tablets 40mg [HILLCROSS]
FUROSEMIDE tablets 40mg [TEVA]
LASIX tablets 40mg [BORG]
TOREM tablets 10mg [MEDA]
bumetanide sugar free liquid 1mg/5ml
bumetanide tablets 1mg
bumetanide tablets 5mg
furosemide injection 20mg/2ml
furosemide injection 50mg/5ml
furosemide sugar free oral solution 40mg/5ml
furosemide tablets 20mg
furosemide tablets 40mg
torasemide tablets 10mg
torasemide tablets 5mg

[1]Product Name as entered in CPRD by the general practice

BNF chapter: Loop diuretics/Potassium-sparing diuretics and aldosterone antagonists/Potassium-sparing diuretics with other diuretics Potassium-sparing diuretics and aldosterone antagonists

Product Name[1]
ALDACTONE film coated tablets 25mg [PHARMACIA]
ALDACTONE film coated tablets 50mg [PHARMACIA]
amiloride hydrochloride tablets 5mg
co-amilofruse (amiloride and furosemide) tablets 2.5mg+20mg
co-amilofruse (amiloride and furosemide) tablets 5mg+40mg
eplerenone tablets 25mg
eplerenone tablets 50mg
spironolactone oral suspension 25mg/5ml
spironolactone sugar-free suspension 10mg/5ml
spironolactone sugar-free suspension 25mg/5ml
spironolactone tablets 100mg
spironolactone tablets 25mg
spironolactone tablets 50mg

[1]Product Name as entered in CPRD by the general practice

BNF chapter: Supraventricular arrhythmias/Calcium channel blockers

Product Name[1]
HALF SECURON SR tablets 120mg [ABBOTT]
SECURON SR tablets 240mg [ABBOTT]
SECURON tablets 120mg [ABBOTT]
UNIVER sustained release capsules 240mg [CEPHALON]
VERAPRESS modified release tablet 240mg [DEXCEL]
VERTAB SR tablets 240mg [CHIESI]
verapamil hydrochloride modified release capsules 120mg
verapamil hydrochloride modified release capsules 240mg
verapamil hydrochloride modified release tablet 120mg
verapamil hydrochloride modified release tablet 240mg
verapamil hydrochloride tablets 120mg
verapamil hydrochloride tablets 160mg
verapamil hydrochloride tablets 40mg
verapamil hydrochloride tablets 80mg

[1]Product Name as entered in CPRD by the general practice

BNF chapter: Centrally-acting antihypertensives

Product Name[1]
ALDOMET tablets 250mg [SIGMA]
PHYSIOTENS tablets 200micrograms [ABBOTT]
clonidine tablets 100micrograms
clonidine tablets 300micrograms
methyldopa tablets 125mg
methyldopa tablets 250mg
methyldopa tablets 500mg
moxonidine tablets 200micrograms
moxonidine tablets 300micrograms
moxonidine tablets 400micrograms

[1]Product Name as entered in CPRD by the general practice

BNF chapter: Antihypertensive vasodilators

Product Name[1]
hydralazine tablets 25mg
hydralazine tablets 50mg
minoxidil tablets 2.5mg

[1]Product Name as entered in CPRD by the general practice

BNF chapter: Centrally-acting antihypertensives/Clonidine

Product Name[1]
clonidine tablets 25micrograms

[1]Product Name as entered in CPRD by the general practice

APPENDIX E

Additional statistical model fitting details about the analyses presented in Chapter 4

Table 4.9 – Model fitting details

- The unadjusted all-cause mortality survival model for $\log_e$BNP indicated a proper fit. The Breslow method was used for handling ties. The maximum likelihood algorithm converged. The model fit statistics, as well as the global null hypothesis tests showed that having $\log_e$-BNP in the model was better than no variables in the model. With $\log_e$BNP in the Cox regression model, the negative two times log likelihood was 5247.056, while the Akaike Information and Schwartz criteria were 5251.056 and 5259.094, respectively. The likelihood ratio test was statistically significant (chi-square test statistic=59.8889, degrees of freedom=2, p-value < 0.0001). The proportional hazards assumption was met. The interaction between $\log_e$BNP and time was not statistically significant (chi-square test statistics=0.7393, degrees of freedom=1, p-value=0.3899). The plot of cumulative martingale residuals against $\log_e$BNP showed reasonable linearity of the continuous covariate. The maximum absolute value of a Kolmogorov-type supremum test for $\log_e$BNP was 18.5227 based on a sample of 1000 simulated residual patterns (seed=607134001, p-value=0.07). The deviance residuals were symmetrically distributed about zero.
- Regarding the unadjusted Cox regression model for $\log_e$NT-proBNP, the model fit statistics for logNT-proBNP as well as the global null hypothesis tests showed that having $\log_e$-NT-proBNP in the model was better than no variables in the model. With $\log_e$NT-proBNP in the Cox regression model, the negative two times log likelihood was 12265.197, while the Akaike Information and Schwartz criteria were 122267.197 and

12271.939, respectively. The likelihood ratio test was statistically significant (chi-square test statistic=169.8114, degrees of freedom=1, p-value < 0.0001). Although the maximum likelihood model converged, the proportional hazards and linearity assumptions were not met. The maximum absolute value of the supremum test for testing the proportional hazard assumption for $\log_e$NT-proBNP was 2.9070 based on 1000 replications and the seed of 19, while the associated p-value was less than 0.0001. The maximum absolute value of the supremum test for the functional form of $\log_e$NT-proBNP was 39.9563 based on 1000 replications and a seed of 176575001, while the corresponding p-value was 0.0020. Alternative seed values were tried; however, the conclusion remained the same.

Table 4.9 – Model fitting details for the adjusted analyses

- LogBNP (adjusted model) - The maximum likelihood algorithm converged. The model fist statistics showed better fit when additional covariates were included in the survival model. For example, the negative two times likelihood, Akaike information and Schwartz criteria were: 4376.987, 4410.987 and 4476.956, respectively when all covariates were included in the model. The likelihood ratio for testing the global null hypothesis was statistically significant (chi-square test statistic=148.1475, degrees of freedom=17, p-value<0.0001). The adjusted survival analysis model for log-BNP also presented some problems. Adding time-dependent interactions with each of covariates resulted in non-estimable parameters. Therefore, Kolmogorov- type supremum tests were performed to assess the proportional hazards assumption. The results indicated that the proportional hazard assumption was satisfied for $\log_e$-BNP, gender, body mass

index, creatinine, smoking, exposure to hypertension and/or heart failure medication and the following regions: East Midlands, North East, North West, Northern Ireland, South Central, South West and Wales. The proportional hazards assumption was violated for age, diabetes status at the baseline, as well as the following regions: East of England, London and South East Coast. The supremum tests for functional forms indicated adequate linearity only for $\log_e$BNP, age, body mass index, and creatinine. Evidence of problems with linearity was observed for gender, smoking, exposure to hypertension and/or heart failure medication, and diabetes status at the baseline. Sensitivity analyses were performed by including only significant covariates in the final adjusted model for $\log_e$BNP while removing the highest 5% of the observation as described in the Section 4.3.4., Sensitivity Analyses.

- LogNT-proBNP (adjusted analyses): The maximum likelihood algorithm converged, while the model fit statistics and testing the global null hypothesis showed better fit when additional covariates were included in the survival model. For example, the negative two times likelihood, Akaike information and Schwartz criteria were: 9833.829, 9873.829 and 9964.879, respectively when all covariates were included in the model. The likelihood ratio for testing the global null hypothesis was statistically significant (chi-square test statistic=250.285, degrees of freedom=20, p-value<0.0001). The adjusted survival analysis model for $\log_e$NT-proBNP revealed some problems. The Kolmogorov type supremum tests indicated that the proportional hazard assumption was satisfied for age, body mass index, creatinine, smoking, exposure to hypertension and/or heart failure medication and for the following regions: East Midlands, East of England, London, North West, Northern Ireland, Scotland, South Central, South East

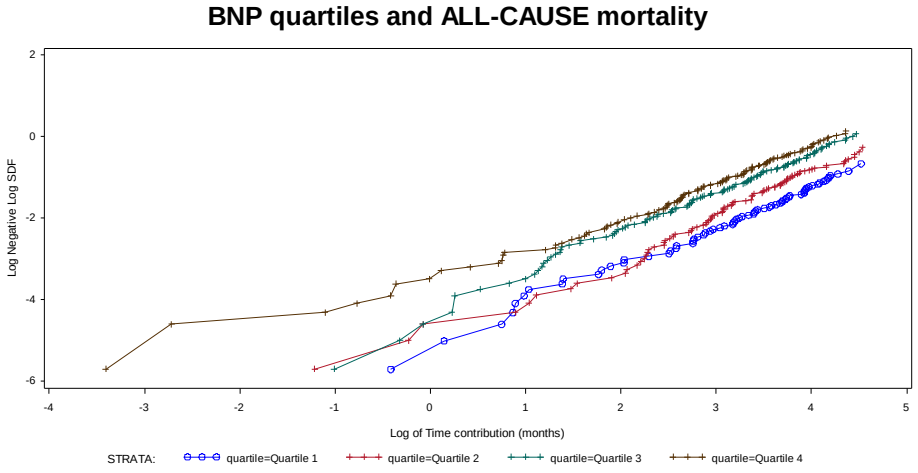
Coast, South West, Wales, and violated for $\log_e$NT-proBNP, gender, North East, and West Midlands regions. Both the visual inspection of the cumulative martingale residuals, as well as the supremum tests for functional forms indicated adequate linearity only for the body mass index among all covariates. The deviance residuals were not consistently symmetric about zero, while the standard deviation was often greater than 1. Sensitivity analyses were performed by including only significant covariates in the final adjusted model for $\log_e$NT-proBNP while removing the highest 5% NT-proBNP levels and they are described in the Section 4.3.4., Sensitivity Analyses.

Table 4.10 – Model fitting details

- BNP categorical quartiles (unadjusted) - The unadjusted all-cause mortality Cox regression model for BNP quartiles indicated a proper statistical fit. The maximum likelihood algorithm converged. The model fit statistics, as well as the global null hypothesis tests showed that having BNP quartiles in the model was better than no variables in the model. With BNP quartiles in the Cox regression model, the negative two times log likelihood was 5245.794, while the Akaike Information and Schwartz criteria were 5251.794 and 5263.849, respectively. The likelihood ratio test was statistically significant (chi-square test statistic=6.1518, degrees of freedom=3, p-value < 0.0001). For all BNP quartiles, the proportional hazards assumption was met based on the supremum test. Considering 1000 replications and a seed of 29, the maximum absolute values of the supremum test for proportional hazards assumption for the second, third and fourth quartiles were: 1.3480 (p-value=0.2290), 0.7166 (p-

value=0.918) and 1.034 (p-value=0.5820), respectively. The proportional hazards assumption is also visualized in the figure below, which shows the log negative log of the survival distribution function by the log-e transformed time. The deviance residuals were symmetrically distributed about zero.

The proportional hazard assumption for BNP quartiles from the unadjusted Cox regression model



- BNP quartiles (adjusted) - The adjusted Cox regression model for BNP quartiles revealed several issues. While the proportional hazards assumption was met for quartiles, gender, body mass index, smoking, serum creatinine, hypertension and/or heart failure medication, and the following geographical regions: East Midlands, North East, North West, Northern Ireland, South Central, South West, it was not met for age, diabetes status at the baseline, as well as the following geographical regions: East of Midland, London, South East Coast, and Wales. While the linearity was acceptable most of the time, the maximum absolute value of the supremum test for the functional form of the body mass index was 19.0741 with the corresponding p-value of 0.021,

based on 1000 replications and a seed of 463319001.

- NT-proBNP quartiles (unadjusted) - The maximum likelihood algorithm converged. The model fit statistics as well as the global null hypothesis tests showed that having NT-proBNP quartiles in the Cox regression model was better than no variables in the model. The proportional hazards assumption was not met neither in the unadjusted nor adjusted, all-cause mortality Cox regression model for the NT-proBNP quartiles. Considering 1000 replications and a seed of 33 in the unadjusted Cox regression model for the NT-proBNP quartiles, the maximum absolute values of the supremum test for proportional hazards assumption for the second, third and fourth NT-proBNP quartiles were: 2.4697 (p-value=0.0010), 1.1972 (p-value=0.3770) and 3.7940 (p-value <0.0001), respectively. The violation of the proportional hazards assumption is also visualized in the figure below, which shows the log negative log of the survival distribution function by the log-e transformed time.

The proportional hazard assumption for NT-proBNP quartiles from the unadjusted Cox regression model

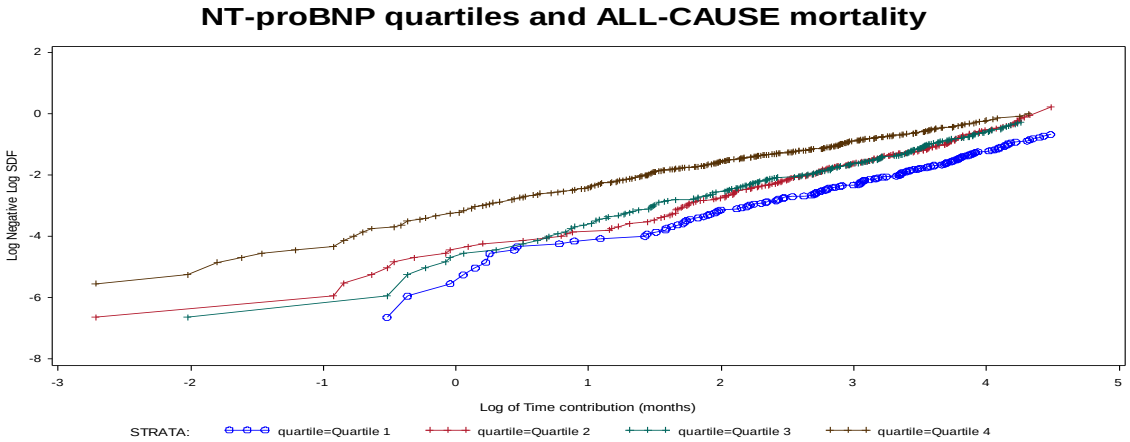


Table 4.11 – Model fitting details

- The unadjusted statistical model fit for BNP continuous quartiles was good. The proportional hazards and linearity assumptions were met. The maximum absolute value for the supremum test for proportional hazards assumption was 0.7798 for the quartiles with the corresponding p-value of 0.4630 based on 1000 simulations. The supremum test for quartile's functional form had a maximum absolute value of 6.631and a p-value of 0.3910 based on 1000 replications and a seed of 933,883,000.
- BNP continuous quartile adjusted model: The maximum likelihood algorithm converged. The model fit statistics, as well as the global null hypothesis tests showed that having the baseline characteristics in the model was better than no variables in the model. Some problems were still present. For example, the proportional hazards assumption was met for all the variables except for age, diabetes status at baseline and the following geographic regions: East of England, London, South East Coast and Wales. All continuous variables had an adequate functional form except for the body mass index.
- NT-proBNP continuous quartile unadjusted model: As with previous statistical models for NT-proBNP, the proportional hazard and linearity assumptions were not met. The supremum test of proportional hazards assumption led to a maximum absolute value of 2.3873 based on 1000 replications and a seed of 33, which was statistically significant ($p<0.0001$). The supremum test for the functional form was also statistically significant ($p<0.0001$) and had a maximum absolute value of 25.7817 based on 1000 replications and a seed of 651,958,001.
- NT-proBNP continuous quartile adjusted model: While the proportional hazards

assumption was met for age, body mass index, smoking status, geographical region, heart failure and/or hypertension medication exposure, diabetes status, it was violated for quartile, gender, and serum creatinine. The linearity assumption was not met for quartile, age, and creatinine. Plots of martingale and deviance residuals against the linear predictors were not symmetric around zero.

- Combined continuous quartiles adjusted model: The model fitting continued to pose some critical issues. For example, the proportional hazard assumption was met for most of the variables except for the combined quartiles, age, gender, serum creatinine and the following regions: East of England, North East, and South East Coast.

T**able 4.12 – model fitting details**

- Model fitting diagnostics indicated a proper fit for the BNP unadjusted analyses; however, the violation of the proportional hazards assumption for NT-proBNP continued to persist. The maximum absolute value of the supremum test for the proportional hazards assumption for loge-NT-proBNP was 2.7634 based on 1000 simulations and a seed of 24, yielding to a significant p-value < 0.0001.

Table 4.13 – model fitting details

- Evaluating the fit of the adjusted Cox regression model for logBNP revealed the correct, functional linear form of the continuous variables. While the proportional hazards assumption was met for logBNP, gender, body mass index, creatinine, smoking status, exposure to heart failure and/or hypertension medication, and most geographic regions, it was violated for the diabetes status at baseline as well as the following geographic regions: East of England, Northern Ireland, South West, and Wales. This was based on

both the examination of the cumulative score residuals against time, as well as the supremum test for the proportional hazards assumption. The martingale and deviance residuals were not symmetrically distributed around zero.

Table 4.14 – model fitting details

- Model fit diagnostics showed that the correct functional form of the continuous variables was used. The proportional hazards assumption was met for age, body mass index, smoking, creatinine, diabetes status at the baseline, and the following regions: East Midlands, East of England, London, South Central, South East Coast, South West and West Midlands. However, the supremum test for proportional hazards as well as the cumulative score residuals against time revealed that this assumption was not met for $\log_e$NT-proBNP, gender, exposure to heart failure and/or hypertension medication, and the following regions: North East, North West, Northern Ireland, Scotland and Wales. The martingale and deviance residuals were not symmetrically distributed around zero.

Table 4.16 – model fitting details

- Model diagnostics for the BNP unadjusted, continuous quartile indicated a proper statistical fit. The proportional hazards assumption and linearity were met. The maximum absolute value for the proportional hazard assumption was 0.874 with the corresponding p-value of 0.316 based on 1000 simulations and a seed of 21. The maximum absolute value of the functional form of quartiles was 3.6522 with the associated p-value of 0.603 based on 1000 simulations and the seed of 584688000. The martingale and deviance residuals were symmetrically distributed around zero.
- BNP continuous quartile, adjusted Cox regression - The diagnostic modeling showed

mixed results. The maximum likelihood algorithm converged, the model fit statistics and well as the global null hypothesis tests indicated that having the baseline characteristics in the model was better than no variables in the model. The linearity assumption was met for all continuous variables such as age, body mass index, serum creatinine, and continuous BNP quartiles. Although the proportional hazard assumption was met for the BNP continuous quartiles, gender, body mass index, smoking, serum creatinine, exposure to the hypertension and heart failure medication, and the following geographic regions: East Midlands, London, North East, North West, South Central, and South East Coast, it was violated for East of England, Northern Ireland, South West, Wales as well as the diabetes status.

- The proportional hazards assumption was not met for the NT-proBNP quartiles either in the unadjusted nor adjusted Cox regression models. Furthermore, in the unadjusted model, the maximum absolute value of the supremum test for the proportional hazard assumption for NT-proBNP quartiles was 2.2413 (p-value < 0.0001) based on 1000 simulation and a seed of 21. In the adjusted model, the maximum absolute value of the supremum test was 1.9566 (p-value=0.004).
- Evaluating the fit of the adjusted Cox regression model for the combined B-type natriuretic peptides revealed that the proportional hazards assumption was met for the second and third combined quartiles, age, body mass index, creatinine, smoking and diabetes status, exposure to heart failure and/or hypertension medication, and most geographic regions such as: East Midlands, London, South Central, South East Coast, South West, and West Midlands. However, it was violated for the fourth combined quartile, male gender, serum creatinine, and the following geographic regions: East of

England, North East, North West, Northern Ireland, Scotland, and Wales. This was based on both the examination of the cumulative score residuals against time, as well as the supremum test for the proportional hazards assumption. The martingale and deviance residuals were not symmetrically distributed around zero.

Model 4.17 – model fitting details

- BNP continuous quartiles: The unadjusted statistical model fit was good. The proportional hazards and linearity assumptions were met. The maximum absolute value for the supremum test for proportional hazards assumption was 0.874 for the BNP quartiles with the corresponding p-value of 0.316 based on 1000 simulations and a seed of 21. The supremum test for the BNP quartile's functional form had a maximum absolute value of 3.652 and a p-value of 0.603 based on 1000 replications and a seed of 584,688,000. The martingale and deviance residuals were symmetrically distributed around zero.
- BNP continuous quartiles (adjusted model) - Although the maximum likelihood algorithm converged and the model fit statistics, as well as the global null hypothesis tests showed that having the baseline characteristics of the model was better than no variables in the model, some problems were still present. For example, the proportional hazards assumption was met for all the variables except for age, diabetes status at baseline and the following geographic regions: East of England, Northern Ireland, South West and Wales. All continuous variables had an adequate functional form.
- NT-proBNP continuous quartiles (unadjusted) - As with previous statistical models for NT-proBNP, the linearity and proportional hazards assumptions were not met,

especially for the first 50 months into the study. The supremum test of proportional hazards assumption led to a maximum absolute value of 2.2413 based on 1000 replications and a seed of 21, which was statistically significant ($p<0.0001$). The supremum test for the functional form was also statistically significant ($p<0.0001$) and had a maximum absolute value of 16.4152 based on 1000 replications and a seed of 677,739,001.

- NT-proBNP continuous quartile (adjusted model) - The proportional hazards assumption was met for age, body mass index, smoking status, serum creatinine, diabetes status, and the following geographical regions such as: East Midlands, East of England, London, North West, South Central, South East Coast, South West, and West Midlands. It was violated for NT-proBNP continuous quartile, gender, heart failure and/or hypertension medication exposure and the following geographic regions: North East, Northern Ireland, Scotland and Wales. The linearity assumption was met for all continuous variables such as: NT-proBNP continuous quartile, age, body mass index and creatinine. Plots of martingale and deviance residuals against the linear predictors were not symmetric around zero.

