

**Neurohormonal and Inflammatory
Markers in Valvular Heart Disease**

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DOCTOR OF MEDICINE

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ABSTRACT

Chronic valvular heart disease is characterised by compensatory mechanisms that result in a long asymptomatic phase associated with variable disease progression. After the development of symptoms or left ventricular dysfunction, mortality is high without surgical intervention. Currently there is no known medical therapy that influences disease progression or clinical outcome. While the development of symptoms or left ventricular dysfunction are the cardinal indications for valve surgery, routine echocardiography may not detect early left ventricular dysfunction and the development of early symptoms may not be appreciated. Numerous studies demonstrate that increased natriuretic peptide plasma levels, including atrial natriuretic peptide (ANP), B-type natriuretic peptide (BNP) and amino-terminal BNP (N-BNP) reflect left ventricular dysfunction, correlate with symptoms of cardiac failure and are independent prognostic markers for clinical outcomes in diverse cardiac conditions, but very few studies address natriuretic peptides in patients with valvular heart disease.

The aims of this thesis are firstly, to determine the clinical utility of measuring natriuretic peptide plasma levels in patients with valvular heart disease, and secondly, to provide supportive biochemical evidence to established histological evidence that aortic stenosis is an inflammatory disease.

One hundred and sixty three patients with chronic valvular heart disease, including aortic stenosis (n=74), aortic regurgitation (n=40) and mitral regurgitation (n=49) underwent independent assessment of symptoms, transthoracic echocardiography and measurement of plasma levels of ANP, BNP and N-BNP. Natriuretic peptide levels were significantly higher in symptomatic compared with asymptomatic patients after adjustment for echocardiographic measures of disease severity and left ventricular function. Of 29 asymptomatic patients with aortic stenosis followed for a mean of 18

months, patients with an N-BNP level above the normal range or with a greater increase in N-BNP/year were at increased risk of symptomatic deterioration. In 33 patients with aortic stenosis who underwent aortic valve replacement, N-BNP levels decreased and symptoms consistently improved by 6 months postoperatively in patients with a preoperative N-BNP level above the normal range, but N-BNP levels did not decrease and symptoms less reliably improved in patients with a preoperative N-BNP level within normal limits.

In contrast to the established theory that aortic stenosis is a degenerative process not amenable to medical therapy, recent histological studies suggest that aortic stenosis may be an inflammatory disease with similarities to coronary atherosclerosis. To further address this issue, high sensitivity C-reactive protein (CRP) was measured in 20 patients with non-rheumatic aortic stenosis, 19 patients with non-rheumatic aortic regurgitation and 31 healthy controls, as well as 6 months after valve replacement in aortic stenosis. CRP was significantly increased in aortic stenosis, but not aortic regurgitation compared with controls and decreased after valve replacement in aortic stenosis. These observations are consistent with histological evidence that the aortic valve is the site of active inflammation.

In conclusion, measurement of plasma natriuretic peptide levels complement clinical and echocardiographic evaluation of patients with valvular heart disease and may assist with the timing of valve surgery. Novel evidence that aortic stenosis may be an inflammatory disease is presented and suggests further studies are required to determine whether agents with anti-inflammatory actions may have a role in delaying disease progression. Following on the studies presented in this thesis, a large multicentre study has commenced in New Zealand to confirm these findings that has the potential to change clinical practice.

SUMMARY

Chapter 1 provides an introduction and literature review on valvular heart disease, the natriuretic peptides and CRP as a background to the studies described in subsequent chapters. Despite numerous publications on the diagnostic and prognostic utility of natriuretic peptide levels in diverse cardiac conditions, there are very few publications on the natriuretic peptides in valvular heart disease. These studies are reviewed.

Chapter 2 covers important aspects of methodology common to the studies, including an outline of the studies design, exclusion criteria, study patients and control subjects, clinical assessment, descriptions of the assays for the natriuretic peptides, performance and interpretation of the echocardiograms, and statistical analyses used throughout the studies.

Chapter 3.1 examines the associations between plasma levels of the natriuretic peptides, ANP, BNP and N-BNP with disease severity, left ventricular function and cardiac symptoms in 74 patients with aortic stenosis. The natriuretic peptide levels were higher in symptomatic patients compared with asymptomatic patients and remained higher after adjustment for age, gender, serum creatinine, aortic valve area and left ventricular ejection fraction. Increased natriuretic peptide levels reflected the presence of symptoms with greater accuracy than any echocardiographic measure of aortic stenosis severity or left ventricular function. These data suggest that measurement of natriuretic peptides may complement clinical and echocardiographic evaluation of patients with aortic stenosis. Although the study was not powered to distinguish between the natriuretic peptides, N-BNP appeared to have the best

discriminatory value and was therefore the focus of the further studies on aortic stenosis.

Chapter 3.2 examines whether serial measurements of N-BNP levels in 29 asymptomatic patients with aortic stenosis may predict the development of symptoms. Patients with an N-BNP level above the normal range at baseline were more likely to develop symptoms during an average of 18 months follow-up compared to patients with an N-BNP level within normal limits. The average increase in level of N-BNP/year was greater for patients who became symptomatic compared to patients who remained asymptomatic. Aortic valve area, peak aortic velocity and left ventricular ejection fraction were less reliable predictors of symptom onset. These data suggest that measurement of plasma N-BNP levels in addition to clinical assessment and echocardiography may allow more reliable follow-up and timing of aortic valve replacement for severe aortic stenosis.

Chapter 3.3 examines the effects of aortic valve replacement on N-BNP levels in 33 patients with severe aortic stenosis. N-BNP levels were higher in patients with aortic stenosis before valve replacement compared with controls and decreased but remained elevated 6 months after valve replacement despite favourable echocardiographic changes. N-BNP levels decreased by 6 months after aortic valve replacement in patients with elevated preoperative N-BNP levels, but did not decrease in patients with preoperative N-BNP levels within normal limits. While symptoms consistently improved in patients with elevated preoperative N-BNP levels, there was less reliable improvement of symptoms in patients with a preoperative N-BNP level within normal limits.

Taken together, the studies presented in chapter 3 suggest that larger and longer-term studies need to be performed to determine whether asymptomatic patients with aortic stenosis and an N-BNP level above the normal range should be followed more closely for the development of symptoms, while patients with an N-BNP level within the normal range and apparently mild symptoms may be managed with close follow-up or further objective testing such as an exercise stress test.

Chapter 4.1 examines the associations between plasma levels of ANP, BNP and N-BNP with echocardiographic measures of left ventricular size, function and wall stress, and cardiac symptoms in 40 patients with moderate-severe chronic aortic regurgitation. Correlations between natriuretic peptide levels and left ventricular size, ejection fraction and systolic wall stress were weak, but natriuretic peptide levels were significantly higher in symptomatic patients compared to asymptomatic patients after adjustment for age, gender and left ventricular ejection fraction. Although natriuretic peptide levels were higher in asymptomatic patients compared with age- and gender-matched controls, there was substantial overlap with most asymptomatic patients having natriuretic peptide levels within the normal range despite increased left ventricular volume.

These novel findings suggest that in patients with moderate-severe chronic aortic regurgitation, the major stimulus for the release of natriuretic peptides is left ventricular decompensation and not increased left ventricular volume as suggested in studies on the natriuretic peptides in patients with cardiac failure due to cardiomyopathy and coronary artery disease.

Chapter 5.1 examines the associations between plasma levels of ANP, BNP and N-BNP with echocardiographic measures of mitral regurgitation severity and symptoms

in 49 patients with mitral regurgitation and left ventricular ejection fractions of >55%. Each of the natriuretic peptides increased with increasing severity of mitral regurgitation but there was no significant correlation between any natriuretic peptide and left ventricular dimensions or ejection fraction. Natriuretic peptide levels were higher in symptomatic patients compared with asymptomatic patients after adjustment for echocardiographic measures of left ventricular function and severity of mitral regurgitation. The results of this study suggest that measurement of natriuretic peptide levels may add to the clinical and echocardiographic evaluation of patients with mitral regurgitation. Further studies are required to determine whether natriuretic peptide levels measured before mitral valve surgery predict postoperative left ventricular function and clinical outcomes, and whether serial measurements of natriuretic peptide levels predict the development of symptoms or left ventricular dysfunction in patients with mitral regurgitation, thereby improving the timing of mitral valve surgery.

Chapter 6 examines CRP plasma levels in 20 patients with non-rheumatic aortic stenosis, 19 patients with non-rheumatic aortic regurgitation, and in 31 healthy controls. CRP was also measured 6 months after valve replacement in 15 patients with aortic stenosis and after 6 months follow-up in the controls. CRP was significantly higher in aortic stenosis than in controls and aortic regurgitation. CRP decreased 6 months after valve replacement for aortic stenosis but did not change in normal controls. These observations that CRP levels are elevated in aortic stenosis and decrease after valve replacement are consistent with histological evidence that the aortic valve is the site of active inflammation.

Chapter 7 describes a large multicentre study recently commenced in New Zealand that follows from the studies described in this thesis. Dr Ralph Stewart is the Principle Investigator of the study and I was involved in the design of the study and recruitment of patients. The New Zealand Heart Valve Study, with expected enrolment of 600 patients with valvular heart disease including aortic stenosis (n=150), aortic regurgitation (n=150), mitral regurgitation (n=150), mitral stenosis (n=50), and mixed valvular disease (n=100), will determine whether serial measurement of natriuretic peptides predict disease progression and symptom onset and whether natriuretic peptide levels measured before valve surgery may predict postoperative left ventricular function and clinical outcome. This study will be adequately powered to further determine whether any single one or combination of the natriuretic peptides are most predictive. Serum, plasma and genetic material will be stored for future analysis of other potential markers that may be useful in the evaluation of patients with valvular heart disease and may provide valuable new information on the pathophysiology of valvular heart disease.

Chapter 8 provides a summary of the results and draws conclusions from each of the studies presented in this thesis. These studies suggest measurement of natriuretic peptide levels may complement clinical and echocardiographic evaluation of patients with valvular heart disease, and may improve the timing of surgical intervention. Data is also provided in support of histological evidence that the aortic valve is the site of active inflammation, and suggest further studies are warranted to determine whether agents with anti-inflammatory actions may have a role in delaying disease progression in patients with aortic stenosis.

Chapter 9 lists all references quoted in the thesis.

PUBLICATIONS

The following papers have been published or submitted from this thesis:

1. Gerber, I.L., Stewart, R.A., Legget, M., West, T., French, R., Yandle, T., French, J., Sutton, T., Richards, A., White, H. Increased natriuretic peptide plasma levels reflect symptom onset in aortic stenosis. *Circulation*. 2003, 107: 1884-1890.
2. Gerber, I.L., Stewart, R.A., Legget, M., Greaves, S., West, T., Kerr, A., French, J., Richards, A.M., White, H. Associations between plasma natriuretic peptide levels, symptoms and left ventricular function in aortic regurgitation. *American Journal Cardiology*. 2003, 92 (6): 755-8.
3. Sutton, T., Stewart, R.A., Gerber, I.L., West, T., Richards, A.M., Yandle, T., Kerr, A. Plasma natriuretic peptide levels increase with symptoms and severity of mitral regurgitation. *Journal of the American College of Cardiology*. 2003, 41: 2280-2287.
4. Gerber, I.L., Stewart, R.A., Hammett, C., Legget, M., Oxenham, H., West, T., French, J., White, H. Effect of aortic valve replacement on high sensitivity C-reactive protein in isolated non-rheumatic aortic stenosis. *American Journal of Cardiology*. 2003, 92 (9): 1129-1132.
5. Gerber, I.L., Stewart, R.A. Neurohormonal assessment of aortic stenosis. *Cardiovascular Reviews and Reports*. 2004, 25: 161-167.
6. Gerber, I.L., Legget, M., Richards, A.M., West, T., Stewart, R.A. Usefulness of serial measurement of amino-terminal B-type natriuretic peptide plasma levels in asymptomatic patients with aortic stenosis to predict symptomatic deterioration. *American Journal of Cardiology*. 2004. In press.
7. Gerber, I.L., Legget, M., Richards, A.M., West, T., Stewart, R.A. Plasma levels of amino-terminal B-type natriuretic peptide before and after aortic valve replacement for severe aortic stenosis. Submitted to the *Journal of Heart Valve Disease*. March 2004.

ABSTRACTS

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American College of Cardiology Meeting, Chicago 2002

- Sutton, T., Kerr, A., Gerber, I.L., Stewart, R.A., Richards, A.M. Amino-terminal B-type natriuretic peptide plasma levels predicts symptomatic status and severity of mitral regurgitation in patients with mitral regurgitation. *Journal American College Cardiology* 2002; 39 (Supplement A): 866.

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- Gerber, I.L., Stewart, R.A., French, R., Legget, M., Greaves, S., Richards, A.M., Yandle, T., French, J., Sutton, T., Kerr, A., White, H.D. Natriuretic peptides are elevated in aortic regurgitation but correlate poorly with disease severity. *Journal American College Cardiology* 2002, 39(9) (Supplement B): 350B-4094.

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- Gerber, I.L., Hammett, C., Stewart, R.A., Oxenham, H., French, R., French, J., West, T., Legget, M., White, H.D. C-reactive protein is increased in patients with aortic stenosis but not aortic regurgitation - Evidence for aortic stenosis as an inflammatory disease. *NZ Med Journal* 2002, 115(1162): 35-35.
- Gerber, I.L., Stewart, R.A., Legget, M., French, R., French, J., Richards, A.M., White, H. Effects of aortic valve replacement for severe aortic stenosis on natriuretic peptides. *NZ Med Journal* 2002, 115(1162): 18-18.
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- Sutton, T., Kerr, A., Gerber, I.L., Stewart, R.A., Richards, A.M. Natriuretic peptides predict symptom status and severity of mitral regurgitation in patients with non-ischaemic mitral regurgitation. *NZ Med Journal* 2002, 115(1162): 26-26.

American College of Cardiology Meeting, Chicago 2003

- Gerber, I.L., Hammett, C., Stewart, R.A., Oxenham, H., French, J., West, T., Legget, M., White, H.D. C-Reactive Protein is increased in patients with aortic stenosis but not aortic regurgitation - Evidence for aortic stenosis as an inflammatory disease. *Journal of the American College of Cardiology* 2003, 41(6 Suppl A): 497A: 1012-24.
- Gerber, I.L., Stewart, R.A., Legget, M., West, T., French, J., Richards, A.M., White, H. Plasma natriuretic peptide levels remain elevated six months after aortic valve replacement for severe aortic stenosis. *Journal of the American College of Cardiology* 2003, 41(6 Suppl A): 5080A: 1134-21.

American Heart Association Meeting, Orlando 2003

- Gerber, I.L., Stewart, R.A., Legget, M., West, T., Richards, A.M. Higher baseline and increasing serial amino-terminal B-type natriuretic peptide levels predict symptom onset in asymptomatic aortic stenosis. *Circulation* 2003, 108 Supplement IV-512.

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1) Plasma natriuretic peptide levels remain elevated six months after aortic valve replacement for aortic stenosis.

2) Plasma high sensitivity C-reactive protein levels are elevated in aortic stenosis but not in aortic regurgitation.

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LIST OF ABBREVIATIONS

Abbreviations used throughout this thesis:

ACC	-	American College of Cardiology
AHA	-	American Heart Association
ANP	-	Atrial Natriuretic Peptide
AR	-	Aortic Regurgitation
AS	-	Aortic Stenosis
AVR	-	Aortic Valve Replacement
BNP	-	B-type Natriuretic Peptide
CI	-	Confidence Interval
CRP	-	High Sensitivity C-Reactive Protein
IQR	-	Interquartile range
Ln	-	Natural Logarithm
LV	-	Left Ventricle
MR	-	Mitral Regurgitation
N-ANP	-	Amino-terminal Atrial Natriuretic Peptide
N-BNP	-	Amino-terminal B-type Natriuretic Peptide
NYHA	-	New York Heart Association

CHAPTER 1

Introduction and Literature Review

1.1	Valvular Heart Disease	2
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1.1 Valvular Heart Disease

Valvular heart disease is the diagnostic term used to describe cardiac dysfunction caused by structural or functional abnormalities in heart valves.¹ World-wide, valvular heart disease remains an important cause of morbidity and mortality today as it did a half century ago, although the causes, diagnostic modalities, and therapeutic options available have changed dramatically.

In 1950, rheumatic heart disease was the most common cause of mitral stenosis, mitral regurgitation and aortic stenosis and tertiary syphilis was a common cause of isolated severe aortic regurgitation.^{2,3} Mitral valve prolapse, the commonest cause of severe mitral regurgitation in the western world today, was not recognised as a clinical entity.⁴ As a consequence, penicillin was the mainstay of medical therapy for the treatment and prophylaxis of valvular heart disease, and closed-chest mitral commissurotomy for mitral stenosis was the most common cardiac surgical intervention.² The severity of the various valve lesions was predominantly determined by bedside clinical skills together with the chest x-ray and electrocardiogram. Non-invasive diagnostic imaging modalities including echocardiography and invasive haemodynamic assessment from cardiac catheterisation were not yet available.

