DURBAN UNIVERSITY of TECHNOLOGY

THE PREDICTIVE VALUE OF PRO BRAIN NATRIURETIC PEPTIDE (ProBNP) LEVELS TO DETERMINE THE PRESENCE AND SEVERITY OF CORONARY ARTERY DISEASE IN PATIENTS WITH A POSITIVE OR INCONCLUSIVE EXERCISE STRESS TEST

By

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AUTHORS DECLARATION

I declare that this research study: **The predictive value of proBNP levels to determine the presence and severity of coronary artery disease in patients with a positive or inconclusive exercise stress test** represents original work by the author. All the theoretical information and related sources that have been used or quoted have been duly acknowledged by means of complete references. It is further declared that this dissertation has not previously been submitted to any institution for degree purposes.

The research described in this dissertation was supervised by Prof J K Adam (M Med Sc, PhD: Clinical Technology), in the department of Biomedical and Clinical Technology, at the Durban University of Technology and Dr A Pearce (Cardiologist) in the Department of Cardiology, at St Anne's Hospital, Pietermaritzburg, South Africa.

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ABSTRACT

INTRODUCTION: Cardiovascular disease (CVD) is one of the major causes of premature deaths worldwide. In South Africa, approximately 195 people die from cardiovascular diseases each day. The earlier coronary artery disease (CAD) is detected, the better the prognosis. NT- pro- brain natriuretic peptide (NT-proBNP) is a cardiac neurohormone that is secreted in the cardiac ventricles in response to excessive stretching of heart muscle cells. Brain natriuretic peptide (BNP) is currently being used as a marker of left ventricular dysfunction but limitations are evident in patients with sepsis, volume overload, stroke and acute mitral regurgitation.

OBJECTIVES: The main objective of this study was to identify a possible value of NT- proBNP level which indicates CAD. It also aimed to compare NT- proBNP levels with the number of diseased vessels; to assess the association between proBNP levels and patients' age and gender; to determine the percentage of false positive proBNP levels; to determine the probability of false positive exercise stress testing and to correlate NT- proBNP levels with LVEDP.

METHODS: Sixty patients were recruited from the Cardiology Department at St Anne's hospital to participate in this trial. They were divided into two groups; Group A, the control group, consisted of thirty patients with a positive EST and Group B, the experimental group, consisted of thirty patients with an inconclusive EST. After the EST, all patients from both groups were required to have a NT- proBNP blood test, a left and right coronary angiogram and a left ventriculogram.

RESULTS: Results of the study showed that post EST NT- proBNP levels, in both groups, increased in the presence of CAD ($p<0.001$). For the positive EST group, the area under the ROC curve was 0.975 which was highly statistically significantly different from the null hypothesis value of 0.5 ($p<0.001$) and a cut- off value of 120 pg/ml was identified with the highest sensitivity (95.7%) and specificity (100%). For patients in the inconclusive EST group, the area under the ROC curve was 0.912 which was highly statistically significantly different from the null hypothesis value of 0.5 ($p<0.001$) and a cut-off value of 85 pg/ml was identified with the highest sensitivity (87.5%) and specificity (86.4%). There was a statistically significant difference between the median NT- proBNP values of males and females in the group of patients with positive EST ($p=0.048$). The values were higher in males. However, there was no significant difference between the genders in the group with an inconclusive EST. A strong and significant correlation ($p<0.001$) between left ventricular end diastolic pressures (LVEDP) and number of disease vessels was demonstrated. The probability of a false positive result for EST was 24.1%. and the probability of a false negative result was 25.8%.

CONCLUSION: Results of the study showed that post EST NT- proBNP levels, in both groups, increased in the presence of CAD and could accurately predict the presence of CAD. Cut- off values of 120 pg/ml for the positive EST group and 85 pg/ml for the inconclusive EST group were identified with the highest sensitivity and specificity. In the positive EST group, a trend of increasing NT-proBNP with age was and NT-proBNP values were higher in males. The positive EST was relatively accurate at predicting CAD; however, 75.9% of patients with an inconclusive EST did not have CAD.

Exercise stress testing in this regard, is therefore relatively inaccurate at predicting CAD in patients with inconclusive ESTs, and the need for an additional tool, such as NT-proBNP measurements post inconclusive EST is warranted in the determination of the presence of CAD.

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ABBREVIATIONS

AMI	Acute Myocardial Infarction
BNP	Brain Natriuretic peptide
CABG	Coronary artery bypass grafts
CVD	Cardiovascular disease
COPD	Chronic Obstructive Pulmonary Disease
CHF	Congestive heart failure
CAD	Coronary artery disease
ECG	Electrocardiogram
EST	Exercise Stress Test
HR	Heart Rate
ICU	Intensive Care Unit
LCA	Left coronary artery
LV	Left Ventricle
LVEDP	Left Ventricular End Diastolic Pressure
LVEF	Left Ventricular Ejection Fraction
LVF	Left ventricular failure
MI	Myocardial Infarction
NT-proBNP	N- Terminal ProBNP
PCI	Percutaneous Coronary Intervention
ProBNP	Pro- Brain Natriuretic Peptide
RCA	Right coronary artery

CHAPTER ONE: INTRODUCTION

Diseases arising from cardiovascular (CV) causes are still the most common cause of death worldwide (Howie- Esquivel, 2008). Coronary artery disease (CAD), also known as ischaemic heart disease, is the most common manifestation of cardiovascular disease and accounts for approximately 17 million deaths a year, which is almost one- third of all deaths globally (Lloyd-Jones, Adams and Carnethon, 2009). By 2020, cardiovascular disease (CVD) will become the leading cause of both death and disability worldwide, with the number of fatalities projected to increase to over 20 million a year and by 2030 to over 24 million a year (Lloyd-Jones, Adams and Carnethon, 2009). Although advances in cardiovascular treatment has greatly improved the prognosis of patients with CAD, it is still the first cause of death and the World Health Organization (WHO) has also predicted that for the next 20 years it will remain as such (Graham, Atar and Borch-Johnsen, 2007).

Improper diagnosis of coronary artery disease (CAD) leads to incorrect or late treatment of the disease. Early diagnosis of this disease may provide patients with effective and less costly treatment as well as adequate time to make necessary lifestyle changes that will help to slow down the progression of CAD and prolong the patient's survival. Despite lifestyle modifications, current treatment options, and surgical procedures, cardiovascular disease still remains a major problem and there is clearly a need for new, more cost- effective and easily usable tools in the early detection and prevention of CAD (Graham, Atar and Borch-Johnsen, 2007).

Exercise stress testing (EST) is currently the most cost effective, non invasive test for diagnosing coronary artery disease. However, unreliability of the stress test approach as a means of diagnosing CAD has been well documented.

While changes in the ECG during EST can be helpful in diagnosing CAD, the interpretation of the results are limited as they are affected by several conditions, including but not limited to hypertension, left ventricular hypertrophy, cardiac rhythm disorders, medications and gender (Berger, 2001).

According to Fletcher, Balady, Amsterdam, Chaitman, Eckel,. Froelicher, Leon, Piña, Rodney, Simons-Morton, Williams and Bazzarre (2001), cardiac stress tests are only capable of detecting medium to high-grade coronary stenoses and not atheroma and vulnerable plaques which are the primary causes of myocardial infarctions. Most of the "vulnerable plaques" cause less than 40% lumen narrowing, a degree of stenosis too small for most stress testing methods to detect (Fletcher et al., 2001). Clinical trials conducted in the late 1990s have shown that vulnerable plaques are commonly present within many regions of the coronary arteries, yet are typically relatively flat and do not protrude into the arterial lumen sufficiently to produce enough stenosis (usually less than 50%, average 20% by some IVUS studies) to be detectable by current stress testing methods. Since current stress testing methods requires medium to high- grade stenosis to detect the risk of myocardial infarction, stress testing alone is seen as an unreliable approach of detecting CAD (Fletcher et al., 2001).

MIBI is an acronym for 2- methoxy isobutyl isonitrile and is currently the sought after non-invasive method of identifying coronary artery disease or myocardial infarction (MIBI scan, 2010). However, this procedure is too costly and time consuming for many patients and the expertise, in this setting, is fairly limited. Therefore, this procedure was not employed in this study. There is also a variety of other imaging techniques that have been recently introduced in detecting vulnerable plaques, such as high resolution computer tomography (CT) myocardial perfusion magnetic resonance imaging (MRI). The sensitivity and specificity of

these nuclear tests (sensitivity 81%, specificity 85-95%) are much higher than treadmill stress testing (sensitivity 67% and specificity 70%) (Jerosch-Herold, Seethamraju, Swingen, Wilke and Stillman, 2004), however, they are still too costly and there is limited expertise is this field.

A limitation of treadmill exercise stress testing is that it encounters a fair amount of false positive and false negative results. The results from a study conducted by Gibbons, Balady, Timothybricker, Chaitman, Fletcher, Froelicher, Mark and McCallister in 2002, confirmed this when compared with other clinical tests.

Reduction of blood flow to the cardiac muscle results in myocardial ischaemia which usually occurs as a result of coronary atherosclerosis, but it may also reflect dynamic components of coronary vascular resistance. Figure 1 illustrates the temporal sequence of the ischemic cascade leading to angina.

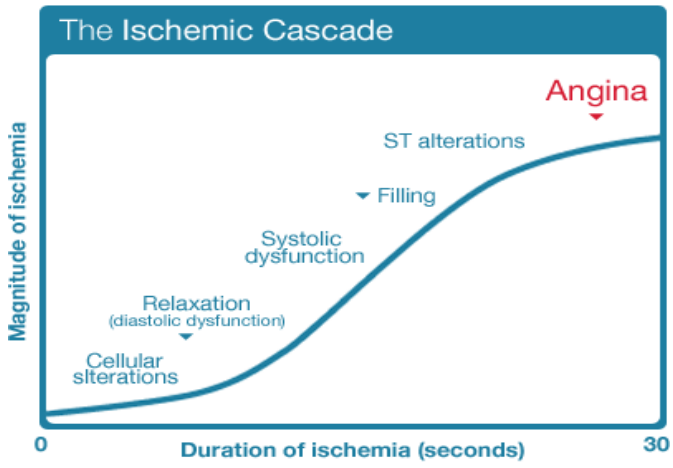


Figure 1: Sequence of the ischaemic cascade leading to angina (Cannon and Lee, 2008).

Atherosclerosis, which typically begins in later childhood, predominantly produces artery wall thickening and artery enlargement, not lumen narrowing. Lumen narrowing typically

occurs as part of healing responses after vulnerable plaque ruptures. Most of these ruptures being clinically silent. Thus lumen narrowing represents only a symptom of very advanced disease, a state which typically requires several decades to develop. Additionally, no stress methods quantitatively measure actual or needed blood flow to the heart muscle. They only detect imbalances of blood flow between regions of the left ventricular muscle which are beyond the capabilities of the arterioles in the downstream vascular bed to fully compensate (Aviles, Messerli, Askari, Penn and Topol, 2004).

The reduction of oxygenated blood flow may cause cardiac dysfunction which may manifest as angina (Cannon and Lee, 2008). According to the angina cascade (Figure 1), as the level of exercise increases, the myocardium first shows diastolic stiffness, then progressive depression of contractility, before the onset of pain. This is why dyspnoea develops as a feature of painless ischaemia. This suggests that chest pain is a late manifestation of ischaemia, and that other events such as cardiac dysfunction, have already occurred. Therefore, measurements by biomarkers, like NT-proBNP, may be of great use in the detection of coronary artery disease.

The early appearance of a perfusion defect helps explain why nuclear stress tests are such a good stratifier and why perfusion images in other modalities are keenly developed. The late appearance of systolic contraction abnormalities does mean that false positives are unusual in dobutamine and physical stress imaging (Cannon and Lee, 2008).

N- Terminal pro brain natriuretic peptide (NT- proBNP) is a cardiac neurohormone that is produced by cardiac myocytes. The main stimulus for peptide synthesis and secretion is myocyte stretch which is caused due to volume expansion and increased filling pressure of the ventricles (Kragelund, Grønning, Omland, Køber, Strande, Steffensen and Hildebrandt,

2006). NT- proBNP can be measured by immunoassay in human blood (The future of cardiac biomarkers, 2009).

During exercise stress testing, changes in the electrocardiogram (ECG) maybe suggestive of CAD. In this study, patients with a strong positive exercise stress test (see pg. 45) are required to have a coronary angiogram. For those patients with an inconclusive EST (see pg. 46) there was doubt that CAD was present (Ellestad, 2003). In this case further costly diagnostic testing such as a nuclear/ 2- methoxy isobutyl isonitrile (MIBI) scan was required.

In this study, coronary angiography was used as the "gold standard" of detecting coronary artery disease in patients with a positive and an inconclusive exercise stress test. After each EST, patients that met the inclusion criteria had their NT- proBNP levels measured and then underwent coronary angiography to determine the presence and severity of CAD. The results of this study might determine whether NT- proBNP levels increases sensitivity and specificity of predicting the presence and severity of coronary artery disease. This cardiac biomarker can possibly be of great use in complementing the tools used in the diagnosis of patients with important coronary artery disease.

CHAPTER TWO: STUDY BACKGROUND AND LITERATURE REVIEW

2.1 INTRODUCTION

Cardiovascular disease is a major global health problem, with the majority of the burden occurring in developing countries (Yusuf, Phil, Reddy, Ôunpuu and Anand, 2001). Currently, Sub-Saharan African (SSA) countries are experiencing one of the most rapid epidemiological transitions characterized by increasing urbanization and changing lifestyle factors resulting in an increase of cardiovascular diseases (BeLue, Okoror, Iwelunmor, Taylor, A. Degboe, Agyemanq and G. Ogedegbe, 2009). Studies indicate that urbanization and economic development have resulted in nutritional transitions characterized by a shift to a higher caloric diet and/or reduction of physical activity.

According to statistics from the South African Heart and Stroke Foundation, 195 people had died per day, between 1997 and 2004, as a result of some form of heart and blood vessel disease (Heart disease in SA, 2010).

Globally, and including South Africa, risk factors (such as, smoking, alcohol consumption, obesity, high caloric diet, increased physical inactivity, psychosocial factors, diabetes, hypertension and high lipid levels) have been found to account for up to 90%, of myocardial infarctions and other poor CVD outcomes such as stroke. In South Africa, CVD is the second leading cause of death after HIV accounting for up to 40% of deaths among adults (BeLue et al., 2009). The current transitions experienced by South Africans can create major challenges in future public health care, and failure to address and correct the problems may impose significant burden for the health sector and the economy of sub-Saharan African countries (BeLu et al., 2009).

Most of our knowledge pertaining to CVD and its prevention, diagnosis and treatment are derived from studies conducted in developed countries and predominantly among the white populations. Therefore, it is vital that we establish appropriate research studies, tailored to the South African population to increase awareness of the CVD burden, and develop better diagnostic and preventive therapies in South Africa (Yusuf et al., 2001).

At present, exercise stress testing is being utilized as the method of choice in diagnosing coronary artery disease. According to Fletcher et al., 2001, the sensitivity and specificity of treadmill testing are 67% and 70% respectively. The sensitivity and specificity of nuclear testing is 81% and 85-95% respectively (of advanced lumen narrowing). However, these percentages refer to the detection of coronary artery luminal narrowing as assessed by stress methods compared with coronary angiography as the gold standard (Fletcher et al., 2001). Since luminal narrowing is not the main foundation of most heart attacks, clinical cardiology experience reveals that the actual sensitivity and specificity values for detecting the probability of a future heart attack, as opposed to lumen narrowing, are much lower than stated above. Whatever the actual numbers, the value of stress tests has increasingly been recognized as limited (Fletcher et al., 2001).

A positive EST [i.e. hypotension (a drop of greater than 10 mmHg in systolic pressure) or greater than 2 mm ST-segment depression] has been reported to strongly suggest the presence of coronary artery disease (Aviles, Messerli, Askari, Penn and Topol, 2004). However, it is well known that patients develop more symptoms on exertion than at rest, but not all patients are symptomatic or develop ischaemia on exercise, resulting in an inconclusive EST. For those patients with an inconclusive EST (i.e. ECG changes that are not diagnostic of ischaemia) there is still doubt whether CAD is present (Essig, 2007). So, to rule out any possibility of CAD, a coronary angiogram will have to be performed to determine the

presence, location and severity of disease (Aviles et al., 2009) or alternatively a CT or MIBI scan can be done. Therefore, a further cost effective and less traumatic diagnostic tool other than more expensive methods of risk stratification, is necessary.

Newer emerging cardiac biomarkers, such as proBNP, can be of great use in complementing the tools available in the diagnosis of patients with myocardial ischaemia (Howie- Esquivel, 2008).

Considerable research is currently being undertaken on this cardiac biomarker and some of the key questions are whether this new biomarker assay can contribute to patient care and will the assay provide any information for early detection, diagnosis, risk stratification, monitoring of disease progression, or can it assist in the selection of the appropriate therapies? (Howie- Esquivel, 2008).

In the present study, some of these important questions are answered and hope to provide cardiologists with vital information in diagnosing coronary artery disease.

2.2 CORONARY ARTERY ANATOMY

The heart is composed mainly of cardiac muscle tissue that continuously contracts and relaxes, requiring a continuous supply of oxygen and nutrients (Figure 2). Coronary arteries deliver blood to the heart muscle and are therefore very important (Figure 3) (Moore and Agur, 2002).