APPENDIX F

Table 1 - Patients with BNP values ≥ 3,500 pg/ml removed from the logBNP sensitivity analysis (n=57)

Test	Value (pg/ml)	Log of value	Age yrs)	Sex	BMI category	Geographic region	Smoking status	HF med	Diabetes mellitus
BNP	24872.00	10.1	78	Male	Normal weight	London	Ex-smoker	Yes	No
BNP	4497.00	8.4	66	Male	Normal weight	London	Ex-smoker	Yes	No
BNP	3923.64	8.3	99	Male	Obese	London	Ex-smoker	Yes	No
BNP	4612.18	8.4	86	Female	Normal weight	East Midlands	Ex-smoker	Yes	No
BNP	6076.00	8.7	77	Male	Obese	South Central	Ex-smoker	Yes	No
BNP	5095.00	8.5	80	Male	Normal weight	South Central	Ex-smoker	No	No
BNP	5553.30	8.6	69	Male	Normal weight	East Midlands	Yes	Yes	No
BNP	4013.00	8.3	89	Female	Normal weight	South Central	No	Yes	No
BNP	4124.32	8.3	80	Female	Normal weight	London	Ex-smoker	Yes	No
BNP	4090.00	8.3	83	Male	Normal weight	East of England	No	Yes	No
BNP	6574.00	8.8	79	Male	Overweight	East of England	No	Yes	No
BNP	4657.00	8.4	91	Male	Underweight	East of England	Ex-smoker	Yes	No
BNP	6450.00	8.8	84	Female	Obese		Ex-smoker	Yes	No
BNP	5559.00	8.6	82	Male	Normal weight	East of England	Yes	Yes	No
BNP	5169.00	8.6	94	Female	Normal weight	East of England	No	Yes	No
BNP	5882.00	8.7	78	Male	.	East Midlands	Ex-smoker	.	No
BNP	19870.00	9.9	86	Male	Normal weight	South East Coast	No	Yes	No
BNP	10088.00	9.2	60	Male	Overweight	East of England	Ex-smoker	Yes	No
BNP	3653.76	8.2	80	Male	Obese	East Midlands	No	Yes	No
BNP	4596.00	8.4	79	Female	Normal weight	Northern Ireland	No	Yes	No
BNP	4116.00	8.3	80	Female	Normal weight	South East Coast	Yes	Yes	No
BNP	3785.24	8.2	62	Female	Obese	South East Coast	Ex-smoker	Yes	No
BNP	3809.46	8.2	82	Female	.	East Midlands	Yes	Yes	No
BNP	5940.82	8.7	71	Male	Overweight	East Midlands	Ex-smoker	Yes	No
BNP	4178.00	8.3	67	Male	Normal weight	South West	No	Yes	No
BNP	5419.00	8.6	77	Female	Normal weight	London	No	Yes	No
BNP	29117.00	10.3	54	Male	Obese	South Central	No	Yes	No
BNP	12798.00	9.5	81	Male	Overweight	South Central	Ex-smoker	Yes	No
BNP	6807.00	8.8	87	Male	Overweight	East of England	Ex-smoker	Yes	No
BNP	5691.70	8.6	86	Male	Normal weight	East Midlands	Ex-smoker	Yes	No
BNP	4311.00	8.4	64	Male	Obese		Ex-smoker	Yes	Yes
BNP	5341.00	8.6	68	Male	Overweight	London	Ex-smoker	Yes	No
BNP	6075.76	8.7	81	Male	Obese	East Midlands	No	Yes	Yes
BNP	5516.00	8.6	66	Male	Obese	South Central	Ex-smoker	No	No
BNP	32765.00	10.4	63	Male	Normal weight	South Central	Ex-smoker	No	No
BNP	3742.00	8.2	81	Female	Overweight	South East Coast	No	Yes	No
BNP	4871.68	8.5	60	Male	Overweight	East Midlands	Yes	Yes	No
BNP	6154.00	8.7	79	Female	Normal weight	South East Coast	No	Yes	No
BNP	5312.00	8.6	84	Male	Normal weight	South East Coast	Ex-smoker	Yes	No
BNP	4899.00	8.5	75	Male	Normal weight	South Central	Ex-smoker	No	No
BNP	15346.00	9.6	89	Female	Normal weight	Wales	No	Yes	No
BNP	4283.00	8.4	80	Female	Normal weight	South Central	No	No	No
BNP	7737.00	9.0	81	Male	Overweight	London	Ex-smoker	Yes	No
BNP	9127.48	9.1	94	Male	Overweight	East Midlands	No	Yes	No
BNP	4333.00	8.4	86	Female	Obese	London	No	Yes	No
BNP	4591.00	8.4	95	Female	Normal weight		No	Yes	No
BNP	5533.00	8.6	66	Male	Normal weight	South Central	Ex-smoker	No	No
BNP	4247.00	8.4	85	Female	Overweight		No	Yes	No
BNP	7120.68	8.9	62	Male	Normal weight	East Midlands	Ex-smoker	Yes	No
BNP	3912.00	8.3	93	Male	Normal weight	London	Ex-smoker	Yes	No
BNP	3666.00	8.2	90	Female	.	London	No	Yes	No
BNP	7609.00	8.9	93	Male	Normal weight	South Central	Ex-smoker	Yes	No
BNP	17359.00	9.8	84	Female	Normal weight	South East Coast	No	Yes	No
BNP	5000.00	8.5	80	Female	Underweight	South East Coast	No	Yes	Yes
BNP	15127.00	9.6	67	Male	Obese	London	Ex-smoker	Yes	No
BNP	5067	8.5	74	Male	Obese	London	No	Yes	No
BNP	4788	8.5	87	Male	.	North West	No	No	No

Table 2 - Patients with NT-proBNP values ≥ 10,500 pg/ml removed from the logNT-proBNP sensitivity analysis (n=148)

Test	Value (pg/ml)	Log of value	Age (yrs)	Sex	BMI category	Geographic region	Smoking status	HF med	Db*
NT-proBNP	10802.00	9.3	81	Female	Obese	North East	Ex-smoker	Yes	No
NT-proBNP	13002.00	9.5	83	Male	Overweight	Northern Ireland	Ex-smoker	Yes	No
NT-proBNP	35062.72	10.5	80	Male	Normal weight	West Midlands	No	Yes	No
NT-proBNP	15862.00	9.7	78	Female	Obese	Northern Ireland	No	Yes	Yes
NT-proBNP	10752.00	9.3	86	Male	Overweight	South West	Ex-smoker	Yes	No
NT-proBNP	14159.00	9.6	89	Female	Normal weight	South Central	Ex-smoker	Yes	No
NT-proBNP	24606.00	10.1	81	Male	Normal weight	Northern Ireland	No	Yes	No
NT-proBNP	11895.00	9.4	78	Male	Normal weight	North West	Ex-smoker	Yes	No
NT-proBNP	20023.00	9.9	84	Female	Normal weight	South Central	Ex-smoker	Yes	No
NT-proBNP	16263.00	9.7	100	Female	Overweight	Northern Ireland	No	Yes	No
NT-proBNP	20898.00	9.9	87	Male	Overweight	South West	Ex-smoker	Yes	No
NT-proBNP	22000.00	10.0	56	Male	Obese	Northern Ireland	Yes	Yes	No
NT-proBNP	10645.00	9.3	51	Male	Obese	Northern Ireland	No	.	No
NT-proBNP	16378.00	9.7	79	Male	Overweight	Wales	Ex-smoker	Yes	No
NT-proBNP	16132.00	9.7	97	Female	.	South Central	Ex-smoker	Yes	No
NT-proBNP	13035.00	9.5	78	Male	Normal weight	North West	Ex-smoker	Yes	No
NT-proBNP	14765.92	9.6	76	Male	Normal weight	West Midlands	Yes	Yes	No
NT-proBNP	35000.00	10.5	54	Female	Overweight	Northern Ireland	No	Yes	No
NT-proBNP	22284.00	10.0	86	Male	Normal weight	South Central	Ex-smoker	Yes	No
NT-proBNP	14903.00	9.6	69	Male	Normal weight	Northern Ireland	No	Yes	No
NT-proBNP	31629.18	10.4	56	Female	Normal weight	West Midlands	No	Yes	No
NT-proBNP	13810.28	9.5	77	Female	Overweight	West Midlands	No	Yes	No
NT-proBNP	15300.00	9.6	90	Female	Overweight	South West	Ex-smoker	Yes	No
NT-proBNP	30859.59	10.3	79	Female	Overweight	West Midlands	Ex-smoker	Yes	No
NT-proBNP	13954.05	9.5	89	Male	Normal weight	West Midlands	No	No	No
NT-proBNP	13854.00	9.5	94	Male	Normal weight	South West	Ex-smoker	No	No
NT-proBNP	11423.00	9.3	77	Female	Normal weight	South Central	No	Yes	.
NT-proBNP	34927.41	10.5	86	Female	.	West Midlands	No	Yes	No
NT-proBNP	14854.00	9.6	86	Male	.		Ex-smoker	Yes	No
NT-proBNP	18403.00	9.8	84	Male	Overweight	East of England	No	Yes	No
NT-proBNP	14077.00	9.6	79	Male	Overweight	Northern Ireland	Ex-smoker	Yes	No
NT-proBNP	23361.00	10.1	93	Female	.	East of England	No	Yes	No
NT-proBNP	11172.00	9.3	52	Male	Obese	Northern Ireland	Ex-smoker	Yes	Yes
NT-proBNP	23508.00	10.1	94	Male	Overweight	Northern Ireland	Ex-smoker	Yes	No
NT-proBNP	14324.00	9.6	76	Female	Overweight	Northern Ireland	Yes	Yes	Yes
NT-proBNP	25884.00	10.2	87	Male	Obese	South Central	No	Yes	No
NT-proBNP	18178.00	9.8	80	Female	Normal weight	East of England	No	Yes	No
NT-proBNP	18245.00	9.8	83	Male	Normal weight	North East	Ex-smoker	Yes	No
NT-proBNP	35000.00	10.5	67	Female	Normal weight	North West	Ex-smoker	Yes	No
NT-proBNP	15381.00	9.6	64	Male	Obese		Ex-smoker	Yes	No
NT-proBNP	16525.00	9.7	80	Male	Overweight	Northern Ireland	Yes	Yes	No
NT-proBNP	10662.00	9.3	74	Female	Overweight	South Central	Ex-smoker	No	No
NT-proBNP	23907.94	10.1	90	Female	Overweight	West Midlands	No	Yes	No
NT-proBNP	16028.00	9.7	88	Male	Normal weight	North West	No	Yes	No
NT-proBNP	33498.18	10.4	77	Male	Normal weight	London	Ex-smoker	Yes	No
NT-proBNP	11230.00	9.3	82	Male	Overweight	South Central	No	Yes	No
NT-proBNP	14588.33	9.6	71	Male	Overweight		Ex-smoker	No	No
NT-proBNP	21616.00	10.0	66	Female	Overweight	South Central	No	Yes	No
NT-proBNP	11222.44	9.3	80	Male	Obese	West Midlands	No	Yes	No
NT-proBNP	18585.00	9.8	69	Male	Overweight	South West	Ex-smoker	Yes	No
NT-proBNP	29981.00	10.3	78	Female	Normal weight	South Central	Yes	Yes	No
NT-proBNP	12142.00	9.4	71	Female	Overweight	North East	Yes	Yes	No
NT-proBNP	26664.92	10.2	86	Male	Normal weight	London	No	Yes	No
NT-proBNP	11998.00	9.4	72	Female	Overweight	East Midlands	No	Yes	No
NT-proBNP	20156.00	9.9	76	Male	Overweight	South Central	Ex-smoker	Yes	No
NT-proBNP	19355.00	9.9	88	Male	Normal weight	Yorkshire & The Humber	Ex-smoker	Yes	No
NT-proBNP	11112.00	9.3	86	Female	Overweight	London	No	Yes	No
NT-proBNP	13666.51	9.5	85	Female	Underweight	West Midlands	Ex-smoker	Yes	No
NT-proBNP	13520.00	9.5	83	Female	Normal weight	Northern Ireland	No	Yes	No
NT-proBNP	35000.00	10.5	82	Male	Overweight	South Central	Ex-smoker	Yes	No
NT-proBNP	11985.00	9.4	66	Male	Obese	Northern Ireland	No	Yes	No
NT-proBNP	15475.00	9.6	90	Female	Normal weight	South West	Ex-smoker	Yes	No

Test	Value (pg/ml)	Log of value	Age (yrs)	Sex	BMI category	Geographic region	Smoking status	HF med	Db*
NT-proBNP	14929.00	9.6	90	Male	Underweight	South West	No	Yes	No
NT-proBNP	15124.00	9.6	62	Female	Overweight	North West	No	Yes	No
NT-proBNP	11754.00	9.4	92	Female	.	Northern Ireland	No	Yes	No
NT-proBNP	11826.00	9.4	96	Female	Overweight	East Midlands	No	Yes	No
NT-proBNP	16561.00	9.7	82	Male	Normal weight	Northern Ireland	No	Yes	No
NT-proBNP	17810.00	9.8	82	Female	Overweight	North West	Yes	Yes	No
NT-proBNP	11789.06	9.4	87	Male	Overweight	Yorkshire & The Humber	No	Yes	Yes
NT-proBNP	30500.00	10.3	81	Male	Overweight	North East	No	Yes	No
NT-proBNP	12671.00	9.4	94	Male	Normal weight	South Central	Ex-smoker	Yes	No
NT-proBNP	21222.00	10.0	87	Female	Underweight	South East Coast	Ex-smoker	Yes	No
NT-proBNP	10676.00	9.3	90	Male	Overweight	South West	No	Yes	Yes
NT-proBNP	35000.00	10.5	83	Male	Overweight	South Central	Ex-smoker	Yes	No
NT-proBNP	24107.00	10.1	66	Male	Overweight	North East	Yes	Yes	Yes
NT-proBNP	35000.00	10.5	89	Male	.	Northern Ireland	No	Yes	No
NT-proBNP	17015.48	9.7	92	Male	Obese	West Midlands	Ex-smoker	Yes	No
NT-proBNP	29687.00	10.3	71	Male	Normal weight	Northern Ireland	Ex-smoker	Yes	No
NT-proBNP	16558.81	9.7	96	Female	Overweight	West Midlands	No	Yes	No
NT-proBNP	29707.00	10.3	76	Male	Normal weight	Northern Ireland	Ex-smoker	Yes	No
NT-proBNP	15697.00	9.7	78	Male	Normal weight	South Central	Ex-smoker	Yes	No
NT-proBNP	12690.00	9.4	88	Female	Overweight	Northern Ireland	Ex-smoker	Yes	No
NT-proBNP	13997.00	9.5	88	Female	Normal weight	Scotland	Ex-smoker	Yes	No
NT-proBNP	12327.00	9.4	89	Male	Overweight	South West	Ex-smoker	Yes	No
NT-proBNP	15081.00	9.6	81	Male	Overweight	North West	No	Yes	No
NT-proBNP	23738.80	10.1	94	Male	Overweight	South West	No	Yes	No
NT-proBNP	14574.00	9.6	71	Male	Normal weight	Northern Ireland	No	Yes	No
NT-proBNP	14194.00	9.6	89	Male	Normal weight	South West	No	No	Yes
NT-proBNP	16347.00	9.7	91	Female	Obese	London	No	Yes	No
NT-proBNP	13319.78	9.5	72	Male	Overweight	London	No	Yes	No
NT-proBNP	13099.89	9.5	86	Female	Overweight	West Midlands	Ex-smoker	Yes	No
NT-proBNP	16064.00	9.7	99	Male	Overweight	West Midlands	No	Yes	No
NT-proBNP	15646.00	9.7	75	Female	Normal weight	North East	Yes	Yes	No
NT-proBNP	29963.15	10.3	100	Male	Normal weight	West Midlands	No	Yes	No
NT-proBNP	13696.00	9.5	59	Male	Normal weight	South Central	Ex-smoker	.	No
NT-proBNP	31389.00	10.4	90	Male	Normal weight	North West	No	No	No
NT-proBNP	17514.45	9.8	75	Male	.	London	Ex-smoker	Yes	No
NT-proBNP	11666.00	9.4	65	Female	Normal weight	Northern Ireland	Ex-smoker	Yes	No
NT-proBNP	18839.00	9.8	63	Female	Normal weight		Yes	Yes	No
NT-proBNP	11669.00	9.4	88	Male	Obese	South Central	Ex-smoker	Yes	No
NT-proBNP	33649.00	10.4	81	Female	Normal weight	North East	No	Yes	No
NT-proBNP	14080.91	9.6	84	Male	Overweight	West Midlands	Ex-smoker	Yes	No
NT-proBNP	34927.41	10.5	72	Male	Normal weight	West Midlands	Ex-smoker	Yes	No
NT-proBNP	10626.00	9.3	88	Male	Normal weight	South Central	No	Yes	No
NT-proBNP	19118.00	9.9	88	Female	Normal weight	South East Coast	No	Yes	No
NT-proBNP	13250.00	9.5	90	Female	Normal weight	Northern Ireland	Ex-smoker	Yes	No
NT-proBNP	18575.00	9.8	79	Female	Normal weight	South Central	No	Yes	No
NT-proBNP	11473.00	9.3	78	Male	Normal weight	South Central	Ex-smoker	Yes	No
NT-proBNP	16465.78	9.7	82	Male	Overweight	London	Ex-smoker	Yes	Yes
NT-proBNP	11893.00	9.4	79	Female	Overweight	North West	No	No	No
NT-proBNP	12334.00	9.4	93	Female	Normal weight	South West	No	Yes	No
NT-proBNP	17480.62	9.8	80	Female	Overweight	London	No	Yes	No
NT-proBNP	10551.00	9.3	85	Male	Normal weight	South West	Ex-smoker	Yes	No
NT-proBNP	10975.00	9.3	79	Female	Obese	Northern Ireland	No	Yes	No
NT-proBNP	19787.00	9.9	86	Female	Normal weight	South Central	No	Yes	No
NT-proBNP	26716.00	10.2	88	Female	Normal weight	Northern Ireland	No	No	No
NT-proBNP	24238.00	10.1	78	Female	Normal weight	North West	Ex-smoker	Yes	No
NT-proBNP	13639.00	9.5	89	Male	Overweight	Northern Ireland	Ex-smoker	Yes	No
NT-proBNP	11183.00	9.3	91	Female	Underweight	South Central	No	Yes	No
NT-proBNP	10782.68	9.3	64	Male	Obese	West Midlands	No	Yes	No
NT-proBNP	14356.00	9.6	47	Male	Overweight	South Central	No	Yes	Yes
NT-proBNP	35000.00	10.5	80	Female	Normal weight	South Central	Yes	Yes	No
NT-proBNP	20359.00	9.9	92	Male	Normal weight	South West	Ex-smoker	Yes	No
NT-proBNP	13457.00	9.5	93	Female	Normal weight	South Central	No	Yes	No
NT-proBNP	13934.00	9.5	75	Male	Normal weight	South West	No	Yes	No
NT-proBNP	27515.00	10.2	87	Female	Underweight	London	No	Yes	No
NT-proBNP	18047.24	9.8	88	Female	Normal weight	West Midlands	Ex-smoker	Yes	No
NT-proBNP	29960.00	10.3	91	Female	Overweight	South Central	No	Yes	No
NT-proBNP	11688.00	9.4	77	Female	Normal weight	London	No	Yes	No
NT-proBNP	35000.00	10.5	72	Male	Normal weight	South Central	Ex-smoker	Yes	No
NT-proBNP	48458.61	10.8	74	Female	Overweight	London	No	.	No
NT-proBNP	12756.00	9.5	82	Male	Normal weight	South Central	No	Yes	No
NT-proBNP	19036.00	9.9	92	Female	.	South Central	No	Yes	No
NT-proBNP	12941.00	9.5	72	Female	Normal weight	South East Coast	Ex-smoker	Yes	No

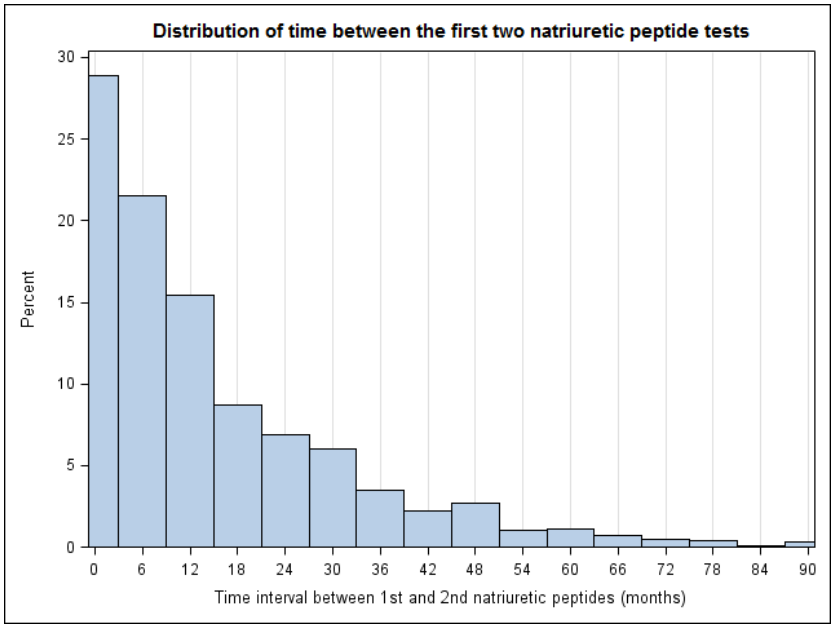
Test	Value (pg/ml)	Log of value	Age (yrs)	Sex	BMI category	Geographic region	Smoking status	HF med	Diabetes mellitus
NT-proBNP	14406.00	9.6	65	Male	.	London	Ex-smoker	Yes	No
NT-proBNP	13877.94	9.5	87	Female	Normal weight	London	No	Yes	No
NT-proBNP	13861.02	9.5	73	Male	Overweight	West Midlands	Ex-smoker	Yes	No
NT-proBNP	31412.00	10.4	81	Male	Overweight	South Central	Ex-smoker	Yes	No
NT-proBNP	30276.06	10.3	89	Female	.	West Midlands	.	Yes	No
NT-proBNP	16787.15	9.7	75	Female	Underweight	West Midlands	Yes	Yes	No
NT-proBNP	20397.00	9.9	71	Male	Overweight	South West	Ex-smoker	Yes	No
NT-proBNP	35000.00	10.5	94	Female	.	West Midlands	Ex-smoker	Yes	No
NT-proBNP	12220.37	9.4	69	Male	Overweight	London	Ex-smoker	Yes	No
NT-proBNP	32939.00	10.4	95	Female	Overweight	London	No	Yes	No
NT-proBNP	47663.65	10.8	75	Female	Underweight	South West	Ex-smoker	Yes	No
NT-proBNP	263063.44	12.5	97	Female	Underweight	South West	No	Yes	No
NT-proBNP	17139.00	9.7	92	Male	.	South Central	.	Yes	No
NT-proBNP	23098.00	10.0	86	Male	Normal weight	South Central	Ex-smoker	Yes	No

Log of value = The natural logarithm of the test value
BMI=Body mass index
HF med= Hypertension and/or heart failure medication
Db= Diabetes mellitus
. = missing data
Age, sex, body mass index, smoking status, hypertension and/or heart failure medication, diabetes mellitus were addressed prior to the study start according to the study design