In 1951, Gorlin and Gorlin used information derived from hydraulic systems to derive equations for the calculation of the aortic valve area⁵ and the mitral valve area.⁶ This led to a series of attempts to measure intracardiac pressures directly. In 1953, Seldinger described the percutaneous approach into a systemic artery for access to the LV⁷ that led to the invasive assessment of valvular heart disease and the calculation of LV volumes and ejection fraction.⁸ Although Edler and Hertz first

recorded movements of cardiac structures with ultrasound in 1954,⁹ it was not until the 1970's with the development of 2D scanning which allowed real-time tomographic images of cardiac morphology and function, and Doppler echocardiography that allowed non-invasive haemodynamic assessment of valvular heart disease.¹⁰ Since valve replacement surgery was first described in 1960,¹¹ there has been a proliferation of mechanical and bioprosthetic valves available as well as improvements in techniques for mitral and aortic valve repair. There has also been improvements in cardiac anaesthesia and perioperative care, so that today most patients can expect a long-term survival after valve surgery similar to an age-matched population - provided the timing of operative intervention is optimal.¹²

Based on the results of predominantly retrospective natural history studies, the major indications for valve surgery are the presence of a severe valve lesion, cardiac symptoms (including symptoms of heart failure, angina, and syncope), and echocardiographic evidence of impaired LV systolic function.¹² Echocardiography provides detailed, noninvasive information about the anatomy and etiology of the valve lesion, the severity of valve stenosis and/or regurgitation, the impact of the valvular lesion on the LV size and function, and any associated cardiac abnormalities. Thus, echocardiographic evaluation is the standard diagnostic approach to the patient with suspected or known valvular heart disease. For most patients with valvular heart disease, information derived from the history, physical examination, and echocardiography is adequate for clinical decision making, including referral for valvular surgery.¹³ Cardiac catheterisation continues to play an important diagnostic role in selected patients with valvular disease, for example coronary angiography for evaluation of coronary disease, for the assessment of complex multivalve disease, and whenever there are significant discrepancies

between clinical assessment and echocardiographic data or when disease severity is uncertain.

In patients with valvular heart disease, the LV response to chronic volume or pressure overload, rather than the severity of the valve lesion alone, is often the primary determinant of clinical outcome. In this sense, the LV is the “end-organ” damaged by underlying valvular heart disease.¹ The LV compensates for the increased pressure load in aortic stenosis by concentric hypertrophy, for the increased volume and pressure load in aortic regurgitation by an increase in LV volume and eccentric hypertrophy, and for the increased volume load in mitral regurgitation by an increase in LV and left atrial volume. During this often prolonged compensated phase patients remain asymptomatic with very high event-free survival. Eventually the compensatory mechanisms are insufficient and LV dysfunction and/or symptoms develop with a poor outcome with medical therapy.^{1,12}

One of the key goals in the management of patients with valvular heart disease is to identify the onset of hemodynamic decompensation and intervene prior to the development of irreversible changes. However, assessment of LV function is particularly problematic in patients with valvular heart disease because loading conditions are uniformly abnormal. There is no ideal independent clinical measure of contractility and echocardiographically derived LV ejection is the most frequently used measure in clinical practice. The need for improved methods for evaluating myocardial mechanics in valvular heart disease results from some fundamental limitations in using ejection fraction to assess LV function. Ejection fraction measurements are dependent on heart rate and intravascular volume. For example, if LV function is truly normal in a patient with severe mitral regurgitation, the ejection

fraction ought to be hyperdynamic due to the unique loading conditions of mitral regurgitation. Conversely, patients with severe mitral regurgitation and a low normal ejection fraction may already have significant depression of LV function, which would be unmasked after successful surgical elimination of the leak.¹⁴ In many cases of concentric LV hypertrophy such as occurs in aortic stenosis, an ejection fraction of 55% indicates substantial muscle dysfunction.¹⁵

Due to the limitation of the ejection fraction in the assessment of LV contractility, other less load dependent measures have been evaluated such as LV end-systolic stress.¹⁶⁻¹⁹ However, some of the simplified calculations are less valid in mitral regurgitation and it is not used in routine clinical practice for aortic valve disease. Exercise stress testing has been proposed as a method to further assess LV systolic function in patients with a normal ejection fraction. Studies on exercise stress echocardiography in patients with mitral regurgitation have shown that failure of the normal increase in ejection fraction and failure to reduce end-systolic volume during preoperative exercise (reduced contractile reserve) occurred in a group of patients who later developed lower resting ejection fractions after successful surgical correction.²⁰ In patients with aortic regurgitation, a change in LV ejection fraction from rest to exercise has been shown to be predictive for clinical outcome in asymptomatic patients with a normal ejection fraction.²¹ Recently, measurement of longitudinal axis function by pulsed-wave tissue Doppler imaging at rest and stress has been shown to be a marker of contractile reserve in the absence of regional LV dysfunction in patients with mitral regurgitation,²² aortic regurgitation²³ and aortic stenosis.²⁴ At present however, the LV ejection fraction continues to be the most frequently used measure of LV systolic function in clinical practice. Perhaps the greatest limitation of relying on the measurement of ejection fraction for the

assessment of LV systolic function is that most patients with valvular heart disease develop symptoms of heart failure necessitating valve surgery with a normal ejection fraction.¹² Therefore, clinicians often need to rely on clinical skills to determine whether the patient has developed symptoms and whether the symptoms are due to the valve disease.

In some patients the early cardiac symptoms may not be recognised by the patient or the clinician, while in other patients the reported non-specific symptoms of dyspnea and fatigue may not be due to the valve disease but rather due to other causes such as respiratory disease or deconditioning, especially in the elderly. Exercise stress testing is sometimes recommended for an objective measure of exercise capacity in an asymptomatic patient, or for the clarification of symptom status in a patient with an equivocal history.²⁵ However, exercise stress testing remains controversial especially in patients with severe aortic stenosis, and requires close monitoring and a physician in attendance.¹² Also, many patients, especially the elderly or those with musculoskeletal limitations such as arthritis are unable to exercise. Currently, there is no consensus on the definition of an abnormal test in patients with valve disease with regards to exercise capacity, and exercise capacity has not been shown to predict clinical outcome, at least not in aortic stenosis.²⁶

In summary, a non-invasive, reproducible and readily available marker that reflects early LV decompensation, ideally before the development of symptoms or echocardiographically detected overt LV dysfunction in patients with valvular heart disease is desirable and is the focus of the studies presented in this thesis.

A second major limitation in the management of patients with valvular heart disease is the lack of placebo controlled trials for medical therapy to delay or prevent disease

progression and improve clinical outcome.⁴ No medical therapy has been shown to be effective in the management of patients with mitral regurgitation, and only mitral valve repair or mitral valve replacement have been shown to improve survival.²⁷ Although a randomised trial in asymptomatic patients with severe aortic regurgitation and a normal ejection fraction suggested improved clinical outcomes in patients randomised to long-acting nifedipine compared to digoxin, there was no placebo arm. It is possible that digoxin therapy masked early LV dysfunction that when detected had already progressed to beyond afterload mismatch resulting in an adverse clinical outcome.²⁸ Angiotensin converting enzyme inhibitors are commonly prescribed instead of nifedipine but these agents have never been assessed in a randomised clinical trial for patients with moderate aortic regurgitation.²⁹

Non-rheumatic aortic stenosis has been regarded a progressive degenerative condition that increases in prevalence with increasing age. No therapy has been shown to reduce disease progression and the only recommended definitive treatment for symptomatic patients with severe aortic stenosis is aortic valve replacement.¹² Stimulated by accumulating evidence that aortic stenosis may be an inflammatory disease similar to atherosclerosis,³⁰⁻³² non-randomised retrospective studies suggest disease progression is slower in patients receiving statin treatment,³³⁻³⁵ consistent with the reported anti-inflammatory effects of these agents.³⁶ These studies potentially have major clinical implications for the medical management of aortic stenosis, but randomised placebo controlled studies confirming these effects are currently lacking.³⁵ This issue is addressed further in this thesis including novel biochemical data which support histological evidence that aortic stenosis is an inflammatory disease.

1.2 The Natriuretic Peptides

1.2.1 Historical Perspective

Following a report in 1964 that the failing heart releases norepinephrine, Eugene Braunwald wrote an editorial entitled "The Heart as an Endocrine Organ" that included the following comment: 'When this finding is viewed in the light of the observation that the isolated mammalian heart is capable of biosynthesis and storage of norepinephrine, the concept that the heart can function as a neuroendocrine organ must be considered seriously. Whether this actually occurs in the intact organism, and if so what the physiologic or pathologic conditions are which predispose to the heart's activity as an endocrine organ, would appear to be worthy of further investigation.'³⁷ These prophetic comments were preceded by a series of experimental observations in the 1950's that helped establish the heart as an endocrine organ.

In 1956, Kisch and colleagues³⁸ detected secretory granules by electron microscopy in guineapig atria. In the same year, Henry and Pearce³⁹ described increased urinary flow after balloon stretch of the canine left atrium. While these observations suggested the possible role of the atrium in regulating intravascular volume, it was not until 1981 when de Bold demonstrated diuresis and natriuresis in rats injected with atrial homogenate that the theory of the heart as an endocrine organ was more convincingly demonstrated.⁴⁰ In 1984, the structure of atrial natriuretic peptide (ANP) was identified and its natriuretic and diuretic properties were demonstrated.⁴¹ Since these seminal observations, a large body of research has radically expanded the perception of the heart as a mechanical pump alone to that of a neuroendocrine organ as well.

In 1988 a compound was isolated from pig brain that caused natriuretic and diuretic responses similar to ANP.⁴² This peptide was therefore referred to as brain natriuretic peptide (BNP). Because the primary site of BNP synthesis and release was subsequently shown to be the ventricular myocardium,^{43,44} it is now more commonly referred to as B-type natriuretic peptide. Subsequently, other peptides with a similar ring structure and biological properties have been recognised, and named in alphabetical order – CNP⁴⁵ and DNP.⁴⁶

1.2.2 Biochemical Structure and Molecular Biology

The natriuretic peptides are a group of structurally related but genetically distinct peptides that exert diverse actions on cardiovascular, renal and endocrine function and have emerged as important contributors to the control of cardiovascular haemodynamics, ventricular remodelling, and sodium and water balance.⁴⁷ The natriuretic peptide family comprises four principle peptides whose prohormones are encoded by at least three separate genes. These include ANP, BNP, CNP, and DNP. Each natriuretic peptide shares considerable sequence homology, particularly within their 17-amino acid disulfide ring structure with a highly conserved sequence (FGXXXDRIGXXSGL) (Figure 1.2.1).⁴⁸

The ANP precursor peptide (NPPA) gene and the BNP precursor peptide (NPPB) gene reside in tandem on chromosome 1p36.2. The NPPA gene encodes a 126-amino-acid precursor peptide, pro-ANP, which is stored in atrial granules. Upon secretion pro-ANP is proteolytically cleaved by a serine protease into equimolar amounts of the mature biologically active 28-amino-acid carboxy-terminal fragment, ANP and the inactive 98-amino-acid amino-terminal fragment, N-ANP.⁴⁹ The NPPB gene encodes a 108-amino acid pro-BNP, which is cleaved into equimolar amounts

of the active 32-amino acid mature peptide, BNP and an inactive 76 amino-terminal fragment, N-BNP.⁵⁰ Although ANP and BNP are the active hormones and N-ANP and N-BNP are the inactive hormones, all four peptides circulate in the plasma under normal conditions and may be measured for diagnostic and prognostic purposes.^{48,51}

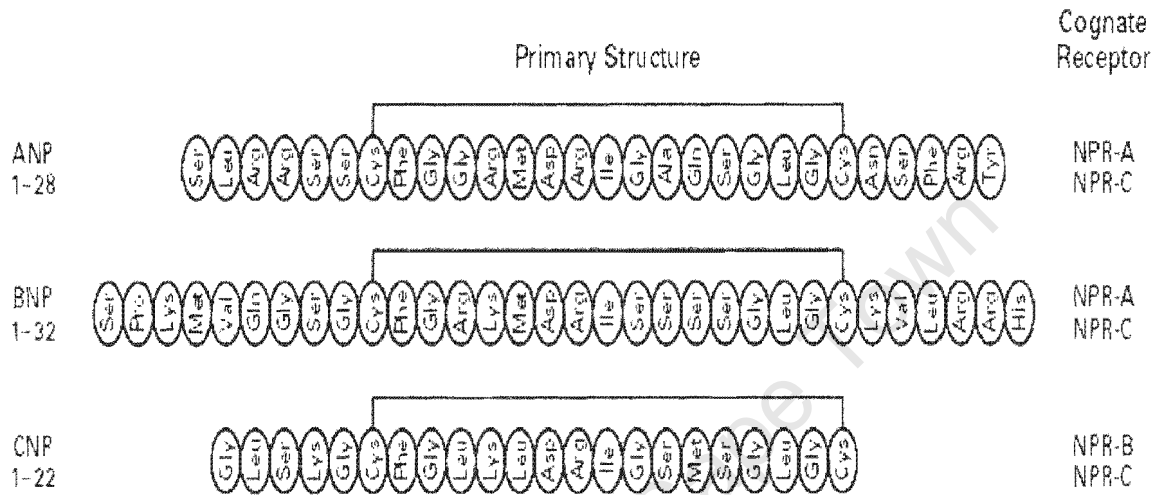


Figure 1.2.1: Amino acid sequences of ANP, BNP and CNP. Adapted from Levin et al.⁴⁸

1.2.3 Synthesis and Secretion of ANP and BNP

The stimulus for ANP and BNP release is myocyte stretch, rather than transmural pressure load.⁵²⁻⁵⁵ In addition to primary regulation via myocyte stretch, natriuretic peptide synthesis can be augmented by several hormones and neurotransmitters, such as endothelin, arginine vasopressin, and catecholamines, independent of the hemodynamic effects of these factors.^{53,54} Regulation of ANP secretion seems to occur at the level of release from storage granules, whereas regulation of BNP release takes place during gene expression.⁵⁶ ANP is produced primarily in the cardiac atria. In healthy individuals little ANP is produced in ventricular tissue, whereas in heart failure, the ventricles are recruited to contribute to constitutive synthesis of ANP.⁴³ BNP is primarily a ventricular hormone where it is constitutively synthesised and secreted, although a small amount is co-stored and secreted from granules together with ANP in the atria.⁵⁷⁻⁶⁰ In contrast to ANP, BNP shows little response to acute stimuli such as intravenous saline loading or supine posture but does respond more slowly to chronic increases in ventricular load, such as those occurring in hypertension and cardiac failure.⁴⁷

1.2.4 Natriuretic peptide Receptors

Natriuretic peptide binding for biological effects

The natriuretic peptides exert their effects through interaction with high-affinity receptors on the surface of target cells (Figure 1.2.2).⁴⁸ Three natriuretic peptide receptors, NPR-A, NPR-B and NPR-C, have been identified.⁶¹ Binding of natriuretic peptides to the NPR-A and NPR-B receptors on the surface of target cells leads to generation of the second messenger cyclic guanosine monophosphate which mediates most of the biological effects of the natriuretic peptides.⁶¹ The NPR-A

gene product is abundantly expressed in the vasculature, kidneys, and adrenal glands. The NPR-B gene product is strongly expressed in the brain, including the pituitary gland, and may have a role in neuroendocrine regulation. ANP and BNP bind preferentially to NPR-A receptors while CNP binds preferentially to NPR-B receptors.⁶²

Natriuretic peptide clearance

Clearance of ANP and BNP from the blood is effected in two ways: through a clearance receptor, NPR-C⁶³ and through enzymatic degradation by neutral endopeptidase (NEP) (Figure 1.2.2).⁶⁴ NPR-C interacts with ANP and BNP with equal affinity. It is the most widely and abundantly expressed natriuretic peptide receptor, with a tissue distribution that includes most of the major endocrine glands, the lungs, the kidneys, and the vascular wall.⁶³ Natriuretic peptides that are bound by the receptor are internalised and enzymatically degraded, after which the receptor returns to the cell surface.⁶⁴ The lower affinity of NPR-C for BNP contributes to a longer plasma half-life of BNP compared with ANP.⁶⁵ The inactive N-terminal fragments, N-ANP and N-BNP have no specific clearance receptors. As a result they have a longer half-life, circulate in plasma in much higher concentrations, and are more stable being less influenced by short bursts of secretion.⁴⁷ Circulating ANP and BNP are also inactivated by cleavage by NEP that is present on the surface of endothelial cells, smooth-muscle cells, cardiac myocytes, renal epithelium, and fibroblasts.⁶⁴ The relative contribution of each system of natriuretic peptide turnover is unknown in humans.