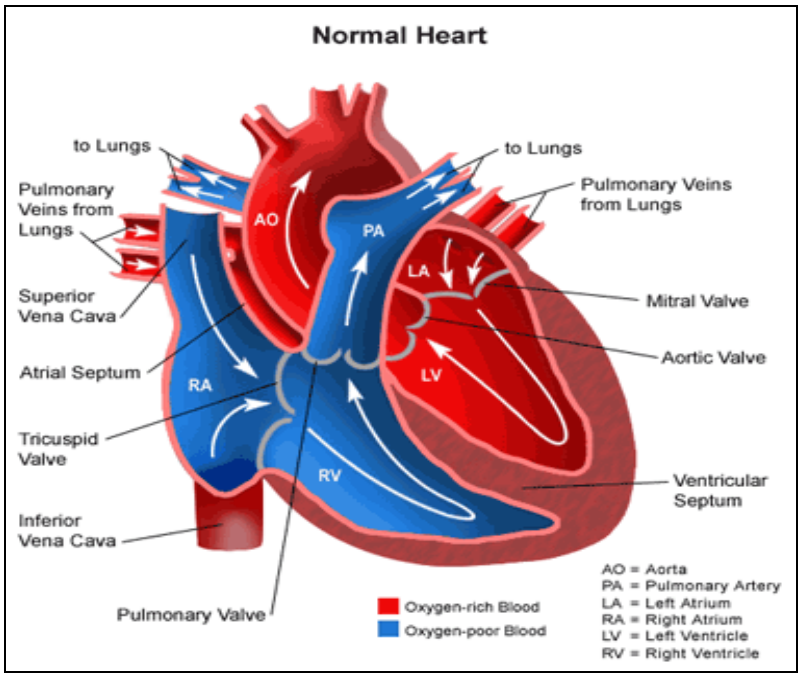


Figure 2: Anatomy of a Normal Heart (Moore and Agur, 2002)

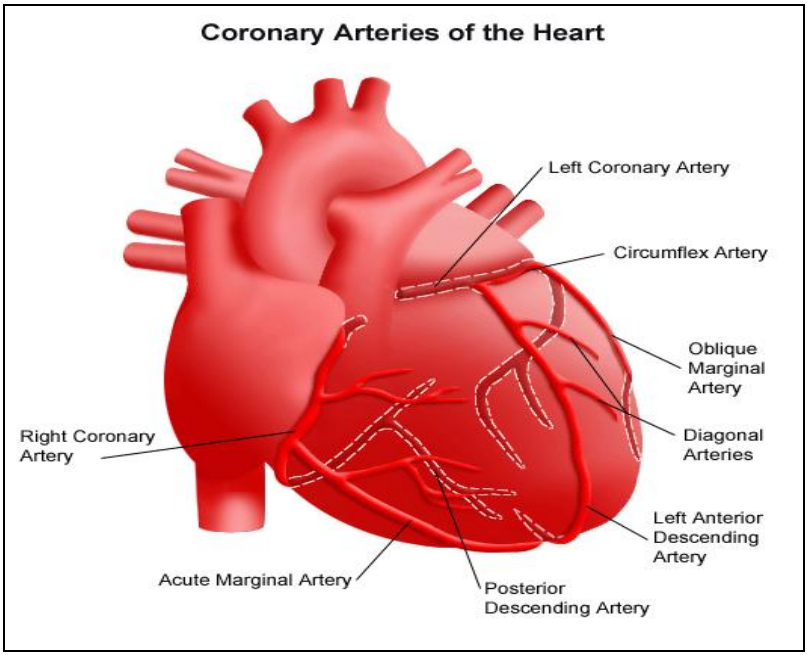


Figure 3: Anatomy of the Coronary Arteries that Supply the Heart (Grabowski, 2003)

2.3 CORONARY ARTERY DISEASE

2.3.1 Pathophysiology of Coronary artery disease

Coronary heart disease is caused by atherosclerosis, which is the progressive narrowing of the coronary arteries due to the accumulation of fatty plaque and is likely to cause angina pectoris and/or heart attack (Nora, Berg and Nora, 1991). This plaque is a combination of inflammatory and immune cells, fibrous tissue, and fatty material. Obstruction of the lumen of the artery causes a decrease its ability to deliver oxygen and nutrients to the heart muscle, resulting in myocardial infarction, angina, unstable angina, and sudden ischaemic death as a result of heart failure (Detecting coronary heart disease with BNP during stress testing, 2008).

Figure 4 shows the structure of a normal artery and an artery with plaque formation (atherosclerosis) which hinders blood flow to the heart muscles resulting in CAD.

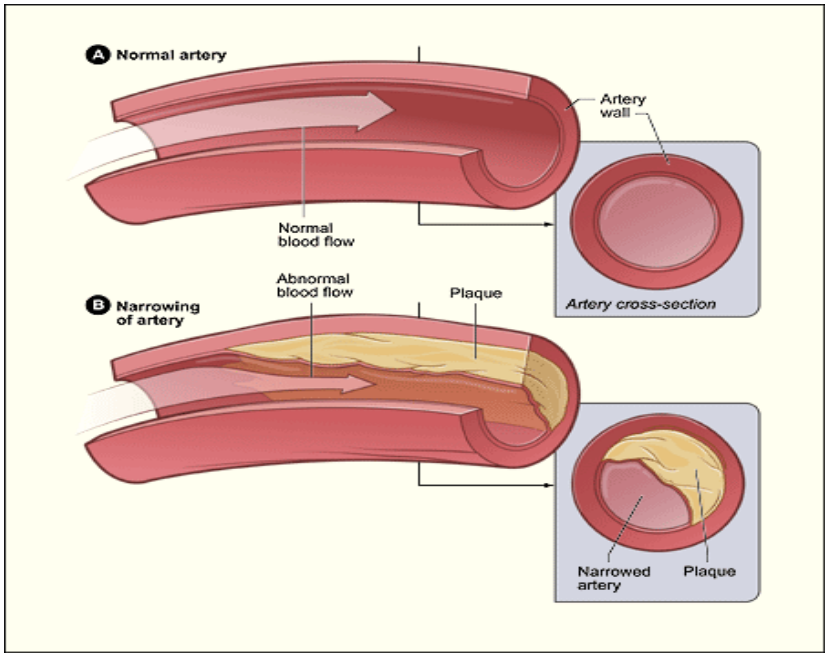


Figure 4: A- Shows a normal artery with normal blood flow.
B- Shows an artery with plaque accumulation (Atherosclerosis).
(www.web-books.com)

2.3.1.1 Pathogenesis of Coronary Artery Disease

The definite cause of CAD is still not known. However, there are two widely accepted theories that attempt to explain this phenomenon, i.e., the response- to- injury and thrombogenic theories.

- **The response- to- injury theory** suggest that factors such as hypertension, tobacco smoke, vasoconstriction and elevated levels of cholesterol and triglycerides in the blood causes endothelial injury within the intimal layer of the artery (Figure 5). This intimal layer is susceptible to tearing due to the force of blood flow against the irregularly shaped lumen of the artery. Intimal tearing causes platelet aggregation, smooth muscle proliferation and lipid deposition at the site of the injury, in response to repair of the site (Bucher and Melander, 1999).

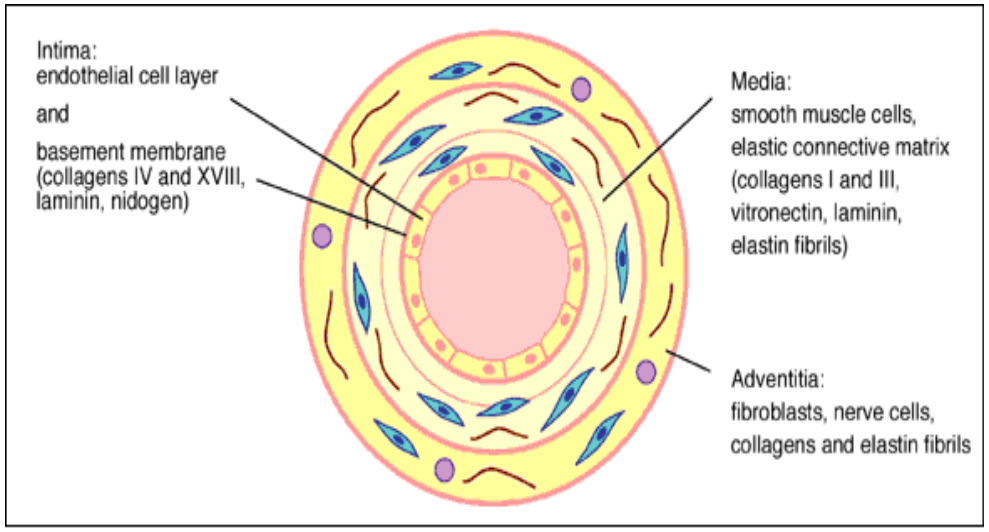


Figure 5: Schematic view of a normal arterial wall in cross- section (Fox, 2004)

- **The thrombogenic theory** describes the process of atherosclerosis in which three levels of plaque lesions form. The first lesion formed is the fatty streak. The second lesion is known as the fibrous plaque which extends into the intima from the medial layer, appears yellowish grey and may obstruct blood flow. This lesion is considered

the culprit in CAD. The third lesion formed is the complicated lesion which is caused by haemorrhage into a fibrous plaque. This plaque formation is commonly associated with myocardial infarction (Bucher and Melander, 1999).

- **A third theory** is a combination of the first two theories. This theory suggests plaque rupture from the artery's intimal wall which is caused by stress on the plaque from hypertension, stress hormones and cellular proliferation. This rupture causes clot formation in order to repair the site as well as further platelet formation and aggregation. The resulting clot causes significant interlumen narrowing and may even completely occlude the artery causing a myocardial infarction (Figure 6) (Bucher and Melander, 1999).

When any of the coronary arteries become partially or completely narrowed, oxygen and nutrients to the cardiac muscle tissue is minimized. This area of cardiac muscle tissue functions less optimally and patients usually present with chest pain or dyspnoea (McPhee, Lingappa and Ganong, 2003). As CAD progresses, without any treatment, the coronary artery becomes completely occluded resulting in damage to the cardiac muscle tissue it supplies (Figure 6). This condition is called a myocardial infarction, commonly known as a heart attack. Coronary artery disease progression could be slowed if it is treated effectively and efficiently (McPhee and Ganong, 2006).

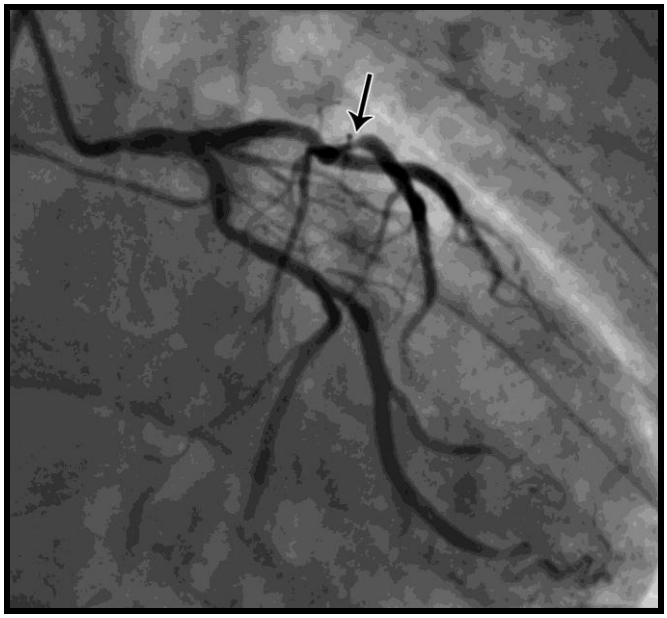


Figure 6: Coronary angiogram showing multivessel stenosis of the left coronary artery (Aviles, Messerli, Askari, Penn, and Topol, 2004).

2.3.1.2 Etiology of CAD

Atherosclerosis of the epicardial coronary arteries is the leading cause of CAD. In 1948, the Framingham Heart Study identified the correlation between certain risk factors and the development of CAD (Framingham heart study, 2009). Over the years, several other studies have been conducted and have concluded similar findings. Hypertension, hyperlipidaemia, smoking, obesity, diabetes, activity levels, age, race, gender and family history have all been identified as risk factors in CAD. It has also been found that the possibility of developing CAD increases with the number of risk factors one may possess (Bucher and Melander, 1999).

Table 1 shows the risk factors categorised into two groups i.e. modifiable and non-modifiable.

Table 1: Modifiable and non-modifiable risk factors (McPhee, Lingappa and Ganong, 2003)

Modifiable	Non- Modifiable
Hyperlipidemia	Age
Tobacco smoking	Gender
Hypertension	Heredity
Obesity	Race
Diabetes	
Physical inactivity	

In Table 2, the different causes of coronary artery disease are identified and comments are provided.

Table 2: Causes of Coronary Artery disease (McPhee, Lingappa and Ganong 2003)

Type	Comment
Atherosclerosis	Most common cause with the above mentioned risk factors.
Spasm	Spasm of the coronary arteries is mediated by serotonin, histamine, catecholamines and endothelium-derived factors. Spasm can occur in any population and at any time.
Emboli	Rare cause but can occur as a result of vegetations in patients with endocarditis.
Congenital	Rarely, congenital abnormalities can cause CAD.

2.3.1.3 Clinical Presentation of Coronary artery disease

Angina pectoris, commonly known as chest pain, is the classic symptom associated with CAD. Angina is a perceived sensation that is often described as a dull pain that radiates from the jaw, down the back and arm and is associated with dyspnoea and nausea. All this is caused by a decrease in oxygen supply to the myocardium (McPhee and Ganong, 2006). Angina can be classified according to the frequency of symptoms and causes (Table 3).

Table 3: Angina Classification (McPhee, Lingappa and Ganong 2003)

Types	Description
Acute coronary insufficiency	Angina that is changing, often with increasing intensity and duration. This angina can lead to a myocardial infarction if left untreated.
Angina decubitus	Angina associated with lying in the recumbent position.
Crescendo angina	Angina that is increasing with frequency with less intense precipitating factors.
Nocturnal angina	Angina that has its onset associated with the REM (rapid eye movement) phase of sleep.
Preinfarction angina	Unstable angina the progresses to myocardial infarction.
Prinzmetal's or variant angina	Angina associated with coronary artery vasospasm. It is often severe and no induced with effort. It can occur at the same time every day. It may or may not occur in the presence of atherosclerosis.
Progressive angina	Angina that is newly diagnosed and increasing in severity.
Stable (chronic) angina	Angina that has not changed in intensity, duration or frequency for at least two months. It is effort induced and can be mild or severe.
Unstable (acute) angina	Angina that changes, often with increasing intensity and duration. Can lead to a myocardial infarction if untreated.

2.4 N- TERMINAL PROBRAIN NATRIURETIC PEPTIDE (NT- proBNP)

2.4.1 Background

Pro brain natriuretic peptide (proBNP) is a cardiac neurohormone that is secreted in the cardiac ventricles in response to myocardial stretch (De Lemos, McGuire and Drazner, 2003). The human BNP gene is located on chromosome 1 and encodes the prohormone proBNP. Cleavage of proBNP by the protease furin produces BNP which is a biologically active 32 amino acid peptide and a 76 amino acid N-terminal fragment (NT-proBNP) which is biologically inactive (Figure 7). ProBNP is the inactive N-terminal portion of the precursor proBNP molecule and can be measured by immunoassay in human blood (Bhatia, Nayyar and Dhindsa, 2003).

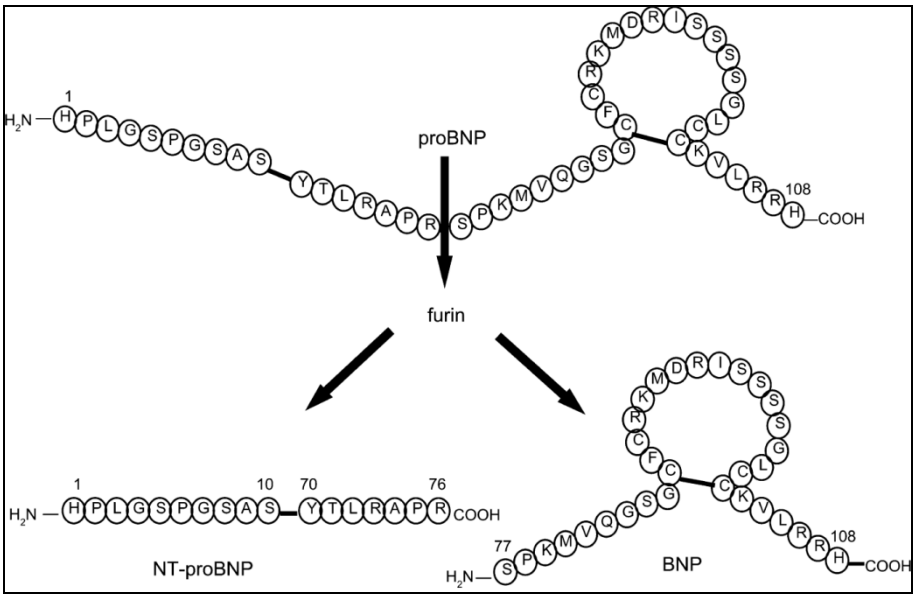


Figure 7: Schematic drawing of proBNP showing enzymatic cleavage into biologically active BNP and NT-proBNP (Hall, 2004)

Cardiac BNP production may be stimulated by other neurohormones in different cardiac cell types. In contrast to atrial natriuretic peptides (ANPyNT-proANP), which originate mainly from atrial tissue, BNP related peptides are produced mainly from ventricular myocytes. Ventricular NT-proBNP production is strongly upregulated in cardiac failure and locally in the area surrounding a myocardial infarction (The future of cardiac biomarkers, 2009).

The functions of NT- proBNP include regulation of vascular tone, regulation of arterial pressure, arterial and venous vasodilatation, and inhibition of sympathetic nerve activity (Bhatia, Nayyar and Dhindsa, 2003). Thus, NT- proBNP is secreted by the ventricles in order to reduce intraventricular pressure in congestive heart failure (CHF).

Many studies support a decision threshold of 125 pg/ml for NT- proBNP (Pfister, Tan, Thhekkanal, Erdmann and Scheider, 2008). A NT- proBNP value of less than 125 pg/ml excludes cardiac dysfunction and heart failure. A NT- proBNP value of greater than 125 pg/ml may indicate cardiac dysfunction and are associated with an increased risk of cardiac complications such as myocardial infarction, heart failure and death (Cardiac Biomarkers, 2009).

Increased NT- proBNP levels could result from: abnormal renal function, emphysema or chronic obstructive pulmonary disease (COPD). Increased levels of proBNP have also been observed in patients on diuretics, patients with atrial fibrillation and patients who have had a recent MI (Essig, 2007). NT-proBNP levels decrease in most patients who have been taking drug therapies for heart failure, such as ACE inhibitors, beta blockers, and diuretics. Levels of both BNP and NT-proBNP tend to increase with age (Essig, 2007).

Brain natriuretic peptide is currently being used as an indicator of left ventricular dysfunction, seeing that a number of studies have shown that the blood level of BNP compares well with left ventricular function (Berger, 2001). The study, conducted by Berger, also showed elevated BNP levels in patients with acute myocardial infarction. Therefore, patients with myocardial infarctions were excluded from this study. Kragelund, Kober, Hildebrandt and Steffensen, (2006), also suggested that the levels of the natriuretic peptides are increased in heart failure, acute coronary syndromes, and chronic coronary disease and are closely linked to prognosis.