APPENDIX G

Supporting statistical analyses for Chapter 5

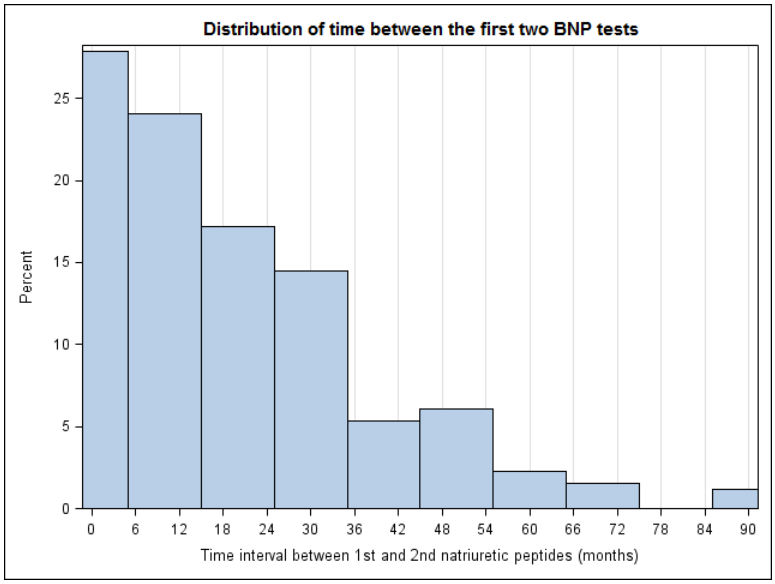
Distribution of the time interval between two consecutive B-type natriuretic peptide tests (n=1094)



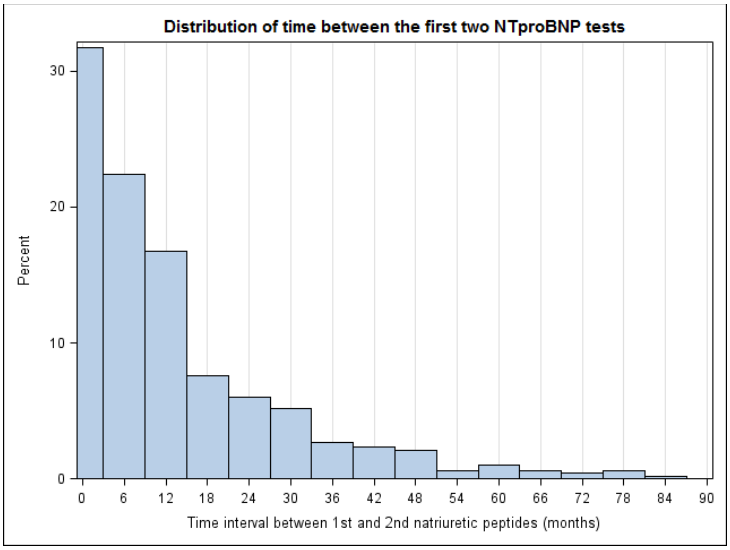
Summary statistics for the time interval (months) between the first two consecutive BNP and NT-proBNP tests

Summary statistics	Patients with 2 consecutive BNP tests (n=262)	Patients with 2 consecutive NT-proBNP tests (n=715)
Lower quartile (Q1)	4.5	1.9
Median (Q2)	13.2	7.6
Upper quartile (Q3)	27.7	17.9
Interquartile range (Q3-Q1)	23.2	10.3
Mean	18.9	13
Standard deviation	18.4	15.2

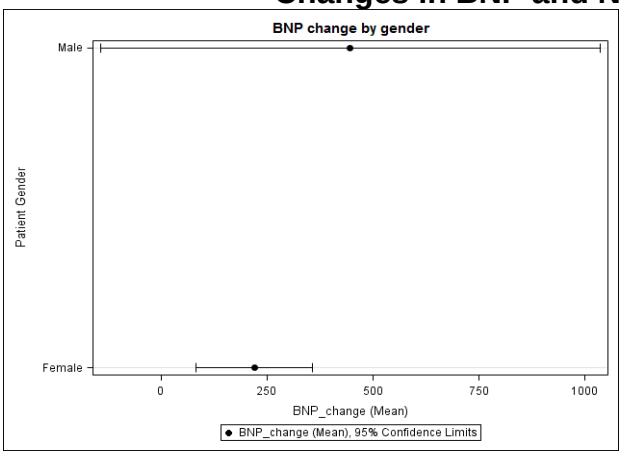
Distribution of the time interval between two consecutive BNP tests (n=262)

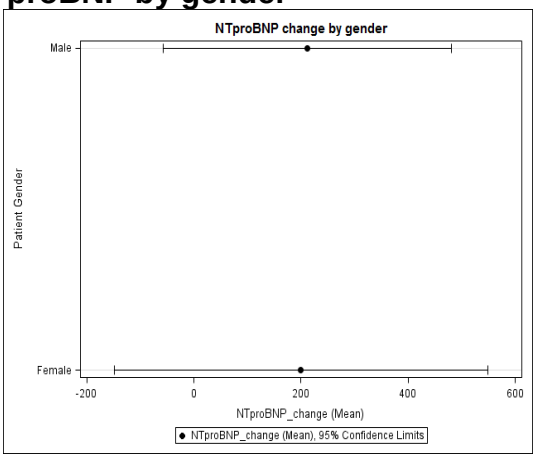


Distribution of the time interval between two consecutive NT-proBNP tests (n=715)

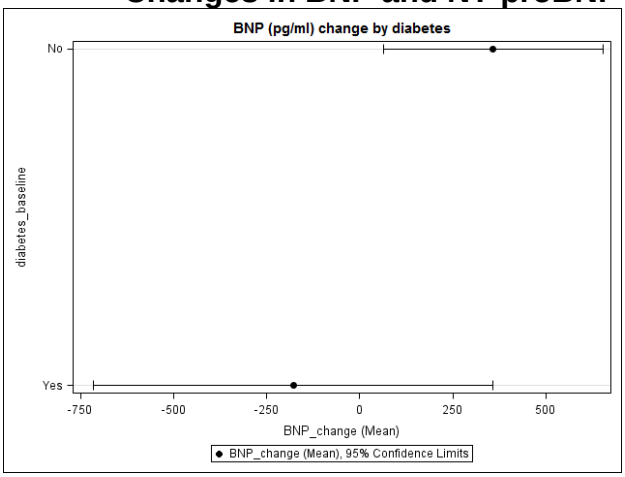


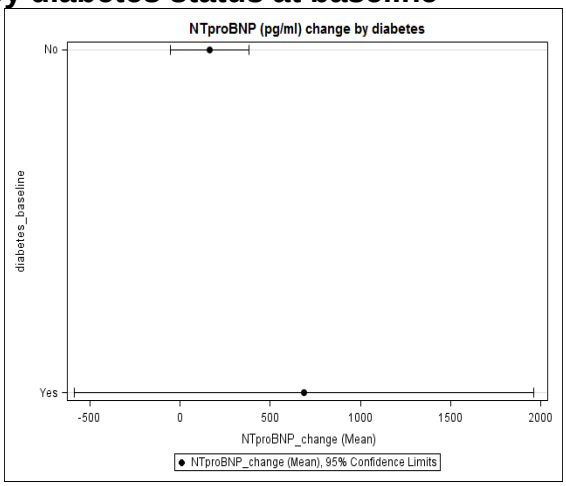
Changes in BNP and NT-proBNP by gender



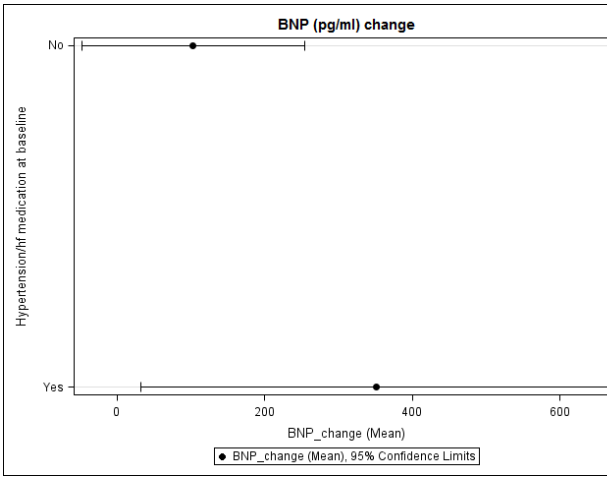


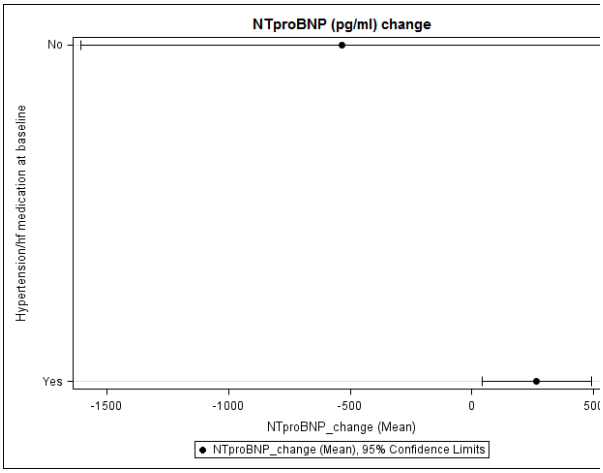
Changes in BNP and NT-proBNP by diabetes status at baseline





Changes in BNP, NT-proBNP and baseline exposure to hypertension

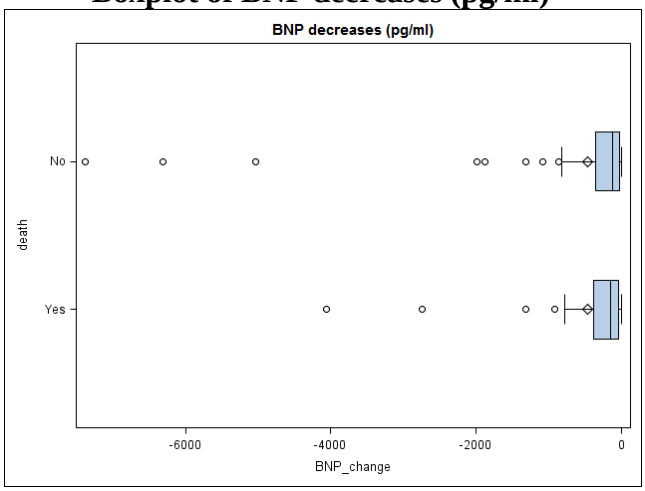




Percentage of patients with BNP decreases by mortality status (any-cause)

Death	Frequency	Percent	Cumulative Frequency	Cumulative Percent
No	83	73.45	83	73.45
Yes	30	26.55	113	100.00

Boxplot of BNP decreases (pg/ml)



Magnitude of BNP decrease (pg/ml)

Death	N Obs	N	N Miss	Mean	Std Dev	Variance	Std Error	Minimum
No	83	83	0	-463.3	1197.5	1433899.0	131.4	-7380.2
Yes	30	30	0	-464.5	873.8	763490.2	159.5	-4058.6

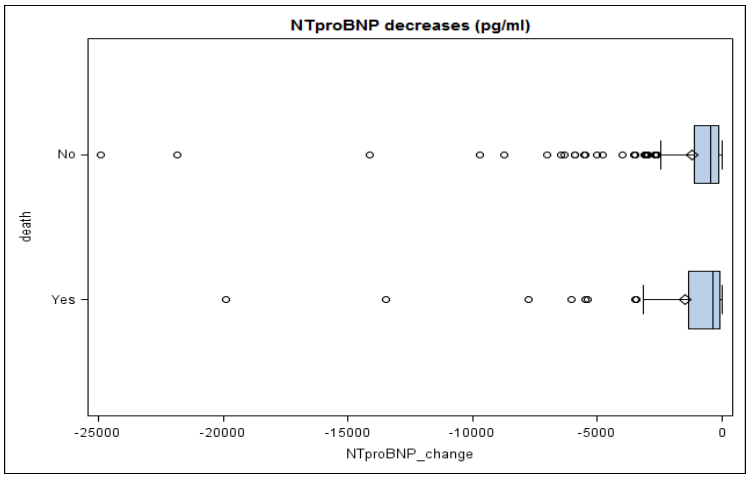
Analysis Variable : BNP_change (pg/ml)

Death	N Obs	Maximum	Range	Lower Quartile	Median	Upper Quartile	Quartile Range
No	83	-2.0	7378.2	-353.0	-119.0	-34.0	319.0
Yes	30	-1.0	4057.6	-388.0	-156.5	-37.0	351.0

Statistical test	Test statistic	p-value
Wilcoxon two-sample test	1636.5	1-sided=0.318 2-sided=0.636
Median two-sample test	14	1-sided=0.356 2-sided=0.713

Percentage of patients with NT-proBNP decreases by mortality status (any-cause)

Death	Frequency	Percent	Cumulative Frequency	Cumulative Percent
No	237	76.95	237	76.95
Yes	71	23.05	308	100.00



Magnitude of NT-proBNP decreases

Death	N Obs	N	N Miss	Mean	Std Dev	Variance	Std Error	Minimum
No	237	237	0	-1230.7	2676.4	7162984.8	173.8	-24894.0
Yes	71	71	0	-1480.2	3091.7	9558309.2	366.9	-19881.0

Analysis Variable : NT-proBNP_change (pg/ml)

Death	N Obs	Maximum	Range	Lower Quartile	Median	Upper Quartile	Quartile Range
No	237	-2.0	24892.0	-1100.0	-451.0	-151.0	949.0
Yes	71	-0.4	19880.6	-1343.0	-374.0	-73.0	1270.0

Statistical test	Test statistic	p-value
Wilcoxon two-sample test	11322	1-sided=0.296 2-sided=0.593
Median two-sample test	36	1-sided=0.446 2-sided=0.892

APPENDIX H

Supplementary analyses for Chapter 6

Table 6.1. Summary statistics for $\log_e$-BNP over time

Summary measure	Week 1	Week 7	Week 13
N	30	30	28
Arithmetic mean	4.1	4.1	3.9
Standard deviation	1.2	1.3	1.3
Between-subject coefficient of variation (CV_b%)	29.5%	30.4%	32.6%
Median	4.1	4.3	4.1
Minimum	1.6	1.8	1.1
Maximum	6.4	6.4	6.6
Range	4.8	4.6	5.5
Lower quartile (Q1)	3.3	3.5	3.1
Upper quartile (Q3)	5	5.1	5
Inter-quartile range (Q3-Q1)	1.7	1.7	1.9
Note: All data available included; log scale			

Table 6.2. Summary statistics for $\log_e$-BNP over time while excluding the two subjects with missing data at week 13

Summary measure	Week 1	Week 7	Week 13
n	28	28	28
Arithmetic mean	4	4	3.9
Standard deviation	1.2	1.2	1.3
Between-subject coefficient of variation (CV_b%)	29.9%	30.8%	32.6%
Median	3.9	3.9	4.1
Minimum	1.6	1.8	1.1
Maximum	6.4	6.4	6.6
Range	4.8	4.6	5.5
Lower quartile (Q1)	3.2	3.2	3.1
Upper quartile (Q3)	4.9	5.1	5
Inter-quartile range (Q3-Q1)	1.7	1.6	1.9
Note: Table excludes the two subjects with missing BNP at week 13; log scale			

Table 6.3. Summary statistics for BNP (ng/l) over time while excluding the two subjects with missing data at week 13

Summary measure	Week 1	Week 7	Week 13
n	28	28	28
Arithmetic mean	107.2	103.5	103.5
Standard deviation	137.8	120.3	141.6
Between-subject coefficient of variation (CV_b%)	128.6%	116.2%	136.8%
Median	48	65.5	61
Minimum	5	6	3
Maximum	605	601	721
Range	600	595	718
Lower quartile (Q1)	25	31	21.5
Upper quartile (Q3)	133.5	158	145.5
Inter-quartile range (Q3-Q1)	108.5	127	24
Note: Table excludes the two subjects with missing BNP at week 13; original scale ng/l			

Table 6.4 – BNP (ng/l) values over time by gender

Gender	Summary Statistics	Week 1	Week 7	Week 13
Female	N	9	9	9
	Arithmetic mean (95%CI)	75.9 (-10.8, 162.6)	71.6 (8.9, 134.2)	58.6 (-4.6, 121.7)
	Geometric mean (95% CI)	33.1 (12.1, 99.4)	36.6 (12.1, 99.4)	27.1 (9.1, 81.4)
	Standard deviation	112.8	81.5	82.2
	Standard deviation – log	1.3	1.4	1.4
	Coefficient of variation	148.6%	113.9%	140.4%
	Median (range)	33 (351)	33 (247)	25 (261)
	Interquartile range	44	77	39
Male	N	21	21	19
	Arithmetic mean (95%CI)	131.4 (65.4, 197.5)	130.7 (70.2, 191.2)	124.8 (47.7, 201.9)
	Geometric mean (95% CI)	73.6 (44.7, 121.5)	81.4 (44.7, 134.2)	73.6 (40.4, 121.5)
	Standard deviation	145.1	132.9	160
	Standard deviation - log	1.1	1.2	1.1
	Coefficient of variation	110.4%	101.7%	128.2%
	Median (range)	97 (593)	120 (595)	75 (711)
	Interquartile range	120	138	135
Unless otherwise noted (standard deviation on natural logarithmic scale), the data was reported in the original scale.				

Table 6.5– BNP (ng/l) values over time by body mass index groups

Gender	Summary statistics	Week 1	Week 7	Week 13
Normal weight	n	5	5	5
	Arithmetic mean (95%CI)	131.4 (-65.8, 328.6)	90.6 (-10.1, 191.3)	91.2 (-33.4, 215.8)
	Geometric mean (95% CI)	73.6 (16.4, 330.2)	49.4 (8.1, 298.8)	44.7 (8.1, 270.4)
	Standard deviation	158.8	81.1	100.4
	Standard deviation – log	1.2	1.4	1.4
	Coefficient of variation	120.9%	89.5%	110%
	Median (range)	55 (381)	85 (168)	51 (235)
	Interquartile range	115	156	124
Overweight	n	11	11	11
	Arithmetic mean (95%CI)	119.1 (6.7, 231.5)	143.1 (32.8, 253.4)	144.9 (12.4, 277.4)
	Geometric mean (95% CI)	66.7 (33.1, 134.2)	81.4 (36.5, 181.2)	90.1 (49.4, 146.2)
	Standard deviation	167.3	164.1	197.2
	Standard deviation - log	1.1	1.2	0.9
	Coefficient of variation	140.5%	114.7%	136.1%
	Median (range)	69 (594)	80 (595)	75 (696)
	Interquartile range	92	123	104
Obese	n	14	14	12
	Arithmetic mean (95%CI)	105.4 (41.6, 169.3)	97.2 (42.5, 152)	70.7 (16.6, 124.7)
	Geometric mean (95% CI)	49.4 (22.1, 109.9)	54.5 (24.5, 109.9)	33.1 (12.1, 81.4)
	Standard deviation	110.6	94.8	85.1
	Standard deviation - log	1.4	1.3	1.4
	Coefficient of variation	104.9%	97.6%	120.4%
	Median (range)	63.5 (351)	39 (256)	26 (261)
	Interquartile range	153	123	107.5

Unless otherwise noted (standard deviation on natural logarithmic scale), the data was reported in the original scale.

Table 6.6 – BNP (ng/l) values over time by weight

Group	Summary statistics	Week 1	Week 7	Week 13
Normal/ Overweight	n	16	16	16
	Arithmetic mean (95%CI)	122.9 (38, 207.9)	126.7 (50.7, 202.7)	128.1 (36.9, 219.3)
	Geometric mean (95% CI)	66.7 (40.4, 121.5)	73.7 (36.6, 134.2)	73.7 (40.4, 134.2)
	Standard deviation	159.4	142.6	171.1
	Standard deviation – log	1.1	1.3	1.1
	Coefficient of variation	129.7%	112.6%	133.5%
	Median (range)	62 (594)	82.5 (595)	73.5 (710)
	Interquartile range	100	131	99.5
Obese	n	14	14	12
	Arithmetic mean (95%CI)	105.4 (41.6, 169.3)	97.2 (42.5, 152)	70.7 (16.6, 124.7)
	Geometric mean (95% CI)	44.7 (22.2, 109.9)	54.5 (24.5, 109.9)	33.1 (12.2, 81.4)
	Standard deviation	110.6	94.8	85.1
	Standard deviation - log	1.4	1.3	1.4
	Coefficient of variation	104.9%	97.6%	120.4%
	Median (range)	63.5 (351)	39 (256)	26 (107.5)
	Interquartile range	153	123	107.5
Unless otherwise noted (standard deviation on natural logarithmic scale), the data was reported in the original scale.				

Table 6.7 – BNP (ng/l) values over time by age groups

Age group (years)	Week	n	Arithmetic mean (95% CI)	Geometric mean (95%CI)	Std dev	Std de log	CVb (%)	Med. range	IQR
< 45	Week 1	4	56.5 (-17, 130)	33.1 (3.6, 298.8)	46.2	1.4	81.8	62 (92)	78
	Week 7	4	86 (-36.1, 208.1)	49.4 (5.4, 492.7)	76.7	1.4	76.7	87.5 (153)	131
	Week 13	4	65.8 (-42.5, 174)	29.9 (2.1, 492.7)	68	1.7	68	52.5 (152)	104.5
45 – 54.9	Week 1	9	130.7 (-15.5, 276.8)	54.5 (20.1, 164)	190.2	1.4	145.5	34 (594)	126
	Week 7	9	122.9 (-24.1, 269.9)	44.7 (14.8, 148.4)	191.3	1.5	155.6	38 (595)	146
	Week 13	9	133.4 (-41.1, 308)	49.4 (16.4, 164)	227.1	1.5	170.2	58 (716)	117
55-64.9	Week 1	13	91.4 (33.6, 149.2)	54.5 (29.9, 109.9)	95.7	1	104.7	55 (344)	96
	Week 7	13	101 (50.7, 151.3)	66.6 (33.1, 121.5)	83.2	1.2	82.4	80 (245)	122
	Week 13	12	84.9 (32.2, 137.6)	49.4 (24.5, 109.9	82.9	1.1	97.7	57 (254)	113.5
65-74.9	Week 1	4	213.3 (-38.7, 465.2)	148.4 (29.9, 812.4)	158.4	1	74.3	207.5 (364)	248.5
	Week 7	4	156.3 (19.6, 292.9)	134.2 (44.7, 403.4)	85.9	0.7	55	154.5 (208)	119.5
	Week 13	3	138.3 (-94.5, 371.2)	121.5 (24.5, 601.8)	93.7	0.6	67.8	94 (171)	171

Unless otherwise noted (standard deviation on natural logarithmic scale), the data was reported in the original scale.

Table 6.8 – BNP (ng/l) values over time by age groups

Age	Summary statistics	Week 1	Week 7	Week 13
< 55 years	n	13	13	13
	Arithmetic mean (95%CI)	107.8 (10.6, 205.1)	111.5 (13.8, 209.3)	112.6 (-3, 228.2)
	Geometric mean (95% CI)	49.4 (22.2, 109.9)	49.4 (20.1, 109.9)	44.7 (16.4, 109.9)
	Standard deviation	161	161.8	191.3
	Standard deviation – log	1.4	1.4	1.5
	Coefficient of variation	149.3%	145%	169.9%
	Median (range)	34 (600)	38 (595)	58 (718)
	Interquartile range	104	131	117
≥ 55 years	n	17	17	15
	Arithmetic mean (95%CI)	120.1 (58.3, 181.8)	114 (70.5, 157.5)	95.6 (48.8, 142.4)
	Geometric mean (95% CI)	73.7 (40.4, 121.5)	73.7 (44.7, 134.2)	60.3 (33.1, 109.9)
	Standard deviation	120	84.6	84.6
	Standard deviation - log	1.1	1.1	1.1
	Coefficient of variation	100%	74.2%	88.4%
	Median (range)	69 (389)	85 (254)	72 (254)
	Interquartile range	113	116	154
Unless otherwise noted (standard deviation on natural logarithmic scale), the data was reported in the original scale.				