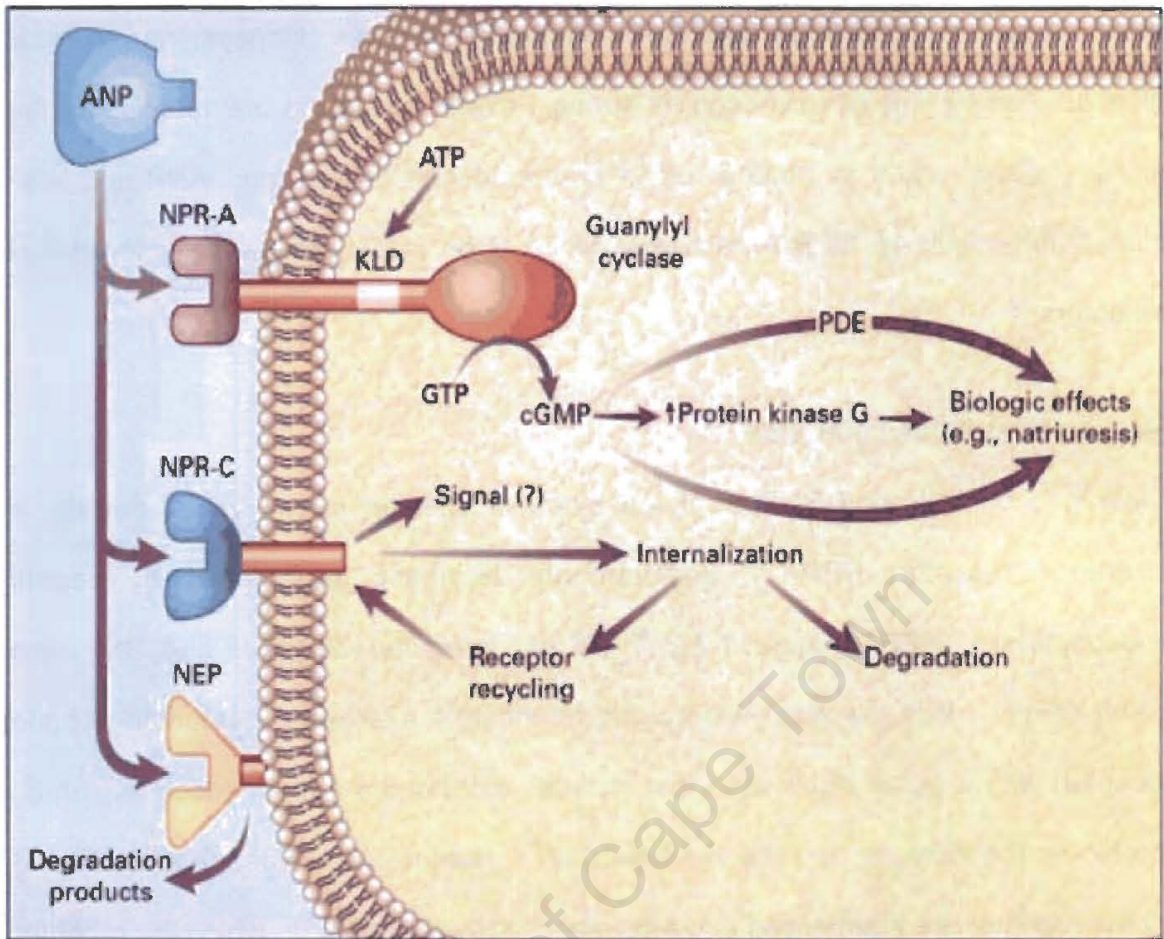


Figure 1.2.2: Schematic representation of the natriuretic peptide receptors, NPR-A, NPR-C and neutral endopeptidase (NEP). Adapted from Levin et al.⁴⁸

1.2.5 Actions of Natriuretic Peptides

Cardiovascular

The principal function of ANP and BNP is to protect the cardiovascular system from volume overload primarily by natriuretic and diuretic effects (Figure 1.2.3).⁴⁸ In addition, they cause intravascular volume contraction by inducing a shift of fluid from the capillary bed to the interstitium, resulting in a decrease in preload and blood pressure.⁶⁶ ANP and BNP also reduce sympathetic tone in the peripheral vasculature by dampening of baroreceptors, by suppression of the release of catecholamines from autonomic nerve endings, and especially by suppression of sympathetic outflow from the central nervous system.^{67,68} ANP and BNP relax vascular smooth muscles, causing arterial and venous dilatation leading to reduced blood pressure and ventricular preload.⁶⁹ BNP has direct lusitropic properties in the myocardium, and might have antiproliferative and antifibrotic effects in vascular tissues.⁷⁰ By contrast with ANP and BNP, CNP does not function as a circulating hormone, but acts locally in the vasculature as a vasodilator and inhibitor of vascular cell proliferation, and acts in the central nervous system where it has several functions.⁷¹

Renal

The natriuretic peptides may be regarded as the natural antagonists of the renin-angiotensin system. ANP and BNP inhibit aldosterone release from adrenal cells and inhibit renal renin release.⁷² ANP, unlike BNP, effects its natriuretic action by increasing the glomerular filtration rate. ANP also directly inhibits sodium transport in the proximal tubule and in the inner medullary collecting duct.^{69,72}

Antimitogenic effects

Each natriuretic peptide has antimitogenic activity in both the cardiovascular system and other organ systems. ANP inhibits mitogenesis in cultured vascular cells and in balloon-injured carotid arteries in rats.⁷³ This implies that the natriuretic peptides may modulate growth within the vascular wall in disorders such as atherosclerosis, hypertension and post-angioplasty restenosis.⁴⁸

Central nervous system

Both ANP and BNP are produced in the brain. Although ANP and BNP do not cross the blood-brain barrier, they reach sites in the central nervous system outside this barrier, such as the subfornical organ, hypothalamic median eminence, and the postrema.⁴⁸ The actions of the natriuretic peptides in the brain reinforce those in the periphery. For example, the peripheral natriuretic effects are amplified by the central inhibition of salt-appetite and water drinking, which complements the renal diuretic effects of the natriuretic peptides.^{74,75} Furthermore, ANP inhibits the secretion of vasopressin and corticotrophin through effects on the brain and the pituitary gland.⁷⁶ Each of these effects implies co-ordinated central and peripheral actions in controlling fluid and electrolyte homeostasis. Natriuretic peptides also act in the brain stem to decrease sympathetic tone.⁷⁷

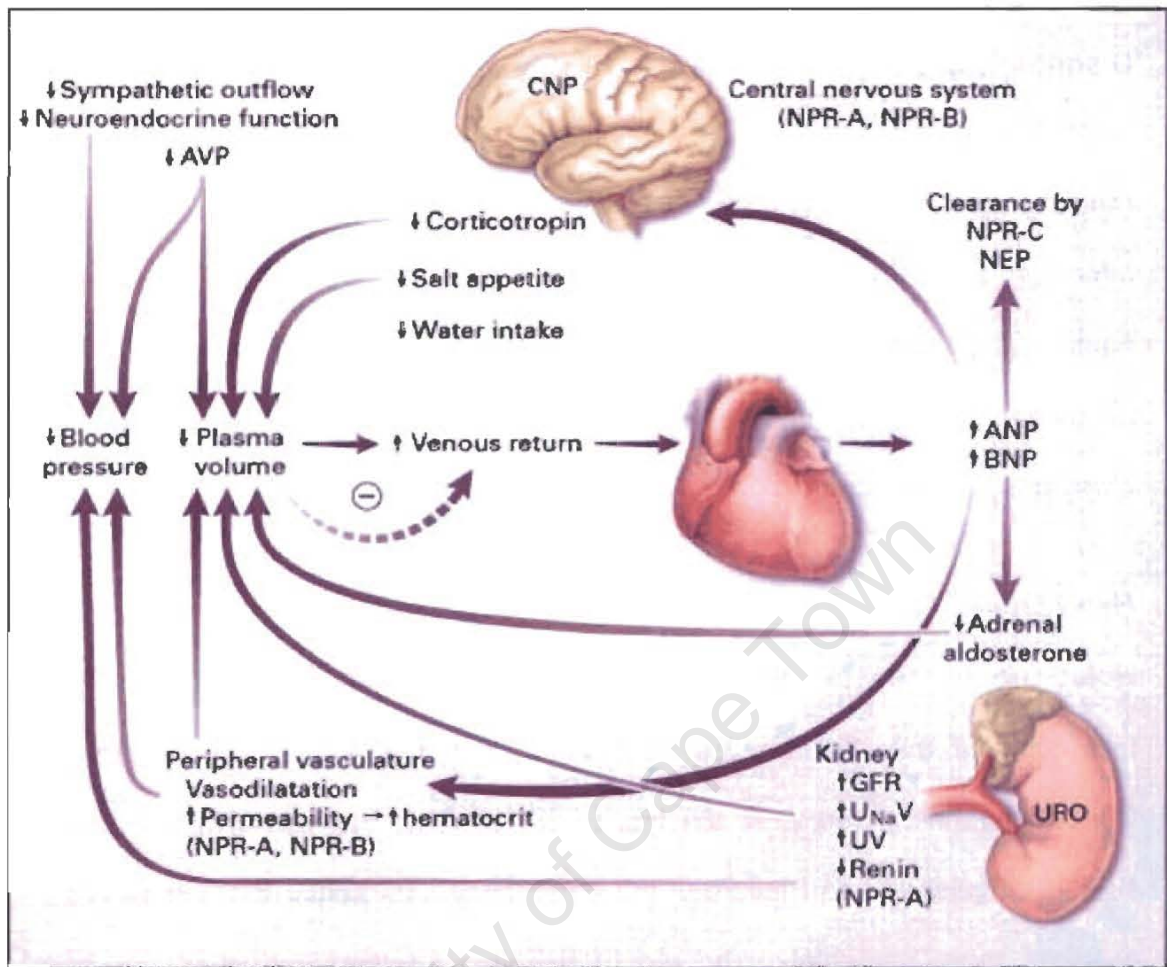


Figure 1.2.3: Schematic representation of the actions of the natriuretic peptides.

Adapted from Levin et al.⁴⁸

1.2.6 Clinical Uses of Natriuretic Peptides

Diagnostic Uses

ANP, BNP and their amino-terminal peptides, N-ANP and N-BNP are elevated in a range of conditions including, but not limited to heart failure, acute myocardial infarction, hypertrophic cardiomyopathy, hypertension, primary and secondary right ventricular disorders, and renal failure.⁷⁸ Most published literature to date on the diagnostic utility of the natriuretic peptides considers heart failure and asymptomatic LV systolic dysfunction.

Heart failure

Heart failure may be difficult to diagnose accurately because the signs and symptoms of this disorder are neither sensitive nor specific. The Christchurch Cardioendocrine group were the first to demonstrate that raised concentrations of BNP distinguished heart failure in the emergency care setting from other causes of dyspnea more accurately than did LV ejection fraction and other neurohumoral markers including ANP and N-ANP.⁷⁹ Similar data was subsequently demonstrated by Cowie et al. for the diagnosis of heart failure in the primary care setting.⁸⁰ These earlier data with relatively few numbers of patients have recently been validated by Maisel et al.⁸¹ in a multicenter study of 1586 patients presenting to the emergency room with dyspnea. Using a threshold of greater than 100 pg/ml to diagnose heart failure, BNP did better than all other clinical variables and the clinical judgement of the emergency room physician. BNP was especially useful for ruling out heart failure; at a BNP threshold of 50 pg/ml, the negative predictive value was 96%.

For patients diagnosed with heart failure in primary care, only a minority has that diagnosis confirmed after cardiologic assessment. The recently published Natriuretic

peptides in the Community Study (NPC Study) by Wright et al.⁸² was the first prospective, randomised controlled trial to determine the effect of N-BNP on the accuracy of heart failure diagnosis in the community. They demonstrated that N-BNP measurement significantly improves the diagnostic accuracy of heart failure by General Practitioners over and above clinical review. In that study, N-BNP rather than BNP was measured. Adequately powered head-to-head comparisons have not been performed between BNP, N-BNP, ANP or N-ANP for the diagnosis of heart failure, but commercial assays are now available for both BNP and N-BNP for the diagnosis of heart failure in the community and emergency care settings.

Asymptomatic left ventricular systolic dysfunction

Asymptomatic LV dysfunction is at least as common as symptomatic heart failure. BNP is less accurate in the detection of asymptomatic LV dysfunction than for the clinical diagnosis of heart failure with substantial overlap in BNP levels between normal individuals and patients with especially mild LV dysfunction.⁸³ Although BNP is a sensitive indicator of cardiac abnormality, it is not specific. The routine measurement of BNP for the detection of asymptomatic LV dysfunction is therefore not currently recommended.

Prognostic Uses

A growing body of data suggests a prognostic role for the measurement of BNP or N-BNP as part of a multi-biomarker assessment, especially in patients with heart failure, post myocardial infarction and in the setting of troponin negative acute coronary syndromes.⁸⁴⁻⁸⁹

Heart failure

In patients with chronic heart failure, higher concentrations of BNP or N-BNP are associated with increased cardiovascular and all-cause mortality, independent of age, NYHA class, previous myocardial infarction, and LV ejection fraction.⁹⁰⁻⁹³ Raised concentrations of BNP are independently associated with sudden cardiac death and readmission in patients with chronic heart failure.⁹⁴ BNP also provides similar prognostic information in diastolic heart failure.⁹⁵ Patients with persistently high levels of BNP or N-BNP despite aggressive treatment for heart failure are at especially high risk for adverse outcomes.^{91,92,96}

Acute coronary syndromes

After acute myocardial infarction, raised BNP or N-BNP levels identifies patients at risk of adverse LV remodelling, LV dysfunction, heart failure and death, independent of age, history of heart failure and LV ejection fraction.^{84,97} The Christchurch Cardioendocrine group demonstrated a sensitivity and specificity of 91% and 72% for N-BNP measured 2-4 days post-MI in predicting death over 24 months of follow-up.⁹⁷ In a cohort of 2525 patients, the OPUS-TIMI 16 study group demonstrated that a single measurement of BNP obtained in the first few days after the onset of ischaemic symptoms provides predictive information for use in risk stratification across the spectrum of acute coronary syndromes.⁸⁹ The TIMI group also showed that N-BNP levels are associated with early death in patients with non-ST segment elevation acute coronary syndromes, and provides complementary information to conventional risk indicators, including the Troponins.⁹⁸

Therapeutic Uses

The natriuretic, diuretic and vasodilatory (arterial and venous) properties make the natriuretic peptides attractive as therapeutic agents in heart failure. In contrast with the loop diuretics and many vasodilators, natriuretic peptides cause inhibition rather than stimulation of the renin-angiotensin-aldosterone system and the sympathetic nervous system.⁹⁹ There may also be important additional beneficial effects of natriuretic peptides in heart failure, such as reducing cardiac ischaemia and modulating vascular growth.¹⁰⁰ The two major approaches for the modulation of the natriuretic peptide hormone system include the administration of exogenous BNP and the inhibition of the natriuretic peptide degradation enzyme, NEP.

Exogenous administration

Since the peptide structure of ANP and BNP preclude their use as oral preparations, their potential use is limited to parenteral administration. Recent studies have demonstrated that administration of BNP via the intravenous or subcutaneous route has beneficial effects in decompensated or stable heart failure, including lowering pulmonary capillary wedge pressure and systemic arterial pressure, improved cardiac index, producing a diuresis and reducing norepinephrine and aldosterone concentrations.¹⁰¹ Although BNP (nesiritide) is now available for intravenous use for the treatment of decompensated heart failure, it remains unclear when this agent should be used instead of established inotropic or vasodilator agents such as dobutamine, milrinone, sodium nitroprusside, or nitroglycerin given the lack of large randomised trials on major adverse clinical outcomes.¹⁰² Furthermore, the cost-effectiveness of using nesiritide rather than less expensive agents remains to be determined. These issues currently limit its broader use in clinical practice.

Vasopeptidase inhibitors

Candoxatril, an orally active prodrug that is metabolised to candoxatrilat, is a specific inhibitor of NEP that is responsible for natriuretic peptide degradation. A study of candoxatril in patients with heart failure has demonstrated a comparable natriuresis to that produced by frusemide, but greater falls in pulmonary capillary wedge pressure and a more favourable neuroendocrine profile than frusemide.⁴⁸ The vasopeptidase inhibitor, omapatrilat, has combined NEP inhibition and angiotensin-converting-enzyme inhibition and is more effective at lowering blood pressure than most available antihypertensive agents. The IMPRESS study,¹⁰³ comparing the effects of omapatrilat with lisinopril on functional status in subjects with heart failure, showed a lower combined clinical end-point of death, worsening heart failure or withdrawal from treatment in patients randomised to omapatrilat. However, two subsequent trials, OVERTURE¹⁰⁴ and OCTAVE¹⁰⁵ did not demonstrate improved survival with omapatrilat compared with enalapril. A further concern for the use of omapatrilat is the increased incidence of angioedema. Therefore, although there is sound theoretical rationale for the use of NEP's, they are not currently in routine clinical use.