A study conducted by Roche Diagnostics also found that ProBNP levels in healthy individuals increased with age and that females have a higher proBNP level when compared to males (Table 4) **(**Prontera, Emdin, Zucchelli, Ripoli, Passino and Clerico, 2003).

Table 4: Reference Range of BNP and ProBNP levels in healthy individuals (Diagnostic BNP ranges, 2008)

Gender	BNP (pg/ml)	ProBNP (pg/ml)
Healthy Males	< 100	≤ 60
Healthy Females	< 100	12 – 150

2.4.2 Clinical Use of NT- proBNP

For a biomarker to be of any value in a clinical setting, it should be able to be measured quickly and precisely at an affordable cost. In addition, it must provide further diagnostic or prognostic information to already existing methods and help guide physicians in the future management of patients. As seen from previous studies, BNP and N-terminal BNP fulfil most

of these criteria in patients with suspected heart failure. N-terminal BNP seems to provide much the same information as BNP (De Lemos, McGuire and Drazner, 2003).

Currently, NT- proBNP measurements are being used in diagnosing acute congestive heart failure (CHF) as the plasma concentration of NT- proBNP is typically elevated in "patients with asymptomatic or symptomatic left ventricular dysfunction". **(**Maisel, Krishnaswamy, Nowak, McCord, Hollander, Duc, Omland, Storrow, Abraham, Wu, Clopton, Steg, Westheim, Knudsen, Perez, Kazanegra, Herrmann and McCullough, 2002). N- terminal pro brain natriuretic peptide is also being used as a prognostic indicator for patients with CHF and in predicting short- and medium–term prognosis across the spectrum of acute coronary syndromes (Kelly and Suthers, 2001). The plasma concentration levels of NT- proBNP are normally higher in patients with a worse outcome in heart failure, and are therefore of prognostic significance.

As shown in previous studies, there is a remarkable correlation between NT- proBNP values and left ventricular dysfunction. In a similar study conducted by Roche Diagnostics, data from 4,625 individuals demonstrated that NT- proBNP levels correlated well with the severity of CHF (Brain Natriuretic Peptide, 2009). However, there is no specific NT- proBNP value that completely separates those patients with heart failure from those without. Brain natriurectic peptide and NT-proBNP tests have high sensitivity but a rather low specificity, meaning that low values are accurate at excluding heart failure as a diagnosis, but high values are not conclusive in identifying heart failure (Maisel et al., 2002).

Brain natriurectic peptide values will generally be above 100 pg/ml (picograms/millilitre) in patients with CHF, however, in order to achieve adequate sensitivity, it is considered that

normal BNP values are less than 50 pg/ml. A BNP value between 100 and 500 pg/ml, is considered inconclusive as it in the diagnostic ‘gray area’. Values above 500 pg/ml are generally considered to be positive **(**Maisel et al., 2002).

However, increased NT- proBNP levels from hypertension, ischaemia, stroke, renal failure and heart failure can sometimes be beneficial to the patient. NT- proBNP causes the blood vessel to dilate which increases the flow of blood to the kidneys, decreases myocardial oxygen demand and blood pressure. "The net effect is a reduced cardiac workload," explains Foote (2008). "It has so many good properties that it's been synthesized and it's now being used as a drug to treat severe heart failure" (Foote, 2008).

There has been considerable number of studies in patients with heart failure as well as in patients with acute coronary syndromes, to determine the prognostic importance of BNP and NT pro- BNP. Both markers have been shown to be strong predictors of morbidity and mortality (Kragelund et al., 2006).

The Elecsys proBNP II assay is indicated as an additional tool in the diagnosis of patients suspected of having congestive heart failure and is further indicated for the risk stratification of patients with acute coronary syndrome. It may also be used in the assessment of patients at risk for heart failure who have stable coronary artery disease, for increased risk of cardiovascular events and mortality. (Sokoll, Baum, Collinson, Gurr, Haas, Luther, Morton, Nowatzke and Zingler, 2004).

2.4.3 Role of NT- proBNP in detecting coronary artery disease

Cardiac myocytes constitute the major source of BNP related peptides. The main stimulus for peptide synthesis and secretion is myocyte stretch which is caused due to volume expansion

and increased filling pressure of the ventricles (Kragelund, Grønning, Omland, Køber, Strande, Steffensen and Hildebrandt, 2006). The natriuretic peptides and their receptors are abundantly present in atherosclerotic plaques in human coronary arteries (Casco, Veinot and Kuroski de Bold, 2002), as has been bourne out by several smaller clinical studies that have shown increased levels of NT- proBNP during episodes of ischaemia (Sabatine, Marrow and Lemos, 2002). Consistent with this observation, both BNP and NT-proBNP correlate to severity, location, and extent of angiographic coronary disease (Weber, Dill and Arnold, 2004). However, it is has not yet been established if BNP levels can be used as a screening tool in identifying significant stenoses during coronary angiography in patients with stable coronary disease (Kragelaund et al., 2006).

Coronary artery disease (CAD) is the most common autopsy finding in cases of sudden cardiac death (Struthers and Lang, 2007). It now appears that NT- proBNP is not just an indicator of increased intracardiac pressure but also of ischaemic heart disease. It was first seen in 1997 when NT- proBNP was found to be more closely related to the presence of CAD than it was to intracardiac pressure. The reason being that ischaemic or injured myocardial tissue releases extra BNP irrespective of haemodynamic factors. A study conducted by Goetze, Christoffersen, Perko, Arendrup, Rehfeld, Kastrup and Nielsen (2003), demonstrated that in- vitro ischaemic tissue expresses increased NT- proBNP. Thus, increased levels of NT- proBNP may result from myocardial ischaemia, irrespective of haemodynamic considerations. This is why myocardial ischaemia per se can be identified by increased NT-proBNP levels (Goetze et al., 2003). Measurement of this cardiac biomarker (NT- proBNP) may be a new way to detect silent myocardial ischaemia and in doing so may possibly even prevent future cardiac events.

According to Foote (2008), NT- proBNP blood level measurements may be a better way of detecting CAD than exercise stress testing as this often fails in detecting ischaemia. Foote (2008), demonstrated a correlation between ischaemia during exercise and elevated levels of BNP in a study that was funded by the Hitchcock Foundation and published in the Journal of the American College of Cardiology. He made use of nuclear perfusion imaging, in patients with coronary artery disease, and then precisely measured blood flow to the heart. Nuclear perfusion imaging is a procedure whereby a radioisotope is administered intravenously during an exercise stress test. It then distributes itself through the heart muscle in proportion to blood flow. Images taken by a special camera then show areas of reduced perfusion indicating myocardial ischemia.

2.4.4 The Effect of Exercise on NT- proBNP

The NT-proBNP levels of seventy four patients with coronary artery disease, in conjunction with exercise stress testing, were studied. Study results showed that over 90% of patients displayed an abnormal increase in NT- proBNP after exercise and only 37.5% of their ECG's showed abnormal patterns. He further established average levels of NT- proBNP in healthy athletes to be 25 pg/ml (members of the Dartmouth women's hockey team), in patients with coronary heart disease but no ischaemia to be 55 pg/ml, in patients with CAD and ischaemia to be 125 pg/ml, and in patients with heart failure to be >1,000 pg/ml (Foote, 2008).

In a study that used single photon emission computed tomography (SPECT), the percentage change in NT- proBNP levels were calculated in 60 patients undergoing the procedure. Pro brain natriuretic peptide levels were measured pre- exercise stress test (baseline), immediately post exercise and 10-15 minutes after exercise. In this study 10 patients were

found to have ischaemic perfusion defects (Win, Chang, Raizner, Shah, Basky, Desai, Plana, Mahmarian, Quinones and Zoghbi, 2005).

In the same study, patients with no evidence of ischaemia, the mean BNP value at baseline was 15.05 pg/ml and increased significantly immediately post exercise (mean level was 34.7 pg/ml) and decreased toward baseline values within 10 to 15 minutes after exercise (20.3 pg/ml). This transient rise in BNP values during exercise was more pronounced in patients with ischaemia. Percent change in BNP values from baseline for each minute of exercise was predominantly higher in patients with ischaemia as compared to those without. Patients with and without ischaemia did not differ in maximal heart rate, peak systolic or diastolic blood pressure, age, exercise time or any other baseline characteristics. A change in BNP value of less than 10% from rest per minute of exercise had an 80%, sensitivity and 71% specificity to detect ischaemia by SPECT (Win et al., 2005). The study concluded that ephemeral elevation in BNP occurs during exercise stress testing and is more pronounced in patients with ischaemia. Therefore, BNP testing may be used in conjunction with exercise stress testing in the evaluation of coronary artery disease (Win et al., 2005).

Leers, Schepers and Baumgarten (2006) investigated changes in BNP and NT- proBNP in 25 male and 2 female long distance runners (age 34-64 years). Results showed a slight increase in brain natriuretic peptide (BNP); however, this was not statistically significant. On the contrary, the N-terminal fragment of BNP (NT-pro-BNP) was significantly increased immediately after the run and was normalized 24 h later in 96% of the sample. The study also suggested that BNP and NT-pro-BNP levels increased with the athletes age and concluded that strenuous exercise significantly increases NT-pro-BNP levels in healthy adults and this could be partially attributed to cardiac stress. However, the transient increases in BNP and

NT-pro-BNP are more likely to reflect myocardial stunning than cardiomyocyte damage. It seems that the magnitude of the increase in BNP could serve as a marker of the biological age of the myocardium.

According to Sahlén, Gustafsson , Svensson, Marklund, Winter, Linde and Braunschweig (2005), cardiac biomarkers play an important role in the diagnosis of cardiovascular disease. Their study analysed NT- proBNP levels in 185 senior runners before and after the marathon. The study demonstrated NT- proBNP elevation was common in senior runners. It also showed that a high baseline NT-proBNP level is predictive of a large release during exercise, suggesting that the factors that control the at rest levels also determine its release with exertion.

2.4.5 The Effects of Age, Gender and Hypertension on NT- proBNP

Although NT- proBNP measurements are approved for clinical use in the diagnosis of heart failure and may aid in the detection of left ventricular dysfunction. Information regarding the impact of age, sex, and other physiologic characteristics on natriuretic peptide levels is limited. For accurate interpretation of NT- proBNP, it is essential that NT- proBNP range should be observed in patients without cardiovascular disease or cardiac dysfunction first (Redfield, Rodeheffer, Jacobsen, Mahoney, Bailey and Burnett, 2002).

A population- based study (Redfield et al., 2002) examined the effects of age and gender on proBNP concentration. The study sample consisted of 767 subjects in normal sinus rhythm without any cardiovascular, renal, or pulmonary disease or diabetes; on no cardiovascular medications; and with normal systolic, diastolic, and valvular function. Two BNP assays (Shionogi and Biosite) and doppler echocardiography, were then performed. Results showed that NT- proBNP increased with age and was higher in females than males.

Since NT- proBNP production occurs in the atria and ventricles, age-related changes in cardiac size could influence NT- proBNP. However, in the study, the effect of age and gender on BNP was independent of atrial volume, left ventricular dimension and mass. These data suggests that undefined alterations in NT- proBNP production, secretion, or degradation occur with age, and further studies are needed to explain the mechanism/s responsible for this effect. An alternative explanation was that NT- proBNP increased in response to age-related alterations in cardiac structure or function that are not detectable by current techniques.

The association of increased NT- proBNP in females appeared to be related to oestrogen levels, as NT- proBNP levels were higher in women using hormone replacement therapy (HRT). There have been previous reports that there is an association between gender-related differences in endothelin and angiotensin-converting enzyme activity with hormonal status. Although preliminary, these data suggest that NT- proBNP production may be sensitive to oestrogen regulation and represent an area for further study (Redfield et al., 2002). The study concluded that the interpretation of NT -proBNP should include consideration of age, gender, and assay-specific partition values (Redfield et al., 2002).

A similar study (Wang, Larson, Levy, Leip, Benjamin, Wilson, Sutherland, Omland, and Vasan, 2002) examined a healthy reference sample of 911 subjects (mean age 55 years, 62% women) from the Framingham Heart Study. The selected study population was also free from hypertension, valvular disease, diabetes, atrial fibrillation, obesity, coronary artery disease, heart and renal failure, and had normal left ventricular systolic function. The NT- proBNP levels were measured and multivariable regression was used to assess correlates of natriuretic peptide levels. Increase in age and female sex was highly significant predictors of increased NT- proBNP levels. Reference limits, based on the empirical distribution, of proBNP levels by gender both across all ages and partitioned by age were then formulated. This study, also,

concluded that interpretation of NT- proBNP levels should take into consideration gender and possibly age.

A prospective cross-sectional study of 121 patients, investigated the confounding effects of age and gender on NT- proBNP concentrations in critically ill patients. The study demonstrated that concentrations of NT- proBNP were higher in females than in males and increased with age. And although the presence of cardiac disease was the most important determinant for NT- proBNP variations, age and gender also contributed significantly. The results of this study therefore suggest that age and gender need to be taken into account when interpreting NT- proBNP concentrations in critically ill patients (McLean, Huang, Nalos, Tang, Stewart, 2003).

Raymond, Groenning, Hildebrandt, Nilsson, Baumann, Trawinski and Pedersen (2003),conducted a study at the Copenhagen University Hospital on 472 patients with heart failure to identify potentially confounding variables for the interpretation of plasma N-terminal pro brain natriuretic peptide. Each patient had to fill in a heart failure questionnaire and underwent pulse and blood pressure measurements, ECG, echocardiography, and blood sampling. Results demonstrated that female sex ($p < 0.0001$), increased age ($p < 0.0001$), increasing dyspnoea ($p = 0.0001$), diabetes ($p = 0.01$), valvular heart disease ($p = 0.002$), low heart rate ($p < 0.0001$), left ventricular ejection fraction $\leq 45\%$ ($p < 0.0001$), abnormal ECG ($p < 0.0001$), high levels of creatine ($p = 0.0009$), low levels of glycosylated haemoglobin A1c ($p = 0.0004$), and high urine albumin levels ($p < 0.0001$) were independently associated with an increased NT-proBNP level by multiple linear regression analysis. The study concluded that a single reference interval for the normal value of NT-proBNP is not adequate. There are several confounders for the interpretation of a given NT-proBNP

concentration and at the very least adjustment should be made for the independent effects of age and sex.

Studies conducted in a multi-ethnic population in the United Arab Emirates documented the determinants and NT-proBNP levels among hypertensive and non- hypertensive subjects. The study showed that NT-proBNP levels were significantly ($p<0.001$), and several-fold higher among hypertensives (median 5.92, inter quartile range: 1.79–18.48 pmol/l) than non-hypertensives (median 1.78, 0.59–4.32 pmol/l). The reason for this could be that hypertension increases wall stress and this, in turn, causes an increase in NT- proBNP production. Also, for all subjects combined, NT-proBNP levels correlated positively and significantly with age ($p<0.01$) and gender, and inversely associated with current exercise. In conclusion, the study found that circulating levels of NT-proBNP was significantly increased in hypertensive subjects as compared to non- hypertensive subjects and independently related to age, gender and possibly exercise. Results further suggest a possible modulating effect of ethnicity on NT-proBNP levels.

2.4.6 Patient Prognosis using NT- proBNP

Unfortunately, morbidity and mortality could not be assessed in the present study due to time constraints. However, studies give strong evidence that NT- proBNP is an excellent reliable marker of cardiac function and provide significant prognostic information in patients with coronary artery disease and heart failure.

In a cohort study that used a combination of clinical risk profile, EST and biomarkers to evaluate 422 patients with chest pain but without any ST- elevation or troponin elevation,

found that there was a strongly significant relationship with NT-pro-BNP (p- value 0.0001). By Cox regression including clinical risk score, exercise testing results and cardiac biomarkers, the exercise stress test was the independent predictor of revascularisation (p-value 0.0001), whereas risk score (p- value 0.03) and NT-proBNP (p- value 0.0004) predicted death or MI. The inclusion of NT-proBNP improved the accuracy of the model for death or MI. "The combination of clinical score and NT-proBNP afforded the stratification in high (17.2%, p- value 0.0001), intermediate (5.3%) and low (1.1%) risk categories of death or myocardial infarction" **(**Sanchis**,** Bosch**,** Bodi, Bellera**,** Núñez**,** Benito**,** Ordóñez**,** Consuegra**,** Heras and Llècer, 2004). The study concluded that BNP testing provides "incremental prognostic information above that given by clinical history" and exercise testing in patients with negative troponin and angina without ST- segment deviation (Sanchis et al., 2004).

Recently, data from the Framingham Heart Study identified NT- proBNP as a strong predictor of morbidity and mortality in the general population even when NT- proBNP levels were below the threshold of 100 pg per milliliter normally used to identify patients with heart failure (Cardiovascular Disease Statistics, 2009).

Early diagnosis of CAD before myocardial infarction, which could replace existing methods, is needed. Furthermore reducing cost and trauma to the patient is vital. If this study can prove that NT- proBNP used in conjunction with EST can determine the presence and severity of CAD, then this study will be of benefit worldwide.

2.5 EXERCISE STRESS TESTING

2.5.1 Background

Coronary artery disease (CAD) is one of the major causes of death worldwide. Appropriate evaluation of chest pain or its equivalent is necessary in the diagnosis and management of CAD (McCaffery and Geraci, 2007). Clinical exercise testing has wide application in medicine, including the assessment of functional capacity, ventilatory function, gas exchange, muscle function, and endocrine and metabolic function (Freedman, 1996). However, an exercise stress test can be a useful tool for detecting coronary artery disease and has been employed in this study.

In 1928, the significance of cardiovascular exercise stress testing was first noted by Feil and Seigel (1928). They reported ST and T wave changes in three patients with chronic stable angina following EST. Shortly afterwards, in 1929, Master and Oppenheimer introduced an exercise protocol that was standardized to determine the functional capacity and haemodynamic response of patients. Further research into causal mechanisms of ST displacement, modification of exercise protocols, and assessment of diagnostic and prognostic exercise variables in clinical patient subsets have since continued to evolve (Akinpelu, 2009).

Treadmill stress testing is the oldest and most extensively used stress testing method to date and can be reliably performed in patients who are clinically stable and who have an interpretable resting electrocardiogram. It can determine if the blood supply is reduced in the coronary arteries by examining a patient's symptoms and ECG changes. Sometimes cardiac patients are asymptomatic at rest, but when under cardiac stress become symptomatic. Although changes in the ECG during EST can be helpful in diagnosing CAD, the

interpretation of the results are limited as they are affected by several conditions, including but not limited to hypertension, left ventricular hypertrophy, cardiac rhythm disorders, medications and gender (Berger, 2001). For those patients who are suspected of having myocardial ischaemia and cannot exercise adequately, myocardial perfusion imaging or multi- slice CT scanning of the coronary arteries may be used but this test is very costly for patients and was therefore not used in this study. Also, exercise stress echocardiography could be used, however the expertise, in this setting was limited.