Table 6.9 – BNP (ng/l) values over time by chronic kidney disease groups based on GFR (Glomerular Filtration Rate)

Subgroup	Summary statistics	Week 1	Week 7	Week 13
CKD Stage I GFR ≥ 90 ml/min	n	9	9	8
	Arithmetic mean (95%CI)	100 (39.7, 160.3)	128.4 (68.2, 188.7)	88.9 (33.3, 144.4)
	Geometric mean (95% CI)	73.6 (33.1, 148.4)	99.4 (60.3, 221.4)	54.5 (20.1, 164.1)
	Standard deviation	78.5	78.4	66.5
	Standard deviation – log	1	0.7	1.2
	Coefficient of variation	78.5%	61%	74.8%
	Median (range)	94 (263)	142 (230)	76.5 (187)
	Interquartile range	69	111	110.5
CKD Stage II GFR 60-89.9 ml/min	n	14	14	13
	Arithmetic mean (95%CI)	113.2 (14.1, 212.4)	117.3 (22.6, 211.9)	124.5 (5.9, 243.2)
	Geometric mean (95% CI)	44.7 (20.1, 99.4)	44.7 (18.1, 109.9)	49.4 (18.1, 121.5)
	Standard deviation	171.7	163.9	196.3
	Standard deviation - log	1.4	1.5	1.5
	Coefficient of variation	151.7%	139.7%	157.6%
	Median (range)	31 (600)	43.5 (595)	59 (718)
	Interquartile range	131	165	148
CKD Stage III GFR 30-59.9 ml/min	n	7	7	7
	Arithmetic mean (95%CI)	136.9 (14.9, 258.8)	84.3 (28.2, 140.3)	81.1 (8.5, 153.7)
	Geometric mean (95% CI)	90.1 (36.5, 221.4)	60.3 (22.1, 164.1)	54.5 (22.1, 134.2)
	Standard deviation	131.9	60.6	78.5
	Standard deviation - log	1	1.1	1
	Coefficient of variation	96.4%	71.9%	96.7%
	Median (range)	121 (371)	80 (162)	58 (234)
	Interquartile range	158	106	70

Unless otherwise noted (standard deviation on natural logarithmic scale), the data was reported in the original scale.

Table 6.10 – BNP (ng/l) values over time by prior Beta-Blocker medication exposure

Subgroup	Summary statistics	Week 1	Week 7	Week 13
Prior beta blocker	n	27	27	25
	Arithmetic mean (95%CI)	110.4 (56.9, 163.9)	111.7 (62.4, 161)	99.5 (39.1, 159.9)
	Geometric mean (95% CI)	60.3 (36.5, 90)	60.3 (36.5, 99.4)	49.4 (27.1, 81.4)
	Standard deviation	135.3	124.5	146.3
	Standard deviation – log	1.2	1.3	1.3
	Coefficient of variation	122.5%	111.5%	147%
	Median (range)	55 (600)	80 (595)	58 (718)
	Interquartile range	124	140	117
No prior beta blocker	n	3	3	3
	Arithmetic mean (95%CI)	154 (-282.4, 590.4)	124 (-153.9, 401.9)	137 (-136.2,410.2)
	Geometric mean (95% CI)	99.4 (5.4, 1808)	99.4 (12.1, 812.4)	109.9 (18.1, 735.1)
	Standard deviation	175.7	111.9	110
	Standard deviation - log	1.2	0.8	0.7
	Coefficient of variation	114.1%	90.2%	80.3%
	Median (range)	69 (319)	65 (199)	75 (192)
	Interquartile range	319	199	192

Unless otherwise noted (standard deviation on natural logarithmic scale), the data was reported in the original scale.

Table 6.11 – BNP (ng/l) values over time by prior ACE inhibitor medication exposure

Subgroup	Summary statistics	Week 1	Week 7	Week 13
ACE inhibitor	n	22	22	20
	Arithmetic mean (95%CI)	113.8 (48.2, 179.4)	106.4 (47, 165.7)	93.2 (19.3, 167.1)
	Geometric mean (95% CI)	54.6 (33.1, 99.4)	54.6 (29.9, 99.4)	44.7 (24.5, 81.4)
	Standard deviation	148	133.9	158
	Standard deviation – log	1.2	1.3	1.2
	Coefficient of variation	130%	125.9%	169.5%
	Median (range)	39 (594)	59.5 (595)	58.5 (716)
	Interquartile range	126	131	66
No ACE inhibitor	n	8	8	8
	Arithmetic mean (95%CI)	117.4 (26.6, 208.1)	131 (61.3, 200.7)	129.3 (52, 206.5)
	Geometric mean (95% CI)	73.6 (24.5, 200.3)	90.1 (33.1, 244.6)	73.6 (20.1, 270.4)
	Standard deviation	108.5	83.4	92.4
	Standard deviation - log	1.3	1.2	1.5
	Coefficient of variation	92.5%	63.6%	71.5%
	Median (range)	110.5 (351)	148.5 (245)	159 (261)
	Interquartile range	93.5	127	149.5

Unless otherwise noted (standard deviation on natural logarithmic scale), the data was reported in the original scale.

Table 6.12 – BNP (ng/l) values over time by prior ALDOSTERONE medication exposure

Subgroup	Summary statistics	Week 1	Week 7	Week 13
Aldosterone	n	20	20	18
	Arithmetic mean (95%CI)	114.3 (42.2, 186.4)	111.3 (45.4, 177.1)	102.2 (18, 186.5)
	Geometric mean (95% CI)	54.5 (29.9, 99.4)	49.4 (27.1, 99.4)	40.4 (22.1, 81.4)
	Standard deviation	154.1	140.7	169.5
	Standard deviation – log	1.3	1.4	1.4
	Coefficient of variation	134.8%	126.4%	165.8%
	Median (range)	37.5 (600)	59 (595)	38.5 (718)
	Interquartile range	126	149.5	121
Remainder of the cohort/ Assumed no Aldosterone	n	10	10	10
	Arithmetic mean (95%CI)	115.7 (43.7, 187.7)	116.3 (61.6, 171)	105.8 (50.8, 160.8)
	Geometric mean (95% CI)	81.4 (36.5, 164.1)	90.1 (50.5, 164.1)	73.6 (33.1, 164.1)
	Standard deviation	100.6	76.4	76.8
	Standard deviation - log	1	0.7	1.1
	Coefficient of variation	87%	65.7%	72.6%
	Median (range)	107.5 (345)	93 (221)	78 (259)
	Interquartile range	89	123	100

Unless otherwise noted (standard deviation on natural logarithmic scale), the data was reported in the original scale.

Table 6.13 – BNP (ng/l) values over time by prior ANGIOTENSIN medication exposure

Subgroup	Summary statistics	Week 1	Week 7	Week 13
Angiotensin	n	8	8	8
	Arithmetic mean (95%CI)	117.4 (26.6, 208.1)	131 (61.3, 200.7)	129.3 (52, 206.5)
	Geometric mean (95% CI)	73.6 (24.5, 200.3)	90.1 (33.1, 244.6)	90.1 (20.1, 270.4)
	Standard deviation	108.5	83.4	92.4
	Standard deviation – log	1.3	1.2	1.5
	Coefficient of variation	92.5%	63.6%	71.5%
	Median (range)	110.5 (351)	148.5 (245)	159 (261)
	Interquartile range	93.5	127	149.5
No prior Angiotensin	n	22	22	21
	Arithmetic mean (95%CI)	113.8 (48.2, 179.4)	106.4 (47, 165.7)	93.2 (19.3, 167.1)
	Geometric mean (95% CI)	54.5 (33.1, 99.4)	54.5 (29.9, 99.4)	44.7 (24.5, 81.4)
	Standard deviation	148	133.9	158
	Standard deviation - log	1.2	1.3	1.2
	Coefficient of variation	130%	125.9%	169.5%
	Median (range)	39 (594)	59.5 (595)	58.5 (716)
	Interquartile range	126	131	66

Unless otherwise noted (standard deviation on natural logarithmic scale), the data was reported in the original scale.

Table 6.14 – BNP (ng/l) values over time by prior DIURETICS medication exposure

Subgroup	Summary statistics	Week 1	Week 7	Week 13
Prior Diuretic Exposure	n	21	21	20
	Arithmetic mean (95%CI)	130.5 (58.2, 202.8)	118.4 (55, 181.8)	113.6 (32.4, 194.8)
	Geometric mean (95% CI)	60.3 (33.1, 109.9)	60.3 (33.1, 109.9)	49.4 (22.1, 99.4)
	Standard deviation	158.8	139.2	168.5
	Standard deviation – log	1.4	1.4	1.5
	Coefficient of variation	121.7%	117.6%	148.4%
	Median (range)	41 (600)	66 (595)	58 (718)
	Interquartile range	140	138	148
No prior Diuretic exposure	n	9	9	9
	Arithmetic mean (95%CI)	78.1 (38.9, 117.4)	100.1 (46.6, 153.6)	82.2 (39.9, 124.5)
	Geometric mean (95% CI)	60.3 (29.9, 109.9)	66.6 (29.9, 164.1)	66.6 (33.1, 121.5)
	Standard deviation	51.1	69.5	66.9
	Standard deviation - log	0.8	1.1	0.9
	Coefficient of variation	65.4%	69.5%	66.9%
	Median (range)	69 (133)	85 (183)	75 (181)
	Interquartile range	87	107	43

Unless otherwise noted (standard deviation on natural logarithmic scale), the data was reported in the original scale.

Table 6.15 – BNP (ng/l) values over time by prior history of arrhythmia

Subgroup	Summary statistics	Week 1	Week 7	Week 13
History of Arrhythmia	n	17	17	16
	Arithmetic mean (95%CI)	99.2 (51.2, 147.2)	109.9 (74.3, 145.5)	92.9 (56.5, 129.3)
	Geometric mean (95% CI)	66.6 (36.6, 109.9)	81.4 (49.4, 134.2)	66.6 (36.6, 109.9)
	Standard deviation	93.3	69.3	68.3
	Standard deviation – log	1.1	1	1.1
	Coefficient of variation	94.1%	63%	73.5%
	Median (range)	94 (396)	120 (219)	78 (243)
	Interquartile range	96	116	106
No history of Arrhythmia	n	13	13	12
	Arithmetic mean (95%CI)	143.6 (25.1, 262)	126 (14.5, 237.5)	127.2 (-16.1, 270.5)
	Geometric mean (95% CI)	60.3 (22.1, 148.4)	49.4 (20.1, 134.2)	44.7 (16.4, 121.5)
	Standard deviation	186.4	175.5	213.3
	Standard deviation - log	1.5	1.5	1.5
	Coefficient of variation	129.8%	139.3%	167.7%
	Median (range)	37.5 (594)	36.5 (595)	49 (716)
	Interquartile range	216.5	182	171

Unless otherwise noted (standard deviation on natural logarithmic scale), the data was reported in the original scale.

Table 6.16 – BNP (ng/l) values over time by history of hypertension

Subgroup	Summary statistics	Week 1	Week 7	Week 13
History of Hypertension	n	16	16	14
	Arithmetic mean (95%CI)	108.1 (49.4, 166.8)	100.2 (55.6, 144.8)	78.2 (34.2, 122.2)
	Geometric mean (95% CI)	60.3 (29.9, 121.5)	60.3 (33.1, 109.9)	40.4 (18.1, 90.1)
	Standard deviation	110.2	83.8	76.2
	Standard deviation – log	1.3	1.2	1.4
	Coefficient of variation	101.9%	83.6%	97.5%
	Median (range)	81.5 (396)	65.5 (256)	61 (243)
	Interquartile range	132	133	70
No history of Hypertension	n	14	14	14
	Arithmetic mean (95%CI)	129.2 (26, 232.5)	136.7 (40.8, 232.6)	137.8 (22.9, 252.6)
	Geometric mean (95% CI)	66.6 (33.1, 134.2)	73.6 (33.1, 134.2)	73.6 (36.5, 148.4)
	Standard deviation	170.9	158.8	190
	Standard deviation - log	1.2	1.3	1.2
	Coefficient of variation	132.2%	116.1%	137.9%
	Median (range)	55 (594)	85 (595)	75 (710)
	Interquartile range	113	138	127

Unless otherwise noted (standard deviation on natural logarithmic scale), the data was reported in the original scale.

Table 6.17 – BNP (ng/l) values over time by history of angina

Subgroup	Summary statistics	Week 1	Week 7	Week 13
History of Angina	n	4	4	3
	Arithmetic mean (95%CI)	154.5 (25.6, 283.4)	198.8 (128.1, 269.4)	145.3 (2.3, 288.3)
	Geometric mean (95% CI)	148.4 (66.6, 298.8)	200.3 (134.2, 270.4)	134.2 (44.7, 445.8)
	Standard deviation	81	44.4	57.6
	Standard deviation – log	0.5	0.2	0.5
	Coefficient of variation	52.4%	22.3%	39.6%
	Median (range)	125 (180)	186 (101)	163 (111)
	Interquartile range	91	59.5	111
No history of Angina	n	25	25	24
	Arithmetic mean (95%CI)	111.7 (51.5, 171.8)	103.4 (51.5, 155.3)	102.1 (38.5, 165.6)
	Geometric mean (95% CI)	54.5 (33.1, 90.1)	54.5 (33.1, 90.1)	49.4 (27.1, 81.4)
	Standard deviation	145.7	125.8	150.5
	Standard deviation - log	1.3	1.2	1.3
	Coefficient of variation	130.5%	121.7%	147.4%
	Median (range)	41 (600)	65 (595)	58.5 (718)
	Interquartile range	124	110	93.5

Unless otherwise noted (standard deviation on natural logarithmic scale), the data was reported in the original scale.

Table 6.18 – BNP (ng/l) values over time by prior history of ischaemic cardiomyopathy

Subgroup	Summary statistics	Week 1	Week 7	Week 13
History of ischaemic cardiomyopathy	n	11	11	10
	Arithmetic mean (95%CI)	137.9 (43.4, 232.4)	128.9 (67.4, 190.4)	119 (54.7, 183.3)
	Geometric mean (95% CI)	81.4 (36.5, 164.1)	90.1 (44.7, 181.2)	90.1 (44.7, 164.1)
	Standard deviation	140.6	91.5	89.9
	Standard deviation – log	1.1	1	0.9
	Coefficient of variation	102%	71%	75.6%
	Median (range)	94 (386)	161 (250)	78 (245)
	Interquartile range	246	157	133
No history of ischaemic cardiomyopathy	n	18	18	17
	Arithmetic mean (95%CI)	105.2 (35.8, 174.5)	109 (39.6, 178.4)	99.8 (12.8, 186.7)
	Geometric mean (95% CI)	54.5 (27.1, 99.4)	54.5 (29.9, 109.9)	40.4 (20.1, 90.1)
	Standard deviation	139.4	139.5	169.1
	Standard deviation - log	1.3	1.3	1.4
	Coefficient of variation	132.6%	128%	169.5%
	Median (range)	62 (600)	72.5 (595)	51 (183.3)
	Interquartile range	62	72.5	51

Unless otherwise noted (standard deviation on natural logarithmic scale), the data was reported in the original scale.

Table 6.19 – BNP (ng/l) values over time by prior history of non-ischaemic cardiomyopathy

Subgroup	Summary statistics	Week 1	Week 7	Week 13
History of non-ischaemic cardiomyopathy	n	19	19	19
	Arithmetic mean (95%CI)	118.4 (47.4, 189.3)	116.6 (49.3, 183.8)	108.9 (25.1, 192.7)
	Geometric mean (95% CI)	60.3 (29.9, 109.9)	60.3 (33.1, 109.9)	44.7 (22.1, 90.1)
	Standard deviation	147.2	139.5	168.6
	Standard deviation – log	1.1	1	0.9
	Coefficient of variation	124.4%	119.7%	154.8%
	Median (range)	69 (600)	80 (595)	54.5 (718)
	Interquartile range	128	123	121
No history of non-ischaemic cardiomyopathy	n	10	10	9
	Arithmetic mean (95%CI)	116.1 (25.1, 207.1)	116.5 (54.8, 178.2)	102.9 (42.5, 163.3)
	Geometric mean (95% CI)	66.6 (33.1, 148.4)	81.4 (40.4, 164.1)	73.6 (40.4, 148.4)
	Standard deviation	127.1	86.2	78.6
	Standard deviation - log	1.3	1.3	1.4
	Coefficient of variation	109.5%	74%	76.4%
	Median (range)	65.5 (386)	113.5 (250)	75 (227)
	Interquartile range	98	139	104

Unless otherwise noted (standard deviation on natural logarithmic scale), the data was reported in the original scale.

Table 6.20 – BNP (ng/l) absolute changes to weeks 7 and 13 by age subgroups

Age group (years)	Week	n	Arithmetic mean (95% CI)	Std dev	CVb (%)	Median (Range)	IQR
0-44	Week 7	4	29.5 (-21.2, 80.2)	31.9	108.1 %	24 (64)	53
	Week 13	4	9.3 (-43, 61.5)	32.8	354.8 %	-4 (-71)	37.5
45-54	Week 7	9	-7.8 (-52, 36.5)	57.5	-739 %	-4 (202)	26
	Week 13	9	2.8 (-47, 52.6)	64.8	2332 %	-6 (249)	34
55-64.9	Week 7	13	9.6 (-16.8, 36.1)	43.8	455.3 %	9 (174)	42
	Week 13	12	-0.1 (-25.6, 25.4)	40.1	-48121.1 %	0.5 (160)	39
65-74.9	Week 7	4	-57 (-242.6, 128.6)	43.8	-205 %	-7 (248)	129
	Week 13	3	-54.7 (-295, 185.6)	40.1	-177 %	-47 (193)	193

Change to week 7 is defined as week 7 – baseline
Change to week 13 is defined as week 3 – baseline
Data reported in the original scale

Table 6.21 – BNP (ng/l) absolute changes to weeks 7 and 13 by age subgroups

Age group (years)	Week	n	Arithmetic mean (95% CI)	Std dev	CVb (%)	Median (Range)	IQR
<55	Week 7	13	3.1 (-28.2, 35.6)	52.8	1428.7%	3 (218)	32
	Week 13	13	4.8 (-28.8, 38.3)	55.5	1163.1%	-6 (249)	34
≥55	Week 7	17	-6.1 (-41.8, 29.7)	69.5	-1147.8%	-1 (302)	36
	Week 13	15	-11 (-41.9, 19.9)	55.8	-507%	-2 (223)	71

Change to week 7 is defined as week 7 – baseline
Change to week 13 is defined as week 3 – baseline
Data reported in the original scale

Table 6,22– BNP (ng/l) percent changes from baseline to weeks 7 and 13 by age groups

Age group (years)	Week	n	Arithmetic mean (95% CI)	Std dev	Median	Min	Max
0-44	Week 7	4	46.9% (-73%, 66%)	26.6 %	53.2%	10%	71.3%
	Week 13	4	-3.5% (-73%, 66%)	43.7 %	-16.9%	-40%	59.8%
45-54	Week 7	9	8.5% (-54%, 70.9%)	81.3 %	-0.7%	-79.1%	71.3%
	Week 13	9	5.9% (-43.1%, 4.9%)	63.7 %	-8.1%	-69.6%	59.8%
55-64.9	Week 7	13	30.6% (-12.6%, 73.8%)	71.5 %	6.2%	-75.8%	175%
	Week 13	12	10.6% (-24.6%, 45.8%)	55.4 %	-1.5%	-63.6%	125%
65-74.9	Week 7	4	-4.4% (-71.7%, 63%)	42.3 %	-2.9%	-57.6%	45.9%
	Week 13	3	10.2% (-188.8%, 209.3%)	80.1 %	-33.3%	-38.7%	102.7%
Data reported in the original scale							

Table 6.23 – BNP (ng/l) percent changes to weeks 7 and 13 by age subgroups

Age group (years)	Week	n	Arithmetic mean (95% CI)	Std dev	Median	Min	Max
<55	Week 7	13	20.3% (-22.1%, 62.7%)	70.1 %	18.9%	-79.1%	190.9%
	Week 13	13	3% (-31.2%, 37.2%)	56.6 %	-13.8%	-69.6%	127.3%
≥55	Week 7	17	22.4% (-11.8%, 56.5%)	66.4 %	-0.8%	-75.8%	175%
	Week 13	15	10.5% (-21.4%, 42.5%)	57.7 %	-7.3%	-63.6%	125%

Table 6.24 – BNP (ng/l) absolute changes to weeks 7 and 13 by gender

Group	Week	n	Arithmetic mean (95% CI)	Std Dev	CVb (%)	Median (Range)	IQR
Female	Week 7	9	-4.3 (-36.5, 27.8)	41.8	-964.5%	3 (142)	26
	Week 13	9	-17.3 (-42.8, 8.1)	33.1	-190.9 %	-6 (106)	19
Male	Week 7	21	-0.8 (-32.5, 31)	69.8	-9160.3%	9 (302)	42
	Week 13	19	2.8 (-27.5, 33.1)	62.8	2251%	3 (271)	49
Change to week 7 is defined as week 7 – baseline Change to week 13 is defined as week 3 – baseline Data reported in the original scale							

Table 6.25 – BNP (ng/l) percent changes to weeks 7 and 13 by gender

Group	Week	n	Arithmetic mean (95% CI)	Std dev	Median	Min	Max
Female	Week 7	9	28.8% (-34%, 91.6%)	81.7 %	10%	-75.8%	190.9%
	Week 13	9	-10.9% (-55%, 33.3%)	57.4 %	-25.8%	-63.6%	127.3%
Male	Week 7	21	18.3% (-9.6%, 46.3%)	61.4 %	6.2%	-79.1%	175%
	Week 13	19	15.5% (-11.1%, 42.1%)	55.2 %	4.3%	-63.6%	125%

Table 6.26 – Absolute BNP (ng/l) changes to weeks 7 and 13 by body mass index groups

Group	Week	n	Arithmetic mean (95% CI)	Std Dev	CVb (%)	Median (Range)	IQR
Normal weight	Week 7	5	-40.8 (-175.9, 94.3)	108.8	-266.8	-6 (261)	53
	Week 13	5	-40.2 (-120.3, 39.9)	64.5	-160.4	-12 (151)	12
Overweight	Week 7	11	24 (3.7, 44.3)	30.2	125.9	17 (76)	55
	Week 13	11	25.8 (-2.7, 54.4)	42.5	164.6	25 (163)	35
Obese	Week 7	14	-8.2 (-40, 23.6)	55.1	-670.7	3 (210)	33
	Week 13	12	-15.5 (-48.5, 17.5)	51.9	-334.7	-6 (191)	28