Guide to Treatment

Plasma BNP or N-BNP levels may help identify high-risk subjects with heart failure or other cardiovascular conditions who might benefit from more intensive treatment. A recent study of treatment of heart failure with carvedilol demonstrated that the major benefit from this drug was seen in patients with above median levels of BNP.⁹³ Similarly, in VHEFT II,¹⁰⁶ the greatest benefit from ACE inhibitor therapy was seen in subjects with the greatest neurohormonal activation. In a randomised trial of N-BNP guided therapy versus clinically guided therapy for patients with heart failure, the

Christchurch Cardioendocrine Group demonstrated that N-BNP levels can be readily reduced in patients with heart failure by intensification of drug therapy and that treatment guided by plasma N-BNP levels reduced the total number of cardiovascular events compared to clinically guided treatment using a similar range of drug therapies.¹⁰⁷ Although these studies are intriguing, large prospective trials are needed to define the role of BNP or N-BNP in therapeutic titration for the management of patients with heart failure.

1.2.7 Factors That Influence Natriuretic Peptide Levels

Natriuretic peptides are typically measured for diagnostic or prognostic purposes for a specific condition, such as heart failure,^{82,90} acute coronary syndromes^{84,89,108-110} or acute pulmonary embolism.¹¹¹⁻¹¹³ However, there are a number of cardiac and non-cardiac conditions that may influence natriuretic peptide levels making interpretation of these levels complex. These conditions include but are not limited to hypertension with left ventricular hypertrophy,¹¹⁴ hypertrophic cardiomyopathy,¹¹⁵ thyroid disorders,^{116,117} hepatic cirrhosis^{118,119} post cardio-thoracic surgery,^{120,121} patients with permanent pacemakers,¹²²⁻¹²⁵ and acute and chronic pulmonary conditions.¹²⁶⁻¹³⁰ A number of other clinically relevant factors discussed in detail below need to be considered when evaluating natriuretic peptide levels.

Age

Each of the natriuretic peptides increases with age in the absence of cardiovascular and renal disease.¹³¹⁻¹³⁶ The mechanism(s) for the increase in natriuretic peptide levels with age is uncertain but may relate to reduced renal excretion, increased production due to age-related changes in atrial and ventricular mechanics,¹³¹ or due to age-related increase in myocardial mass.^{137,138} It is uncertain whether age-

adjusted reference ranges should be used when the natriuretic peptides are measured for clinical and research purposes.

Gender

Plasma levels of natriuretic peptides are higher in women compared to men.^{132,136,139}

The mechanism(s) for the gender difference is uncertain, but hormonal mechanisms, particularly the effects of oestrogen, have been implicated.^{132,140} The effect of gender may diminish with increasing age, as studies conducted in older populations have shown no significant differences between the genders.¹⁴¹

Body mass index

Natriuretic peptide levels are lower in obese subjects and show significant negative correlation with body mass index in both health and disease.^{142,143} Since adipose tissue contains the clearance receptor for the natriuretic peptides and the degradation enzyme NEP, it is likely that there is both an increase clearance and increased degradation of the natriuretic peptides in obese subjects.

Renal function

Natriuretic peptide levels correlate with serum creatinine and creatinine clearance in normal subjects and patients with renal failure.^{144,145} However, the elevation of natriuretic peptide levels in renal failure may be due to sub-clinical cardiac disease such as LV hypertrophy or LV dysfunction¹⁴⁵ and not decreased renal clearance.¹⁴⁵⁻¹⁴⁸

Medication

Commonly prescribed cardiovascular medications may affect natriuretic peptide levels. Diuretics such as frusemide and spironolactone reduce natriuretic peptide

levels by reducing ventricular filling pressures,^{107,149-151} and angiotensin converting enzyme inhibitors may also decrease BNP and N-BNP levels.¹⁵²⁻¹⁵⁴ Data on the effects of digoxin^{144,155,156} and beta-blockers^{92,157} on natriuretic peptide levels are conflicting.

University of Cape Town

1.3 Natriuretic Peptides in Valvular Heart Disease

Although the natriuretic peptides have been extensively studied as diagnostic and prognostic markers in many cardiac and non-cardiac conditions, with data suggesting they may be of use in detecting latent LV dysfunction, it is very surprising that there is limited data available on the potential role for measuring natriuretic peptide levels in patients with valvular heart disease. To date, excluding work published from this thesis, there have been only six published reports in peer reviewed journals on the natriuretic peptides in patients with aortic valve disease or mitral regurgitation.

1.3.1 Aortic Stenosis

Ikeda et al.¹⁵⁸ were the first authors to report increased plasma natriuretic peptide levels in patients with aortic stenosis. ANP and BNP levels were higher in patients with aortic stenosis (n=13) compared with controls (n=12): ANP mean 78 ± 23 pg/mL vs. 26 ± 4 pg/mL and BNP 367 ± 143 pg/mL vs. 17 ± 4 pg/mL; ($p < 0.0001$ for both). Patient symptom status was not reported. A novel finding was the very strong correlation between LV systolic meridional wall stress and Ln BNP, $r=0.96$ and Ln ANP, $r=0.95$, ($p < 0.0001$ for both). Nine patients had a repeat echocardiogram and measurement of ANP and BNP levels 1-13 months after AVR. There were moderate correlations between the difference of LV wall stress and the difference of the natriuretic peptide levels; Ln BNP ($r=0.76$; $p=0.014$) and Ln ANP ($r=0.81$; $p=0.006$). The authors concluded that in patients with aortic stenosis, secretion of ANP and BNP appears to be stimulated by increased LV systolic wall stress.

Prasad et al.¹⁵⁹ similarly reported increased plasma ANP and BNP levels in patients with aortic stenosis (n=30) compared with controls (n=14): ANP mean 30.2 ± 1.4

pmol/L vs. 7.3 ± 1.3 pmol/L and BNP mean 13.9 ± 4.5 pmol/L vs. 3.7 ± 0.5 pmol/L; ($p < 0.004$ for both). Seven patients were asymptomatic (NYHA class I), and 23 patients were symptomatic (NYHA II, $n=13$; NYHA III, $n=10$, respectively). There were moderate correlations between the NYHA class and BNP level (correlation coefficient 0.66; $p < 0.001$) and ANP level (correlation coefficient 0.41; $p < 0.03$). Both ANP and BNP levels correlated with LV mass index, peak-to-peak aortic valve gradient, and mean aortic gradient, although the correlation coefficients were stronger for BNP than for ANP. BNP, but not ANP correlated significantly with the LV end-diastolic pressure measured at cardiac catheterisation in a small subset of patients ($n=8$). The authors concluded that measurement of plasma BNP levels may be a useful indicator of the severity of aortic stenosis.

ANP and BNP levels were also measured in 7 patients one month after AVR. Overall, they found no significant reduction in either ANP or BNP levels. The authors speculated that the continued elevation of ANP and BNP levels in some patients after AVR whose symptoms improved may be caused by continued asymptomatic LV dysfunction, a reflection of the small increased gradient across the prosthetic valve, or the continuing effects of the surgery.

Talwar et al.¹⁶⁰ were the first investigators to assess N-BNP in patients with aortic stenosis. N-BNP levels were higher in patients with aortic stenosis ($n=15$, mean 253 [range 79-542] fmol/ml) compared with control subjects ($n=10$, mean 157 [105-237] fmol/ml), $p < 0.005$. Consistent with the data on ANP and BNP by Prasad et al.,¹⁵⁹ N-BNP levels correlated with the peak aortic gradient, ($r=0.53$, $p < 0.05$) and were higher in symptomatic ('short of breath') patients ($n=6$) compared with asymptomatic

patients (n=9): N-BNP 299 [243-542] fmol/ml, vs. N-BNP 217 [79-327] fmol/ml, $p=0.01$.

In a more comprehensive study, Qi et al.¹⁶¹ studied 67 patients with aortic stenosis before undergoing AVR. This was the first study on the natriuretic peptides in aortic stenosis to include invasive hemodynamic data in all patients and thereby provided further insight into the potential mechanisms for elevated natriuretic levels in these patients. This was also the first and to date only published study to consider N-ANP levels in aortic stenosis. Four patients were asymptomatic (NYHA class I) and sixty-three patients were symptomatic (NYHA class II, n=32, NYHA class III, n=28 and NYHA class IV, n=3). There were moderate correlations between each of the Ln natriuretic peptides (ANP, N-ANP, BNP, N-BNP) and pulmonary capillary wedge pressure (PCWP), right atrial pressure, LV end-diastolic pressure, LV mass index, aortic valve area index and LV shortening fraction ($p<0.05$ for all). Each of the natriuretic peptides were higher in aortic stenosis with an elevated PCWP (>12 mmHg, n=26) compared with a normal PCWP (≤ 12 mmHg, n=41), respectively, for BNP, mean 130 ± 29 pmol/L vs. 49 ± 12 pmol/L; for N-BNP, mean 371 ± 73 pmol/L vs. 152 ± 24 pmol/L; for ANP, mean 74 ± 11 pmol/L vs. 31 ± 5 pmol/L; and for N-ANP, mean 2156 ± 221 pmol/L vs. 952 ± 80 pmol/L, $p<0.01$ for all. There was no significant difference between the groups with respect to age, aortic valve area index and mean aortic gradient, but patients with a PCWP >12 mmHg had a lower LV shortening fraction ($29 \pm 2\%$ vs. $37 \pm 2\%$, $p<0.01$) and a greater LV mass index (213 ± 13 g/m² vs. 173 ± 10 g/m², $p<0.05$). Natriuretic peptides were higher in patients with a PCWP ≤ 12 mmHg compared to controls (n=19); for BNP, mean 7.0 ± 1.2

pmol/L; for N-BNP, mean 41 ± 3 pmol/L; for ANP, mean 7.6 ± 0.9 pmol/L; and for N-ANP 701 ± 105 pmol/L.

Receiver operating characteristic curves indicated that BNP and N-BNP performed best in the detection of increased LV mass and N-ANP performed best in the detection of increased left atrial pressure. The authors suggested that plasma BNP and N-BNP levels may serve as early markers of LV hypertrophy, whereas ANP and N-ANP increase later and reflect increased left atrial pressure. Therefore, an elevation of BNP with little or no increase in ANP may indicate a relatively early stage of aortic stenosis (compensated), whereas the elevation of both natriuretic peptides may indicate a more advanced stage (decompensated). They concluded that the repeated and combined measurements of natriuretic peptides might provide diagnostic information relevant to the evaluation of the stage of aortic stenosis.

In a subsequent paper by the same group,¹⁶² the effects of AVR on the plasma levels of N-ANP and N-BNP was assessed. At 12 months post-AVR, only patients with elevated preoperative PCWP (n=11) had decreased natriuretic peptide levels. In this subgroup, N-ANP decreased from a median 2102 (IQR 983-3043) pmol/L to 985 (778-1759) pmol/L, $p=0.017$, and N-BNP decreased from 230 (76-447) pmol/L to 137 (71-189) pmol/L, $p=0.058$. Of the patients with a normal preoperative PCWP (n=19), natriuretic peptide levels remained elevated; N-ANP 761 (588-1388) pmol/L vs. 788 (627-1186) pmol/L, $p=ns$ and N-BNP 92 (34-160) pmol/L vs. 83 (51-144) pmol/L, $p=ns$. The patients with the largest postoperative valve area index had the largest reduction of N-BNP (47%), whereas those with the smallest valve area index had no decrease in N-BNP (all patients had a Carbo Medics, Inc., Austin, TX, mechanical valve). They concluded that a reduction in left atrial wedge pressure is

the main factor causing the change of N-ANP levels after AVR, and a small prosthetic valve orifice area with a high aortic valve gradient might prevent regression of LV hypertrophy, thus representing a stimulus for increased cardiac secretion of N-BNP.

1.3.2 Mitral Regurgitation

Brooks et al.¹⁶³ were the first authors to report BNP levels in mitral regurgitation. They studied 22 patients with isolated \geq moderate mitral regurgitation to determine whether BNP could serve as a marker for early LV dysfunction in patients with chronic mitral regurgitation. Eleven patients were asymptomatic (NYHA class I) and eleven patients were symptomatic (NYHA class II/III, n=6, and NYHA IV, n=5). Plasma BNP levels were higher in patients with mitral regurgitation than in eight normal controls (20.9 ± 17 vs. 3.4 ± 0.9 pmol/L), $p=0.007$. BNP levels increased with increasing NYHA class ($p<0.005$), but did not correlate with LV dimensions, LV fractional shortening or left atrial size. Two asymptomatic patients with high BNP levels were subsequently referred for mitral valve surgery within the 12-month follow-up period due to the development of symptoms. The authors concluded that BNP levels are elevated in most patients with chronic mitral regurgitation, including those who were asymptomatic with normal LV dimensions, and that changes in LV physiology associated with increased BNP levels occur early in the disease process before they can be detected echocardiographically. Important limitations were that this study included a relatively high number of patients with advanced symptoms of heart failure (NYHA class IV, n=5), a group where measurement of BNP levels may be less clinically relevant. This study also did not assess the relation between the

severity of mitral regurgitation assessed by echocardiography and natriuretic peptide levels.

There are currently no published manuscripts on BNP levels in patients with mitral regurgitation who undergo mitral valve surgery. An abstract presented at the 2003 ACC Meeting by Troughton et al.¹⁶⁴ from the Cleveland Clinic prospectively studied 22 consecutive asymptomatic patients with severe mitral regurgitation and LV ejection fraction $\geq 55\%$ referred for mitral valve surgery. Patients underwent preoperative and postoperative (day 4) echocardiographic evaluation with concurrent measurement of BNP. LV ejection fraction was calculated by the modified biplane Simpson's method. Preoperative echocardiographic data were consistent with severe mitral regurgitation (mean regurgitant orifice area $0.88 \pm 0.4 \text{ cm}^2$, LV dilatation [mean LV end-systolic volume $69 \pm 23 \text{ ml}$]) and on average normal LV ejection fraction [mean $61 \pm 6\%$]. Preoperative Ln BNP correlated with postoperative LV end-systolic volume ($r=0.50$, $p=0.04$) and postoperative LV ejection fraction ($r=0.50$, $p=0.03$). Preoperative BNP levels were higher in patients who developed postoperative LV dysfunction, defined as an ejection fraction $<50\%$ ($141 \pm 85 \text{ pg/ml}$ vs. $57 \pm 51 \text{ pg/ml}$, $p=0.03$). A preoperative BNP level of 50 pg/ml had a sensitivity of 83%, specificity of 60%, and negative predictive value of 91% for the prediction of post-operative LV dysfunction ($C=0.8$). The authors concluded that a preoperative BNP level below 50 pg/ml may be useful in excluding the likelihood of postoperative LV dysfunction.

Limitations of this study include the small number of study subjects, the modest correlation coefficients and the postoperative echocardiograms were performed very early (day 4). Furthermore, no data was provided whether the preoperative BNP

level predicted the postoperative LV ejection fraction independent of other potential confounders such as preoperative LV ejection fraction, perioperative myocardial infarction, mitral valve repair vs. mitral valve replacement and type and size of the prosthetic valve.

There is limited data on whether BNP levels in asymptomatic patients with chronic mitral regurgitation predict the development of symptoms. Klaar et al.¹⁶⁵ from the University of Austria presented an abstract at the 2003 AHA meeting demonstrating that in 85 patients with severe mitral regurgitation followed for 260 ± 49 days, BNP levels were significantly higher in asymptomatic patients who subsequently developed symptoms than patients who remained asymptomatic; BNP 147 ± 161 pg/ml vs. 33 ± 27 pg/ml, $p=0.002$, thus potentially identifying patients with mitral regurgitation at risk of symptomatic deterioration.

1.3.3 Aortic Regurgitation

With the exception of the study from this thesis on the association between natriuretic peptide levels, LV function and symptoms in patients with chronic aortic regurgitation (chapter 4), there are no published reports on the natriuretic peptides in patients with aortic regurgitation.