The principle of the exercise stress testing is based on the characteristics of coronary artery flow. An EST requires a patient to walk on a treadmill while the gradient and speed increases after every three minutes (using Bruce protocol). This causes an increased oxygen demand of the heart muscle (Bucher and Melander, 1999). In normal coronary arteries, an increase in myocardial oxygen demand is directly proportional to an increase in coronary artery flow. In stenosed coronary arteries, atheroma obstructs blood flow and thus myocardial oxygen demands are not met adequately. This results in myocardial ischaemia.

The ultimate objective of exercise stress testing is to induce ischaemia in a controlled clinical setting, usually by attempting to achieve a maximum exercise threshold, and to measure the physiologic response (Chou and Amidon, 1994). There are numerous studies available in cardiac literature on EST and its use in evaluating CAD, with a mean sensitivity of 67% and a mean specificity of 72% (Gibbons, Balady and Bricker, 2002). Froelicher, Fearon and Ferguson (1999) found a sensitivity of 45% and a specificity of 85% for EST after 814 patients complaining of chest pain underwent both EST and cardiac catheterization. Morise and Diamond (1995) reported that the accuracy of EST for the diagnosis of CAD is lower in women than in men.

The common indications for exercise stress testing worldwide are summarized in Table 5 (Morton, 2001).

Table 5: Indications for Exercise Stress Testing (Morton, 2001)

1. A diagnostic test in patients presenting with atypical angina and are suspected of CAD
2. Assess the functional capacity and prognosis of patients with known CAD
3. Evaluate of patients with symptoms consistent with recurrent, exercise-induced cardiac arrhythmias.
4. Assess the functional capacity of selected patients with congenital or valvular heart disease.
5. Evaluate of patients with rate-responsive pacemakers.
6. Cardiac assessment of individuals with special occupations (airline pilots, bus drivers, etc) some of which may have two or more CAD risk factors.
7. Evaluate individuals (men ≥ 45 years and women ≥ 55 years) with two or more risk factors who plan to enter a vigorous exercise program.
8. Evaluate asymptomatic individuals > 40 years with two or more risk factors for CAD.
9. Assess the response to medical therapy in patients with ischemic heart disease or failure
10. Evaluate patient progress post coronary artery bypass or percutaneous coronary intervention.

There are risks associated with exercise stress testing and a number of cardiac and non cardiac related illnesses where the EST is not recommended (Table 6). Furthermore, there are various factors which indicate when it is necessary to terminate an EST (Table 7). Therefore, it is necessary for informed consent to be obtained from the patient prior to the test. According to previous statistics one in 10, 000 people will die, and two to three in 10, 000 will have a major morbid event such as myocardial infarction, a major arrhythmia requiring resuscitation, severe hypotension, severe heart failure or unstable angina pectoris (Freedman, 1996).

Table 6: Contraindications for Exercise Stress Testing (Akinpelu, 2009)

1. Recent acute myocardial infarction (< 3–4 days).
2. Unstable angina
3. Severe symptomatic left ventricular dysfunction.
4. Untreated life-threatening cardiac arrhythmias.
5. Acute pericarditis, myocarditis, or endocarditis.
6. Acute pulmonary embolus or infarction.
7. Advanced atrioventricular heart block (AV Block)
8. Critical aortic stenosis.
9. Non-cardiac related illness that inhibits physical exertion, i.e. deep vein thrombosis, dissecting aneurysm and neuromuscular or arthritic conditions.

Table 7: When to Terminate an Exercise Stress Test (Akinpelu, 2009)

1. Drop in systolic BP by > 10 mm Hg from baseline BP and accompanied by symptoms or signs of ischemia.
2. Patient complains of moderate to severe angina/ chest pain.
3. Patient complains of dyspnoea, dizziness or near syncope.
4. Signs of poor perfusion (cyanosis or pallor).
5. Patient's desire to stop due to extreme fatigue.
6. Sustained ventricular tachycardia, increasing multifocal ventricular ectopy, supraventricular tachycardia, heart block, or bradycardia.
7. ST elevation (≥ 2.0 mm) on the ECG and may be accompanied by chest pain.
8. ST depression (> 2.0 mm), especially if accompanied by chest pain or signs of ischemia.
9. Excessive BP rise (> 250 mm Hg systolic and > 115 mm Hg diastolic).

2.5.2 Risks associated with exercise stress testing

Palpitations, chest pain, dyspnoea, headache, nausea, or fatigue are major side effects from stress testing. A major risk of stress testing, however, is the possibility of inducing an MI, especially in patients with severe multiple vessel coronary artery disease. However, this risk, is much lower than the risks associated with cardiac catheterization (such as inducing a heart attack, stroke, peripheral artery clot and embolism) (Fletcher, 2001).

2.5.3 Limitations of exercise stress testing

One of the major limitations of exercise stress testing is that it can only detect medium to high-grade flow limitations; this assuming the testing is fully and aggressively performed. (Fletcher, 2001).

It was believed, in the mid-1980s, that detecting high-grade stenoses was the key to recognizing people who would have heart attacks in the future. However, clinical experience found that some patients who were able to achieve maximum exercise thresholds without experiencing any abnormal symptoms and who had completely normal stress test results, died as a result of a massive heart attack within a few days to weeks. This shows that cardiac stress tests are only capable of detecting medium to high-grade coronary stenoses, which may produce recurring angina but not the atheroma that cause heart attacks. While anecdotal and not quantitative, these observations have long demonstrated the unreliability of the stress test approach as a means of diagnosing arterial disease before serious health problems occur (Fletcher et al., 2001).

In a study conducted by Hlatky, Pryor, Harrell, Califf, Mark and Rosati (2009), it was found that the sensitivity and specificity of an exercise electrocardiogram varies with clinical history, significance of disease and treadmill performance. “Unlike the predictive value of a diagnostic test, which depends on the prevalence of disease in the population tested, its sensitivity and specificity have been assumed to be constants” (Hlatky et al., 2009). This was examined in patients who had undergone an exercise stress test and a coronary angiography. The effects on sensitivity of factors from clinical history, coronary angiography, and exercise performance were defined by “multivariable logistic regression analysis in 1,401 patients” (Hlatky, 2009) with coronary artery disease.

Similar analysis was used in 868 patients without coronary artery disease to define the effects on specificity. Results showed that five factors had significant independent effects on exercise ECG sensitivity: maximum heart rate, age and sex of the patient, number of coronary arteries diseased, type of angina. However, only maximal exercise heart rate had a significant, independent effect on exercise ECG specificity.

It is now apparent that exercise stress testing, alone, recognizes most people who at risk for heart attacks too late. It is hoped that future research in cardiac biomarkers, such as NT-proBNP, will allow for earlier detection of CAD and to initiate lifestyle changes and optimal medical therapy before any fatal cardiac events occur.

The principle investigator in consultation with the cardiologist performed all the exercise stress tests in this study and was able to recognise and exclude patients at high risk, and deal effectively with any complications.

2.6 CARDIAC CATHETERIZATION

2.6.1 Background

Cardiac catheterization is a combined haemodynamic and angiographic invasive procedure performed to identify the presence of any heart condition, and if found, then therapeutic procedures have to be performed (Lee, 2005). The first cardiac catheterization was performed in 1929 by Dr. Werner Forssmann on himself in a small hospital in Germany (Hollmann and Wildor, 2006). Almost a century later, cardiac catheterization has evolved into the most definite means of diagnosing and treating various cardiovascular diseases. Different diagnostic procedures in various combinations may be used in cardiac catheterization. These diagnostic procedures include right and left heart catheterization, coronary angiography, ventriculography, aortography and cardiac biopsy (Hollman and Wildor, 2006).

The primary indication of coronary angiography is to identify the coronary artery anatomy and the degree of luminal obstruction. Coronary angiography still remains the gold standard for the assessment of CAD, because no other currently available test can accurately define the extent of coronary luminal obstruction. As with every invasive procedure, there are several complications as well as contraindications associated with it. The procedure is relatively expensive therefore the physician must make reasoned decisions on its use based on the anticipated clinical benefit versus the risks and costs of the procedure.

2.6.2 Coronary Angiography

Coronary angiography is performed using a catheter which is introduced into the femoral artery and advanced towards the heart under fluoroscopic guidance. The catheter is advanced through the aorta to the left and right coronary arteries. Once the catheter is in the appropriate

position, radiopaque contrast/dye (solution containing iodine, which is easily visualized with x-ray images) is injected through the catheter to make the coronary arteries visable (Figure 8). The left and right coronary arteries are injected individually and are viewed from various angles so that the entire circulation can be visualized. Several injections are required to thoroughly visualize the anatomy and patency of the coronary arteries (Aviles et al, 2004). The procedure takes approximately 20-30 minutes. After the procedure is completed, the catheter is removed from the artery and is manually compressed by the nurse to prevent bleeding.

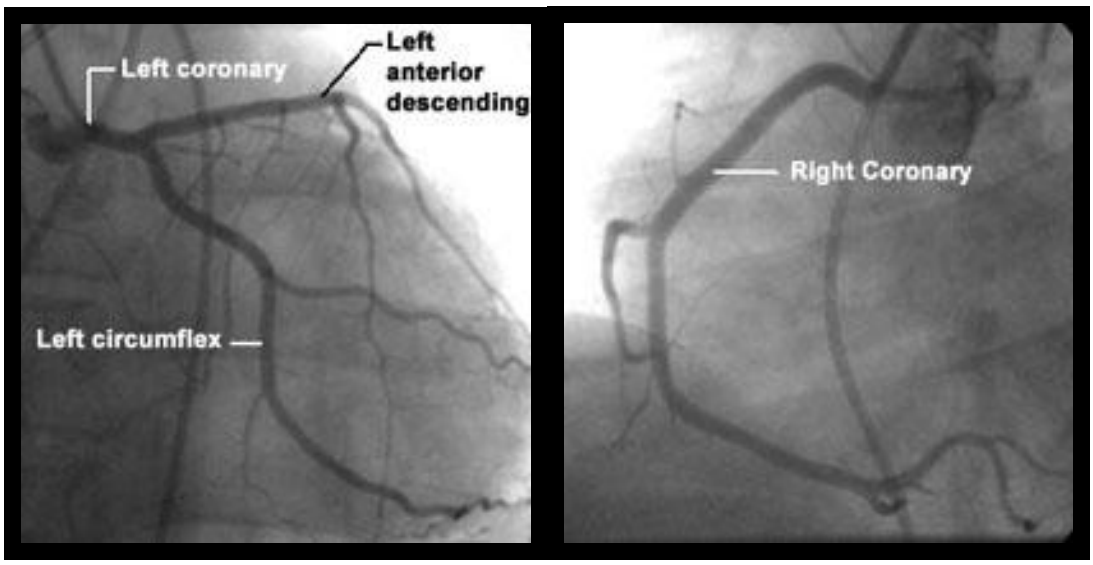


Figure 8: Normal left and right coronary arteries on angiogram (Aviles et al., 2004)

2.7 Rationale

This was a prospective analysis of a cohort undergoing treadmill exercise stress testing to assess the value of post treadmill exercise stress test NT- proBNP levels in detecting the presence and severity coronary artery disease, especially in those patients with an inconclusive EST. Patients included in this study had at least one risk factor for coronary artery disease. These patients, therefore, required further expensive testing to determine the

presence of CAD. In this study, the data was analysed separately for each EST group as the main focus was on reducing the need for those patients with inconclusive ESTs to have a coronary angiogram to determine the presence of CAD (See study design). It is important to note that not all patients with an inconclusive EST required an angiogram.

Considering that there are limitations to exercise stress testing alone, we hypothesized that NT- proBNP blood level measurements could be an important additional tool in the detection of CAD. According to Struthers and Lang (2007), it appears that NT-proBNP is not just an indicator of increased intracardiac pressure but also of ischaemic heart disease. Studies show that ischaemic or injured myocardial tissue releases extra NT- proBNP irrespective of haemodynamic factors (Struthers and Lang, 2007).

If normal NT- proBNP threshold levels are established in the presence and absence of myocardial ischaemia during exercise, the need for all patients, with an inconclusive EST, to undergo further expensive testing or coronary angiography may be eliminated. This method of assessing the presence of CAD will be more cost effective to the patient since a coronary angiogram will not be required. Furthermore, hospital-stay and the additional risk associated with invasive procedures will be reduced.

CHAPTER THREE: MATERIALS AND METHODOLOGY

3.1 Introduction

This prospective, quasi- experimental and quantitative study was conducted at a private hospital (St Anne's) in Pietermaritzburg, Kwa- Zulu Natal. The study sample consisted of 60 patients, both males and females from all race groups and were between the ages of 36 to 85 years old. It must also be considered, when reviewing the results of the study, that the study population drawn on by this practice was more affluent than the general population. The aim of this study was to determine the post exercise stress test predictive value of NT- proBNP to assess the presence and severity of coronary artery disease.

3.2 Aim of the study:

To assess the value of post treadmill exercise stress test NT- proBNP in detecting the presence and severity coronary artery disease.

3.3 Specific objectives:

1. To identify a possible value of NT- proBNP level which indicates CAD.
2. To assess the association between exercise stress test and CAD
3. To Compare the median number of diseased vessels by presence or absence of specific risk factors
4. To compare NT- proBNP levels with the number of diseased vessels
5. To assess the association between proBNP levels and patients' age and gender
6. To determine the percentage of false positive proBNP levels
7. To determine the probability of false positive exercise stress testing

3.4 Sampling

A cohort sample of 60 cardiac patients was selected, from the cardiology unit at St Anne's Private Hospital, after obtaining permission from the consulting cardiologist and consent from the patient. The sample size was calculated as follows:

The StatAdvisor

This procedure determined the sample size required when estimating the mean of a normal distribution. Assuming that the standard deviation of the normal distribution equals 1.0, 60 observations will estimate the true mu to within +/-0.258328 with 95.0% confidence. The Power Curve was obtained using Statgraphics Centurion (Figure 9).

Parameter to be estimated: normal mean

Sample size: 60

Confidence level: 95.0%

Sigma: 1.0 (to be estimated)

The tolerance will be +- 0.258328

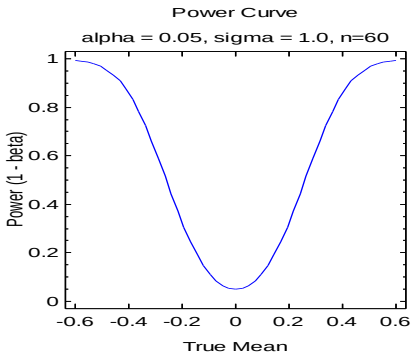


Figure 9: Power Curve

The patient sample was then divided into 2 groups as follows:

Group A - the control group, consisting of 30 patients, all of which had a positive EST and;

Group B - the experimental group, consisting of a further 30 patients, all of which had an inconclusive EST.

All patients recruited into the study were under the consultant care of the cardiologist, who confirmed the inconclusive and positive exercise stress tests.

3.4.1 Inclusion criteria

1. Patients with a left ventricular function of > 55% measured on echocardiography.
2. Patients presenting with symptoms of coronary artery disease, including the classic anginal symptoms of chest pain/ pressure that occurs with or without exertion. Atypical presentations or angina equivalents, such as shortness of breath or dyspnoea on exertion.
3. Patient must possess at least one of the following risk factors
    - Diabetes mellitus
    - History of smoking
    - Obesity
    - Hypertension
    - High serum cholesterol level
    - Family history of heart disease
    - Elevated low density lipoproteins in the blood

3.4.2 Exclusion criteria

1. Any patient that has abnormal renal function
2. Patients with myocardial infarctions in past three months

3. Patients on diuretics

4. Patients with arrhythmias including atrial fibrillation

5. Patients with left ventricular dysfunction

6. Patients with emphysema or chronic obstructive pulmonary disease

3.5 Consent

Before commencing with the experimental investigation a detailed proposal was submitted to and approved by the Faculty of Health Sciences Research Committee (FHSRC) at the Durban University of Technology (DUT). Ethical approval was obtained from the DUT's Ethics Committee. All patients that met the inclusion criteria were asked if they wished to participate in the study. An information and consent form was given to them which defined what the study entailed and how their participation was beneficial to the study. Once the patient agreed to participate in the study the consent form, which was available either in English or Zulu, was signed (Appendix 13 and 14). Patients were also informed that they could withdraw from the study at any time and that their withdrawal would in no way hinder their assessment and treatment. All patient details, i.e. their sex, age, date of birth, initials, diagnosis, etc. were anonymously processed into a study report.

3.6 Procedure

All subjects first consulted the cardiologist, followed by an exercise stress test. Those subjects with positive or inconclusive EST's had their NT- proBNP levels measured 10- 20 minutes post peak exercise stress test. Subjects with negative EST's were not sampled because this would have been unethical as coronary angiography was used as the gold standard in detecting coronary artery disease.