Change to week 7 is defined as week 7 – baseline
Change to week 13 is defined as week 3 – baseline
Data reported in the original scale
Normal weight: 18.5 $kg/m^2 \leq$ body mass index < 25 kg/m^2
Overweight: 25 $kg/m^2 \leq$ body mass index < 29.9 kg/m^2
Obese: body mass index ≥ 30 kg/m^2

Table 6.27 – BNP (ng/l) absolute changes to weeks 7 and 13 by body mass index groups

Group	Week	n	Arithmetic mean (95% CI)	Std Dev	CVb (%)	Median (Range)	IQR
Normal/ Overweight	Week 7	16	3.8 (-32.9, 40.4)	68.8	1833.9	7.5 (302)	43
	Week 13	16	5.2 (-25.5, 35.8)	57.5	1109.2	5.5 (271)	48.5
Obese	Week 7	14	-8.2 (-40, 23.6)	55.1	-670.7	3 (210)	33
	Week 13	12	-15.5 (-48.5, 17.5)	51.9	-334.7	-6 (191)	28

Change to week 7 is defined as week 7 – baseline
Change to week 13 is defined as week 3 – baseline
Data reported in the original scale
Normal weight/overweight: 18.5 $kg/m^2 \leq$ body mass index < 30 kg/m^2
Obese: body mass index ≥ 30 kg/m^2

Table 6.28 – BNP (ng/l) percent changes to weeks 7 and 13 by body mass index groups

Group	Week	n	Arithmetic mean (95% CI)	Std Dev	Median	Min	Max
Normal weight	Week 7	5	-18% (-85.1%, 49.1%)	54%	-30%	-75.8%	54.5%
	Week 13	5	-32.5% (-62.9%, -2.2%)	24.5%	-38.7%	-63.6%	-7.3%
Overweight	Week 7	11	34.6% (-1.3%, 70.6%)	53.5%	40.5%	-45.5%	135.7 %
	Week 13	11	46.2% (9.3%, 83.2%)	55%	29.4%	-33.3%	127.3 %
Obese	Week 7	14	25.2% (-19.9%, 70.3%)	78.1%	8.1%	-79.1%	190.9 %
	Week 13	12	-12.4% (-41.8%, 17%)	46.3%	-27.8%	-69.6%	86.7%

Normal weight: 18.5 kg/m^2 ≤ body mass index < 25 kg/m^2
Overweight: 25 kg/m^2 ≤ body mass index < 29.9 kg/m^2
Obese: body mass index ≥ 30 kg/m^2

Table 6.29 – BNP (ng/l) percent changes to weeks 7 and 13 by body mass index groups

Group	Week	N	Arithmetic mean (95% CI)	Std Dev	Median	Min	Max
Normal weight/ Overweight	Week 7	16	18.2% (-12.5%, 48.9%)	57.6%	9.1%	-75.8%	135.7 %
	Week 13	16	21.6% (-10.3%, 53.6%)	60%	11.8%	-63.6%	127.3 %
Obese	Week 7	14	25.2% (-19.9%, 70.3%)	78.1%	8.1%	-79.1%	190.9 %
	Week 13	12	-12.4% (-41.8%, 17%)	46.3%	-27.8%	-69.6%	86.7%

Normal weight/overweight: 18.5 kg/m^2 ≤ body mass index < 29.9 kg/m^2
Obese: body mass index ≥ 30 kg/m^2

Table 6.30 – BNP (ng/l) absolute changes to weeks 7 and 13 by chronic kidney disease (CKD)

CKD Group	Week	N	Arithmetic mean (95% CI)	Std Dev	CVb (%)	Median (Range)	IQR
Stage I	Week 7	7	28.4 (6.3, 50.6)	28.8	101.4	28 (83)	35
	Week 13	7	10.6 (-16.8, 38.1)	32.8	308.9	-5 (81)	41
Stage II	Week 7	14	4.1 (-18, 26.2)	38.3	941	0 (162)	26
	Week 13	13	-15.5 (-12.2, 43.3)	45.9	295.2	14 (208)	38
Stage III	Week 7	9	-52.6 (-144.3, 39.1)	99.1	-186.6	-2 (270)	154
	Week 13	8	-55.7 (-114.2, 2.8)	63.3	-113.6	-36 (163)	127

Change to week 7 is defined as week 7 – baseline
Change to week 13 is defined as week 3 – baseline
Data reported in the original scale
Stage I: Glomerular filtration rate (GFR) ≥ 90 ml/min
Stage II: Glomerular filtration rate (GFR) between 60 and 89.9 ml/min
Stage III: Glomerular filtration rate (GFR) between 30 and 59.9 ml/min

Table 6.31 – BNP (ng/l) percent changes to weeks 7 and 13 by chronic kidney disease (CKD)

CKD Group	Week	N	Arithmetic mean (95% CI)	Std Dev	Median	Min	Max
Stage I	Week 7	7	51.6% (6.5%, 96.8%)	58.7%	46.4%	-5.8%	190.9 %
	Week 13	7	0.4% (-32.6%, 33.4%)	39.5%	-7.7%	-54.5%	59.8%
Stage II	Week 7	14	20.6% (-19%, 60.3%)	68.7%	2.8%	-72.7%	175%
	Week 13	13	33% (-5.4%, 71.4%)	63.6%	24.7%	-45%	127.3 %
Stage III	Week 7	9	-15.6% (-72.6%, 41.3%)	61.6%	-1.4%	-79.1%	95.1%
	Week 13	8	-33.6% (-61%, -6.3%)	29.6%	-33.3%	-69.6%	19.5%

Data reported in the original scale
Stage I: Glomerular filtration rate (GFR) ≥ 90 ml/min
Stage II: Glomerular filtration rate (GFR) between 60 and 89.9 ml/min
Stage III: Glomerular filtration rate (GFR) between 30 and 59.9 ml/min

Table 6.32 – BNP (ng/l) absolute changes over time by prior cardiovascular medication exposure

Prior medication	Week	N	Arithmetic mean (95% CI)	Std Dev	CVb (%)	Median (Range)	IQR
Beta-blocker	Week 7	27	1.3 (-23.3, 25.9)	62.2	4798.9	3 (302)	43
	Week 13	25	-2.1 (-24.8, 20.6)	55	-2643.5	-4 (271)	37
ACE inhibitor	Week 7	22	-7.5 (-36.3, 21.4)	65.1	-873.7	-2.5 (298)	27
	Week 13	20	-9.9 (-36.5, 16.7)	56.9	-574.5	-6.5 (271)	30.5
Aldosterone Antagonist	Week 7	20	-3.1 (-30.1, 24)	57.9	-1897.8	0.5 (290)	30
	Week 13	18	-0.2 (-26.2, 25.7)	52.2	-23474	-3 (271)	23
Angiotensin 2 Antagonist	Week 7	8	13.6 (-30.8, 58.1)	53.2	390.2	19.5 (174)	45
	Week 13	8	11.9 (-30.6, 54.3)	50.8	427.6	17 (160)	52.5
Diuretic	Week 7	21	-12 (-43.7, 19.6)	69.6	-577.9	3 (290)	33
	Week 13	19	-7.4 (-38, 23.3)	63.5	-862.4	-2 (271)	56

Change to week 7 is defined as week 7 – baseline
Change to week 13 is defined as week 3 – baseline
Data reported in the original scale

Table 6.33 – BNP (ng/l) percent changes over time by prior cardiovascular medication exposure

Prior medication	Week	N	Arithmetic mean (95% CI)	Std Dev	Median	Min	Max
Beta-blocker	Week 7	27	23.4% (-4%, 50.9%)	69.5%	10%	-79.1%	190.9%
	Week 13	25	4.6% (-18.5%, 27.7%)	55.9%	-13.8%	-69.6%	127.3%
ACE inhibitor	Week 7	22	18.1% (-15.6%, 51.8%)	76.1%	-1.1%	-79.1%	190.9%
	Week 13	20	6.1% (-23.4%, 35.5%)	63%	-15.2%	-69.6%	127.3%
Aldosterone Antagonist	Week 7	20	10.9% (-16.9%, 38.6%)	59.3%	2.8%	-75.8%	175%
	Week 13	18	4.1% (-22%, 30.2%)	52.5%	-12.4%	-63.6%	127.3%
Angiotensin 2 Antagonist	Week 7	8	30.7% (4.2%, 57.3%)	31.8%	43.4%	-28.9%	60%
	Week 13	8	9.4% (-22.3%, 41.1%)	37.9%	8.7%	-40%	59.8%
Diuretic	Week 7	21	21.6% (-13.4%, 56.6%)	77%	6.2%	-79.1%	190.9%
	Week 13	19	2.9% (-25.4%, 31.2%)	58.7%	-20%	-69.6%	127.3%

Table 6.34 - BNP (ng/l) absolute changes over time by prior cardiovascular medical history

Prior medical CV history	Week	N	Arithmetic mean (95% CI)	Std Dev	CVb (%)	Median (Range)	IQR
Arrhythmia	Week 7	17	10.6 (-24, 45.3)	67.4	632.8	17 (302)	46
	Week 13	16	-2 (-29.6, 25.6)	51.7	-2585.9	-3 (223)	48.5
Angina	Week 7	4	44.3 (-17, 105.5)	38.5	87	59 (83)	49.5
	Week 13	3	30.7 (-70.9, 132.2)	40.9	133.3	37 (81)	81
Hypertension	Week 7	16	-7.9 (-49.4, 33.6)	77.9	-981.4	3 (302)	37.5
	Week 13	14	-13.8 (-49, 21.5)	61	-442.7	-4 (223)	38
Ischaemic cardiomyopathy	Week 7	11	-9 (-68, 50)	87.9	-976.3	10 (302)	63
	Week 13	10	-5.3 (-54.1, 43.5)	68.2	-1287.6	19 (223)	50
Non-ischaemic cardiomyopathy	Week 7	19	-1.8 (-25.5, 21.9)	49.1	-2743.8	3 (210)	33
	Week 13	18	-6.7 (-33, 19.6)	52.9	-787.2	-5 (249)	20

Change to week 7 is defined as week 7 – baseline
Change to week 13 is defined as week 3 – baseline
Data reported in the original scale

Table 6.35 - BNP (ng/l) percent changes over time by prior cardiovascular medical history

Prior medical CV history	Week	N	Arithmetic mean (95% CI)	Std Dev	Median	Min	Max
Arrhythmia	Week 7	17	30.9% (8.8%, 53.1%)	43.1%	35.7%	-57.6%	135.7%
	Week 13	16	15% (-14%, 44%)	54.4%	-7.7%	-40%	125%
Angina	Week 7	4	41.2% (-11.1%, 93.5%)	32.9%	48.9%	-4.4%	71.3%
	Week 13	3	23.5% (-62.8%, 109.7%)	34.7%	29.4%	-13.8%	54.8%
Hypertension	Week 7	16	31.8% (-11.2%, 74.8%)	80.7%	8.1%	-79.1%	190.9%
	Week 13	14	-2.3% (-33.7%, 29%)	54.3%	-18.3%	-69.6%	125%
Ischaemic cardiomyopathy	Week 7	11	24% (-12.9%, 60.9%)	54.9%	35.7%	-57.6%	135.7%
	Week 13	10	36.2% (-7.3%, 79.9%)	60.8%	42.1%	-38.7%	125%
Non-ischaemic cardiomyopathy	Week 7	19	22.5% (-12%, 57%)	71.6%	6.2%	-79.1%	190.9%
	Week 13	18	-7.1% (-30%, 15.9%)	46.1%	-18.3%	-69.6%	127.3%

Figure 6.1 – LogBNP individual profiles throughout the clinical trial duration – all data included

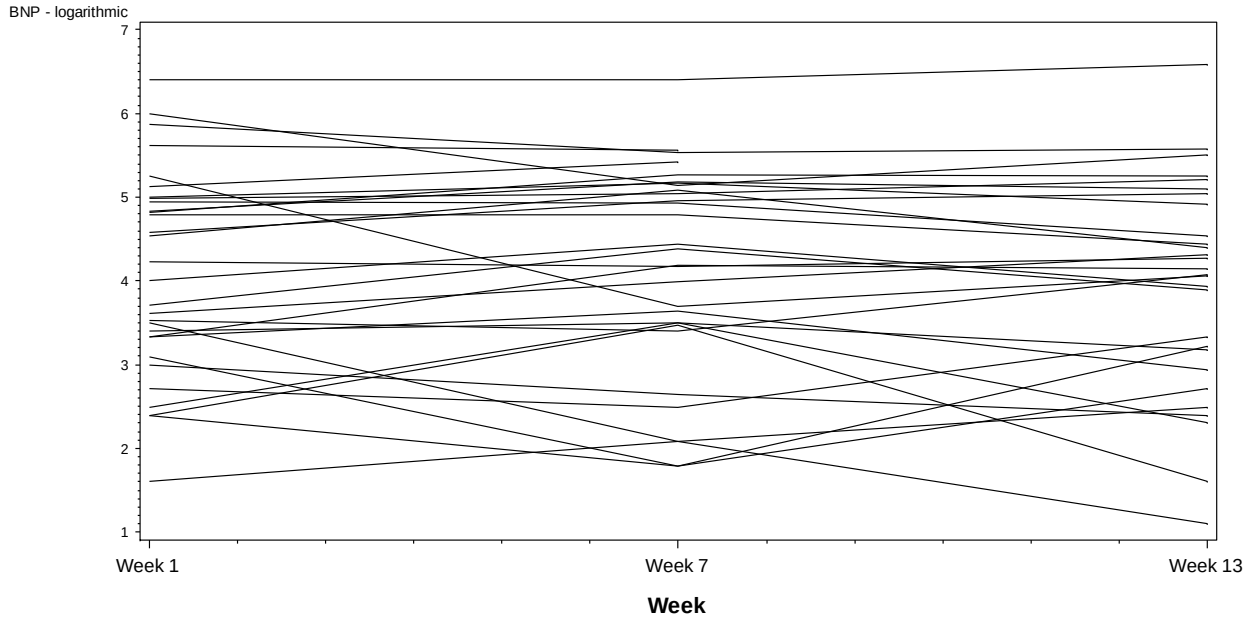


Figure 6.2. – LogBNP individual profiles throughout the clinical trial duration – excluding the two subjects with missing BNP values at week 13

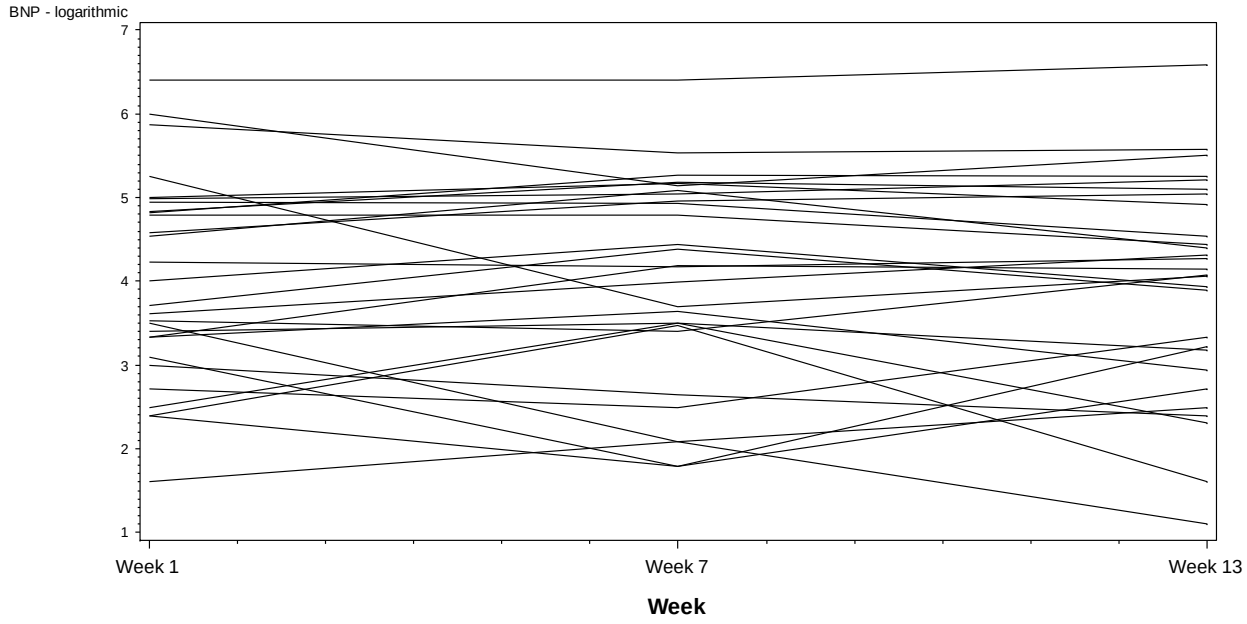


Figure 6.3 – Mean/standard error plot for BNP (ng/l) while excluding the two subjects with missing BNP values at week 13 – BNP in the original scale

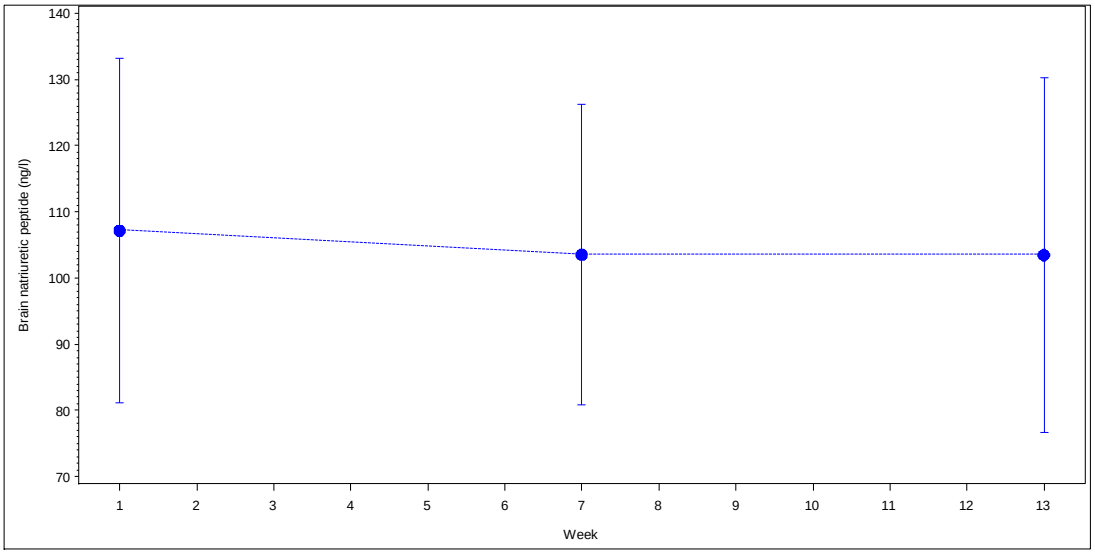


Figure 6.4 – Mean/standard error plot for logBNP over time upon excluding the two subjects with missing BNP values at week 13

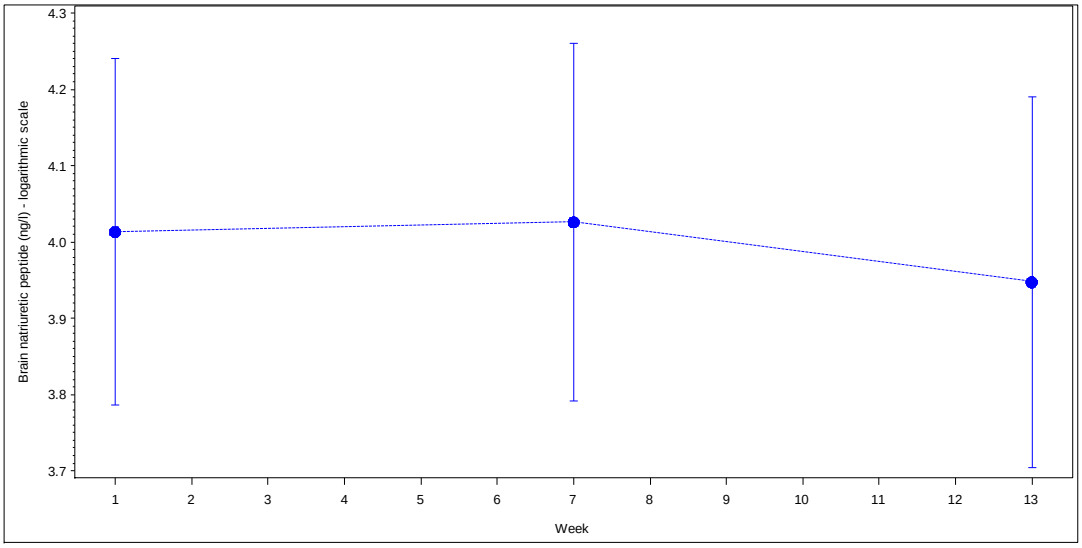


Figure 6.5 – LogBNP and left ventricular ejection fraction at weeks 1 and 13

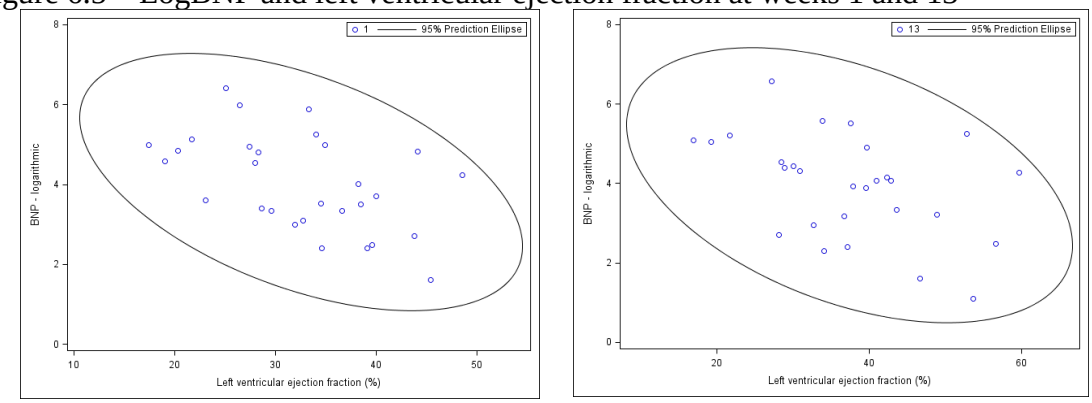
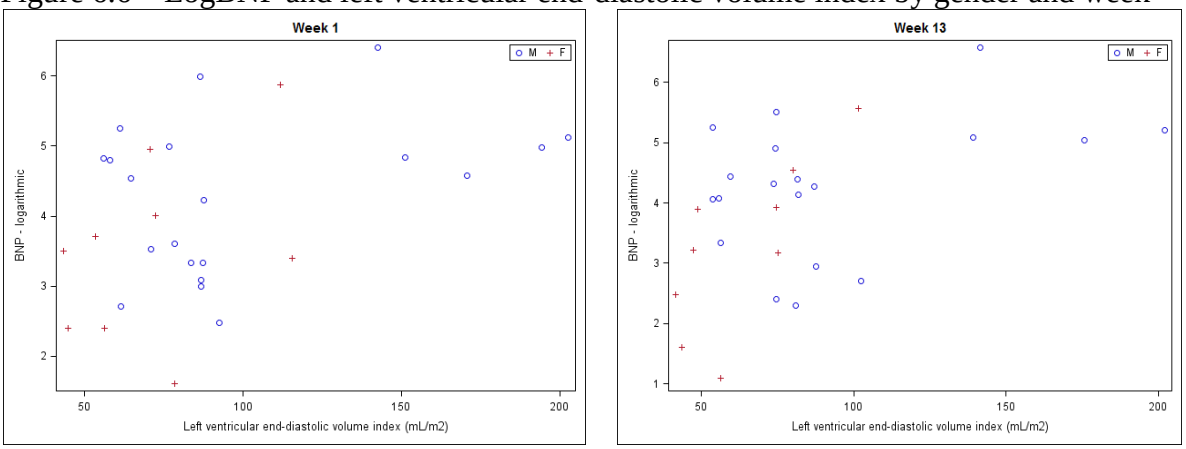
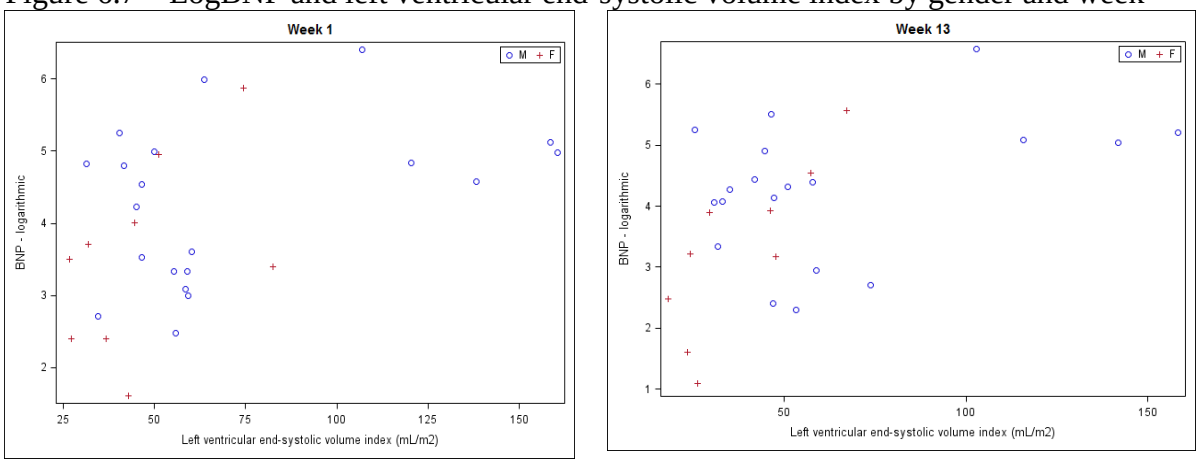