1.3.4 Summary and Critique of the Literature

Symptoms

The importance of symptoms in patients with valvular heart disease is emphasised by the current AHA/ACC guidelines for management of valvular heart disease. These guidelines recommend valve surgery for patients with severe valvular disease in the presence of symptoms, even when mild and associated with apparently normal LV systolic function. This recommendation is based on the well described adverse post-operative prognosis when patients undergo valve surgery with advanced symptoms of heart failure or LV dysfunction.¹² Although some of the previous studies on the natriuretic peptides in valvular heart disease made reference to the association of cardiac symptoms and the natriuretic peptides,^{159,161,163} the studies included relatively small numbers of patients and did not address the symptoms of angina and syncope. Furthermore, they did not adjust for potential confounders such as LV ejection fraction, disease severity, age, gender and serum creatinine when determining the association between the natriuretic peptides and NYHA class. These issues are addressed in the studies presented in this thesis.

Diastolic function

Diastolic dysfunction is considered to be one of the possible mechanisms for the early symptoms of heart failure in patients with aortic stenosis and often persists for many years after aortic valve replacement.^{166,167} Although previous studies on the natriuretic peptides in patients with hypertension and heart failure have demonstrated increased natriuretic peptide levels with diastolic dysfunction,¹⁶⁸⁻¹⁷⁰ none of the aforementioned studies on aortic stenosis addressed the association between natriuretic peptides and echocardiographic measures of LV diastolic

dysfunction. The association of echocardiographic measures of diastolic function with the natriuretic peptides and the presence of symptoms in patients with aortic stenosis is addressed in the studies presented in Chapter 3.

Mechanism(s) of natriuretic peptide production

Many authors suggest that LV volume and/or pressure load is a major stimulus for the increased production of the natriuretic peptides. Most of the referenced studies supporting this assumption have included patients with advanced heart failure and increased LV volumes associated with predominantly severely reduced LV ejection fractions.^{51,171} Chronic aortic regurgitation is a unique condition characterised by a slowly progressive course commonly associated with a marked increase in LV volumes despite preserved LV ejection fraction and the absence of symptoms. Chapter 4 considers natriuretic peptides in predominantly asymptomatic patients with chronic moderate-severe aortic regurgitation and preserved LV ejection fraction, and suggests that it is not compensated increased LV volume that is the predominant stimulus for increased natriuretic peptide levels, but rather LV decompensation associated with symptoms of heart failure. Chapter 3 considers natriuretic peptides in aortic stenosis and demonstrates that increased LV pressure is not the major stimulus for the increased production of natriuretic peptides, but rather LV decompensation associated with the development of symptoms of heart failure.

Serial measurements

Finally, all studies published so far include only single measurements of the natriuretic peptides in asymptomatic patients with aortic stenosis and mitral regurgitation. The potential clinical utility for measurement of the natriuretic peptides in patients with valvular heart disease is to improve the timing of valve surgery by the

association of the natriuretic peptides with early symptoms of heart failure and predicting clinical outcome. It is widely accepted that it is very difficult to predict disease progression in an individual with valve disease by current methods of clinical evaluation and echocardiography. While some patients remain stable and asymptomatic for many years, other patients progress rapidly with the risk of sudden death and advanced symptoms of heart failure before operative intervention.^{172,173} There are currently no published manuscripts on the serial measurement of the natriuretic peptides in asymptomatic patients with valve disease to predict symptomatic deterioration. The results of a prospective longitudinal study of serial measurements of N-BNP in asymptomatic patients with aortic stenosis to predict the development of symptoms are presented in chapter 3.2.

1.4 High-Sensitivity C-Reactive Protein

C-reactive protein (CRP) is a non-specific marker of inflammation. CRP levels are commonly elevated in infective illnesses as well as inflammatory illnesses including rheumatoid arthritis and systemic lupus erythematosus.¹⁷⁴ Standard clinical assays for CRP have a lower detection threshold of 3 to 8 mg/l and lack sensitivity for detection of CRP within the low-normal range. CRP levels lower than this detection limit have been shown to be a strong predictor of future cardiovascular events even after adjustment for traditional risk factors. Recent data also demonstrate an association between low CRP levels and all-cause mortality.¹⁷⁴

Several high-sensitivity assays for CRP are now available with a detection threshold of 0.15 mg/l.¹⁷⁴ In an analysis of >5000 Americans without known cardiovascular disease, the median CRP level was 1.6 mg/l and ranges were 0.1-15.0 mg/l.¹⁷⁴ An elevated CRP level within the low-normal range is considered to represent an inflammatory process present in atherosclerotic plaques and the higher the CRP level the higher the risk for cardiovascular events. Recent evidence suggests that the anti-inflammatory actions of aspirin and statins in particular may be the major mechanism for the reduction in cardiovascular events seen in patients prescribed these agents.³⁶ Although the major clinical use for measurement of CRP is for the prediction of cardiovascular risk, it may also be measured for research purposes to improve our understanding of other conditions considered to have an inflammatory component.

A number of factors need to be considered when interpreting CRP levels in research and clinical practice. For most individuals, CRP levels appear to be stable over long periods of time¹⁷⁵ and no circadian variation appears to exist.¹⁷⁶ However, CRP is a

non-specific marker and increases with acute infection, acute ischaemia and trauma.¹⁷⁷ Other factors may also influence CRP levels. Obesity is associated directly with increased plasma levels of CRP, most likely due to increased secretion of interleukin-6, a primary hepatic stimulant for CRP production.^{178,179} Diabetic patients have increased levels of CRP,¹⁸⁰ as do smokers.¹⁷⁴ Certain medication may affect CRP levels. Statins decrease CRP levels by up to 35% independent of their cholesterol lowering effects, presumably by anti-inflammatory mechanisms.^{36,181-183} The effect of aspirin on CRP is uncertain.^{184,185}

Non-rheumatic aortic stenosis has been considered a degenerative condition that occurs with increasing frequency with increasing age. However, recent histological studies indicate that aortic stenosis represents a chronic inflammatory disease with similarities to atherosclerosis. Both conditions increase in prevalence with age, but neither is thought to be a normal consequence of ageing. The presence of aortic sclerosis or aortic stenosis is associated with the same clinical factors, at a similar level of risk, as has been associated with coronary artery disease. In the population based cardiovascular health study, the presence of aortic sclerosis on echocardiography in adults over age 65 years, with no known coronary artery disease at study entry, was associated with a 50% increased risk of cardiovascular mortality and myocardial infarction over a mean follow up interval of 5.5 years.

In chapter 6, CRP levels in patients with non-rheumatic aortic valve disease and the effects of aortic valve replacement on CRP levels are described. These data provide supportive biochemical evidence to previously published histological reports that aortic stenosis is an inflammatory disease, and that the aortic valve is the likely cause for the elevated CRP. These data also provide further stimulus for studies to

assess the possible role of medications with anti-inflammatory properties in slowing or preventing disease progression in patients with aortic sclerosis and aortic stenosis.

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CHAPTER 2

Methods

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2.1 Contributions to Thesis

The studies presented in this thesis were undertaken during a fellowship in the Department of Cardiovascular Research, Green Lane Hospital, Auckland, New Zealand from December 2000 to June 2003. I was the principal investigator for the studies on the natriuretic peptides in aortic stenosis and aortic regurgitation, and CRP in aortic valve disease. Dr Timothy Sutton, Cardiologist at Middlemore Hospital, Auckland, New Zealand, was the principal investigator for the study on the natriuretic peptides in mitral regurgitation and I was involved in all aspects of that study. I obtained ethics approval for all the studies from the Auckland Regional Ethics Committees in January 2001 and all patients in these studies gave written informed consent. I obtained grants from the National Heart Foundation of New Zealand and the Green Lane Research and Education Fund. With the exception of the study on the natriuretic peptides in mitral regurgitation, I recruited all patients in the studies by telephone interview and written invitation to participate. I screened and reviewed all patients at Green Lane Hospital including the clinical assessment and collection and storage of blood samples. The echocardiograms were performed by either myself or Ms Renelle French, and were analysed at a later time by Ms French blinded to previous echocardiographic results, clinical data and natriuretic peptide levels. I initially recorded all collected data on paper case record forms and subsequently recorded all data electronically for analysis. Ms. Teena West, Biostatistician at Green Lane Hospital, assisted with me with the statistical analysis for the studies.

2.2 Aims

Two major challenges in the management of patients with chronic valvular heart disease include: Firstly, to determine the earliest development of left ventricular decompensation that may allow optimal timing of valve surgery, and secondly, to improve the understanding of the mechanisms involved in the development and progression of aortic stenosis that may allow for medical treatment to slow or even prevent disease progression.

The aims of this thesis are:

1. To determine the association between plasma levels of the natriuretic peptides, ANP, BNP and N-BNP and disease severity, left ventricular function and cardiac symptoms in patients with aortic stenosis, aortic regurgitation and mitral regurgitation.
2. To determine whether serial measurements of N-BNP levels in asymptomatic patients with aortic stenosis predict the development of symptoms.
3. To determine the effect of aortic valve replacement on N-BNP levels in patients with aortic stenosis and the association with symptoms.
4. To determine CRP plasma levels in patients with non-rheumatic aortic stenosis and the effect of aortic valve replacement for severe aortic stenosis on CRP levels.

2.3 Studies Design

A number of methods were common between the studies, including the study patients, exclusion criteria, clinical assessment, normal control subjects, performance and interpretation of echocardiograms, collection and assay of the natriuretic peptides, and statistical analysis. These common methods are outlined in this chapter and specific methods are provided in detail for each study where appropriate.

Patients

Between January 2001 and November 2001, all eligible patients with aortic stenosis, aortic regurgitation, or mitral regurgitation were assessed and enrolled in the study if eligible. All patients on the waiting list for valve surgery at Green Lane Hospital, Auckland were screened for possible participation and all eligible patients were invited to participate. All echocardiograms performed at the 4 major teaching hospitals in Auckland in the 12 months preceding the study were reviewed and all eligible patients were invited to participate. In cross sectional observational studies, the association between natriuretic peptide levels, disease severity, left ventricular function and symptoms was assessed for aortic stenosis (Chapter 3.1), aortic regurgitation (Chapter 4), and mitral regurgitation (Chapter 5). For the longitudinal study on N-BNP in asymptomatic aortic stenosis (Chapter 3.2), the patients were comprised predominantly from the asymptomatic patients in Chapter 3.1 and for the study on N-BNP before and after aortic valve replacement for severe aortic stenosis (Chapter 3.3), the patients were comprised predominantly from the symptomatic patients in Chapter 3.1.

Exclusion Criteria

To more accurately determine the association between natriuretic peptide levels and valvular heart disease, other conditions that are associated with increased natriuretic peptide levels were exclusion criteria. These included myocardial infarction within 6 months, regional wall motion abnormalities on echocardiography, LV ejection fraction <55% considered not due to the valve lesion, prior cardiac surgery, a plasma creatinine level of >0.16 mmol/L, and known severe respiratory disease. For the studies on aortic stenosis, patients with more than mild aortic regurgitation or more than mild mitral valve disease were also excluded. For the study on aortic regurgitation, patients with an aortic valve area <1.4 cm² or more than mild mitral valve disease were also excluded. For the study on natriuretic peptides in mitral regurgitation, patients with a mitral valve area <1.5 cm² or more than mild aortic valve disease were also excluded.

Clinical Assessment

All patients recruited for the studies had been reviewed by their cardiologist prior to enrolment and documented as symptomatic or asymptomatic. Heart failure symptoms were classified according to the NYHA functional class I-IV. For the studies on the natriuretic peptides in aortic stenosis and aortic regurgitation, I clinically assessed all patients and judged them symptomatic if they had a history of symptoms of heart failure (NYHA class ≥II) and/or angina (Canadian Cardiovascular Society class ≥1) and/or exertional presyncope or syncope considered due to aortic valve disease. The heart rate, blood pressure, cardiac rhythm, height, weight, and current medications were recorded. For the studies on the natriuretic peptides in mitral regurgitation, Dr Sutton performed the clinical assessment as described

above. For the very few occasions where there was a discrepancy between the assessment of symptom status, cases were discussed with Dr Ralph Stewart and consensus was achieved. The presence or absence of symptoms was always determined before the study echocardiogram was performed and before the collected blood samples were analysed for natriuretic peptide levels. None of the patients in the studies had undergone an exercise stress test.

Normal Controls

Natriuretic peptide levels in normal controls subjects without clinical symptoms and signs of cardiopulmonary or renal disease by a questionnaire were obtained from Dr Mark Richards, Director of the Cardioendocrine Research Unit, Christchurch, New Zealand. Natriuretic peptide levels for one hundred controls were used for the study on natriuretic peptide in aortic stenosis (Chapter 3.1) and mitral regurgitation (Chapter 5), and normal control subjects were matched 1:1 by age and gender to patients with aortic regurgitation (Chapter 4) and patients with aortic stenosis who underwent aortic valve replacement (Chapter 3.3).

Quality Control Measures

All data was collected on case record forms (CRF's) and entered into an electronic database. Prior to analysis, I double checked all electronically entered data against the CRF's. In the event of a discrepancy, I reviewed the data and made changes as appropriate. All natriuretic peptide levels were sent to me in an electronic format. The Christchurch Cardioendocrine unit have excellent quality control measures in place to ensure the accuracy of the data provided and have provided natriuretic peptide levels for many other studies. The statistical analyses were performed with a statistician and all data provided were checked for accuracy.

2.4 Echocardiography

Echocardiograms for the studies on the natriuretic peptides in aortic stenosis (Chapter 3), aortic regurgitation (Chapter 4) and CRP in aortic valve disease (Chapter 6) were performed by me or Ms Renelle French, senior cardiac echocardiographer at Green Lane Hospital. Echocardiographic data were obtained with the use of a Hewlett Packard 5500 Sonos ultrasound system (Palo Alto, California). All analyses were performed off-line by Ms French blinded to the patients' symptom status, previous echocardiographic results, natriuretic peptide levels and CRP levels.

Echocardiograms performed in the study on the natriuretic peptides in mitral regurgitation (Chapter 5) were performed by Ms Renelle French or Dr Timothy Sutton, Cardiologist at Middlemore Hospital, Auckland. Echocardiographic data were obtained using a standard protocol on commercially available systems (Series 5 or Vivid 5 System, GE Vingmed Ultrasound, Horten, Norway). All analyses were performed offline by Dr Timothy Sutton or Dr Andrew Kerr, Cardiologist at Middlemore Hospital blinded to the patients' symptom status, previous echocardiographic results and natriuretic peptide levels.

All patients underwent comprehensive examination including M-mode, two-dimensional and Doppler echocardiography. Measurements of LV size and systolic function were made according to American Society of Echocardiography guidelines¹⁸⁶ and averaged from 3 cardiac cycles. The LV systolic meridional wall stress (g/cm^2) was calculated as previously described using the cuff brachial systolic blood pressure recorded at the end of the echocardiographic study.¹⁸

For patients with aortic stenosis (Chapter 3), the peak aortic velocity was recorded using continuous-wave Doppler from the window yielding the highest-velocity signal. The mean aortic valve gradient was obtained by tracing the continuous wave flow velocity signal across the aortic valve. The aortic valve area was calculated by using the continuity equation.¹⁶⁷ Assessment of LV diastolic function was made from transmitral (E and A wave velocities and deceleration time) and pulmonary venous flow parameters (S and D wave velocities) and classified as normal, impaired relaxation, pseudonormal or restrictive - normal diastolic function (E/A >1 and S/D > 1), impaired relaxation (E/A <1 and S/D > 1), pseudonormal (E/A > 1 and S/D <1) and restrictive (E/A > 1, S/D <1 and deceleration time < 150 ms.)¹⁸⁷ Tricuspid regurgitation peak velocity was measured in patients with colour Doppler evidence of tricuspid regurgitation and right ventricular systolic pressure (RVSP) was calculated by the equation: $RVSP = 4(\text{tricuspid regurgitation velocity m/s})^2 \text{ mmHg}$.

For patients with mitral regurgitation, the severity of mitral regurgitation was assessed from the regurgitant fraction,¹⁸⁸ vena contracta width,¹⁸⁹ and the mitral regurgitation score described by Thomas and Foster et al.¹⁹⁰ The mitral regurgitation score is calculated from visual assessment of left atrial size, mitral regurgitation jet penetration into the left atrium, mitral continuous wave Doppler characteristics, pulmonary venous flow pattern, tricuspid regurgitation velocity, and proximal isovelocity surface area (PISA) radius.

2.5 Collection and Assays of Natriuretic Peptides

For the measurement of the natriuretic peptides in all studies presented in this thesis, 20mls of venous blood was drawn into chilled EDTA tubes from a peripheral vein after patients had rested for at least 10 minutes in a semi-recumbent position. Blood samples were centrifuged within 10 minutes at 3000 rpm for 10 minutes at 4 degrees Celsius. Plasma was then separated and stored frozen at -80 degrees Celsius. After the completion of each study, the frozen samples were couriered on dry ice to the Christchurch Cardioendocrine Laboratory for measurement of ANP, BNP and N-BNP using established radioimmunoassays^{50,191,192}

Plasma was extracted using Sep-Pak cartridges pre-washed with methanol and 4% acetic acid. The natriuretic peptides were eluted with 3mls of ethanol-water-acetic acid mixture. Triton X 100 (10 uL, 1%) was then added and this mixture was dried under an air stream at 37 degrees Celsius before being dissolved in 0.5 mls of assay buffer. Specific assays for each natriuretic peptide is described below.