The NT- proBNP levels were measured in the blood serum using the Elecsys proBNP II assay. Thereafter, all patients had a coronary angiogram to assess the presence and severity of coronary artery disease. The results of the coronary angiogram were then compared to the NT- proBNP levels to assess the strength of the correlations. See figure 10 below:

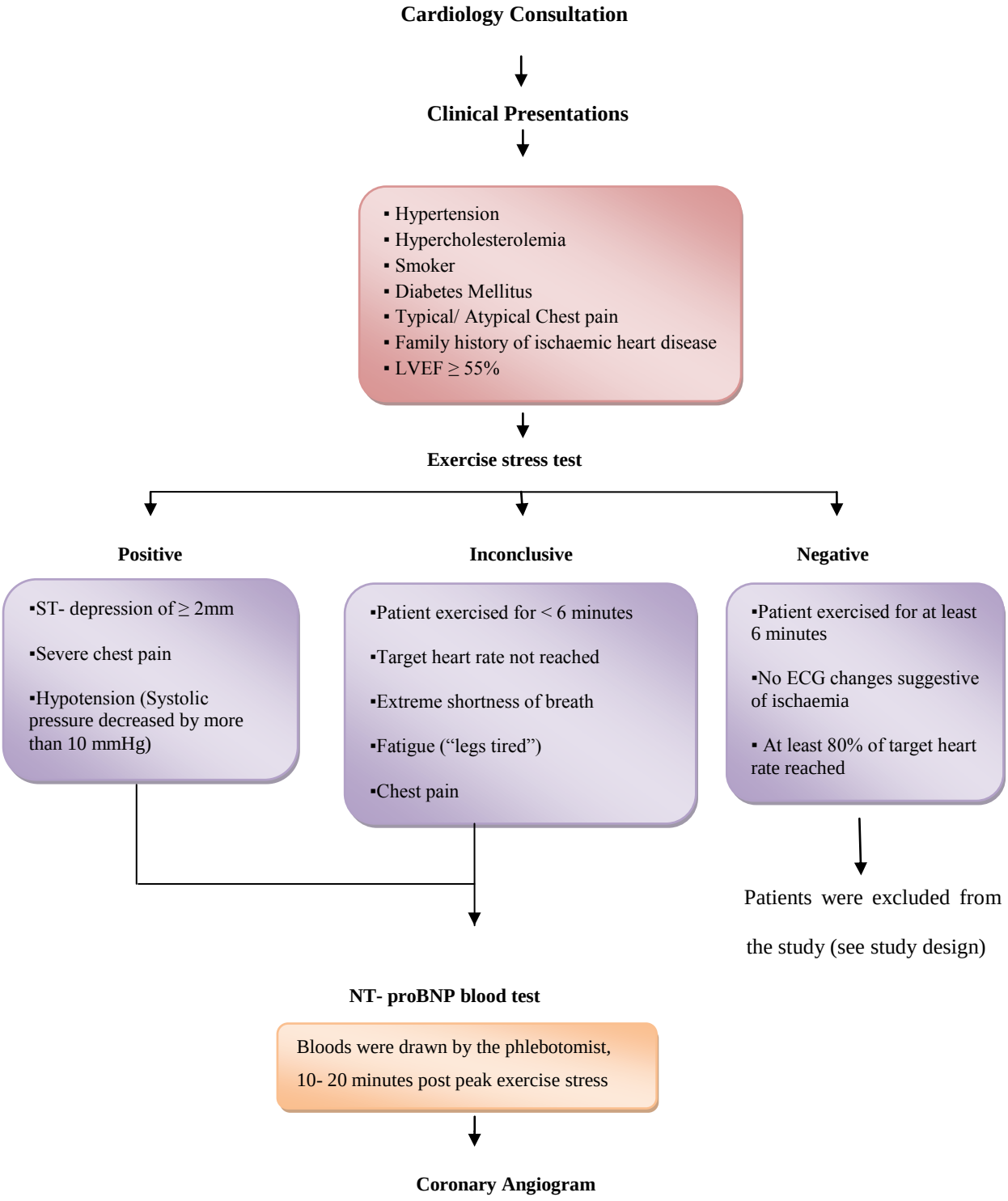


Figure 10: Schematic diagram of sample selection

3.6.1 Exercise Stress Test

The QRS Cardio Suite (ENU 4.05) Exercise Treadmill Stress System was used to conduct the EST for this study. Equipment needed for an exercise stress test included a treadmill, a monitoring system, blood pressure cuff and sphygmomanometer, a resuscitation trolley and a defibrillator. The cardiologist and the clinical technologist (cardiac) performed the tests.

The Bruce protocol was the most commonly used protocol in exercise stress testing (Table 8). However, if a patient was unable to perform the EST due to some sort of debilitation, the Naughton protocol was used. The Naughton Protocol is a lower level exercise stress testing protocol that was developed by John Naughton (Table 9) (Bruce protocol stress test, 2008).

Table 8: Bruce Protocol (Evans and White, 2009)

Stage	*Speed (km/hr)*	*Speed (mph)*	*Gradient %*
1	2.74	1.7	10
2	4.02	2.5	12
3	5.47	3.4	14
4	6.76	4.2	16
5	8.05	5.0	18
6	8.85	5.5	20
7	9.65	6.0	22
8	10.46	6.5	24
9	11.26	7.0	26
10	12.07	7.5	28

Table 9: Naughton Protocol (Evans and White, 2009)

Stage	*Speed (mph)*	*Gradient %*
1	1.2	10
2	1.5	12
3	1.5	14
4	1.5	16
5	1.5	18
6	2.0	20

Before the test began a target maximum heart rate was calculated to assess the adequacy of the stress test in order to induce angina. Not all patients have the same target heart rate. This is due to older patients having a decreased beta- adrenergic response which results in decreased maximum heart rate and cardiac output. Therefore, a target maximum heart rate was calculated, by subtracting the patient's age (in years) from 220, for each patient. Any patient who reached 80% of the target maximum heart rate was considered to have a good test result. (Master and Oppenheimer, 1929).

The patient was then connected to a 12 lead electrocardiogram (ECG) and a blood pressure cuff was attached around the patients left arm. An ECG and blood pressure measurement was printed after every exercise protocol stage was completed.

A resting ECG and blood pressure measurement was done and this served as a baseline during the test. The patient's ECG was continuously monitored and the blood pressure was measured according to the protocol being used.

In this study the Bruce Protocol was mainly used (n = 57). The Naughton protocol was used for those patients who were much older and frail (n = 3) (Table 14).

For the Bruce protocol, the test began at 2.74 km/ hr at a gradient of 10 (using Bruce protocol) and after every three minutes, the speed and gradient was increased. At the end of each stage the BP was measured and an ECG was recorded. The patient was able to stop the test at anytime if they could not continue.

Reasons for stopping the EST included the following:

- Target heart rate was achieved
- Ischaemic changes on the ECG was found (significant ST- depression/ fatal arrhythmias)
- Patient developed hypotension (systolic pressure decrease of more than 10mmHg)
- The patient developed chest pain, extreme shortness of breath or fatigue

Thereafter, the patient was monitored in the recovery stage which usually lasted about 3- 5 minutes. A test report containing comments about the maximum heart rate and blood pressure, level of exercise achieved, symptoms, arrhythmias, ECG changes and vital signs during test was generated.

3.6.1.1 Interpretation of the results of the EST

Positive EST was concluded if the patient developed:

- Hypotension (systolic pressure decreases by more than 10 mmHg)

- Ischaemic changes on ECG (more than 2 mm ST segment depression was used as this is a strong positive result and leaves little clinical doubt that significant coronary artery disease exists.
- Severe disabling chest pain (Ellestad, 2003).

Inconclusive EST was concluded if

- Inadequate heart rate response
- Patient developed chest pain, extreme shortness of breath or fatigue
- Inadequate exercise duration due to deconditioning
- ECG changes were not diagnostic of ischaemia
- The occurrence of unifocal, premature atrial contractions or premature ventricular contractions (fewer than five per minute) was not an indicator for coronary artery disease.
- Development of right bundle branch block and left bundle branch block during exercise.
- Interpretation of an ECG with an intraventricular block maybe difficult due to obscure ischaemic changes (Darrow, 1999).

Negative EST was concluded if:

- There was none of the above mentioned findings
- Patient was able to exercise for more than 6 minutes without any chest pain or ECG changes suggestive of ischaemia
- Patient reached target heart rate (Ellestad, 2003)

The report generated at the end of the EST allowed the cardiologist to determine whether the test results were positive, negative or inconclusive. Further tests were conducted in those patients with positive and inconclusive exercise stress tests to obtain more information about the presence and severity of the coronary artery disease.

3.6.2 NT- proBNP Blood Test

N- terminal pro brain natriuretic peptide blood tests were performed on those patients with a positive or inconclusive exercise stress test. The phlebotomist drew blood within 10 – 20 minutes post peak exercise stress test. Blood samples could not be drawn within 5 minutes post peak EST as there was a time delay for the phlebotomist to arrive. As this is a private practice, patients did not fully consent for the primary investigator to draw bloods and then again have a phlebotomist draw bloods for various other blood tests prior to coronary angiography. The blood specimen was then delivered to the laboratory for analysis of the NT-proBNP level. The method that was used to measure the NT- proBNP levels in the blood was the Elecsys proBNP II assay.

3.6.2.1 Elecsys proBNP II Assay

The Elecsys proBNP II assay is an electrochemiluminescent immunoassay ‘ECLIA" employing two polyclonal NT-proBNP-specific antibodies in a sandwich test format. Results are determined using a calibration curve that is generated specifically on each instrument by a “2 point calibration and a master curve” (Pro-Brain Natriuretic Peptide, 2008).

3.6.2.2 Procedure for analysing NT- proBNP levels:

1. 3.0 ml of blood was collected from each patient in a BD Vacutainer SST II Advance yellow top sampling tube, which contained an acrylic based gel.
2. The specimen was centrifuged at 4000 revs/ minute for 10 minutes. The acrylic based gel, within the tube, formed a barrier between the clot (which contained blood cells) and the serum.
3. The serum was then placed in the sample wheel of the Cobas e 411 analyser.
4. The Elecsys proBNP II reagent kit (Appendix 15) was inserted into the analyser.
5. Patient details were entered and proBNP test was selected. The double sandwich technique (steps 6 – 11) that followed was automatically performed within the analyser.
6. The sample probe drew 20 microliters of sample and placed it in an empty sterile tube.
7. A biotinylted monoclonal NT- proBNP- specific antibody and a monoclonal NT-proBNP-specific antibody labelled with a ruthenlum complex were combined with the 20 microliters of sample to form a sandwich complex. This was incubated for 9 minutes.
8. Thereafter, streptavidin- coated micro particles were added and incubated for a further 9 minutes. As a result of the interaction of biotin and streptavidin, the complex becomes bound to the solid phase.
9. After the second 9 minute incubation, the reaction mixture was aspirated into the measuring cell where the micro particles were captured onto the surface of the electrode by a magnet.
10. Unbound substances were then removed before washing the measuring cell.

11. Thereafter, a voltage was applied to the electrode in the presence of a tri-propylamine (TPA) which induced an electrochemiluminescent emission, which was measured by a photomultiplier.

The total duration of the procedure was 18 minutes. The results were determined via a calibration curve, which were instrument- specifically generated by 2- point calibration and a master curve provided via the reagent barcode (Pro- Brain Natriuretic Peptide, 2008).

3.6.2.3 Cut-off values

Many studies support a decision threshold of 125 pg/ml for NT- proBNP (Pfister, Tan, Thhekkanal, Erdmann and Scheider, 2008). A NT- proBNP value of less than 125 pg/ml excludes cardiac dysfunction and heart failure. A NT- proBNP value of greater than 125 pg/ml may indicate cardiac dysfunction and are associated with an increased risk of cardiac complications such as myocardial infarction heart failure and death (Cardiac Biomarkers, 2009).

3.6.3 Coronary Angiogram

Once the patients decided on an appropriate date to have the coronary angiogram, they were admitted, on that particular day, into the intensive care unit (ICU) of St Anne's hospital. All patients were starved for approximately 12 hours prior to left and right coronary angiography (Figure 11). The procedure was performed by the cardiologist and the principle investigator, in the cardiac catheterization laboratory. The duration of the procedure was about 30 minutes.

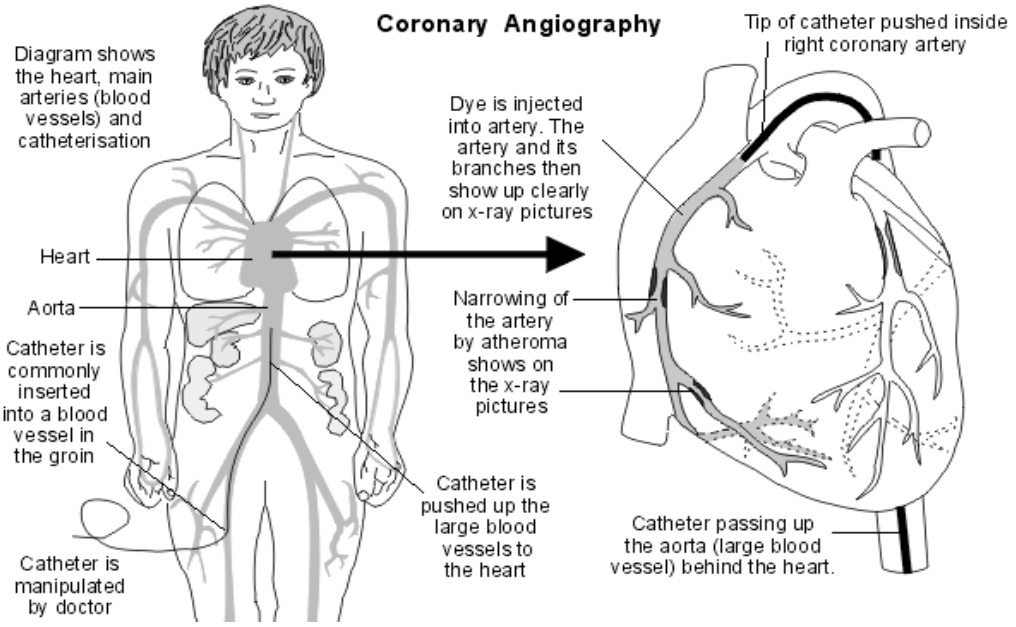


Figure 11: Schematic representation of the heart, main arteries and catheterisation

(Watson and Gorski, 2000)

3.7 Data Analysis

SPSS version 15.0 (SPSS Inc., Chicago, Illinois) was used to analyse the data. A p value <0.05 was considered as statistically significant. In order to identify the optimum cut point of NT- proBNP to indicate the presence of CAD (one or more vessels involved), receiver operator curves (ROC) were constructed in each group separately. The area under the curve was computed and tested for significance. A non parametric distribution was assumed. Cut points were decided on by examining the sensitivity and specificity of each value in the table and choosing a value which optimised these parameters.

CHAPTER FOUR: RESULTS

The results of sixty patients, were studied to determine whether increased NT- proBNP values post EST were indicative of coronary artery disease. These patients were recruited after they had had an inconclusive or positive exercise stress test performed Thereafter NT-proBNP blood level test measurements were done and the patients underwent a diagnostic coronary angiogram.

Patient demographics

Sixty patients were studied (thirty male and thirty female, mean ages and standard deviation = 57.60 +/- 10.337). Thirty patients comprised the group of positive EST (Group A) and thirty in the group of inconclusive EST (Group B) (Table 10 and 11). In each group there were fifteen males and fifteen females with age-group ranging from 36 to 85 years old. There was, however, a significant difference in mean age between the two groups (p=0.004). The mean age of the positive EST group was higher than that of the inconclusive group.

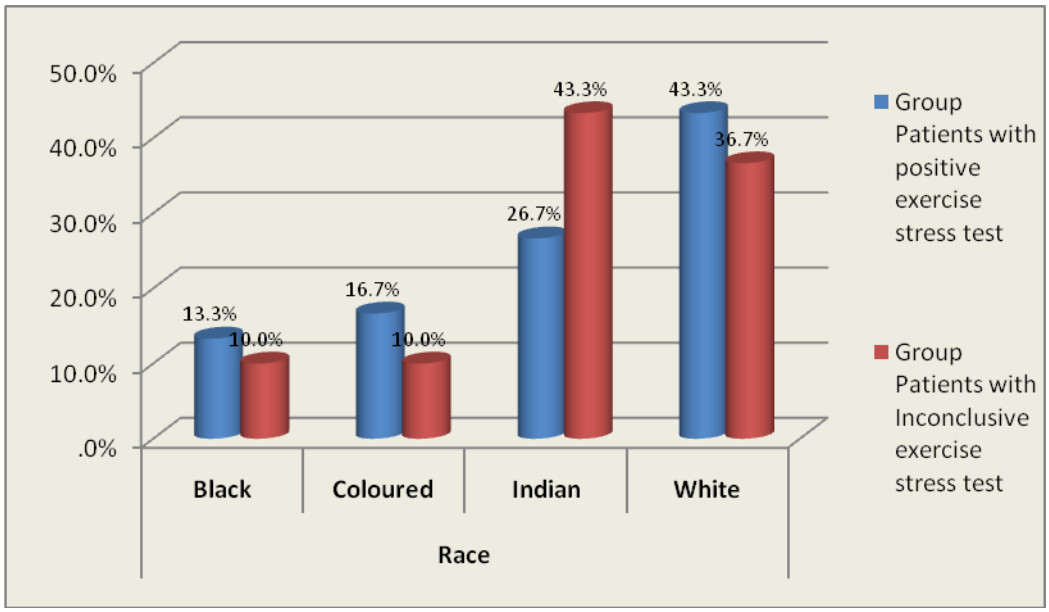
The composition of patients in Group A, were predominantly White (43.3%) followed by Indian (26.7%), Coloured (16.7%) and Black (13.3%). Similarly in Group B there were predominantly Indian (43.3%) followed by White (36.7%), Coloured (10%) and Black (10%) (Graph 1). All patients possessed at least one of the cardiac risk factors.

Table 10: Demographical details of patients with positive EST

Race Groups	N= 30										
			Clinical History							EST Protocol	
	Male	Female	Angina	Dyspnoea	Hypertension	Hypercholesterolaemia	Smoker	Diabetes Mellitus	Family history of ischaemic heart disease	Bruce	Naughton
Black	4	0	4	2	2	1	2	0	2	4	0
Coloured	3	2	2	4	2	1	1	1	2	5	0
Indian	6	2	6	6	7	5	6	5	4	8	0
White	2	11	8	10	7	5	4	0	4	11	2
TOTAL	**15**	**15**	**20**	**22**	**18**	**12**	**13**	**6**	**12**	**28**	**2**

Table 11: Demographical details of patients with inconclusive EST

Race Groups	N= 30										
			Clinical History							EST Protocol	
	Male	Female	Angina	Dyspnoea	Hypertension	Hypercholesterolaemia	Smoker	Diabetes Mellitus	Family history of ischaemic heart disease	Bruce	Naughton
Black	1	2	3	1	0	0	0	0	0	3	0
Coloured	1	2	2	2	2	1	0	2	1	3	0
Indian	5	8	8	9	3	3	2	1	6	13	0
White	8	3	6	7	5	2	4	2	1	10	1
TOTAL	**15**	**15**	**19**	**19**	**10**	**6**	**6**	**5**	**8**	**29**	**1**



Graph 1: Graphic Representation of Race

There was no significant difference between the two groups in terms of race group (p=0.572) (Table 12). However, there were more Whites in the positive EST group and more Indians in the inconclusive EST group. Therefore, correlations could not be done within race groups as the sample size was limited and could skew the results.