Figure 6.6 – LogBNP and left ventricular end-diastolic volume index by gender and week



M=Male; F=Female

Figure 6.7 – LogBNP and left ventricular end-systolic volume index by gender and week



M=Male; F=Female

Variance-covariance structures adopted in the mixed modeling of BNP variability

Variance Components

$$\begin{bmatrix} \sigma_B^2 & 0 & 0 & 0 \\ 0 & \sigma_B^2 & 0 & 0 \\ 0 & 0 & \sigma_{AB}^2 & 0 \\ 0 & 0 & 0 & \sigma_{AB}^2 \end{bmatrix}$$

Compound Symmetry

$$\begin{bmatrix} \sigma^2+\sigma_1 & \sigma_1 & \sigma_1 & \sigma_1 \\ \sigma_1 & \sigma^2+\sigma_1 & \sigma_1 & \sigma_1 \\ \sigma_1 & \sigma_1 & \sigma^2+\sigma_1 & \sigma_1 \\ \sigma_1 & \sigma_1 & \sigma_1 & \sigma^2+\sigma_1 \end{bmatrix}$$

Heterogeneous Autoregressive

$$\begin{bmatrix} \sigma_1^2 & \sigma_1\sigma_2\rho & \sigma_1\sigma_3\rho^2 & \sigma_1\sigma_4\rho^3 \\ \sigma_2\sigma_1\rho & \sigma_2^2 & \sigma_2\sigma_3\rho & \sigma_2\sigma_4\rho^2 \\ \sigma_3\sigma_1\rho^2 & \sigma_3\sigma_2\rho & \sigma_3^2 & \sigma_3\sigma_4\rho \\ \sigma_4\sigma_1\rho^3 & \sigma_4\sigma_2\rho & \sigma_4\sigma_3\rho & \sigma_4^2 \end{bmatrix}$$

Heterogeneous Toeplitz

$$\begin{bmatrix} \sigma_1^2 & \sigma_1\sigma_2\rho_1 & \sigma_1\sigma_3\rho_2 & \sigma_1\sigma_4\rho_3 \\ \sigma_2\sigma_1\rho_1 & \sigma_2^2 & \sigma_2\sigma_3\rho_1 & \sigma_2\sigma_4\rho_2 \\ \sigma_3\sigma_1\rho_2 & \sigma_3\sigma_2\rho_1 & \sigma_3^2 & \sigma_3\sigma_4\rho_1 \\ \sigma_4\sigma_1\rho_3 & \sigma_4\sigma_2\rho_2 & \sigma_4\sigma_3\rho_1 & \sigma_4^2 \end{bmatrix}$$

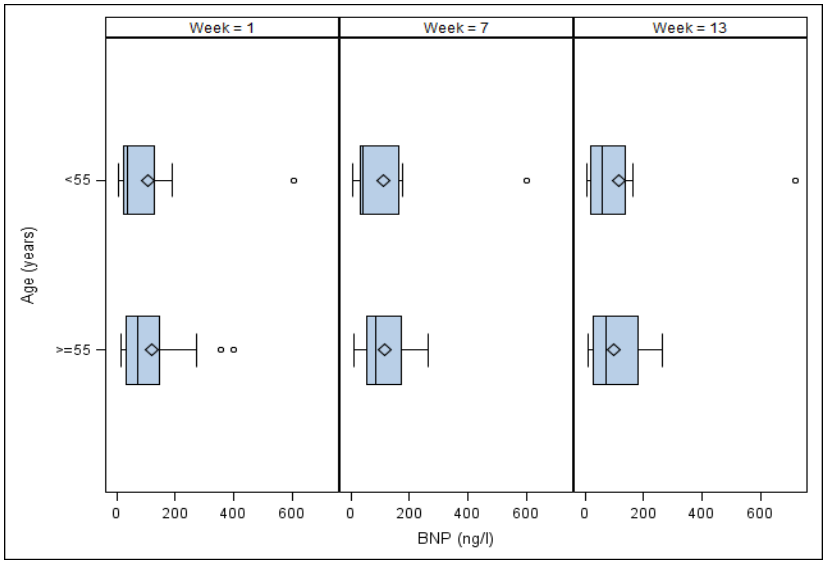
Unstructured

$$\begin{bmatrix} \sigma_1^2 & \sigma_{21} & \sigma_{31} & \sigma_{41} \\ \sigma_{21} & \sigma_2^2 & \sigma_{32} & \sigma_{42} \\ \sigma_{31} & \sigma_{32} & \sigma_3^2 & \sigma_{43} \\ \sigma_{41} & \sigma_{42} & \sigma_{43} & \sigma_4^2 \end{bmatrix}$$

Source: SAS® Online documentation, SAS Institute Inc., Cary, NC

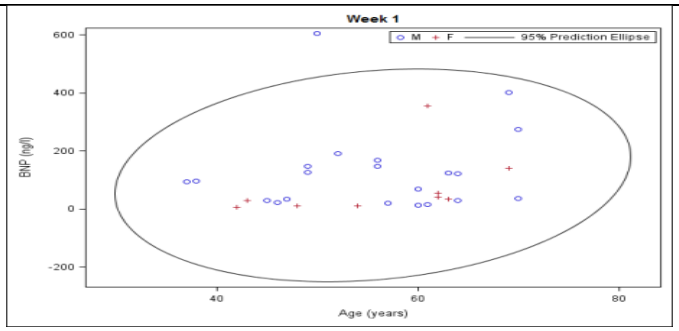
The figure below shows that the BNP interquartile range was slightly higher in the older subgroup. The BNP variability seems higher in subjects younger than 55 years old. Although the means are similar between the two groups, the median BNP values are higher in subjects older than 55 years old.

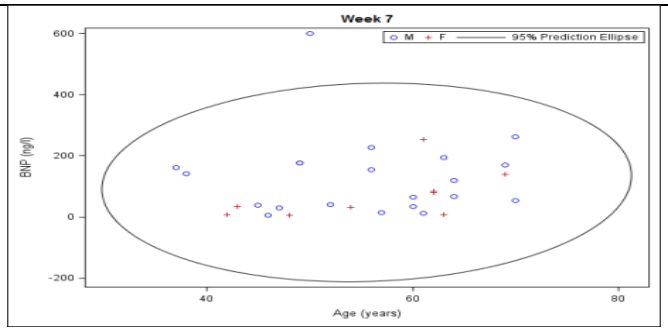
BNP (ng/l) over time by age subroups

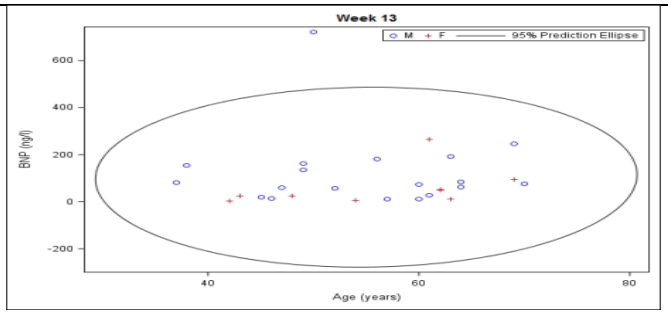


The relationship between BNP and age was also explored with age as a continuous variable. The figure below displays the 95% prediction ellipse for a new observation at week 1, week 7, and week 13, respectively. The ellipse assumed a bivariate normal distribution between BNP and age at each week. It shows a very weak linear correlation between BNP and age at week 1 (Pearson correlation coefficient = 0.176; Spearman correlation = 0.267). The correlation appeared to be stronger for females than males.

BNP values by age and gender over time



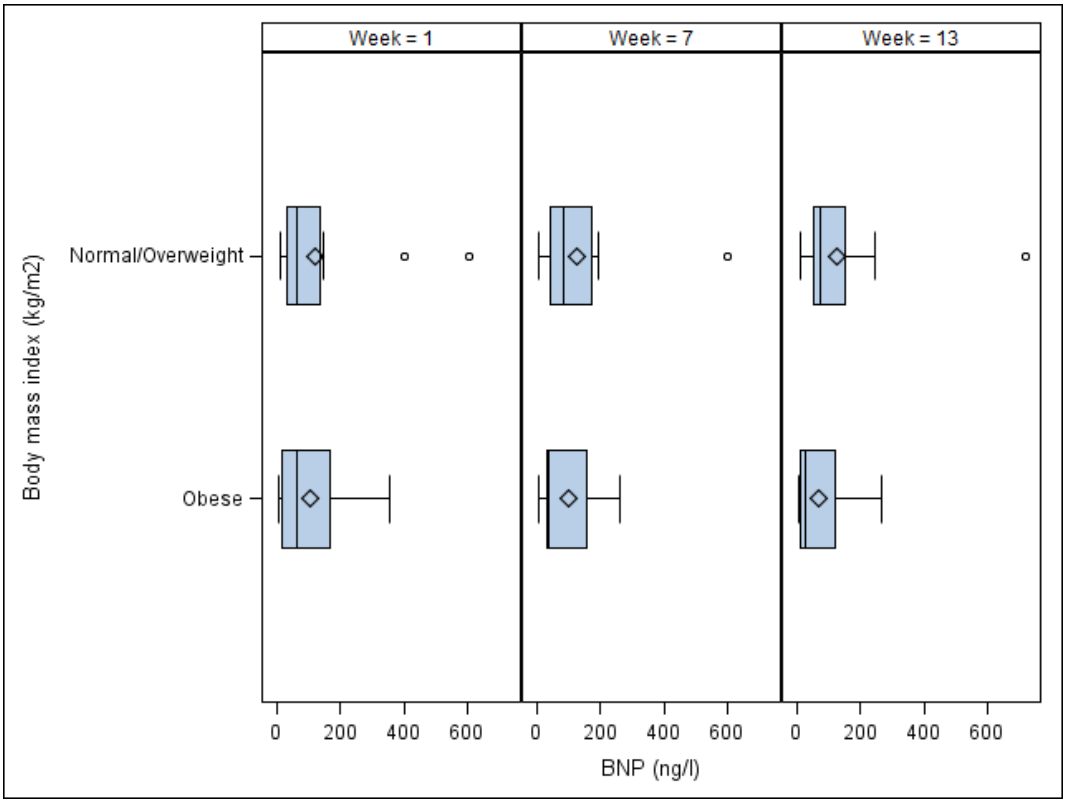




M=Male, F=Female

The figure below displays boxplots of BNP over time in both subgroups. Although the standard deviation and range were higher in the normal and overweight subjects, the inter-quartile range was higher in the obese. As expected, the BNP values were lower in the obese subgroup.

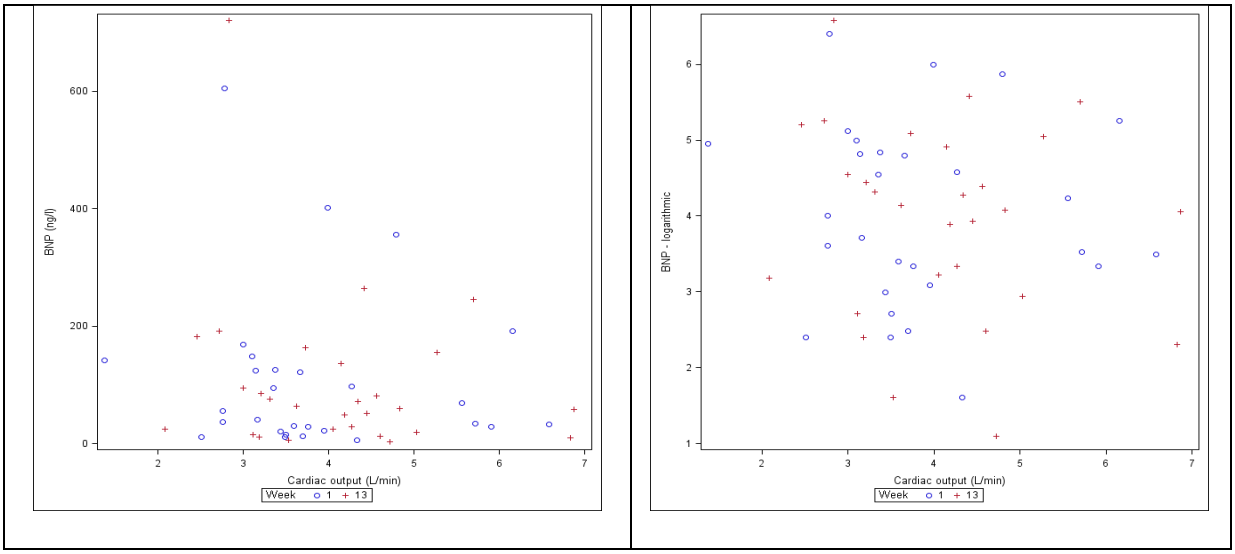
BNP over time by body mass index



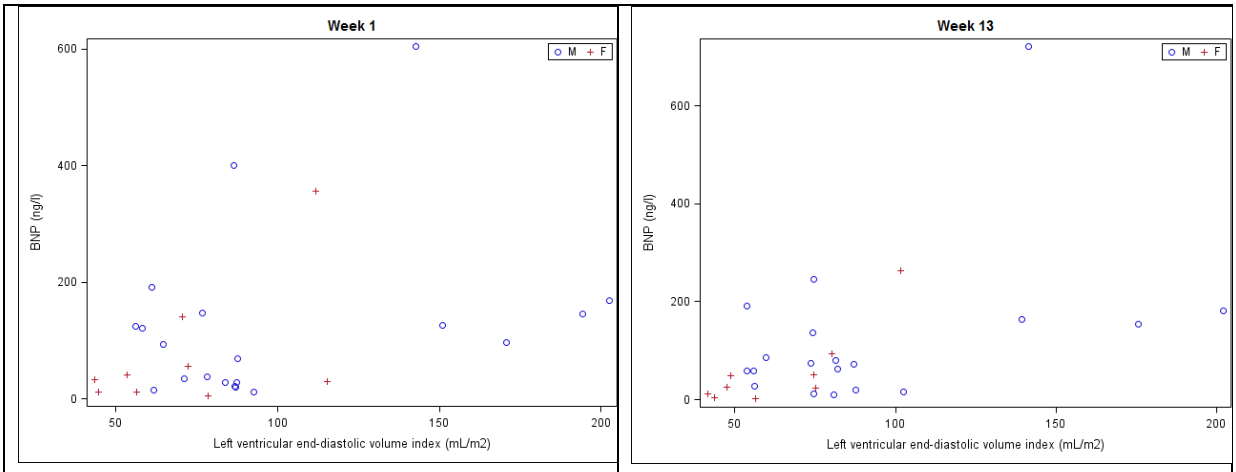
Across all time points, there was a negative correlation between BNP and the body mass index. The Pearson correlation coefficients at week 1, week 7 and week 13 were -0.18, -0.19, and -0.19, respectively. The Spearman correlation coefficients at week 1, week 7, and week 13 were -0.18, -0.11, and -0.21 respectively. None of the correlations were statistically significant at the alpha level of 5%, and this was partly due to the small sample size.

Overall, there was no correlation between the BNP levels and cardiac output at week 1, however a weak, negative linear correlation was observed at week 13, as illustrated in Figure 6.6. It appeared that gender may play a role because of the negative correlation between BNP and cardiac output for males at both time points. For males, the Pearson correlation coefficient was -0.221 at week 1 and -0.295 at week 13, while for females the Pearson correlation coefficients were 0.08 and 0.114 at weeks 1 and 13, respectively.

Scatterplot of BNP and cardiac output



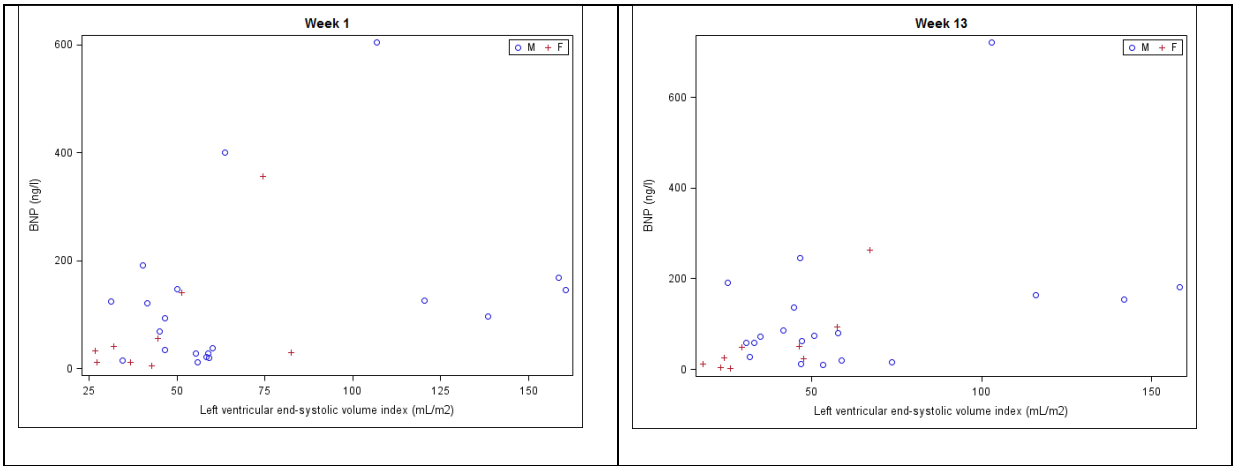
BNP and left ventricular end-diastolic volume index by gender and week



M=Males; F=Females;

Similarly, gender appeared to alter the association between BNP and left ventricular end-systolic volume index. The correlation was much stronger in females, as evidenced by the Pearson correlation coefficient of 0.557 at week 1 and 0.807 (p-value=0.008) at week 13.

BNP and left ventricular end-systolic volume index by gender and week



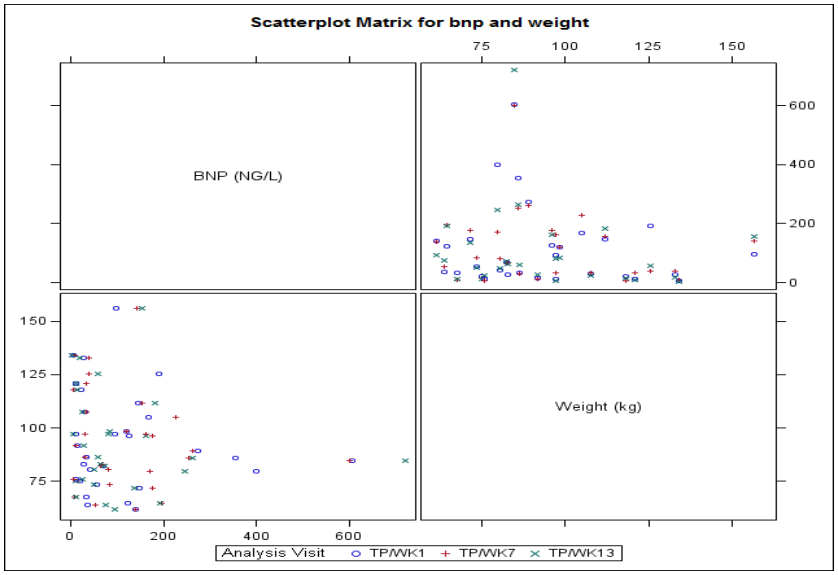
M=Male; F=Female

The serial correlation between the B-type natriuretic peptide and weight, heart rate, blood pressure and creatinine

BNP and weight

The scatterplot matrix shows a weak negative association between BNP and weight at weeks 1, 7 and 13.