Atrial Natriuretic Peptide¹⁹¹

In this assay, 100 uL of plasma extract in assay buffer were incubated with 100 uL of locally raised polyclonal antiserum to ANP for 21 hours at 4 degrees Celsius. Radiolabelled ANP in 100 ul of assay buffer was then added, and this mixture was incubated for a further 24 hours at 4 degrees Celsius. Bound and free ANP were separated by the addition of 100 uL of normal rabbit serum solution and 100 uL of goat anti-rabbit immunoglobulin antiserum. The precipitate formed during this reaction was counted in a gamma counter (Gamma Master; LKB Wallace). The detection limit of the assay was <5 pmol/L. The inter-assay coefficient of variation

(CV) was 7.8% measured at 20 pmol/L and intra-assay CV was 2% at 20 pmol/L. The normal range is <24 pmol/L.

B-type Natriuretic peptide¹⁹²

BNP levels were determined by a competitive radioimmunoassay similar to the method described for ANP, using commercially available human BNP antiserum from Phoenix Pharmaceuticals (catalogue number AB-011-03) and standards for human BNP from Peninsula Laboratories (Belmont, California, USA; catalogue number 9086). The detection limit of the assay was <5 pmol/L. The inter-assay CV was 13.3% measured at 13 pmol/L and intra-assay CV was 4.4% at 10 pmol/L. The normal range is <12 pmol/L.

Amino-terminal B-type Natriuretic Peptide⁵⁰

Plasma extract was incubated with 100 μ l of Radiolabelled tracer and 100 μ l of diluted locally raised polyclonal antiserum. After incubation for 24 hours at 4 degrees Celsius, bound and free N-BNP were separated by the addition of 100 μ l of normal rabbit serum solution and donkey anti-rabbit antibody. The bound radioactivity was counted in a gamma counter (Gamma Master; LKB Wallace). The detection limit of the assay was <8 pmol/L. The inter-assay CV was 8.5% measured at 17 pmol/L, 6.8% measured at 127 pmol/L, and 6% measured at 247 pmol/L. The intra-assay CV was 3.4% at 50 pmol/L. The normal range is <50 pmol/L.

2.6 Statistical Analysis

A SAS release 8.0 statistical software package (Cary, North Carolina) was used for the statistical analyses. Since the distribution of the natriuretic peptide levels was positively skewed, all analyses used natural log-transformed (Ln) levels unless otherwise specified. Natriuretic peptide levels are expressed as median and interquartile range [IQR] unless otherwise stated. Continuous echocardiographic and clinical variables are expressed as mean \pm standard deviation (SD) unless otherwise stated. A p value of <0.05 was considered statistically significant. Specific statistical methods are provided in detail for each study.

CHAPTER 3

Natriuretic Peptides in Aortic Stenosis

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3.1 Associations Between Natriuretic Peptide Plasma Levels, Disease Severity, Left Ventricular Function and Symptoms in Aortic Stenosis.

INTRODUCTION

The natural history of aortic stenosis in adults includes a prolonged asymptomatic period during which morbidity and mortality are very low.^{193,194} Symptom onset signals a dramatic change in outlook, and following the development of angina, syncope or heart failure, the average survival with medical therapy is less than 3 years.^{195,196} Although the clinical outcome is different in symptomatic compared with asymptomatic patients, there are wide overlaps in hemodynamic and echocardiographic measures of severity between these patient groups.²⁶ According to current ACC/AHA guidelines, no single clinical, hemodynamic or echocardiographic measure has been adopted as a class I recommendation for valve replacement in the absence of symptoms in patients with isolated aortic stenosis.¹²

In many patients the development of symptoms is clear, but in others symptoms are difficult to assess because of inactivity or under-reporting. In some patients it may also be unclear whether symptoms are related to the aortic stenosis. A non-invasive marker of early cardiac decompensation would therefore be helpful in monitoring disease progression in patients with aortic stenosis. In this study the association between natriuretic peptide plasma levels, disease severity, LV function, and symptoms were determined in patients with aortic stenosis.

METHODS

Seventy-four consecutive eligible patients with echocardiographic evidence of aortic stenosis (peak aortic velocity ≥ 2.5 m/s) were enrolled in this study. A detailed description of the methods including clinical assessment, exclusion criteria, normal controls, echocardiography and measurements of ANP, BNP and N-BNP is described in chapter 2.

Statistical Analysis

Where the results for N-BNP, BNP and ANP were similar, the results for N-BNP only are given to simplify presentation. Comparisons between groups for continuous variables were made using t-tests or one-way analyses of variance (ANOVA) where appropriate, and the χ^2 test was used for categorical variables. Pearson's correlation coefficient was used to assess the association between natriuretic peptide levels and echocardiographic variables. Two-way ANOVA was used to compare the mean natriuretic peptide levels within each NYHA class. Analyses of covariance were used to assess natriuretic peptide levels in symptomatic and asymptomatic patients after adjustment for confounders. The homogeneity of slopes was assessed by fitting an interaction term. The areas under the receiver-operating characteristic (ROC) curves were used to evaluate the diagnostic performance of natriuretic peptide and echocardiographic variables. The cut-points were chosen when the sensitivity and specificity were maximised. Linear regression models were fitted to the control data to provide normal natriuretic peptide ranges for a given sex and age. Model fits were assessed by plotting residuals. Ninety-five percent confidence intervals for the ratio of the geometric means between the groups (symptomatic/asymptomatic) are reported for each peptide.

RESULTS

Baseline Characteristics of the Patients

Of the 74 patients with aortic stenosis, 41 (55%) were male and 33 (45%) female, with a mean age of 69.2 ± 12.5 years. Of the 100 normal control subjects, 41 were male and 59 female, with a mean age of 55 ± 11 years. The etiology of the aortic stenosis was rheumatic in one, bicuspid aortic valve in 15 and 'calcific degeneration' in 58 patients. The cardiac rhythm was sinus in 69, atrial fibrillation in four and paced in one. Twenty-nine patients (39%) were asymptomatic and 45 (61%) were symptomatic.

Association With Echocardiographic Measurements

There was a statistically significant correlation between the Ln of each of the natriuretic peptides and echocardiographic measures of aortic stenosis severity and LV function. The correlation coefficients (r) for Ln BNP, N-BNP and ANP and the echocardiographic variables were: aortic valve area (r=-0.55, -0.57, -0.55 respectively), peak aortic velocity (r=0.33, 0.35, 0.38 respectively), mean aortic gradient (r=0.36, 0.37, 0.38 respectively), LV mass index (r=0.62, 0.59, 0.46 respectively), LV end-diastolic volume index (r=0.51, 0.41, 0.47 respectively), LV end-systolic volume index (r=0.54, 0.45, 0.48 respectively), LV ejection fraction (r=-0.48, -0.42, -0.39 respectively), LV systolic meridional wall stress (r=0.29, 0.36, 0.37 respectively), RV systolic pressure (r=0.60, 0.59, 0.63 respectively), left atrial diameter (r=0.30, 0.34, 0.35 respectively) and LV end-diastolic posterior wall thickness (r=0.37, 0.38, 0.29 respectively); (p<0.05 for all comparisons).

Natriuretic peptide levels were similar in patients with normal diastolic function (n=24, N-BNP median 59 [IQR 24-136] pmol/L), an impaired relaxation pattern (n=31,

median 58 [35-97] pmol/L) or a pseudonormal pattern (n=5, median 70 [22-150] pmol/L). Natriuretic peptide levels were higher in the four patients (all symptomatic) with a restrictive filling pattern (median 243 [198-348] pmol/L). Three of these patients also had a LV ejection fraction of <50%.

Comparison Between Symptomatic and Asymptomatic Patients

The symptomatic patients were older (72 ± 10 years vs. 65 ± 15 years; $p=0.035$), and had a higher serum creatinine (0.10 ± 0.02 mmol/L vs. 0.09 ± 0.02 mmol/L, $p=0.01$) but there was no difference between these two groups with respect to sex, history of hypertension, diabetes mellitus, smoking or the use of any cardiovascular medication. The natriuretic peptide levels and echocardiographic measures of asymptomatic and symptomatic patients are compared in Table 3.1.1. N-BNP, BNP and ANP levels were higher and the aortic valve area was smaller in symptomatic patients than in asymptomatic patients. After adjustment for age, sex, serum creatinine, aortic valve area and LV ejection fraction, the natriuretic peptide levels remained higher in symptomatic patients than in asymptomatic patients (for N-BNP: geometric mean 1.74 times higher [95% CI 1.12-2.69], $p=0.014$; for BNP 1.51 times higher [1.03-2.23], $p=0.036$; and for ANP 1.27 times higher [0.95-1.69], $p=0.10$ respectively).

Natriuretic peptide levels increased as the aortic valve area decreased, as illustrated for N-BNP in Figure 3.1.1. The median N-BNP levels in the control subjects and in the four groups of patients categorized by aortic valve area, symptom status and LV systolic function are shown in Figure 3.1.2. In a subanalysis of patients with an aortic valve area <1.0 cm² (n=59), the aortic valve area was similar in symptomatic (n=43, mean aortic valve area 0.68 ± 0.19 cm²) and asymptomatic patients (n=16,

mean aortic valve area $0.76 \pm 0.12 \text{ cm}^2$), $p=0.18$, but the natriuretic peptides were higher in symptomatic patients (N-BNP median 142 [IQR 73-210] pmol/L) compared to asymptomatic patients (N-BNP 53 [28-91] pmol/L), $p=0.0007$. The difference remained significant after adjusting for age, gender, serum creatinine and ejection fraction, $p=0.01$. Similar results were obtained for BNP and ANP.

Natriuretic Peptide Levels by NYHA Class, Angina and Syncope

N-BNP, BNP and ANP levels all increased with increasing NYHA class (Table 3.1.2). Within each NYHA class, natriuretic peptide levels were not higher in patients with angina, presyncope or syncope than in those without these symptoms. Coronary angiography was performed in patients scheduled for aortic valve surgery, $n=42$ (57%). There was a significant association between the presence of at least one $\geq 50\%$ angiographic coronary artery stenosis and angina, $p=0.009$.

Sensitivity and Specificity for the Presence of Symptoms

The sensitivity and specificity of natriuretic peptide levels and echocardiographic measures for the presence of symptoms are shown in Table 3.1.3. Natriuretic peptide levels, aortic valve area and peak aortic velocity were stronger predictors of symptoms than measures of LV volumes, mass, ejection fraction, systolic meridional wall stress, posterior wall thickness or diastolic function. Receiver-operator characteristic curves showing the sensitivity and specificity of N-BNP and aortic valve area for the presence of symptoms is shown in Figure 3.1.3.

Age and Sex

The level of each natriuretic peptide increased with age in normal subjects and patients with aortic stenosis ($p<0.0001$ for all), as illustrated for N-BNP in Figure

3.1.4. Levels of natriuretic peptides were higher in women than in men after adjustment for age (N-BNP, 1.70 times higher [95% CI 1.33-2.18]; BNP, 1.33 times higher [1.13-1.57]; and ANP, 1.31 times higher [1.13-1.50]; $p < 0.001$ for all). There was no statistically significant interaction between age and sex. The use of an age- and sex-adjusted normal range did not improve the predictive value of natriuretic peptide levels for symptoms.

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Table 3.1.1: Comparison of plasma natriuretic peptide levels and echocardiographic measures in asymptomatic and symptomatic patients

	Asymptomatic (n=29)	Symptomatic(n=45)	T-Statistic	P Value*
N-BNP, pmol/L [†]	33 (16–58)	112 (70–193)	5.99	<0.0001
BNP, pmol/L [†]	9 (6–14)	28 (14–44)	5.62	<0.0001
ANP, pmol/L [†]	19 (12–24)	32 (22–47)	4.63	<0.0001
Aortic valve area, cm ²	0.99 ± 0.31	0.71 ± 0.23	4.36	<0.0001
Peak aortic velocity, m/s	4.0 ± 0.8	4.6 ± 0.7	3.81	0.0003
Mean aortic gradient, mmHg	39 ± 16	53 ± 17	3.58	0.0006
LV ejection fraction, %	65 ± 5.7	59 ± 11.6	2.97	0.005
LV end-systolic volume index, mL/m ²	18 ± 6	28 ± 20	2.54	0.02
LV mass index, g/m ²	114 ± 29	137 ± 35	2.32	0.03
Posterior wall thickness in diastole, cm	1.10 ± 0.22	1.24 ± 0.23	2.25	0.03
Right ventricular systolic pressure, mmHg	28 ± 6.1	34 ± 7.1	2.28	0.03
LV end-diastolic volume index, mL/m ²	53 ± 11	63 ± 22	2.16	0.04
LV systolic meridional wall stress, g/cm ²	79 ± 27	94 ± 39	1.46	0.15
Diastolic function, n (%)				
Normal	14 (50)	10 (28)		
Impaired relaxation	13 (46)	18 (50)		
Pseudonormal	1 (4)	4 (11)		
Restrictive	0 (0)	4 (11)		0.01 [‡]

*P values were generated from t-tests on natural logarithm transformed values.

[†]Natriuretic peptide levels are expressed median [interquartile range]. Continuous echocardiographic measures are expressed mean ± SD.

[‡]Cochran-Armitage trend statistic=2.51.

Table 3.1.2: Association between natriuretic peptide levels and symptoms

	N	Natriuretic peptide*	N	NYHA class I	N	NYHA class II	N	NYHA class III/IV	F-statistic	P Value [†]
All subjects	74	N-BNP	30	34 [16-58]	28	105 [57-159]	16	202 [87-394]	22.86	<0.0001
		BNP	30	8 [6-14]	28	25 [13-35]	16	40 [18-66]	20.41	<0.0001
		ANP	30	18 [13-24]	28	31 [19-40]	16	47 [24-59]	15.82	<0.0001
No angina	47	N-BNP	30	34 [16-58]	11	91 [33-142]	6	243 [193-298]		
Angina	27	N-BNP	0	-	17	106 [72-176]	10	147 [74-435]	0.64	0.4
No syncope	60	N-BNP	30	34 [16-58]	18	108 [64-176]	12	243 [147-394]		
Presyncope	9	N-BNP	0	-	6	91 [50-115]	3	50 [22-514]		
Syncope	5	N-BNP	0	-	4	101 [64-201]	1	85 [-]	2.30	0.1

NYHA indicates New York Heart Association.

The results for patients with and without angina, presyncope and syncope had similar patterns for BNP and ANP.

*Natriuretic peptide levels are expressed as median [interquartile range] pmol/L.

[†]P values were generated from analysis of variance on natural logarithm transformed peptide levels.

Table 3.1.3: Discriminatory value of natriuretic peptide levels and echocardiographic measures for symptoms in patients with aortic stenosis

Variable	Cut-Point*	Sensitivity (%)	Specificity (%)	AUC
N-BNP (pmol/L [†])	60	78	79	0.84
BNP (pmol/L [†])	14	76	76	0.83
ANP (pmol/L [†])	25	70	82	0.80
Aortic valve area (cm ²)	0.75	56	81	0.76
Peak aortic velocity (m/s)	4.3	73	69	0.76
Mean aortic gradient (mm Hg)	45	67	76	0.75
LV mass index (g/m ²)	128	70	70	0.71
LV systolic meridional wall stress (g/cm ²)	73	67	43	0.60
LV ejection fraction (%)	61	54	76	0.67
LV end-diastolic volume index (mL/m ²)	60	43	79	0.65
LV end-systolic volume index (mL/m ²)	18	67	54	0.65
Diastolic function, n (%)				
Restrictive versus non-restrictive		11	100	-
Normal versus impaired relaxation and pseudonormal		69	50	-

AUC indicates area under receiver-operating characteristic curve.

*The cut-points were chosen when the sensitivity and specificity were maximized.

[†]The upper limits of the normal reference ranges were 50pmol/L for N-BNP, 12pmol/L for BNP and 19pmol/L for ANP.