Table 12: Race count within Groups

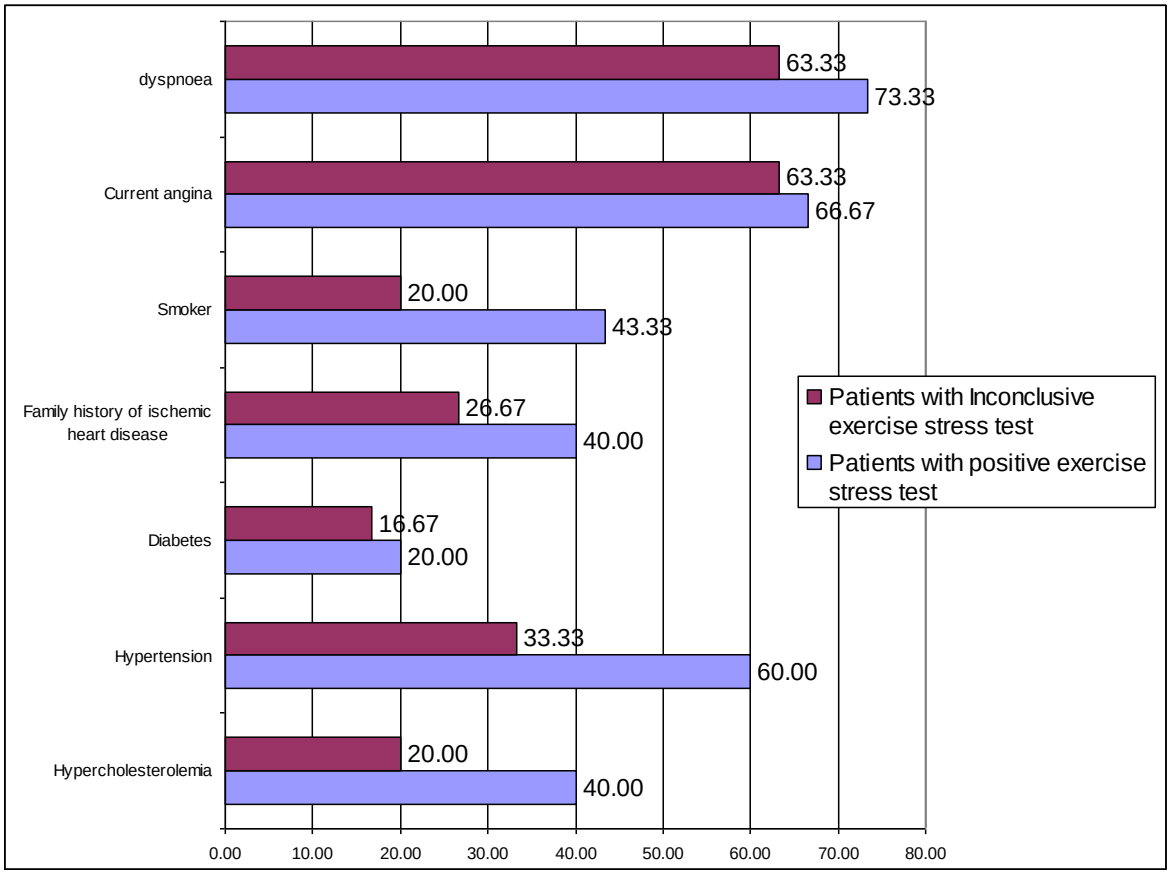
Race		Group		Total
		Patients with positive exercise stress test	Patients with Inconclusive exercise stress test	
Black	Count	4	3	7
Coloured	Count	5	3	8
Indian	Count	8	13	21
White	Count	13	11	24
Total	Count	30	30	60

Chi-Square Tests

	Value	df	P- value
Pearson Chi-Square	2.000(a)	3	0.572
Likelihood Ratio	2.018	3	0.569
Linear-by-Linear Association	.066	1	0.798
N of Valid Cases	60		

a 4 cells (50.0%) have expected count less than 5. The minimum expected count is 3.50.

The presence of risk factors are shown in Graph 2 with the prevalence rates for the two sets of patients. As expected, the general trend that is observed is that the risk factor and symptomatology are higher for patients with positive exercise stress test. The most common factors were angina and dyspnoea in both groups (more than 50% for each group) and the least prevalent is diabetes in both groups.



Graph 2: Risk factors that contribute to coronary artery disease

There was a significant association between number of disease vessels and group (p<0.001). Majority of the patients (n=22) that had normal coronary arteries were in the inconclusive EST group. The remaining 8 patients in the inconclusive group had coronary artery disease but only single vessel disease. In the positive EST group, majority of the patients (n= 12) had single vessel disease (Table 13). Patients with negative ESTs did not undergo coronary angiography as this would've been unethical in this private setting.

Table 13: Angiographic findings

Angiographic findings (number of vessels diseased)	Positive EST	Negative EST	Inconclusive EST
Normal	5	N/A	22
Single vessel disease	12	N/A	8
Double vessel disease	6	N/A	0
Triple vessel disease	5	N/A	0

*N/A: Not studied. See study design

Chi-Square Tests

	Value	df	P- value
Pearson Chi-Square	19.559(a)	3	<0.001
Likelihood Ratio	24.203	3	0.000
Linear-by-Linear Association	18.109	1	0.000
N of Valid Cases	60		

a 4 cells (50.0%) have expected count less than 5. The minimum expected count is 2.50.

Objective 1: To identify a possible value of NT- proBNP levels which indicates CAD

A receiver operator curve was constructed to assess the ability of NT-pro-BNP to detect the presence and severity of coronary artery disease. This analysis was done separately for the two groups of participants (Group A and B). This is because we hypothesized that the cut-off points found in the two groups were very different, indicating that separate cut-off points should be used in the clinical situation depending on whether the patient has a positive or inconclusive stress test.

Patients with a positive EST:

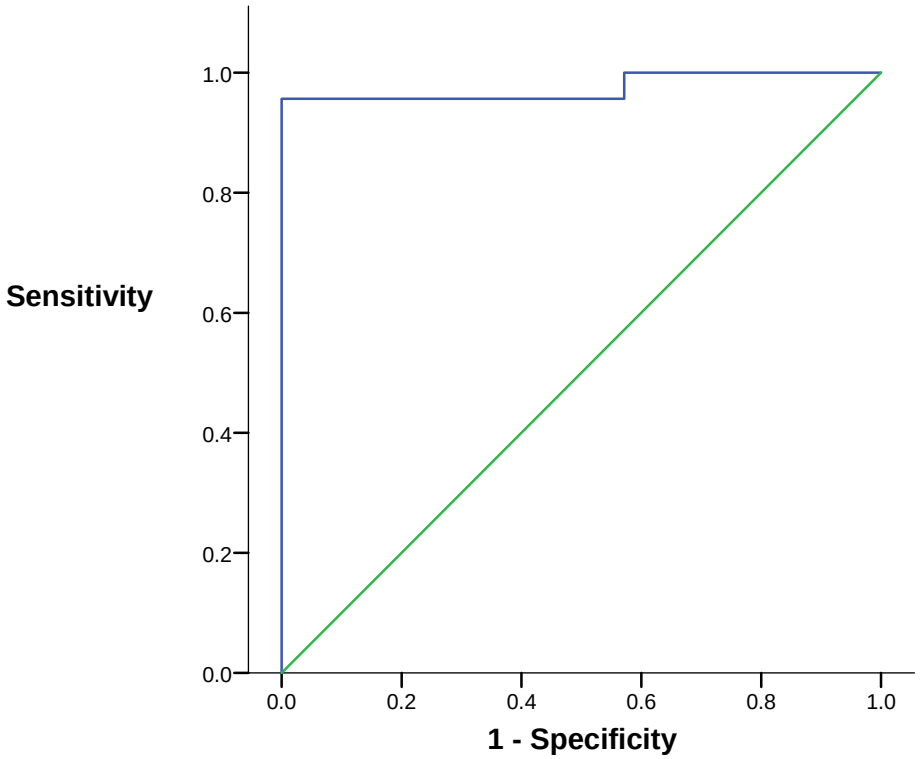
The area under the curve was 0.975 and was highly statistically significantly different from the null hypothesis value of 0.5 ($p<0.001$), indicating that NT- proBNP could very accurately predict the presence of CAD. The cut-off of 120 pg/ml was identified with the highest sensitivity (95.7%) and specificity (100%).

Area under the curve ROC analysis for NT- proBNP to predict CAD in patients with a positive EST

Area	Std. Error(a)	Asymptotic Sig.(b)	Asymptotic 95% Confidence Interval	
0.975	0.027	<0.001	0.923	1.028

(a) Under the nonparametric assumption (b). Null hypothesis: true area = 0.5

Graph 3 shows that the sensitivity of NT- proBNP was very high at all values of 1-specificity.



Graph 3: ROC curve for NT-proBNP vs. CAD in patients with a positive EST

Patients with inconclusive EST:

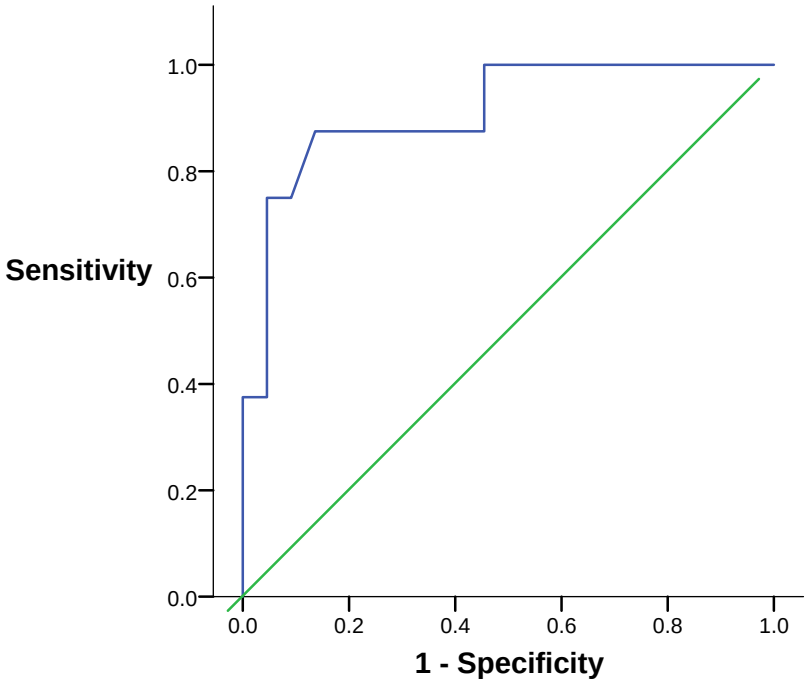
The area under the curve was 0.912 and was highly statistically significantly different from the null hypothesis value of 0.5 ($p<0.001$), indicating that NT- proBNP could very accurately predict the presence of CAD. The cut-off value of 85 pg/ml was identified with the highest sensitivity (87.5%) and specificity (86.4%) (Graph 4).

Area under the curve ROC analysis for NT- proBNP to predict CAD in patients with an inconclusive EST

Area	Std. Error(a)	Asymptotic Sig.(b)	Asymptotic 95% Confidence Interval	
.912	.061	.001	.793	1.031

(a) Under the nonparametric assumption (b). Null hypothesis: true area = 0.5

Graph 4 shows that the sensitivity of NT- proBNP was very high at all values of 1-specificity.



Diagonal segments are produced by ties

Graph 4: ROC curve for NT-proBNP vs. CAD in patients with an inconclusive EST

Objective 2: **To assess the association between exercise stress testing (EST) and CAD**

Excercise Stress Test group and presence of CAD were cross-tabulated and a Pearson's chi square test was performed to assess whether positive EST could predict CAD (Table 14).

There was a highly significant association between EST and CAD ($p<0.001$). The relative risk of having CAD in a positive EST is 2.88 (95% Confidence interval 1.54 to 5.37) conferring a 2.9 times higher risk of having CAD than someone who has an inconclusive EST.

Table 14: Cross tabulation of EST group and CAD

Group	Count	CAD		
		Negative	Positive	Total
Patients with positive EST	Count	7	23	30
Patients with Inconclusive EST	Count	22	8	30
Total	Count	29	31	60

Objective 3: To Compare the median number of diseased vessels by presence or absence of specific risk factors

Within the group of patients with positive EST, Table 15 shows that there was a statistically significantly higher number of vessels diseased in those who were smokers than in the non smokers. Within the group with an inconclusive EST there was a significantly higher number of vessels involved in those with hypercholesterolemia and hypertension compared to those who did not have these risk factors. Family history and current angina did not have an impact on number of diseased vessels in either group.

Table 15: Mann-Whitney test to compare median number of vessels diseased between those with and without specific risk factors

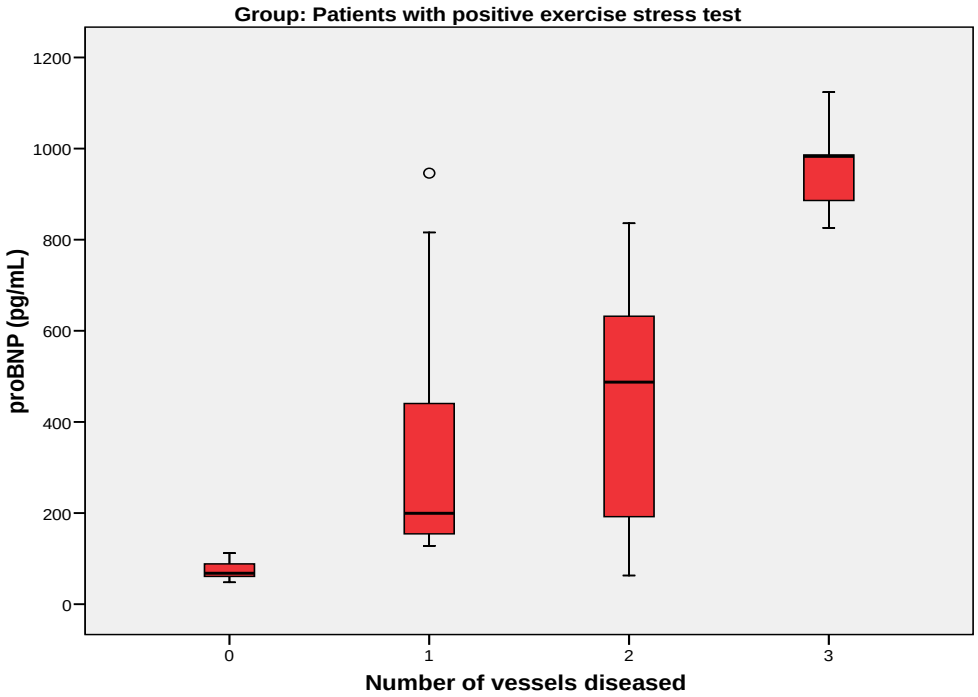
Risk Factors		Number of vessels diseased (median, p value)			
		Patients with positive EST		Patients with inconclusive EST	
Hypercholesterolaemia	Yes	1.0	0.877	1.0	0.015
	No	1.0		0.0	
Hypertension	Yes	1.0	0.825	0.5	0.045
	No	1.0		0.0	
Diabetes	Yes	2.0	0.109	1.0	0.069
	No	1.0		0.0	
Family history of ischemic heart disease	Yes	1.0	0.214	0.5	0.087
	No	1.0		0.0	
Smoker	Yes	2.0	0.016	0.0	0.543
	No	1.0		0.0	
Current angina	Yes	1.0	0.065	0.0	0.103
	No	1.0		0.0	

Objective 4: To compare NT- proBNP levels with number of diseased vessels

The median NT- proBNP level was compared between the groups with different numbers of diseased vessels using Kruskal-Wallis tests. In patients with a positive EST the median NT-proBNP value increased as the number of diseased vessels increased (Table 16). This increase was highly significant ($p<0.001$) (Appendix 10). This trend is shown graphically in graph 5.

Table 16: NT-proBNP levels and number of diseased vessels in the positive EST group

Number of vessels diseased	NT-proBNP (pg/ml)		
	Median	Minimum	Maximum
0	68.00	48	112
1	199.50	128	946
2	487.50	63	836
3	983.00	826	1124
Total	199.50	48	1124

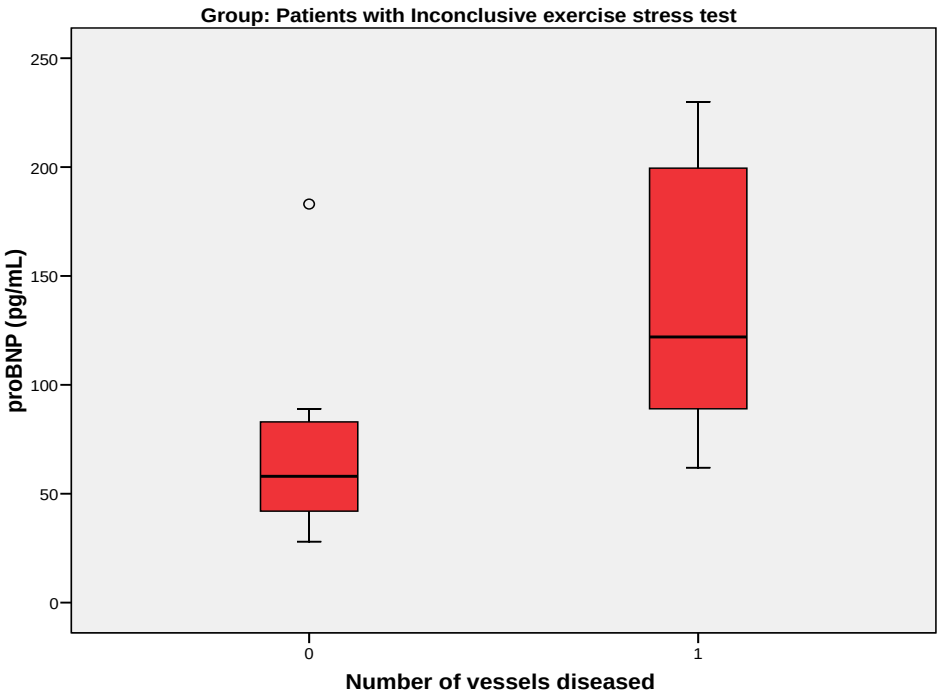


Graph 5: Boxplot of NT-proBNP levels by number of diseased vessels in positive EST group

In patients with an inconclusive EST there were only patients with either 0 or 1 diseased vessel. Predictably, the median NT- proBNP value was higher in those with one vessel diseased compared to those with none (Table 17). This increase was highly significant (p=0.001) (Appendix 11). Graph 6 shows this trend graphically. There was also a highly significant difference in median NT- proBNP between the groups (p<0.001).