Scatterplot matrix of BNP and weight



The correlation coefficients between BNP (ng/l) and weight (kg)

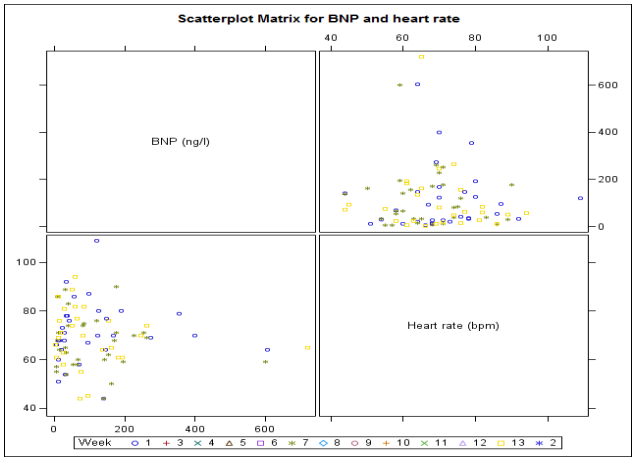
Type of correlation	Week	n	Correlation estimate	95% Confidence interval	p-value
Pearson	1	30	-0.134	(-0.471, 0.237)	0.474
	7	30	-0.169	(-0.499, 0.202)	0.367
	13	28	-0.141	(-0.488, 0.245)	0.469
Spearman	1	30	-0.136	(-0.473, 0.235)	0.468
	7	30	-0.150	(-0.484, 0.221)	0.421
	13	28	-0.247	(-0.568, 0.137)	0.197

Both the Pearson and Spearman correlation coefficients showed a very weak negative correlation between the B-type natriuretic peptides and weight at weeks 1, 7, and 13. The correlation coefficients were approximate -0.15, except for the week 13 when the Spearman correlation was -0.25. At all weeks, the 95% confidence intervals included 0 and the p-values were not statistically significant. The correlation coefficients hardly seemed to change over the three-month period.

BNP and heart rate

The relationship between the B-type natriuretic peptides and heart rate is visualized in the figure below. Despite some outliers due to high BNP values, overall there is no association between the BNP and heart rate at week 1 and a very weak one at weeks 7 and 13.

Scatterplot matrix of BNP and heart rate



The linear and rank correlations between the B-type natriuretic peptides and heart rate over time are presented in the table below. It doesn't appear to be a correlation between BNP and heart rate during the three-month study period. Both the Pearson and Spearman correlation coefficients are close to zero. Their corresponding 95% confidence intervals include zero, while the p-values are not statistically significant.

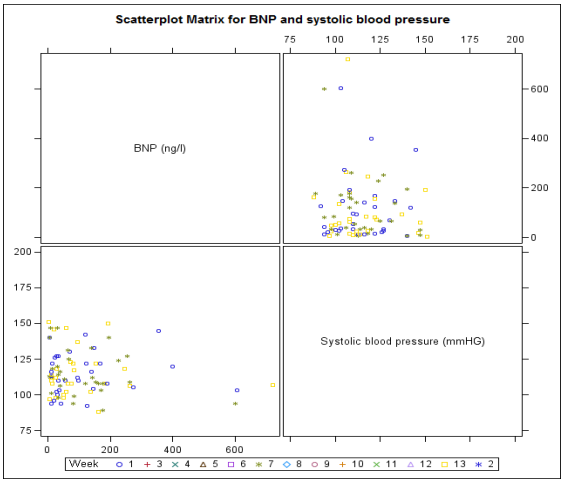
The correlation coefficients between BNP and heart rate

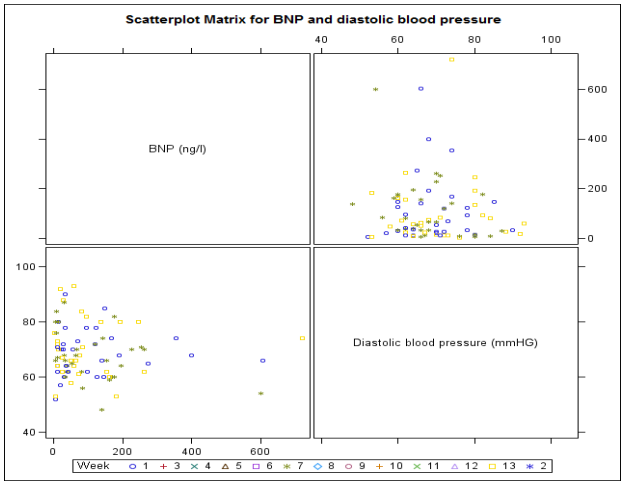
Type of correlation	Week	n	Correlation estimate	95% Confidence interval	p-value
Pearson	1	30	0.018	(-0.343, 0.376)	0.920
	7	30	-0.108	(-0.451, 0.261)	0.563
	13	28	-0.125	(-0.476, 0.259)	0.518
Spearman	1	30	0.004	(-0.116, 0.563)	0.168
	7	30	0.029	(-0.334, 0.385)	0.875
	13	28	-0.147	(-0.493, 0.238)	0.449

BNP and blood pressure

The relationship between the B-type natriuretic peptides and blood pressure was depicted below. The figure shows a random pattern.

Scatterplot matrix of BNP and blood pressure





The correlation coefficients between BNP and blood pressure

Blood pressure	Type of correlation	Week	n	Correlation estimate	95% Confidence interval	p-value
Systolic	Pearson	1	30	0.066	(-0.301, 0.416)	0.724
		7	30	-0.264	(-0.570, 0.106)	0.152
		13	28	-0.09	(-0.449, 0.291)	0.643
	Spearman	1	30	0.049	(-0.316, 0.402)	0.795
		7	30	-0.276	(-0.579, 0.092)	0.132
		13	28	-0.005	(-0.377, 0.368)	0.977
Diastolic	Pearson	1	30	0.017	(-0.344, 0.375)	0.925
		7	30	-0.346	(-0.628, 0.015)	0.056
		13	28	0.011	(-0.363, 0.382)	0.954
	Spearman	1	30	0.149	(-0.223, 0.483)	0.426
		7	30	-0.328	(-0.615, 0.036)	0.071
		13	28	0.016	(-0.358, 0.387)	0.931

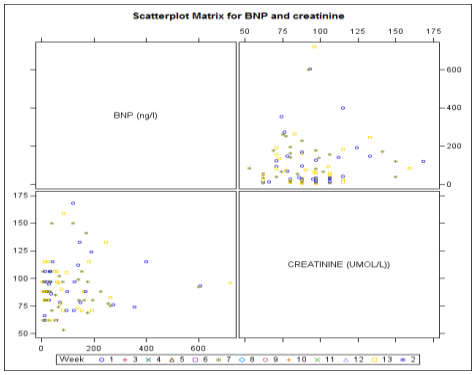
No association between the B-type natriuretic peptides and blood pressure was apparent at weeks 1 and 13. Both the Pearson and Spearman correlation estimates were almost zero. Furthermore, the 95% confidence intervals included zero, while the p-values were not statistically significant. In the middle of the clinical trial, at week 7, a very weak negative

correlation between the B-type natriuretic peptides and blood pressure emerged. At this moment in time, the linear and ranked correlation coefficients were approximate -0.3.

BNP and serum creatinine

The figure below showed no association between the B-type natriuretic peptide and serum creatinine.

Scatterplot matrix of BNP and serum creatinine



No correlations between BNP and serum creatinine were observed at weeks 1, 7, and 13 in all chronic heart failure patients, males, and females. The Pearson and Spearman correlation coefficients were mostly close to zero. The correlations based on ranks indicated a very weak association between the B-type natriuretic peptides and serum creatinine in females only. This inconsistent correlation was mildly positive at the beginning of the trial and negative three months into the study.

The correlation coefficients between BNP and serum creatinine

Group	Type of correlation	Week	n	Correlation estimate	95% Confidence interval	p-value
Overall (all cohort)	Pearson	1	30	0.088	(-0.281, 0.434)	0.640
		7	30	0.001	(-0.358, 0.361)	0.993
		13	28	0.068	(-0.312, 0.430)	0.725
	Spearman	1	30	0.145	(-0.226, 0.480)	0.438
		7	30	-0.026	(-0.382, 0.337)	0.889
		13	28	0.009	(-0.364, 0.381)	0.959
Males	Pearson	1	21	0.079	(-0.365, 0.493)	0.730
		7	21	-0.065	(-0.483, 0.377)	0.776
		13	19	0.116	(-0.356, 0.542)	0.629
	Spearman	1	21	-0.004	(-0.435, 0.428)	0.984
		7	21	-0.089	(-0.502, 0.355)	0.694
		13	19	0.103	(-0.367, 0.532)	0.667
Females	Pearson	1	9	-0.041	(-0.686, 0.640)	0.914
		7	9	-0.013	(-0.671, 0.656)	0.972
		13	9	-0.154	(-0.742, 0.567)	0.683
	Spearman	1	9	0.217	(-0.522, 0.770)	0.564
		7	9	0.114	(-0.594, 0.723)	0.763
		13	9	-0.277	(-0.794, 0.474)	0.457

Changes in BNP and changes in the cardiac structure factors

Changes in BNP (ng/l)	Correlation coefficients (p-value)	
	Pearson	Spearman
Change in Cardiac output (L/min)	-0.15 p-value=0.433 n=27	-0.10 p-val=0.587 n=27
Change in Left ventricular ejection fraction (%)	-0.17 p-value=0.385 n=28	-0.09 p-val=0.643 n=28
Change in Left ventricular ejection fraction index (%/m^2)	-0.10 p-value=0.593 n=28	-0.03 p-val=0.843 n=28
Change in Left ventricular end diastolic volume (mL)	0.11 p-val=0.550 n=28	-0.02 p-val=0.899 n=28
Change in Left ventricular end diastolic volume index (mL/m^2)	0.10 p-val=0.585 n=28	-0.02 p-val=0.887 n=28
Change in Left ventricular end systolic volume (mL)	0.15 p-val=0.419 n=28	0.01 p-val=0.972 n=28
Change in Left ventricular end systolic volume index (mL/m^2)	0.138 p-val=0.482 n=28	-0.01 p-val=0.971 n=28
Change=week 13 – week 1(baseline)		

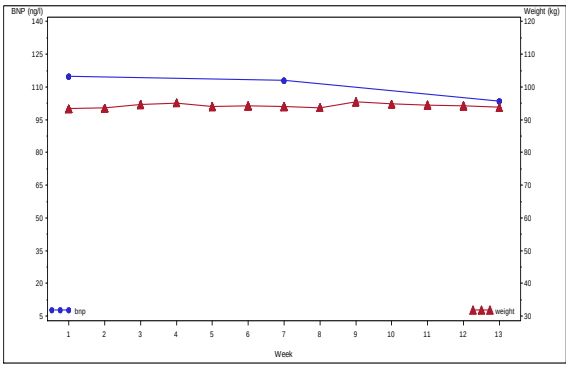
This table showed the weak correlation between changes in BNP and changes in the cardiac structure factors from baseline to week 13.

Weight, heart rate, blood pressure, serum creatinine and BNP over time

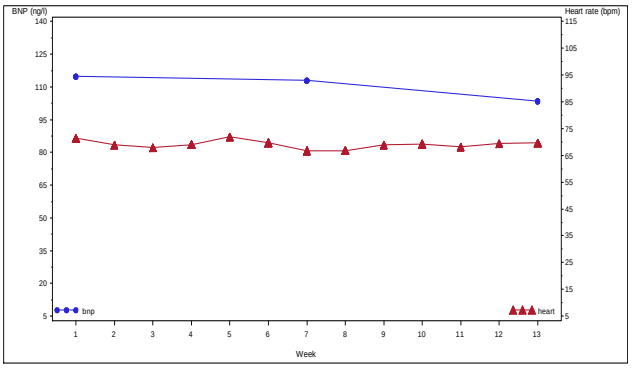
The average weight, heart rate, and BNP levels during the study period were plotted below. While the B-type natriuretic peptides slightly decreased towards the end of the study, the average weight followed mostly a flat pattern. Although the heart rate fluctuated somewhat towards the middle of the study, the final levels were similar to the baseline.

Mean weight, heart rate and BNP over time

Mean weight and BNP



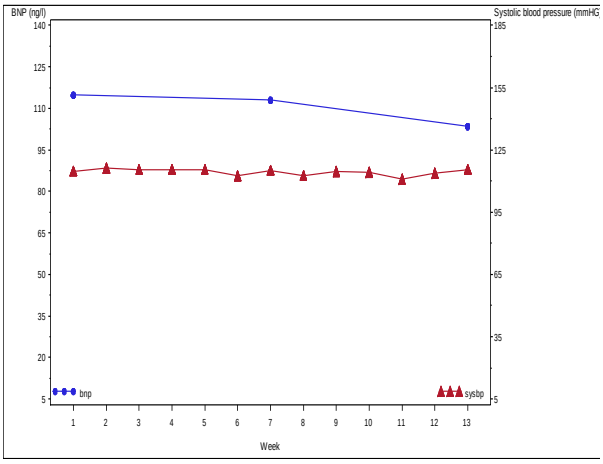
Mean heart rate and BNP



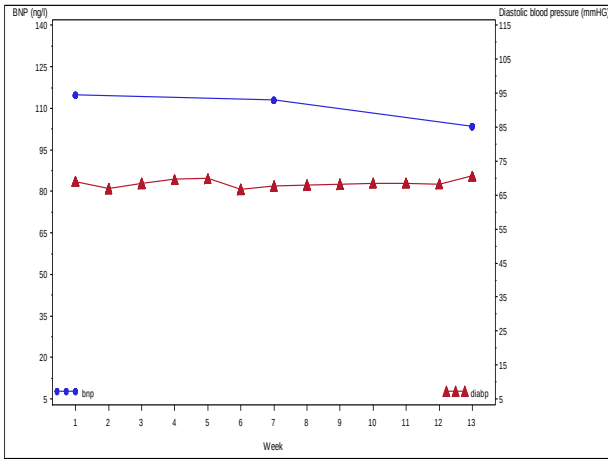
The average blood pressures are illustrated below. The systolic blood pressure follows a flat pattern over time. The diastolic blood pressure slightly oscillated until the middle of the study period, but it appeared flat afterward.

Mean systolic and diastolic blood pressures and BNP

Mean systolic blood pressure and BNP

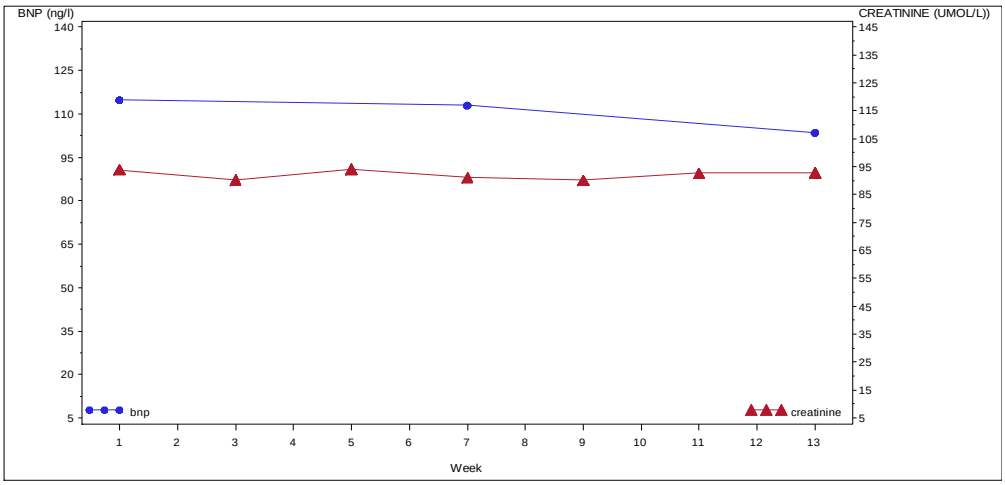


Mean diastolic blood pressure and BNP



As illustrated below, the serum creatinine levels were stable over time. On average, the serum creatinine slightly decreased at the end of the trial compared to the start of the study. However, the decrease was noted only in men, as a slight increase in the serum creatinine was observed in women.

Mean serum creatinine and BNP



The between-subject coefficient of variation was approximately 25% for weight and serum creatinine. When evaluating the serum creatinine by gender, the between-subject coefficient of variation ranged from 21.4% to 26.6% in males and from 22.1% to 33.7% in nine females. During the study period, the between-subject coefficient of variation varied between 14.7% and 19.6% for the heart rate. Regarding the systolic and diastolic blood pressures, the between-subject coefficient of variation ranged from 12.7% to 18.8% and from 11.5% to 16%, respectively.

The between-subject coefficient of variations for weight, heart rate, blood pressure, serum creatinine and B-type natriuretic peptides are presented in the following table by week.

The between-subject coefficients of variation for weight, vital signs, creatinine, and BNP

Week	Weight (kg)	Heart rate (bpm)	Blood pressure (mmHg)		Creatinine* (umol/l)	BNP** (ng/l)
			Systolic	Diastolic		
Week 1	24.8%	17.8%	12.7%	12.2%	24.3%	119.2%
Week 2	24.6%	14.7 %	18.8%	15.9%	-	-
Week 3	24.8%	15.9%	14.9%	11.8%	26.4%	-
Week 4	24%	18.5%	14.4%	12%	-	-
Week 5	24.6%	15.8%	14.8%	12.7%	25%	-
Week 6	25%	19.6%	15.4%	11.5%	-	-
Week 7	24.5%	16.7%	13.5%	13.4%	25.7%	107.7%
Week 8	25.4%	15.3%	15.1%	15.1%	-	-
Week 9	24%	16.2%	16%	16%	23.2%	
Week 10	25.4%	15.5%	14.5%	13.3%	-	-
Week 11	25%	17.3 %	16.9%	17%	24.6%	-
Week 12	25.3%	15.3%	15%	13.3%	-	-
Week 13	25.6%	16.8%	14.5%	15.2%	23.2%	136.8%

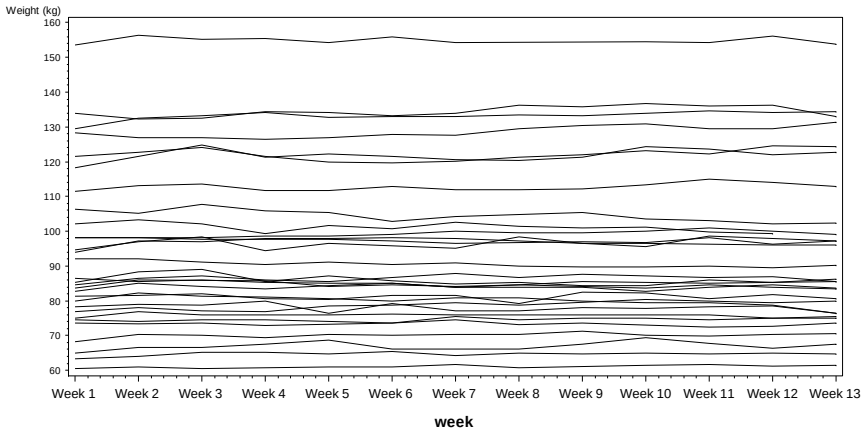
*Serum creatinine was measured biweekly at weeks 1, 3, 5, 7, 9, 11, and 13
**BNP was measured every at weeks 1, 7, and 13

The within-subject variability in weight, heart rate, blood pressure, and serum creatinine

WEIGHT

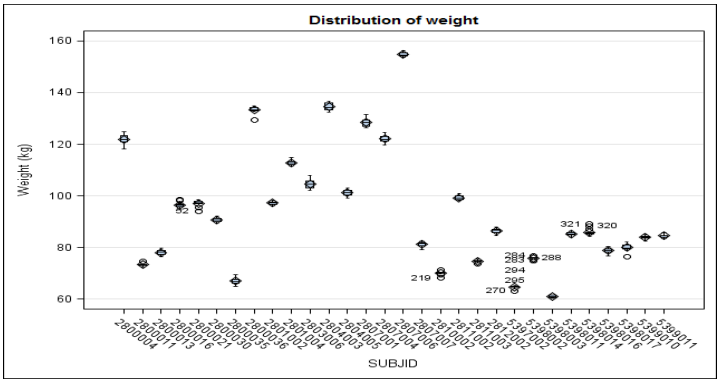
The individual weight profiles over time are displayed in the figure below for the entire cohort. The chronic heart failure patients were heterogeneous regarding their weight. At week 1, the weight ranged from 60.5 kg to 153.5 kg. Half of the patients weighed less than 85 kg. The between-subject variability for weight was approximately 25%. Regardless the patients' baselines, the weight profiles were mostly flat, indicating small variability over time.

Individual weight profiles over time



The weight was measured weekly; thus each patient had up to thirteen weight measurements taken during the study period. The mean and standard deviation from this mean as well as the within-subject coefficient of variation were calculated for each patient. While the average within-subject coefficient of variation was 1.11%, it ranged from 0.58% to 1.82%. The figure below displays the distributions of weights for each patient, indicating the low within-subject variability despite the outliers. Although the patients had different individual weights, the variability was small.

Individual patient's distribution of weight over time



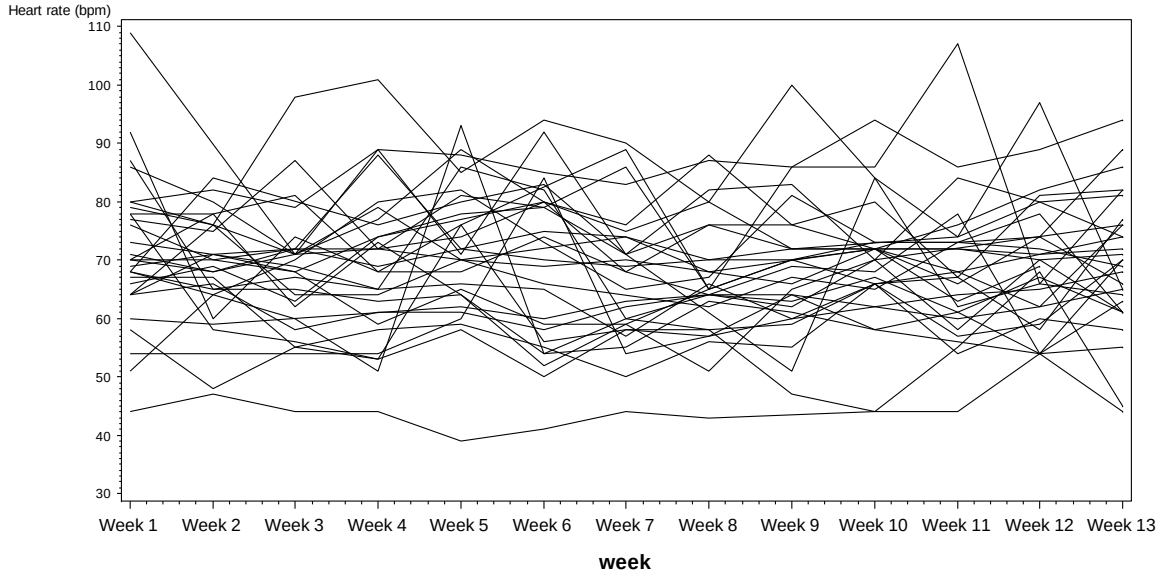
Note: the numbers close to the boxplots denote the number of observation in the dataset

The within-subject coefficient of variability for weight was estimated as 1.2% from the random intercept model. This value was almost identical to the average within-subject coefficient of variation aforementioned. In the random intercept model with weight as the dependent variable, the week was treated as a fixed term and intercept as a random effect. The estimated residual variance was 1.2599. The estimate for the fixed effect weight was 0.02 with a standard error of 0.01, showing that the week did not have a significant effect on the weight. The p-value was 0.1398 based on a t-value of 1.48 and 352 estimated degrees of freedom. The distribution of residuals, conditional residuals, as well as influence diagnostic metrics indicated a reasonable fit of the data.