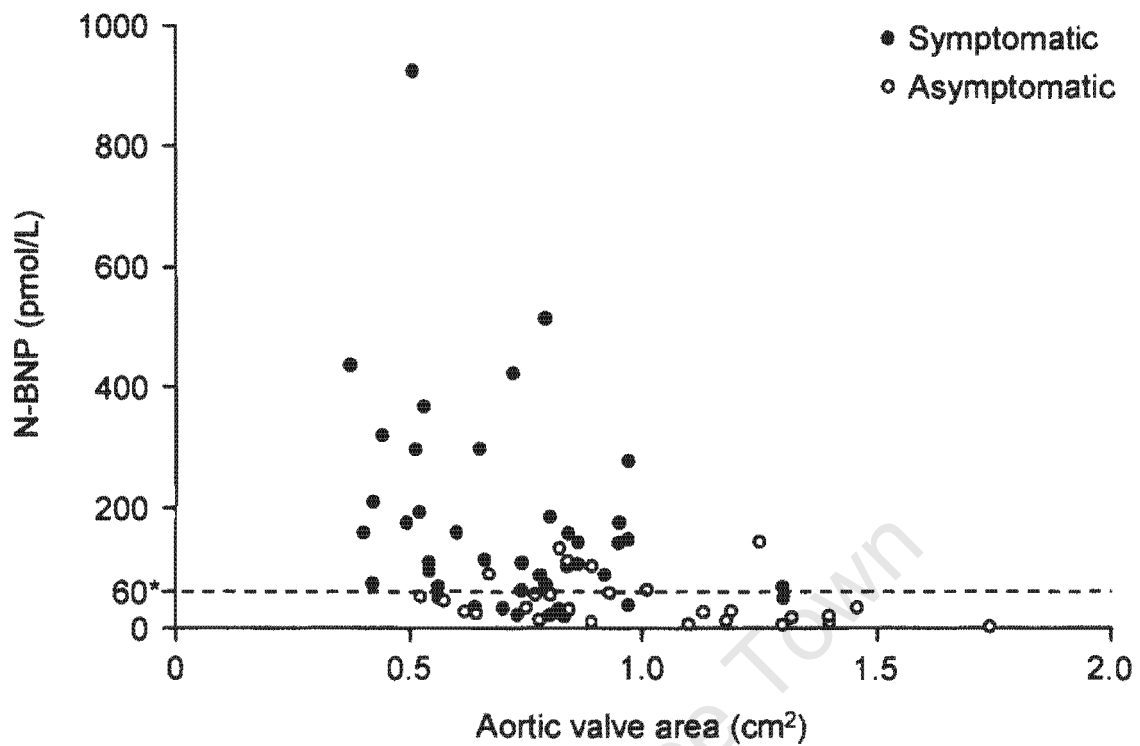


Figure 3.1.1: Association between N-BNP levels and aortic valve area in patients with aortic stenosis. *The line drawn at the N-BNP level of 60pmol/L indicates the cutpoint of maximum sensitivity and specificity for the presence of symptoms.

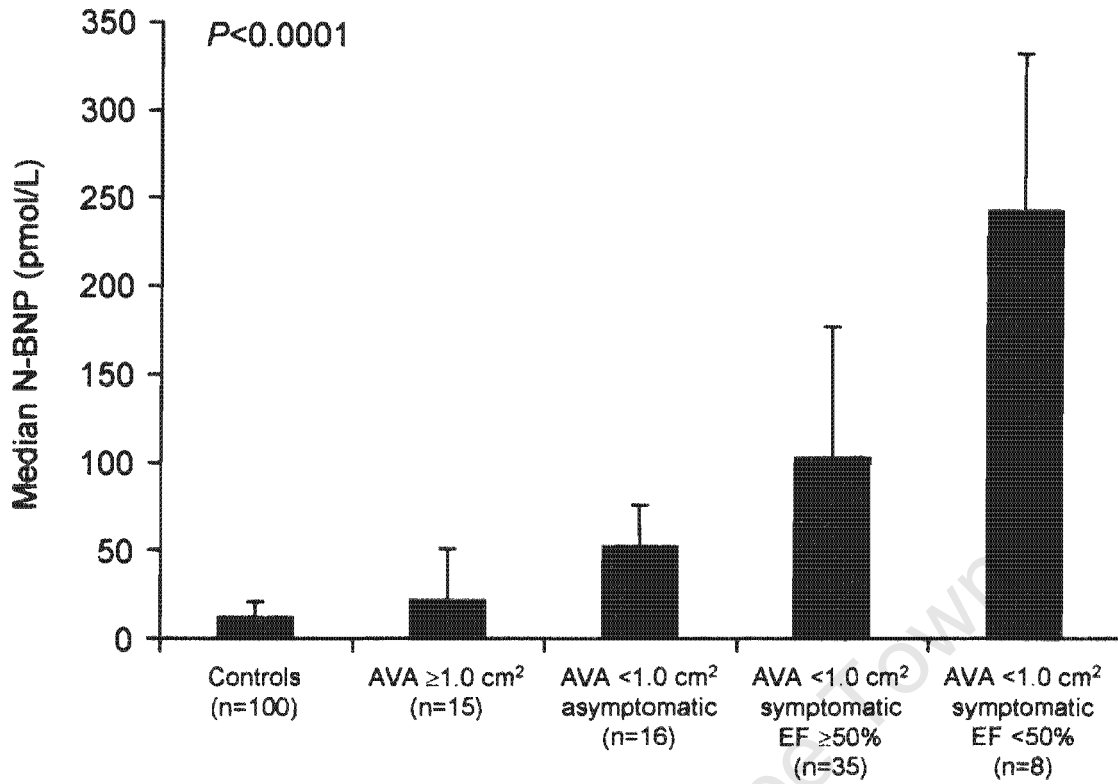


Figure 3.1.2: Association between N-BNP levels and severity of aortic stenosis. The N-BNP levels (median [upper quartile]) in normal control subjects and in subgroups of patients with aortic stenosis by aortic valve area, symptoms, and left ventricular systolic function are shown. AVA indicates aortic valve area; EF, ejection fraction.

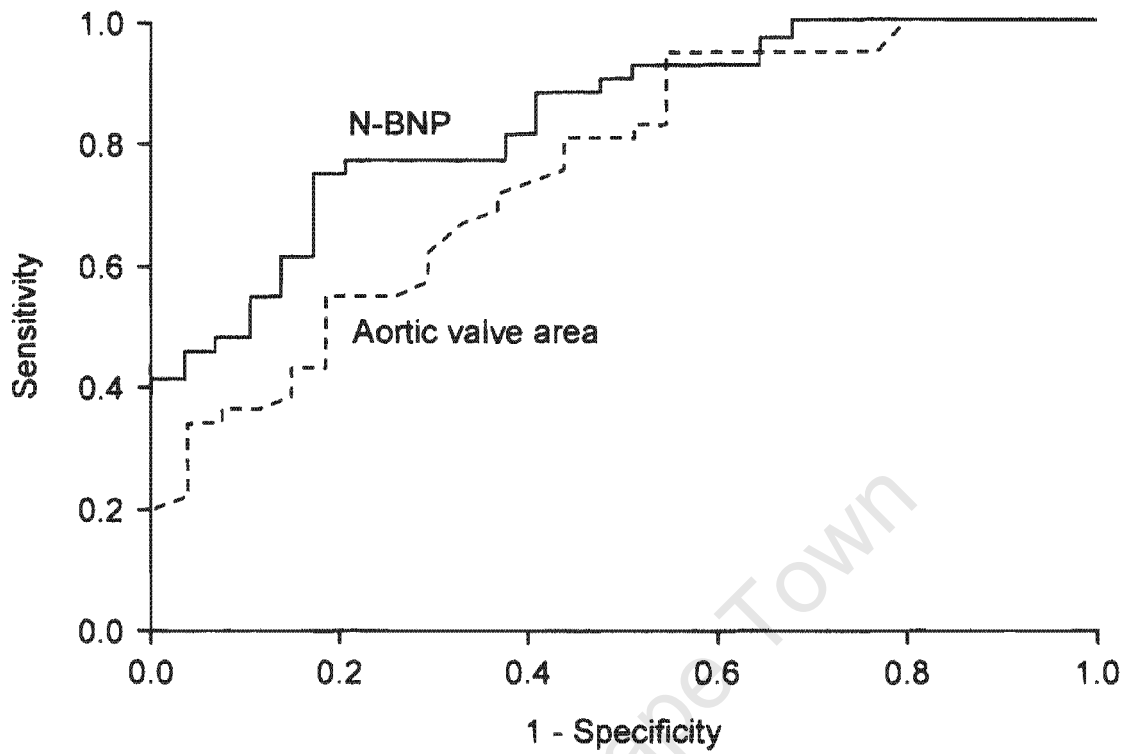


Figure 3.1.3: Receiver-operating characteristic curves of the sensitivity and specificity of N-BNP levels and aortic valve area for the presence of symptoms.

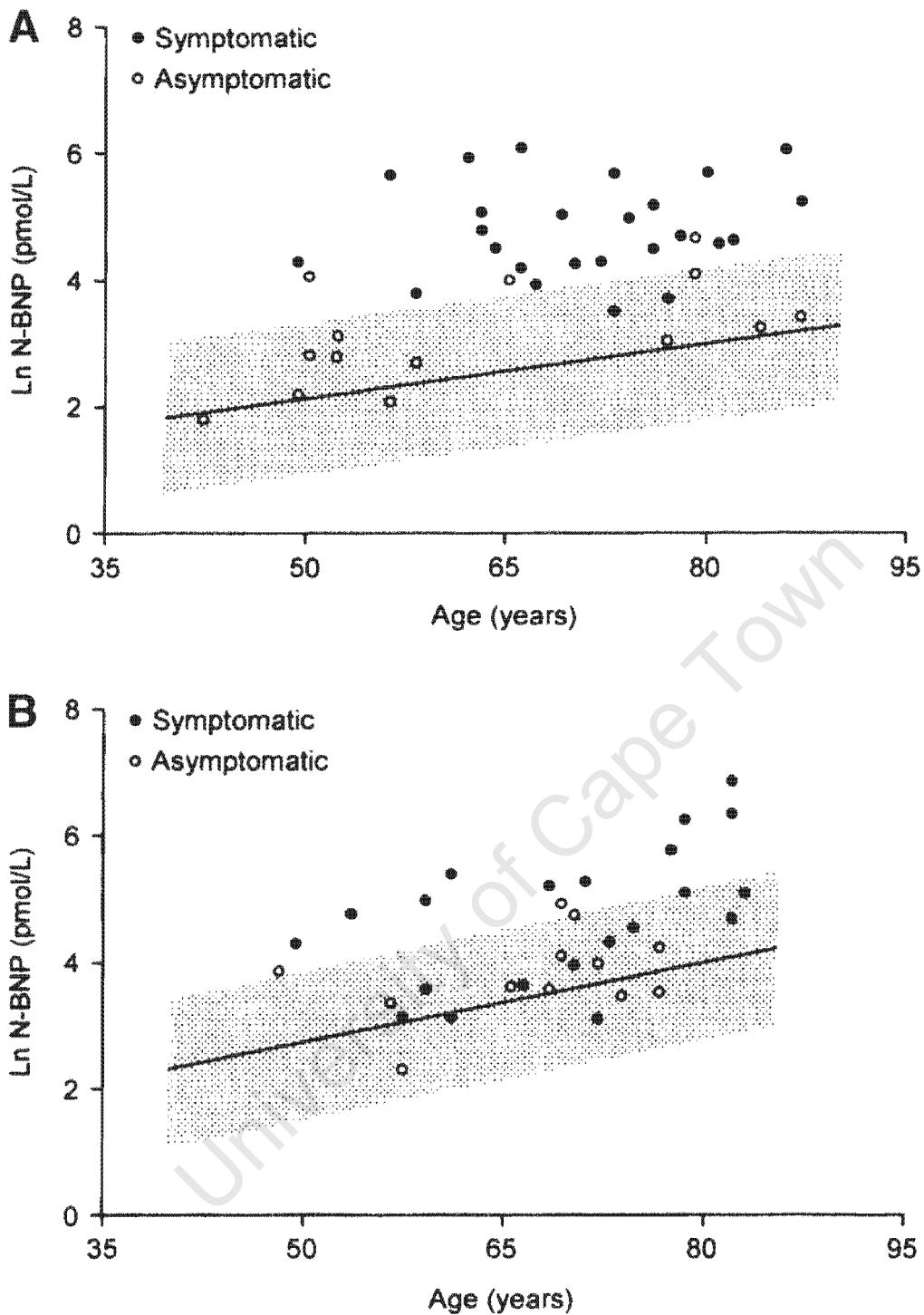


Figure 3.1.4: Association between N-BNP levels and age in men (A) and women (B) with aortic stenosis. The shaded areas represent the 95% prediction limits for age-matched normal control subjects. Ln indicates log-transformed.

DISCUSSION

The importance of early and accurate recognition of symptoms in patients with aortic stenosis is emphasized by the risk of rapid deterioration and sudden death following symptom onset in some patients.^{172,173} An additional reason for identifying early symptoms is that surgery can be performed with low operative mortality compared with the higher surgical risk when symptoms are severe or when surgery is not elective.¹⁹⁷ The most common initial symptoms are exertional dyspnea and fatigue.²⁶ These symptoms are non-specific, subtle at onset and often difficult to evaluate clinically.

In this study there was a strong association between the NYHA class and plasma levels of N-BNP, BNP and ANP. Importantly, natriuretic peptide levels were higher in patients with NYHA class II symptoms than in those with class I symptoms, suggesting that natriuretic peptide levels can be used to discriminate between early symptoms of heart failure and normal effort tolerance. After adjustment for the NYHA class, there was no association between natriuretic peptide levels and syncope or the presence or absence of angina. This suggests that the stimulus for increased secretion of natriuretic peptides by cardiac myocytes is associated with the clinical manifestations of exertional dyspnea. On the other hand, angina¹⁹⁸ and syncope¹⁹⁹ have different pathophysiologies in patients with aortic stenosis, and are not associated with increased secretion of natriuretic peptides.

The compensatory response of the LV to the chronic and gradually progressive pressure overload of aortic stenosis is concentric hypertrophy. Typically, the ejection fraction is preserved until late in the course of the disease. However, there may be significant myocyte dysfunction even when the ejection fraction is normal in the presence of concentric LV hypertrophy.²⁰⁰ In this study there was a progressive

increase in natriuretic peptide levels with decreasing aortic valve area, but a large increase in natriuretic peptide levels in patients with an ejection of <50%, emphasising the important association between LV systolic function and natriuretic peptide levels. Increased LV wall stress has been proposed as a stimulus for the release of natriuretic peptides in aortic stenosis.¹⁶⁸ In this study there was a weaker but significant correlation between wall stress and natriuretic peptide levels and wall stress was higher in symptomatic compared with asymptomatic patients. The development of diastolic dysfunction is another plausible explanation for symptom onset.¹⁶⁷ However, the majority of symptomatic patients in this study had normal diastolic function or an impaired relaxation pattern rather than more severe diastolic dysfunction.

The most widely used measures of aortic stenosis severity in clinical practice are the peak aortic velocity and the aortic valve area as determined by the continuity equation.¹⁶⁷ As in previous studies, these measures were most strongly associated with the presence of symptoms.²⁶ However, there was a large overlap between symptomatic and asymptomatic patients, consistent with the known heterogeneous response to the pressure load of aortic stenosis.²⁰¹ In contrast, there was less overlap in natriuretic peptide levels between symptomatic and asymptomatic patients. Furthermore, the natriuretic peptides — and N-BNP in particular — provided additional predictive value to the aortic valve area and LV ejection fraction for the presence of symptoms. These findings are consistent with observations from experimental models in which increased synthesis and release of BNP occurred with the transition from compensated to decompensated LV function.²⁰² This transition may not be reliably detected by current echocardiographic measures. Although there is increased understanding of the molecular changes that occur during myocardial

decompensation,²⁰³ the signalling pathways that lead to increased natriuretic peptide secretion are currently poorly defined.

Whereas echocardiographic assessment of aortic stenosis requires trained and experienced sonographers with meticulous attention to the technical details of imaging and Doppler flow recording, the introduction of automated assays means that measurement of plasma levels of natriuretic peptides is simple, not operator-dependent, relatively inexpensive and reproducible.⁴⁷ Although N-BNP appeared to have the best discriminatory value for symptoms, the current study did not have sufficient statistical power to reliably distinguish between the different natriuretic peptides. N-ANP has similar biological properties to N-BNP and has been shown to be a more reliable marker than ANP,^{161,204} but was not measured in this study.

Previous studies have shown that natriuretic peptide levels increase with normal ageing and are higher in women than in men with no cardiac disease.¹⁴² Similar age and sex differences were observed in normal control subjects in the current study. These observations suggest that the use of an age- and sex-specific normal range would improve the diagnostic accuracy of natriuretic peptide levels. However, in the current study the association between natriuretic peptide levels and symptoms did not increase after adjustment for age. A possible explanation is that the increase in natriuretic peptide levels reflects age-related changes in myocardial function,¹³⁷ which in themselves increase the likelihood of symptoms. In this case, the absolute level of natriuretic peptides, rather than the age- and sex-adjusted levels, may be most predictive.