Table 17: NT-proBNP levels and number of diseased vessels in the inconclusive EST group

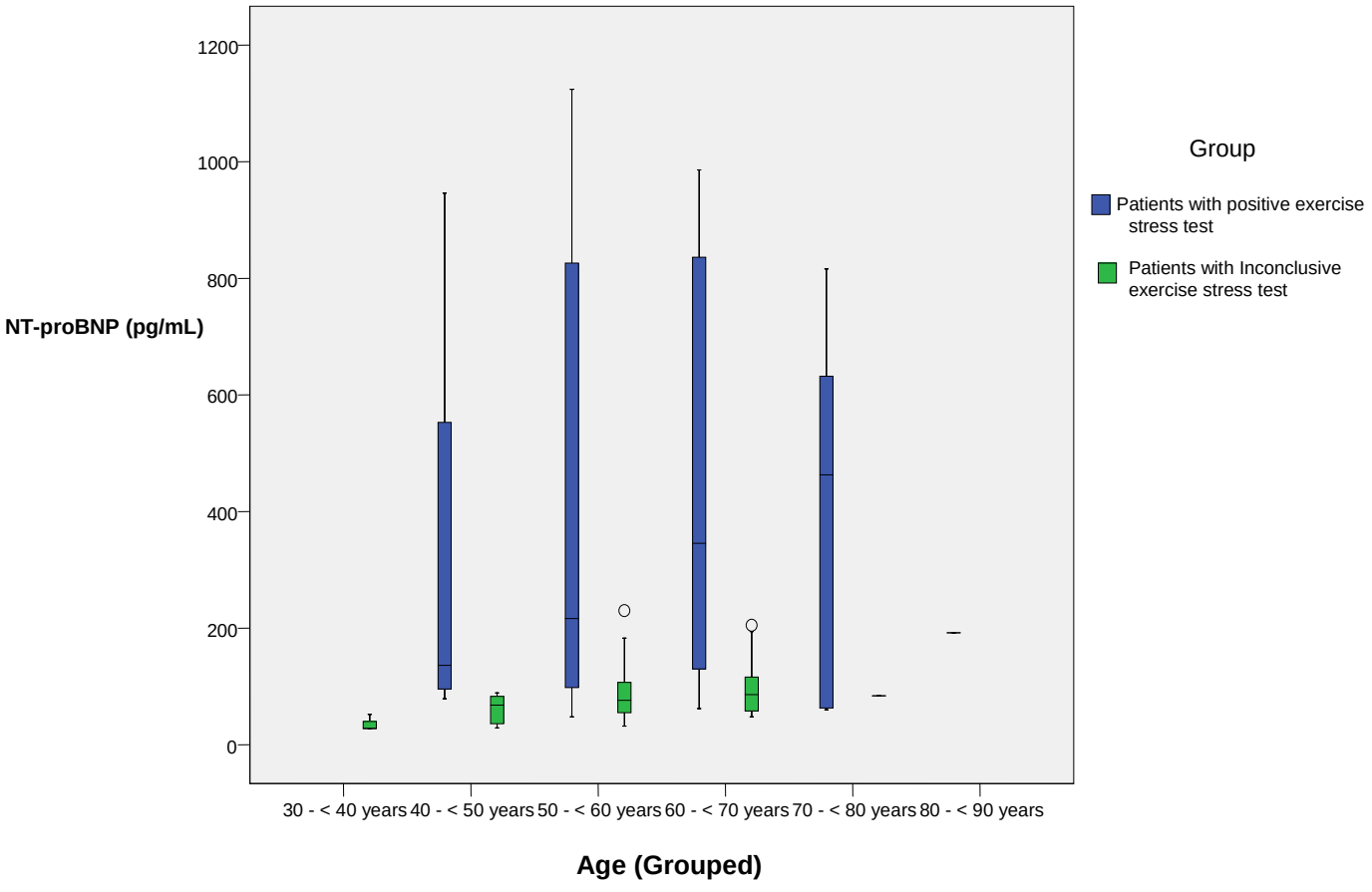
Number of vessels diseased	NT-proBNP (pg/ml)		
	Median	Minimum	Maximum
0	58.00	28	183
1	122.00	62	230
Total	70.50	28	230



Graph 6: Boxplot of NT- proBNP levels by number of diseased vessels in inconclusive EST group

Objective 5: To assess the association between proBNP levels and patients' age and gender

The Kruskal-Wallis test to compare median NT- proBNP value between the age groups was not statistically significant in either of the patient groups. Although a trend of increasing NT-proBNP with age can be noted in the patients with a positive EST, the results failed to reach significance due to the large spread of data around the median at all age groups, which can be seen in the boxplot in Graph 7.



Graph 7: Boxplot of NT- proBNP values by age group and EST group

There was a statistically significant difference between the median NT- proBNP values of males and females in the group of patients with positive EST (p=0.048). The values were higher in males. However, there was no significant difference between the genders in the group with an inconclusive EST (Table 18).

Table 18: Kruskal-Wallis and Mann-Whitney test to compare median NT-proBNP values between age and gender groups

		NT- proBNP (pg/ml) (median, p value)			
		Patients with positive EST		Patients with inconclusive EST	
Age (Grouped)	30 - < 40 years	.	0.859	28.0	0.112
	40 - < 50 years	136.0		68.0	
	50 - < 60 years	216.5		76.0	
	60 - < 70 years	345.5		86.0	
	70 - < 80 years	463.0		84.0	
	80 - < 90 years	192.0		KK.	
Gender	Male	245.0	0.048	58.0	1.00
	Female	179.0		79.0	

Objective 6: To determine the percentage of false positive ESTs determined by NT-proBNP levels

In patients with positive EST a cut-off of 120 pg/ml (identified with the highest sensitivity and specificity in Graph 3) was used to determine the presence or absence of CAD, and showed zero false positive NT- proBNP values (Table 19). These results suggest that the probability of having false positive elevated NT- proBNP values in the presence of CAD is extremely low. Therefore the sensitivity of NT- proBNP was 95.7% and the specificity was 100%. The probability of false negative NT- proBNP value is 12.5% (1/8 at a value of 120 pg/ml in the presence of CAD).

Table 19: Sensitivity and specificity of false positive and negative NT-proBNP levels to determine CAD at a value of 120 pg/ml in the presence of CAD

			CAD		Total
			Positive	Negative	
NT-proBNP	positive	Count	22	0	22
		% within BNP	100.0%	.0%	100.0%
		% within CAD	95.7%	.0%	73.3%
	negative	Count	1	7	8
		% within BNP	12.5%	87.5%	100.0%
		% within CAD	4.3%	100.0%	26.7%
Total		Count	23	7	30
		% within BNP	76.7%	23.3%	100.0%
		% within CAD	100.0%	100.0%	100.0%

In patients with an inconclusive EST a cut-off of 85 pg/ml (identified with the highest sensitivity and specificity in Graph 4) was used to determine the presence or absence of CAD, and showed 3 false positive NT- proBNP values (Table 20). These results suggest that the probability of having false positive elevated NT- proBNP values in the presence of CAD is 30%. Therefore the sensitivity of NT- proBNP was 87.5% and the specificity was 86.4%. The probability of false negative NT- proBNP value is 5% (1/20 at a value of 85 pg/ml in the presence of CAD).

Table 20: Sensitivity and specificity of false positive and negative NT-proBNP levels to determine CAD at a value of 85 pg/ml in the presence of CAD

			CAD		Total
			Positive	Negative	
NT-proBNP	positive	Count	7	3	10
		% within BNP	70.0%	30.0%	100.0%
		% within CAD	87.5%	13.6%	33.3%
	negative	Count	1	19	20
		% within BNP	5.0%	95.0%	100.0%
		% within CAD	12.5%	86.4%	66.7%
Total		Count	8	22	30
		% within BNP	26.7%	73.3%	100.0%
		% within CAD	100.0%	100.0%	100.0%

Objective 7: To assess the probability of a false positive EST

The probability of a false positive result for EST (positive EST but no CAD, ie, zero vessels involved) was 7/29 or 24.1% (Table 21). The probability of a false negative result (ie. Inconclusive EST but presence of CAD) was 8/31 or 25.8%

Table 21: Cross tabulation of EST group and CAD

			CAD		Total
			Negative	Positive	
GROUP	Patients with positive exercise stress test	Count	7	23	30
		% within CAD	24.1%	74.2%	50.0%
	Patients with Inconclusive exercise stress test	Count	22	8	30
		% within CAD	75.9%	25.8%	50.0%
Total		Count	29	31	60
		% within CAD	100.0%	100.0%	100.0%

The other screening parameters of EST versus the gold standard are shown below with their 95% confidence intervals (in brackets).

Sensitivity : 74% [55%, 87%]

Specificity : 76% [56%, 89%]

Accuracy : 75% [62%, 85%]

Predictive value of +ve result : 77% [57%, 89%]

Predictive value of -ve result : 73% [54%, 87%]

The 95% confidence interval are the values between which one can be 95% sure of what the true population parameters fall between. For example, for sensitivity the estimate was 74% but if that estimate is extrapolated to the population from which the sample was taken then

one can be 95% sure that sensitivity will range between 55% and 87%. This shows that the ESTs conducted in this study was therefore relatively accurate at predicting CAD.

CHAPTER FIVE: DISCUSSION

Sixty patients, (thirty male and thirty female), ages (57.60 ± 10.337) were recruited to participate in this study, which attempted to determine whether increased NT- proBNP plasma levels, post- EST, was indicative of coronary artery disease in patients with an inconclusive exercise stress test, and if so what were the specific cut off values. Group A (positive EST group) and Group B (inconclusive EST group) comprised of thirty patients each (Table 10 and 11). In each group there were fifteen males and fifteen females with age-group ranging from 36 to 85 years old. It was hypothesized that research in cardiac biomarkers, such as NT- proBNP, will reduce the number of inconclusive ESTs resulting in normal angiograms and will also allow for earlier detection of CAD to initiate lifestyle changes or optimise medical therapy before any fatal cardiac events occur.

Since current stress testing methods requires medium to high- grade stenosis to detect the risk of myocardial infarction, stress testing alone is seen as an unreliable approach of detecting CAD which many studies have already demonstrated (Fletcher et al., 2001). Myocardial perfusion imaging, multi- slice CT scanning and exercise stress echocardiography may be used as additional tools in diagnosing CAD but these procedures are costly and the expertise in this setting is not readily available.

In this study, 60 patients underwent coronary angiography to determine the presence of CAD. Of the sixty patients, 74.2% in the positive EST group and 25.8% in the inconclusive EST group had CAD (Table 21).

Brain natriuretic peptide is currently being used as a marker of left ventricular dysfunction

but in addition to popular belief that the main pathophysiological process underlying increased BNP and NT-pro-BNP levels, is not only as a result of increased left ventricular wall stress but also as a direct result of cardiac ischaemia (Fletcher et al., 2001). This was demonstrated in the present study by the relationship between NT- proBNP and the presence of CAD using Kruskal-Wallis tests (Table 16 and 17). In patients with a positive EST, the median NT- proBNP value increased significantly as the number of diseased vessels increased ($p<0.001$) (Appendix 9). This increase is shown graphically in graph 5. In patients with an inconclusive EST, there were only patients with either 0 or single vessel disease. Predictably, the median NT- proBNP value was significantly higher ($p=0.001$) in those with single vessel disease compared to those with none (Appendix 10). This increase is shown graphically in Graph 6. The positive EST group had a significantly higher median NT-proBNP level than the inconclusive EST group ($p<0.001$) as well as a significantly higher number of diseased vessels (Table 14). This suggests that NT- proBNP levels will increase in the presence of CAD.

There was no significant difference between the two groups in terms of race ($p=0.572$) (Appendix 11). However, there were more Whites in the positive EST group and more Indians in the inconclusive EST group. As this is a private practice, the population it draws on is more affluent; therefore, correlations could not be done accurately within race groups as the sample size was limited and could skew the results.

The T-test (Appendix 13), showed that there was a significant difference in mean age between the two groups ($p=0.004$). The mean age of the positive EST group was higher than that of the inconclusive group. The Kruskal-Wallis test to compare median NT- proBNP value between the age groups was not statistically significant in either of the patient groups

(Table 18). Although a trend of increasing NT-proBNP with age can be noted in the patients with a positive EST, however, the results failed to reach significance due to the large spread of data around the median at all age groups, which can be seen in the boxplot in Graph 7. Several studies demonstrated similar results where NT- proBNP levels increased with age. A population- based study by Redfield et al., (2002) examined the effects of age on NT-proBNP concentration. The study sample consisted of 767 subjects in normal sinus rhythm without any cardiovascular disease or renal dysfunction. Results showed that NT- proBNP increased with age. Raymond et al., (2003), conducted a study on 472 patients with heart failure to identify potentially confounding variables for the interpretation of plasma NT-proBNP. By means of multiple linear regression analysis their results demonstrated that an increase in age was independently associated with an increased NT-proBNP level.

There was a statistically significant difference between the median NT- proBNP values of males and females in the group of patients with positive EST ($p=0.048$) (Table 18). The values were higher in males. However, there was no significant difference between the genders in the group with an inconclusive EST. This is not in keeping with current medical trends as females generally have higher NT- proBNP levels than males. This result could be due to the small sample size used in this study and can be seen as a limitation. The results of a study conducted, on 767 normal subjects, by Redfield et al., (2002) showed that NT-proBNP was higher in females than males. The association of increased NT- proBNP in females appeared to be related to oestrogen levels, as NT- proBNP levels were higher in women using hormone replacement therapy (HRT). There have been previous reports that suggest that there is an association between gender-related differences in endothelia and angiotensin-converting enzyme activity with hormonal status. Although preliminary, these

data suggest that NT- proBNP production may be sensitive to oestrogen regulation and represent an area for further study (Redfield et al., 2002).

All patients possessed at least one of the cardiac risk factors. The presence of risk factors are shown in Graph 2, with the prevalence rates for the two sets of patients. As expected, the general trend that was observed was that the risk factor and symptomatology were higher in patients with positive exercise stress test. This could be due to the majority of patients (74.2%) in the positive EST group had CAD. This suggests that the likelihood of the presence of CAD is dependent on risk factors and symptomatology. The most common risk factors were angina and dyspnoea in both groups (more than 50% for each group).

The Mann-Whitney test was used to compare the median number of diseased vessels by the presence or absence of specific risk factors. Within the group of patients with positive EST, Table 20 shows that there was a statistically significantly higher number of vessels diseased in those who were smokers than in the non smokers. This is expected as toxins in cigarette smoke causes plaque formation, thus leading to atherosclerosis. According to Shah and Helfant (2006), the adverse effects of smoking are likely to be related to its effects on coronary vasocclusive factors, such as platelet aggregation, vasomotor reactivity and a prothrombotic state rather than to severity of coronary atherosclerosis. There is overwhelming evidence that the cardiovascular risk is substantially reduced among quitters (Shah and Helfant, 2006). Thus, smoking is appropriately the single most preventable risk factor for coronary disease.

Within the group with an inconclusive EST there was a significantly higher number of vessels involved in those with hypercholesterolaemia and hypertension compared to those

who did not have these risk factors. Family history and current angina did not have an impact on the number of diseased vessels in either group. Many studies suggest that hypertension and hypercholesterolaemia are associated with an increased risk of CAD and myocardial infarction (Adnan, Qureshi, Fareed, Suri, Kirmani, Divani and Mohammad, 2005). Hypertension increases the heart's workload, causing the heart to thicken and become stiffer. In a retrospective review of the complete medical records of 223 hypertension patients, it was found that an age of at least 60 years, increased body mass index and hypercholesterolaemia are independent predictors for CAD (Vennamaneni, Miryala and Bearelly, 2002).

There was a significant association between number of diseased vessels and group ($p<0.001$). The positive EST group had a greater number of diseased vessels than the inconclusive EST group. In the positive EST group, 25 patients had CAD, majority of which (n= 12) had single vessel disease (Table 14). In the inconclusive EST group, 8 patients had CAD but only single vessel disease.

The main objective of the study was assessed by constructing a receiver operator curve (ROC) to assess the ability of NT-pro-BNP to detect the presence and severity of coronary artery disease. This analysis was done separately for the two groups of participants (Group A and B). This is because we hypothesized that the cut-off points found in the two groups were very different, indicating that separate cut-off points should be used in the clinical situation depending on whether the patient has a positive or inconclusive stress test. For the positive EST group, the area under the curve was 0.975 and was highly statistically significantly different from the null hypothesis value of 0.5 ($p<0.001$) (Graph 3), indicating that NT-proBNP could very accurately predict the presence of CAD. The cut- off of 120 pg/ml was identified with the highest sensitivity (95.7%) and specificity (100%).

For patients in the inconclusive EST group, the area under the curve was 0.912 and was highly statistically significantly different from the null hypothesis value of 0.5 ($p<0.001$) (Graph 4), indicating that NT- proBNP could very accurately predict the presence of CAD. The cut- off of 85 pg/ml was identified with the highest sensitivity (87.5%) and specificity (86.4%) (Graph 4).

In a similar study, NT-proBNP levels were measured in 781 consecutive patients with normal left ventricular function referred for coronary angiography owing to symptoms or signs of CAD. Results showed that elevated NT-proBNP levels were significantly associated with the extent of CAD and with the female sex. The ability of NT-proBNP to predict significant coronary disease at angiography was assessed separately for males using a cut-off point of 85 pg/ml and 165pg/l for females. The area under the receiver operating characteristic (ROC) curve was 0.72 for males and 0.71 for females (Wolber , Maeder, Rickli, Riesen, Binggeli, Duru and Ammann (2007). In the present study no adjustments were made for age and gender as there was no significant difference between age and gender in both EST groups. Instead, ROC curves were constructed for each group separately since this study is mainly concerned with the large number of inconclusive ESTs produced in this practice.

Another study assessed the relationship between NT-pro-BNP and the extent of ischaemia on stress myocardial perfusion imaging, in stable patients with a normal left ventricular ejection fraction. The study suggested that the post-stress increase in NT-pro-BNP is related to myocardial ischemia and accurately predicts the presence or absence of myocardial perfusion defects. It further established an optimal NT- proBNP cut-off value of 214 pg/ml for predicting CAD (Vanzetto, Jacon, Calizzano, Neuder, Faure, Fagret and Machecourt (2007). Our study differs considerably as the cut-off value for the positive EST group was 120pg/ml and 85pg/ml for the inconclusive group.