HEART RATE

Individual heart rate profiles over time are displayed in the figure below. For most of the patients, the values go up and down during the study period. The change appeared to be more drastic in some patients than others. The between-subject coefficient of variation for heart rate was between 15% and 20% during all weeks. At week 1, the lowest heart rate was 44 beats per minute, while the highest one was 109 beats per minute in a 64-year old obese male patient. At week 13, while the lowest heart rate was the same, the highest observed value was 94 beats per minute. Three patients had single episodes of abnormally elevated heart rates throughout the study period. These high heart rates were between 101 and 109 beats per minute. These three patients were white males with ages between 47 and 64 years old. There were no repeat episodes of high heart rate for the same patient.

Individual heart rate profiles over time

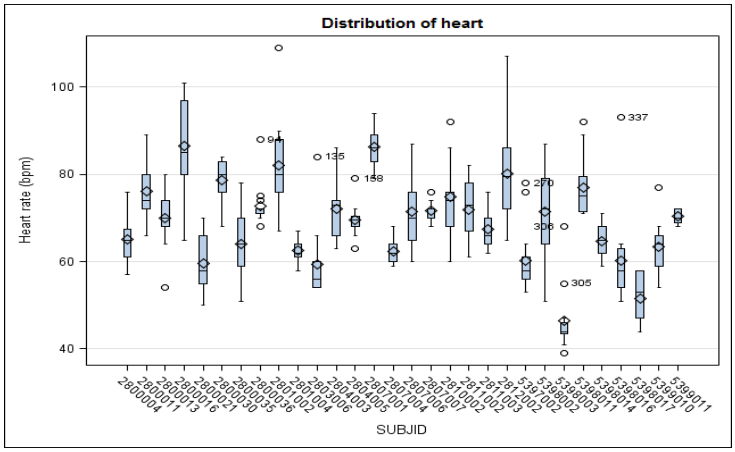


Despite the individual spikes, on average the heart rate was stable. For example, at week1, the mean heart rate was 71.4 beats per minute, while at week 13 it was 69.6 beats per minute. The median heart rates were very close to the average heart rates.

Each patient had thirteen heart rate measurements that were taken weekly during the study period. The average within-subject variability for the heart rate was 9.6%. Half of the patients had a within-subject coefficient of variation less than 9.3%. One 70-year old male patient had very similar heart rates, and his within-subject coefficient of variation was 1.9%. A 48-year old woman had the highest within-subject coefficient of variation of 17.9%. Except for the week 5, when her heart rate was 93 beats per minute, all other times her heart rate levels were in the middle 50s and low 60s.

The individual patient's heart rate distribution is displayed in below. Although there were a few outliers present, the within-subject variability for these patients was close to the average variability of the heart rate.

Distribution of individual patient's heart rate over time



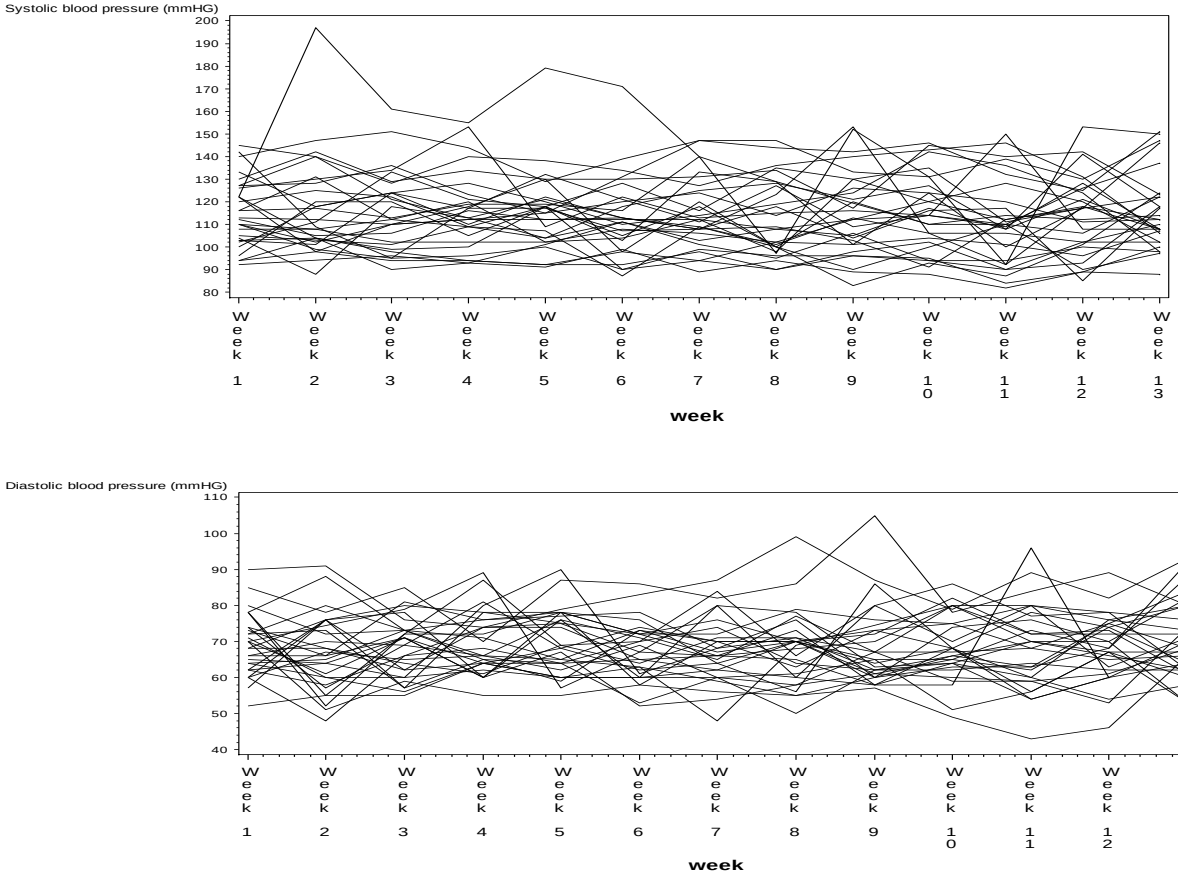
The estimated within-subject coefficient of variation was 10.3% based on the random intercept model. In this statistical model, the heart rate was a dependent variable and week was a fixed term, while the intercept was considered random. The residual variance was 50.392. The week, when the measurement was taken didn't impact the heart rate. The fixed effect for the week was -0.09 with the standard error as 0.096 (e.g. t-value=-0.93, df=353, F-value=0.87, p-value=0.35). The fit statistics and residuals indicated a reasonable fit. The estimated within-subject coefficient of variation (10.3%) was slightly higher than the average within-subject coefficient (9.65%).

BLOOD PRESSURE

The individual patient profiles for the systolic blood pressure are displayed in the figure below. Most of the patients had some pre-high or high systolic blood pressure readings. However, on average the systolic blood pressure was normal.

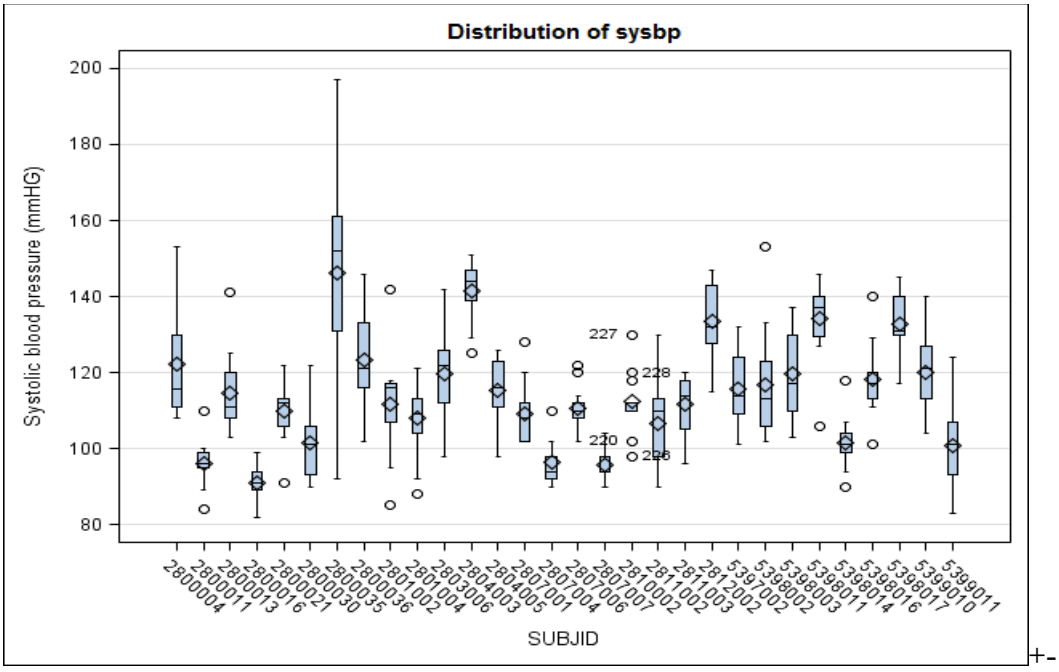
The diastolic blood pressure went up and down from normal to pre-high values and vice versa. For example, a 62-year old female had a very low diastolic blood pressure of 43 mmgHg at week 11, but at week 13 the diastolic blood pressure was normal again. The graph shows three high spikes of diastolic blood pressure values closer to 100 mmHg in the second part of the trial. They belonged to three male patients in their late 40s who also had a high systolic blood pressure of approximately 150 mmHg. These patients were White, African-American and Native-Hawaiian or Other Pacific Islander. Despite the individual profiles, on average the diastolic blood pressure was normal during the clinical trial.

Individual blood pressure profiles over time



Each patient had weekly blood pressure measurements. The individual distributions for the systolic blood pressures are plotted below. The highest systolic blood pressure variation was observed for a 63-year old male patient. The systolic blood pressure readings for this subject ranged from 92 mmHg to 197 mmHg. His within-subject coefficient of variation was 20.6%. There was an additional patient, a 42-year old female who had a systolic blood pressure higher than 140 mmHg, on average. Her systolic blood pressure readings were consistently closer to her average, and therefore the variability in her systolic blood pressure measurements was low. For the entire cohort, the average within-subject coefficient of variation was 8.7%. Half of the chronic heart failure stable patients had the within-subject coefficient of variation higher than 8%. The smallest within-subject coefficient of variation was 3.8%. This 62-year old female patient had her systolic blood pressure measurements between 92 mmHg and 104 mmHg.

Distribution of systolic blood pressure over time

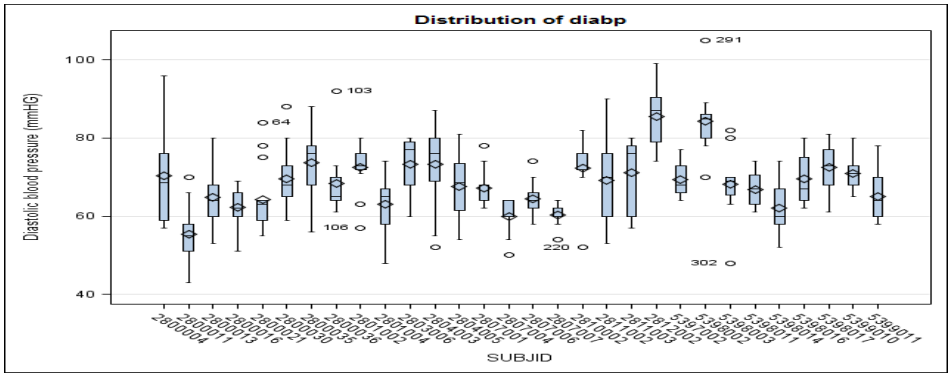


The individual distributions for the diastolic blood pressure are plotted in the figure below. The highest diastolic blood pressure of 105 mmHg was observed in a 49-year old male patient at week 9 into the clinical trial. However, all his other diastolic blood pressure readings were consistently between 70 mmHg and 89 mmHg and on average, his systolic blood pressure measurements were normal. His B-type natriuretic peptide levels were 148 ng/l, 176 ng/l and 136 ng/l at weeks 1, 7, and 13, respectively. His within-subject coefficient of variation was 9.5%. The highest variability of 17.7% was seen in the first patient displayed on the plot. The patient was a 46-year old man with the diastolic blood pressure ranging from 58 mmHg to 96 mmHg. He had one single episode of high diastolic blood pressure at week 11 when his systolic blood pressure was also high. All his other diastolic blood pressure readings were normal, however he did experience a pre-high systolic blood pressure at week 2 and elevated systolic blood pressure of over 150 mmHg at weeks 3 and 11. His B-type natriuretic peptide levels were very low: 22ng/l, 6 ng/l and 15 ng/l at weeks 1, 7, and 13, respectively.

The smallest within-subject coefficient of variation of 4.4% was observed in a 62-year old female patient. Her blood pressure readings were normal and B-type natriuretic peptide levels were low. Her smallest diastolic blood pressure value of 54 mmHg was the observation 220 on the plot.

On average, the within-subject coefficient of variation for the diastolic blood pressure was 10.3% for the entire placebo cohort. It ranged from 4.4% to 17.7%. Half of the placebo patients had a within-subject coefficient of variation less than 9.7%. Patients with a baseline B-type natriuretic peptide of at least100 ng/l or 200 ng/l had a similar within-subject coefficient of variation of approximately 10% for the diastolic blood pressure.

Distribution of diastolic blood pressure over time



Note: Numbers on the plot show the observation id in the dataset.

Based on the random intercept statistical model, the within-subject coefficient of variation for the systolic blood pressure was estimated as 9.7% for the entire placebo cohort. The residual variance was 124.26. The subject with the highest influence on the residual variance was a 64-year old male who had the highest systolic blood pressure of 197 mmHg at week 2. The estimated random intercept was 174.3 mmHg. The week, when the systolic blood pressure was measured did not have an impact on the systolic blood pressure. The fixed effect estimate for week was -0.17 with a standard error of 0.1512 (t-value=-1.15, F-value=1.32, df=353, p-value=0.2516).

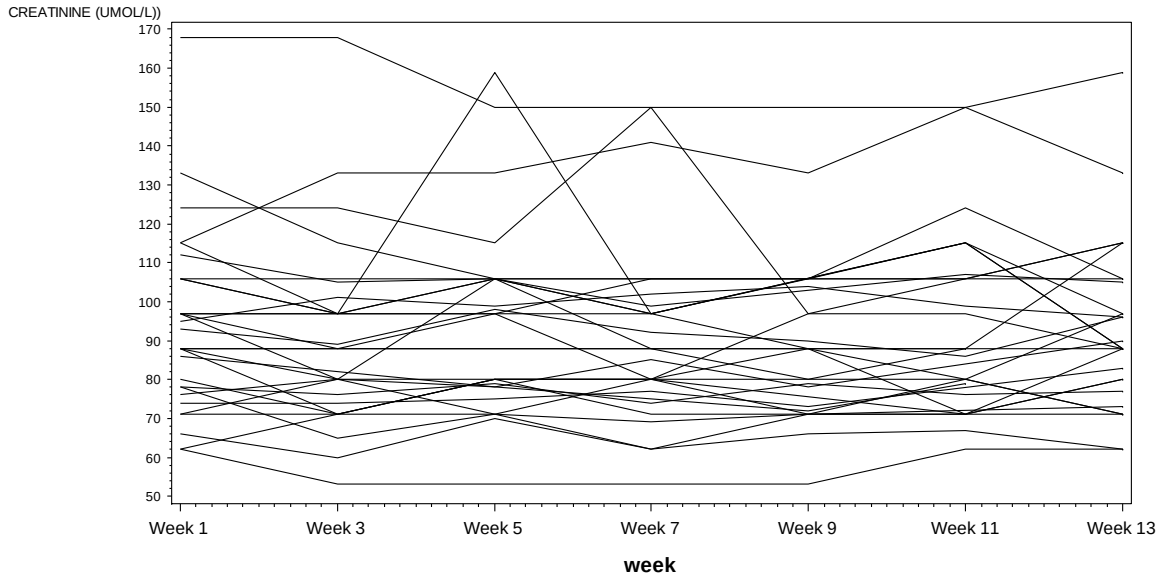
The random intercept statistical model estimated the within-subject coefficient of variation as 10.7% for the diastolic blood pressure. The estimation was very close to the observed average within-subject coefficient of variation reported above. The residual variance was 54.4721. The patient with the highest within-subject coefficient of variation had the highest influence on the residual variance. The estimated intercept was 68.2 mmHg with the corresponding standard error of 1.354 mmHg. Patients had significantly different diastolic blood pressures at the start of the trial. The week, when the diastolic blood pressure was taken did not impact the diastolic blood pressure reading. The fixed effect estimate for the week was 0.05 with a standard error of 0.1.

The estimated within-subject subject coefficient of variation was approximately 10% for the systolic blood pressure when including all placebo patients. It slightly increased to 11.5% in twelve patients with a BNP of at least 100 ng/l at baseline. It slightly decreased to approximately 9% in four patients with a baseline B-type natriuretic peptide of at least 200 ng/l at baseline. The estimated within-subject coefficient of variation was about 10% for the diastolic blood pressure.

SERUM CREATININE

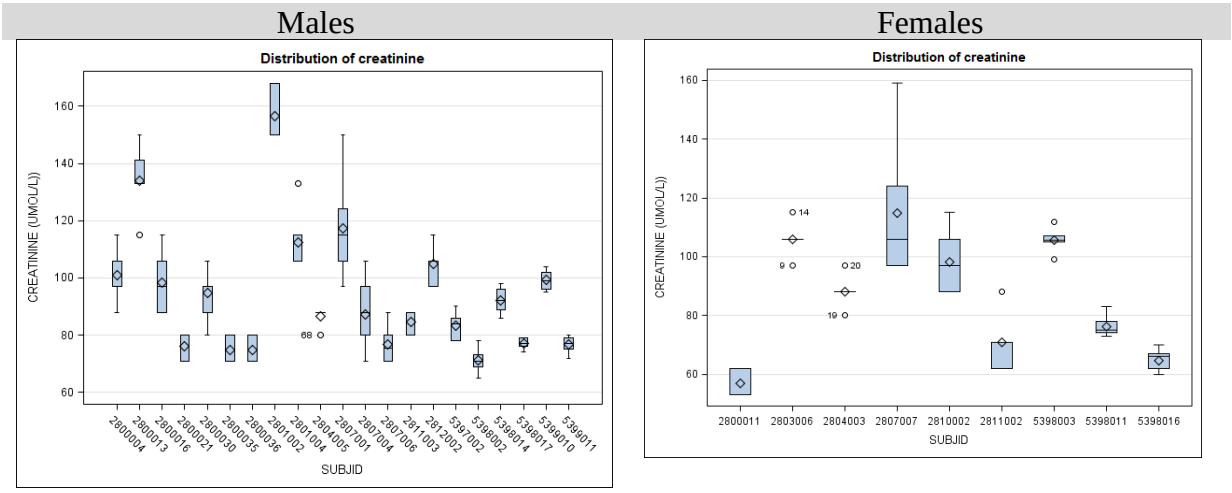
The individual patient profiles for serum creatinine were displayed in the figure below. Patients had very different creatinine levels at the start of the trial. The between-subject coefficient of variation was close to 25%. At week 1, the serum creatinine ranged from 62 umol/l to 168 umol/l. Although the serum creatinine fluctuated during the study, on average, the baseline measurements were very similar to those at the end of the trial. There were four males with high serum creatinine levels.

Individual patient profiles for serum creatinine over time



The figure below displays the patients' distributions for serum creatinine by gender. The plot shows two male patients with all high serum creatinine levels. The average within-subject coefficient of variation among males was 6.9%. The average within-subject coefficient of variation for females was 8.2%.

Distribution of serum creatinine by gender over time



There were only two females with a baseline B-type natriuretic peptide level higher than100 ng/l. One of them was 61-year old with high BNP levels such as: 356 ng/l, 253 ng/l and 264 ng/l at weeks 1, 7 and 13, respectively. The other 69-year old female patient had BNP measurements of 141 ng/l, 139 ng/l and 94 ng/l at weeks 1, 7 and 13, respectively. The average within-subject coefficient of variation for serum creatinine for these two women was 4.2%. Ten out of twenty-one males had a baseline B-type natriuretic level of at least 100 ng/l. Among these male patients, the average within-subject coefficient of variation was 7.1%. There were only three males with a baseline BNP of at least 200 ng/l. The average within-subject coefficient of variation for the serum creatinine in this subgroup of patients was 5.4%.

The random intercept statistical model that included the entire cohort estimated the within-subject coefficient of variation as 8.9%. The residual variance was 67.6543. The statistical model included the intercept as a random factor, week as a fixed term and serum creatinine as the depended variable. The estimate for the fixed effect week was -0.0741 with the standard error of 0.1443. The corresponding F-value was 0.26 and p-value was 0.608. A 41-year old female patient appeared to have the highest impact on the serum creatinine variability. Without this female patient, the estimated within-subject coefficient of variation was slightly smaller,

8.1%. The average within-subject coefficient of variation based on the entire placebo cohort was 7.3%, somewhat lower than the estimated within-subject coefficient of variation of 8.9%. The fit statistics, examination of residuals, as well as the influence analyses indicated a reasonable fit of the random intercept model. While the estimated serum creatinine variability for the entire cohort was similar to those with a baseline B-type natriuretic peptide measurement of 100/ng or higher, it decreased to approximately 6% in the four patients with a baseline BNP of at least 200 ng/l.

Among the twenty one males, the within-subject coefficient of variation was between 2.4% and 14.9%, while the average within-subject coefficient of variation was 6.9%. The estimated within-subject coefficient of variation for males was somewhat higher, 8.1%. This was based on a residual variance of 58.5394. A 52-year old patient with serum creatinine levels ranging from 97 umol/l to 150 umol/l throughout the clinical trial had the highest impact on the serum creatinine variance among males. Without this patient, the estimated within-subject coefficient of variation was 6.6%, very similar to the average within-subject coefficient of variation. Among all men, as well as males with a baseline B-type natriuretic peptide level of at least 100 ng/l, the estimated within-subject coefficient of variation was approximately 8%. It decreases to 6.6% in three male patients who had a baseline BNP of at least 200 ng/l.
The highest variability in the serum creatinine was observed in women. Among all females, the estimated within-subject coefficient of variation was a 10.7%. In the two female patients with a BNP of at least 100 ng/l, the within-subject variability was approximately 4%. There was one female patient with a baseline BNP of over 200 ng/l and her serum creatinine levels ranged from 73 mmol/l to 83 mmol/l throughout the study period. The average within-subject

coefficients of variation were smaller than the within-subject coefficient of variations estimated from the random intercept model for the serum creatinine.

The estimated within-subject coefficient of variation for serum creatinine was 8.9% based on the thirty subjects, 8.1% in twenty one males and 10.7% in nine females.