Diastolic dysfunction was not excluded in this study, however, a strong and significant correlation ($p < 0.001$) was demonstrated between LVEDP and the number of diseased vessels, in both groups (Appendix 15). This suggests that myocardial ischaemia resulted in an elevated LVEDP. This was also demonstrated by the ischaemic cascade in Figure 1. A limitation to this objective, apart from the small sample size, was that LVEDP measurements were obtained using computer averages rather than direct measurements from the catheter tip. A further limitation is that the detection and quantification of diastolic dysfunction by other validated methods like Tissue Doppler Imaging was not performed due to increased cost to the patient. We were therefore not able to project a correlation between diastolic dysfunction and NT- proBNP.

A cut-off of 120 pg/ml was used to determine the presence or absence of CAD, and showed zero false positive NT- proBNP values in the positive EST group (Table 19). These results suggest that the probability of having false positive elevated NT- proBNP values in the presence of CAD is extremely low unless it is due to other causes. Therefore, the sensitivity of NT- proBNP was 95.7% and the specificity was 100%. Other causes of increased NT-proBNP levels are; abnormal renal function, emphysema or chronic obstructive pulmonary disease (COPD). Increased levels of proBNP have also been observed in patients on diuretics, patients with atrial fibrillation, congestive heart failure and patients who had a recent MI (Essig, 2007). However, all these patients were excluded from this study. The probability of false negative NT- proBNP value was 12.5% (1/8 at a value of 120 pg/ml in the presence of CAD).

A cut-off of 85 pg/ml was used to determine the presence or absence of CAD, and showed 3 false positive NT- proBNP values in the inconclusive EST group (Table 20). These results

suggest that the probability of having false positive elevated NT- proBNP values in subjects with an inconclusive exercise stress test is 30%. Therefore, the sensitivity of NT- proBNP was 87.5% and the specificity was 86.4%. The probability of false negative NT- proBNP value is 5% (1/20 at a value of 85 pg/ml in the presence of CAD).

The probability of a false positive result for EST (i.e. positive EST but no CAD) was 24.1% (Table 21). The probability of a false negative result (i.e. inconclusive EST but presence of CAD) was 25.8%. The positive EST was therefore, relatively accurate at predicting CAD. However, only 25.8% of patients with an inconclusive EST had CAD, suggesting that 75.9% of patients with an inconclusive EST had to undergo coronary angiography, unnecessarily. Exercise stress testing in this regard, is therefore relatively inaccurate at predicting CAD in patients with inconclusive ESTs.

CHAPTER SIX: CONCLUSION

At present, exercise stress testing is being utilized as the method of choice in diagnosing coronary artery disease. However, many studies have demonstrated its unreliability. This research study aimed to determine the presence and severity of coronary artery disease using NT- proBNP blood level measurements in conjunction with exercise stress testing.

Results of the study showed that NT- proBNP levels post EST, in both positive EST and inconclusive groups, increased in the presence of CAD. For the positive EST group a cut- off value of 120 pg/ml was identified with the highest sensitivity (95.7%) and specificity (100%). The area under the ROC curve was 0.975 and was highly statistically significantly different from the null hypothesis value of 0.5 ($p<0.001$) indicating that NT- proBNP could very accurately predict the presence of CAD.

For patients in the inconclusive EST group, a cut- off value of 85 pg/ml was identified with the highest sensitivity (87.5%) and specificity (86.4%). The area under the ROC curve was 0.912 and was highly statistically significantly different from the null hypothesis value of 0.5 ($p<0.001$), indicating that NT- proBNP could very accurately predict the presence of CAD.

A trend of increasing NT-proBNP with age was noted in patients with a positive EST, however, the results failed to reach significance due to the large spread of data around the median at all age groups. Also, NT-proBNP values were higher in males. This is not in keeping with current medical trends as females generally have higher NT- proBNP levels than males. However, there was no significant difference between the genders in the group

with an inconclusive EST. This result could be due to the small sample size used in this study and can be seen as a limitation.

As expected, the general trend that was observed in this study was that the risk factor and symptomatology were higher in patients with positive exercise stress test. This was due to the majority of patients (74.2%) in the positive EST group having CAD. This suggests that the likelihood of the presence of CAD is dependent on risk factors and symptomatology. The most common risk factors were angina and dyspnoea in both groups (more than 50% for each group).

Results of the study also showed that, within the group of patients with positive EST, there was a statistically significantly higher number of vessels diseased in those who were smokers than in the non smokers. Within the group with an inconclusive EST there was a significantly higher number of vessels involved in those with hypercholesterolaemia and hypertension compared to those who did not have these risk factors. This confirms that comorbidities such as hypercholesterolaemia, hypertension and smoking increased the risk of CAD.

The current study showed strong and significant correlations between LVEDP and the number of disease vessels. This suggested that although diastolic dysfunction was not excluded from the study, elevated LVEDP was as a result of myocardial ischaemia.

The probability of a false positive result for EST (i.e. positive EST but no CAD) was 24.1%. and the probability of a false negative result (i.e. inconclusive EST but presence of CAD) was 25.8%.

Exercise stress testing in this regard, is therefore relatively inaccurate at predicting CAD in patients with inconclusive ESTs, and the need for an additional tool, such as NT-proBNP measurements post inconclusive EST is warranted in the determination of the presence of CAD.

However, there were certain limitations to the study which include:

1. Negative ESTs were not sampled.
2. NT- proBNP blood measurements were not done prior to and at peak exercise stress test.
3. Study population was more affluent as this study was conducted in a private practice.
4. NT– proBNP blood levels were measured 10 – 15 minutes post peak exercise as opposed to 5 minutes.
5. A direct measurement of the LVEDP at the catheter tip was not used; rather a computer average was measured and LVEDP was not measured at the time of sampling for NT- proBNP.

Future studies will take into consideration the above mentioned limitations.

"I would not be surprised if [BNP] became a routine part of risk assessment," says Foote (2008).

CHAPTER SEVEN: REFERENCES

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APPENDIX 1: Demographic raw data for the positive EST group

Male/ Female	Age	Race	proBNP (pg/ml)	Number of vessels diseased
Male	46	Coloured	946	1
Female	55	White	68	0
Female	58	White	98	0
Female	45	White	79	0 (vasospastic disease)
Male	58	White	245	1
Male	55	Indian	826	3
Male	61	Indian	986	3
Female	62	Coloured	62	0
Female	45	White	112	0 (vasospastic disease)
Female	58	White	230	1
Female	68	Indian	130	1
Male	76	Indian	63	2
Female	73	White	632	2
Male	60	Indian	983	3
Female	55	White	48	0
Male	53	Indian	1124	3
Male	77	Black	60	0
Female	65	White	179	1
Female	59	White	196	1
Male	67	Black	149	1
Male	85	Coloured	192	2
Male	65	Indian	636	1
Female	64	Indian	836	2
Female	50	White	886	3
Male	69	Coloured	128	1
Male	53	White	203	1
Female	73	White	463	2
Male	60	Black	512	2
Female	78	Coloured	816	1
Male	47	Black	160	1

APPENDIX 2: Demographic raw data for the inconclusive EST group

Male/ Female	Age	Race	proBNP (pg/mL)	Number of vessels diseased
Male	68	White	58	0
Male	53	Indian	52	0
Female	53	Indian	86	0
Female	45	Indian	68	0
Female	45	Black	36	0
Female	44	Indian	29	0
Female	51	White	58	0
Male	61	Indian	62	1
Male	56	White	183	0
Female	56	Indian	79	0
Male	61	Indian	48	0
Male	38	Indian	52	0
Female	51	White	83	0
Male	55	White	42	0
Female	58	Indian	68	0
Male	44	White	83	0
Female	54	Black	73	0
Male	37	White	28	0
Male	62	Coloured	116	1
Female	56	Coloured	230	1
Female	62	Coloured	56	0
Male	36	Indian	28	0
Male	51	White	32	0
Male	71	White	84	0
Female	51	Indian	128	1
Male	60	Black	86	1
Female	67	White	92	1
Female	60	Indian	194	1
Male	61	White	205	1
Female	49	Indian	89	0

APPENDIX 3: Race vs. ProBNP in the positive EST group

ProBNP (pg/ml)	Black	Coloured	Indian	White
0 - < 100	3.3%	3.3%	3.3%	13.3%
100 - < 200	6.7%	6.7%	3.3%	10.0%
200 - < 300	.0%	.0%	.0%	10.0%
400 - < 500	.0%	.0%	.0%	3.3%
500 - < 600	3.3%	.0%	.0%	.0%
600 - < 700	.0%	.0%	3.3%	3.3%
800 - < 900	.0%	3.3%	6.7%	3.3%
900 - < 1000	.0%	3.3%	6.7%	.0%
1100 - < 1200	.0%	.0%	3.3%	.0%

APPENDIX 4: Race vs. ProBNP in the inconclusive EST group

ProBNP (pg/ml)	Black	Coloured	Indian	White
0 - < 100	10.0%	3.3%	36.7%	30.0%
100 - < 200	.0%	3.3%	6.7%	3.3%
200 - < 300	.0%	3.3%	.0%	3.3%

APPENDIX 5: Gender vs. proBNP in the positive EST group

ProBNP (pg/ml)	Gender		Total
	Male	Female	
0 - < 100	6.7%	16.7%	23.3%
100 - < 200	13.3%	13.3%	26.7%
200 - < 300	6.7%	3.3%	10.0%
400 - < 500	.0%	3.3%	3.3%
500 - < 600	3.3%	.0%	3.3%
600 - < 700	3.3%	3.3%	6.7%
800 - < 900	3.3%	10.0%	13.3%
900 - < 1000	10.0%	.0%	10.0%
1100 - < 1200	3.3%	.0%	3.3%

APPENDIX 6: Gender vs. ProBNP in the inconclusive EST Group

ProBNP (pg/ml)	Gender		Total
	Male	Female	
0 - < 100	40.0%	40.0%	80.0%
100 - < 200	6.7%	6.7%	13.3%
200 - < 300	3.3%	3.3%	6.7%

APPENDIX 7: Coordinates of the ROC Curve for patients with a positive EST

Positive if Greater Than or Equal To (a)	Sensitivity	1 – Specificity
47.00	1.000	1.000
54.00	1.000	.857
61.00	1.000	.714
62.50	1.000	.571
65.50	.957	.571
73.50	.957	.429
88.50	.957	.286
105.00	.957	.143
120.00	.957	.000
129.00	.913	.000
139.50	.870	.000
154.50	.826	.000
169.50	.783	.000
185.50	.739	.000
194.00	.696	.000
199.50	.652	.000
216.50	.609	.000
237.50	.565	.000
354.00	.522	.000
487.50	.478	.000
572.00	.435	.000
634.00	.391	.000
726.00	.348	.000
821.00	.304	.000
831.00	.261	.000
861.00	.217	.000
916.00	.174	.000
964.50	.130	.000
984.50	.087	.000
1055.00	.043	.000
1125.00	.000	.000

APPENDIX 8: Coordinates of the ROC Curve for patients with an inconclusive EST

Positive if Greater Than or Equal To (a)	Sensitivity	1 – Specificity
27.00	1.000	1.000
28.50	1.000	.909
30.50	1.000	.864
34.00	1.000	.818
39.00	1.000	.773
45.00	1.000	.727
50.00	1.000	.682
54.00	1.000	.591
57.00	1.000	.545
60.00	1.000	.455
65.00	.875	.455
70.50	.875	.364
76.00	.875	.318
81.00	.875	.273
83.50	.875	.182
85.00	.875	.136
87.50	.750	.091
90.50	.750	.045
104.00	.625	.045
122.00	.500	.045
155.50	.375	.045
188.50	.375	.000
199.50	.250	.000
217.50	.125	.000
231.00	.000	.000

The test result variable(s): proBNP (pg/mL) has at least one tie between the positive actual state group and the negative actual state group.

a The smallest cutoff value is the minimum observed test value minus 1, and the largest cutoff value is the maximum observed test value plus 1. All the other cutoff values are the averages of two consecutive ordered observed test values.

b Group = Patients with Inconclusive exercise stress test

APPENDIX 9: Test statistics of NT- proBNP levels with number of diseased vessels in the positive EST group

	proBNP (pg/mL)
Chi-Square	20.191
Df	3
Asymp. Sig.	<0.001

a Kruskal Wallis Test
b Grouping Variable: Number of vessels diseased

APPENDIX 10: Test statistics of NT- proBNP levels with number of diseased vessels in the inconclusive EST group

	proBNP (pg/mL)
Chi-Square	11.576
df	1
Asymp. Sig.	0.001

a Kruskal Wallis Test
b Grouping Variable: Number of vessels diseased

APPENDIX 11: Chi-Square Tests (p value) for race groups

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	2.000(a)	3	.572
Likelihood Ratio	2.018	3	.569
Linear-by-Linear Association	.066	1	.798
N of Valid Cases	60		

a 4 cells (50.0%) have expected count less than 5. The minimum expected count is 3.50.

APPENDIX 12: Chi-Square Tests (p value) for angiographic findings

	Value	df	P- value
Pearson Chi-Square	19.559(a)	3	<0.001
Likelihood Ratio	24.203	3	0.000
Linear-by-Linear Association	18.109	1	0.000
N of Valid Cases	60		

a 4 cells (50.0%) have expected count less than 5. The minimum expected count is 2.50.

APPENDIX 13: T-Test for Equality of Means

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
Age	Equal variances assumed	.701	.406	2.978	58	.004	7.467	2.507	2.448	12.485
	Equal variances not assumed			2.978	56.721	.004	7.467	2.507	2.446	12.487

APPENDIX 14: LVEDP vs. Number of Diseased Vessels

Group	P- value
Patients with Positive EST	<0.001
Patients with Inconclusive EST	<0.001

APPENDIX 15: REAGENTS (WORKING SOLUTIONS)

M Streptavidin- coated microparticles (transparent cap), 1 bottle, 6.5mL: Streptavidin- coated microparticles 0.72mg/mL; preservative.

R1 Anti- NT- proBNP- Ab- biotin (gray cap), 1 bottle, 9mL: Biotinylated monoclonal anti- NT- proBNP antibody (mouse) 1.1µg/mL; phosphate buffer 40mmol/L, pH 5.8; preservative.

R2 Anti- NT- proBNP- Ab~ Ru(bpy) (black cap), 1 bottle, 9mL: monoclonal anti- NT- proBNP antibody (sheep) labelled with ruthenium complex 1.1µg/mL; phosphate buffer 40mmol/L, pH 5.8; preservative (www.probnp.com).

APPENDIX 16: PATIENT INFORMATION LEAFLET AND CONSENT (ENGLISH)

Study Title:

The predictive value of pro BNP levels to determine the presence and severity of coronary artery disease in patients with an inconclusive exercise stress test

Introduction:

My name is Nivashni and I am conducting a study with Dr A Pearce as my supervisor. Before you agree to take part in this study, you should fully understand what it entails. If you have any questions that are not fully explained in this leaflet, do not hesitate to ask.

Purpose of the study:

You have consulted your cardiologist to examine you for any possible heart conditions. After your exercise stress test, you will have to do blood test to measure your pro BNP levels and thereafter you will have a coronary angiogram. The purpose of this study is to determine a possible value of pro BNP levels that indicate presence and severity of coronary artery disease. The results of this test will help the cardiologist, in future, to determine whether or not you have coronary artery disease. This will save you time and money in the future.

Your rights as participant in this study:

Your participation in this study will be entirely voluntary and you can refuse to participate or stop at any time without stating any reason.

Confidentiality:

All information obtained during the course of this study is strictly confidential. Reported data will not include any information that identifies you as a patient in the study. You will be informed of any findings of importance to your health.

Risks involved in the study:

There are no risks involved in this study

For more information call:

Supervisor: Dr Adrian Pearce
033 3455 310 (work)
083 2760 905 (cell)

Student : Nivashni Naidoo
033 3426 522 (work)
073 8300 384 (cell)

Patient signature: ……. Date: .....

APPENDIX 17: PATIENT INFORMATION LEAFLET AND CONSENT (ZULU)

IPHESHANA LOLWAZI NGESIGULI NEMVUME NGESIGULI

Isihloko socwaningo:

Inani lokuqagela amazinga e-BNP ukuze utshengise izinga lesifo semithambo yenhliziyo ezigulini ekade zibe nemiphumela engawona umnqamula juqu ekuhlolweni ngokuzivocavoca.

Isingeniso:

Igama lami ngingu-Nivashni,ngenza ucwaningo nodokotela u- A.Pearce njengenduna/mphathi wami.Phambi kokuba uvume ukuba yingxenye yalolucwaningo kudingeka ukuba uqonde kahle ukuthi lolucwaningo luqukethe.Uma unemibuzo engachazekile kulelipheshana,ungangabazi ukubuza.

Inhloso yocwaningo :

Uhambile wayobona udokotela wezinhliziyo ukuze akuxilongele isifo senhliziyo.Emuva kokuhlolwa ngokuzivocavoca,kuzomele uthathwe amagazi ukukala amazinga e-BNP yakho,emuva kwalokho kuzomele uxilongwe imithambo yenhliziyo..Inhloso yalocwaningo ukuthola ukuthi ngabe likhona yini inani lezinga le –BNP elitshengisa izinga lesifo semithambo yenhliziyo.Imiphumela yalokhu kuhlolwa izosiza udokotela wezinhliziyo,ukuze nangomuso akwazi ukusho ukuthi unaso yini noma awunaso isifo semithambo yenhliziyo.Lokhu kuzokongela isikhathi nemali.

Amalungelo akho njengomuntu ozibandakanye nalolucwaningo:

Ukuzibandakanya kwakho nalolucwaningo kufana nokunikela kanti futhi unganqaba noma nini ukuqhubeka ngaphandle ngokuba uze ubeke isizathu.

Ukugodlwa kolwazi:

Lonke ulwazi olutholakele ngesikhathi kwenzeka lolucwaningo luyimfihlo.Okuyokhishwa ngeke kuqukethe ulwazi oluyokuveza njengesiguli kulolucwaningo.Uyokwaziswa ngokutholakele ikakhukukazi okubalulekile mayelana nempilo yakho.

Uma udinga olunye ulwazi, shaya

Umphathi wocwaningo: **Dokotela Adrian Pearce**
033 3455 310(Emsebenzini)
083 2760 905(Cell)

Umfundi: Nivashni Naidoo
033 3426 522(Emsebenzini)
073 8300 384(Cell)

Isandla sesiguli:…… **Usuku:**.....