The association between natriuretic peptide levels and symptoms was stronger in men than in women. It is not clear whether this sex difference existed because women report symptoms differently or whether there is a sex difference in the response of the LV to the hemodynamic load of aortic stenosis, as other researchers have suggested.^{205,206} In previous studies, women have had greater impairment of functional status and a poorer exercise capacity than men, despite a similar aortic valve area and greater LV fractional shortening.^{205,206}

As in clinical practice, it is likely that some patients' symptoms were not a consequence of aortic stenosis, while others were classified as asymptomatic because they undertook little physical activity or ignored subtle symptoms. Exercise testing has been proposed as a method of identifying patients with aortic stenosis who are at increased risk.²⁵ While a lack of increase in systolic blood pressure with exercise predicts a poor outcome, exercise capacity does not,²⁶ and some patients are unable to exercise. Furthermore, exercise testing needs to be performed with close monitoring and is contraindicated in symptomatic patients,¹² thus it was not undertaken in this study. Further studies comparing the clinical value of exercise testing and natriuretic peptide levels in apparently asymptomatic patients with aortic stenosis are needed.

In conclusion, plasma natriuretic peptide levels are elevated in symptomatic patients with aortic stenosis after adjustment for echocardiographic measures of aortic stenosis severity and LV function. These data suggest that measurement of natriuretic peptides is likely to complement clinical and echocardiographic evaluation of patients with aortic stenosis. Although this study was not adequately powered to distinguish between the natriuretic peptides, N-BNP appeared to have the best

discriminatory value for the presence of symptoms of heart failure. A limitation of the current study is the cross sectional design without clinical outcomes. The following chapter describes a prospective longitudinal study of the measurement of N-BNP levels in asymptomatic patients with aortic stenosis to predict disease progression and the development of symptoms.

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3.2 Measurement of Amino-terminal B-type Natriuretic Peptide Plasma Levels in Asymptomatic Aortic Stenosis to Predict Symptomatic Deterioration

INTRODUCTION

Novel data presented in the preceding chapter demonstrated that plasma levels of N-BNP were higher in symptomatic compared to asymptomatic patients with aortic stenosis even after adjusting for echocardiographic measures of stenosis severity and LV function. Patients with plasma levels of N-BNP above the normal range were much more likely to be symptomatic, but some asymptomatic patients had elevated plasma levels of N-BNP. It is not known whether these patients have a higher risk of symptomatic deterioration during follow-up than asymptomatic patients with plasma levels of N-BNP in the normal range. The aim of this study was to determine whether higher plasma levels of N-BNP predict the development of symptoms during follow-up in initially asymptomatic patients with aortic stenosis.

METHODS

Study Design

Twenty-nine asymptomatic patients with a peak aortic jet velocity ≥ 2.5 m/s were enrolled in the study. At study entry, each patient underwent a comprehensive clinical assessment and echocardiogram and plasma was collected and stored frozen for later analysis of N-BNP. During follow-up, clinical assessment and collection of plasma for measurement of N-BNP was performed at 6 monthly intervals and an echocardiogram was performed at 12 monthly intervals or for presence of symptoms. The pre-specified end-point was the development of symptoms of heart failure (NYHA class \geq II) and/or angina and/or exertional presyncope/syncope

determined by the patients regular cardiologist who was blinded to the N-BNP levels but not to the results of the echocardiograms. A detailed description of the methods including clinical assessment, exclusion criteria, normal controls, echocardiography and measurement of N-BNP is described in chapter 2.

Statistical Analysis

To assess change in echocardiographic measurements and N-BNP during follow-up the difference between measurements at baseline and at the onset of symptoms, or at the final measurement for subjects who remained asymptomatic, were determined. Distribution properties of variables were assessed visually with box and whisker plots and with the Shapiro-Wilk statistical test. Statistical testing was with an unpaired t-test or Mann-Whitney U test for non-parametric data. Categorical comparisons were with Fisher's Exact test as 25% of the cells had expected counts less than 5. The association between baseline N-BNP and development of symptoms was analysed with logistic regression and the results are reported as odds ratio (OR) and 95% confidence intervals (CI).

RESULTS

Clinical Characteristics

The mean age was 65 ± 15 years. Fifteen (52%) patients were male. The cause of aortic stenosis was 'calcific degeneration' in 19 (66%) patients, bicuspid aortic valve in nine (31%) patients and rheumatic heart disease in one patient. The mean serum creatinine was 0.09 ± 0.02 mmol/L. During follow up for a mean of 18 [IQR 13 to 23] months 8 patients developed symptoms and 21 patients remained asymptomatic. Seven patients developed exertional dyspnea and fatigue (NYHA class II) and one patient developed angina. The median time to symptom onset was 17.2 [IQR 14 to

20] months. Symptomatic patients were more likely to be older and male, although these differences were not statistically significant (Table 3.2.1.). There was no significant difference in progression to symptoms by cause of aortic stenosis, blood pressure, creatinine or prescribed cardiac medications.

Echocardiographic Measurements and Outcome

Compared to patients who remained asymptomatic, patients who developed symptoms during follow-up had on average more severe aortic stenosis with a lower aortic valve area, higher peak aortic velocity and higher mean aortic gradient at baseline (Table 3.2.1.). These differences did not all reach conventional levels of statistical significance ($p < 0.05$). LV ejection fraction at baseline was similar for the two groups, and no patients had an ejection fraction $< 50\%$. The majority of patients had an impaired relaxation pattern of diastolic dysfunction. None of the 8 (28%) patients with normal diastolic function developed symptoms during follow-up (Table 3.2.1.). There was a trend ($p > 0.05$) for a greater decrease in aortic valve area and increase in peak aortic velocities during follow-up in patients who became symptomatic, but there was no significant change in LV ejection fraction (Table 3.2.2.).

N-BNP and Outcome

Patients with a plasma level of N-BNP above the normal range (> 50 pmol/L) at baseline were more likely to develop symptoms (6 of 11, 55%) than patients with levels of N-BNP within normal limits (2 of 18, 11%). Two patients with plasma levels of N-BNP < 50 pmol/L at baseline developed symptoms during follow-up. One patient developed angina with important coronary artery disease found on coronary angiography, but plasma levels of N-BNP remained within normal limits during follow-

up (Figure 3.2.1B). The other patient developed exertional dyspnea after 18 months follow-up associated with an increase in plasma N-BNP from 25pmol/L to 44 pmol/L 6 months before symptom onset, then to 114pmol/L at the final visit (Figure 3.2.1B). This patient had severe aortic stenosis with a peak aortic velocity of 5.0 m/s at baseline, which increased to 5.5 m/s at the final visit.

The odds ratio for symptomatic deterioration for the 11 subjects with a plasma level of N-BNP >50 pmol/L compared to the 18 with N-BNP in the normal range at baseline was 9.6 (95% CI 1.5 to 63.5), $p=0.02$. After adjusting for age, peak aortic velocity and ejection fraction at baseline the odds ratio for symptomatic deterioration for patients with a baseline N-BNP >50 pmol/L was 13 (95%CI 1 to 164), $p=0.05$. For comparison, the odds ratio for symptom onset for the 11 patients with a peak aortic velocity >4.2 m/s compared to those with a peak aortic jet velocity <4.2 m/s was 2.0 (95%CI 0.4 to 10), $p=0.41$.

Change in N-BNP during Follow-Up

Serial N-BNP levels at baseline and during follow-up are shown for patients who remained asymptomatic and for those who developed symptoms in Figures 3.2.1A and 3.2.1B respectively. Nine of 11 patients with baseline levels of N-BNP >50pmol/L had at least one follow-up measurement of N-BNP. In eight of these patients all follow-up measurements were also >50pmol/L (20/20 measurements). In one patient the plasma level of N-BNP varied more, but was >50pmol/L on 4 of 5 measurements (Figure 3.2.1A). This patient had labile blood pressure and required changes to anti-hypertensive medication during follow-up. Sixteen of 18 patients with N-BNP <50pmol/L at baseline had follow-up measurements of N-BNP. In 3 of these patients N-BNP increased above 50pmol/L during follow-up. One of these patients

became symptomatic. On average there was a greater increase in N-BNP levels in the patients who developed symptoms compared to patients who remained asymptomatic. The average rate of increase in plasma level of N-BNP/ year for patients who became symptomatic was +26.4 (95% CI 13.4 to 39.0) pmol/L and for asymptomatic patients +7.2 (95% CI 0.8 to 14.5) pmol/L. $p=0.014$.

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Table 3.2.1: Comparison of baseline measurements for patients who remained asymptomatic compared to patients who developed symptoms during follow-up

	Asymptomatic n=21	Symptomatic n=8	P value
Age, years	62 (15)	73 (12)	0.09
Male, number (%)	9 (43%)	6 (75%)	0.2
Echocardiography			
Aortic valve area, cm ²	1.06 (0.55)	0.89 (0.23)	0.2
Peak aortic jet velocity, m/s	3.8 (0.81)	4.4 (0.7)	0.07
Mean aortic gradient, mmHg	35 (15)	49 (17)	0.03
LV ejection fraction, %	66 (6)	64 (6)	0.4
Diastolic function			
Normal, n (%)	8 (38%)	0	
Impaired relaxation, n (%)	13 (62%)	6 (100%)	0.07
N-BNP at baseline			
Median [IQR], pmol/L	30 [16 to 49]	75 [41 to 98]	0.03
>50 pmol/L, n (%)	5 (24%)	6 (75%)	0.03

Numbers reported as mean (SD), or median and interquartile range [IQR], unless otherwise stated.

Table 3.2.2: Change in echocardiographic measures and N-BNP levels during follow-up for patients who remained asymptomatic compared to those who developed symptoms

	Asymptomatic n=21	Symptomatic n=8	P value
Echocardiography			
Aortic valve area, cm ²	0.03 (-0.09, 0.11)	-0.14 (-0.17, -0.03)	0.2
Peak aortic velocity, m/s	0.2 (-0.10, 0.35)	0.5 (-0.20, 0.90)	0.4
Mean aortic gradient, mmHg	2.5 (-1.0, 9)	15 (-5, 18)	0.3
LV ejection fraction, %	-1 (-2, 2)	0 (-2, 2)	0.9
N-BNP			
Median change, pmol/L (IQR)	13 (3, 19)	24 (22, 50)	0.02
>50 pmol/L at baseline, n	5	6	
Increase from < to >50 pmol/L, n	2	1	
Remained <50 pmol/L, n	14	1*	0.03

The difference between the baseline and the final measurement was calculated for each subject. Median and interquartile range [IQR] for each group are given.

*This subject developed angina with significant coronary artery disease on angiography

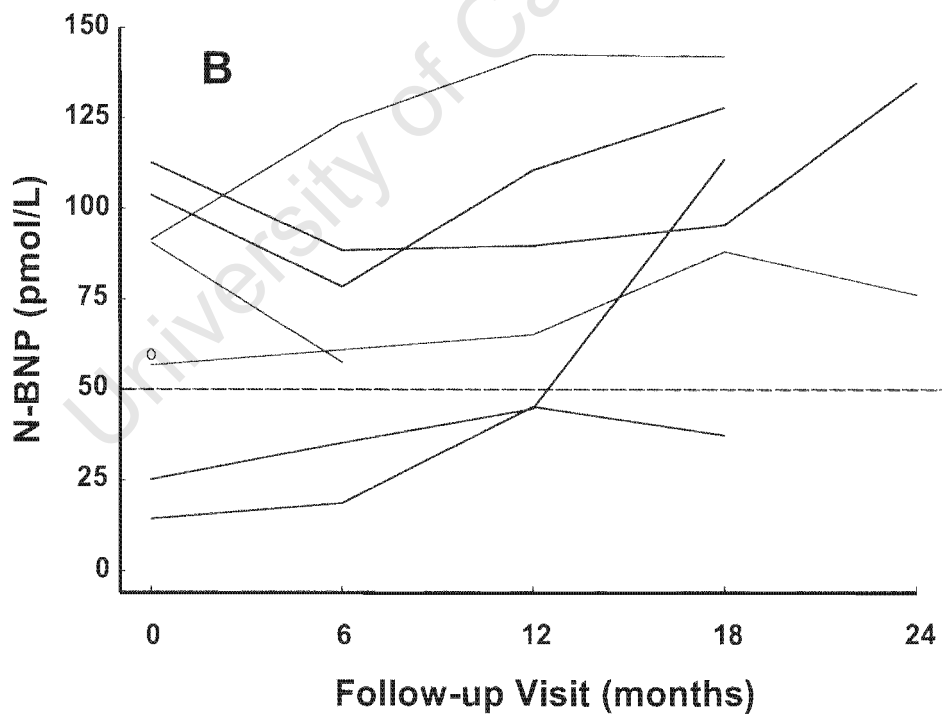
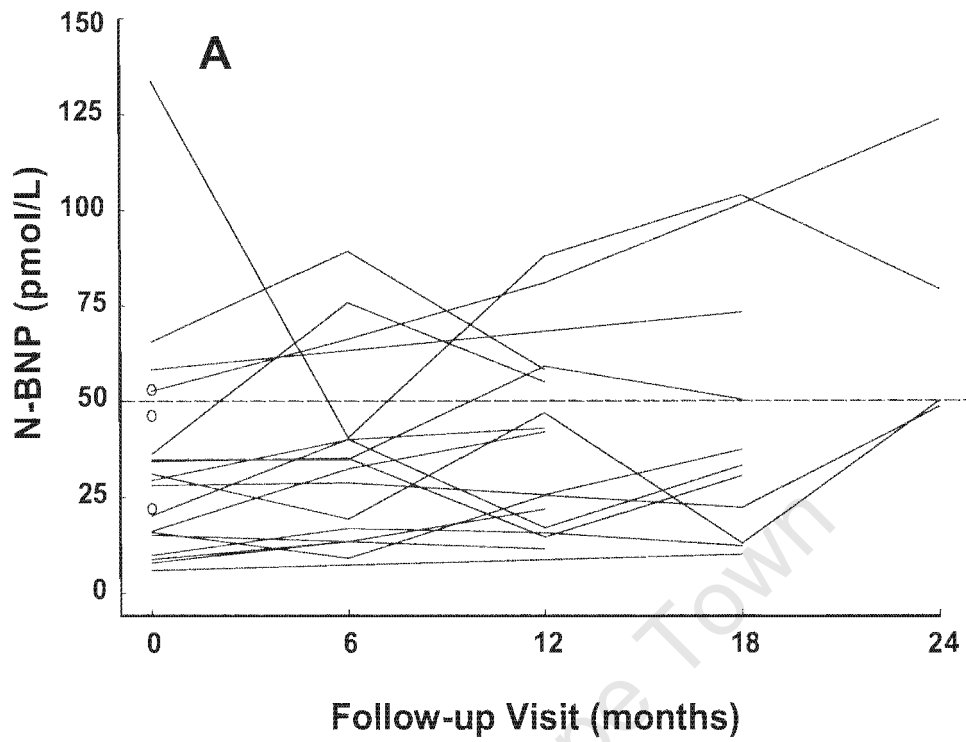


Figure 3.2.1: Serial N-BNP levels in patients who remained asymptomatic at follow-up (A) and patients who developed symptoms at follow-up (B).

DISCUSSION

The current study demonstrated that asymptomatic patients with aortic stenosis and plasma levels of N-BNP above the normal range have a much higher risk of developing symptoms of dyspnea and fatigue during follow-up than patients whose plasma level of N-BNP is within the normal range. During a median follow-up of 18 months about half of patients with a plasma level of N-BNP above the normal range became symptomatic. This implies that in many patients there is a time lag of one or more years between when N-BNP increases above the normal range and when symptoms occur. It is likely that with longer follow-up a greater proportion of patients with raised plasma level of N-BNP would become symptomatic.

Serial measurements of N-BNP may be important because of the potential for levels to increase over time in some patients. In this study N-BNP increased from the normal range to above 50pmol/L in 3 (17%) patients during follow-up. Although the study was too small to reliably assess the value of serial measurements of N-BNP to monitor disease progression, one patient with a low N-BNP at baseline who subsequently developed symptoms of heart failure provides useful information. The plasma level of N-BNP had nearly doubled from baseline to 6 months before symptom onset, but was still within the normal range (44pmol/L). This suggests a clear increase in N-BNP within the normal range during follow-up may predict future onset of symptoms. This patient had very severe aortic stenosis with a peak aortic velocity at the final visit of 5.5 m/s. It is possible the time between N-BNP increase and symptom onset is shorter when aortic stenosis is very severe or when stenosis severity progresses rapidly. Larger studies with a longer duration of follow-up are needed to evaluate this question. The current study does however provide evidence that N-BNP is relatively stable during follow-up. An increase in plasma levels of N-

BNP could occur due to conditions other than aortic stenosis such as acute myocardial infarction⁸⁹ or change in medications such as angiotensin converting enzyme inhibitors or diuretics.¹⁰⁷ These possibilities need to be considered in individual cases.

Previous studies have attempted to identify factors which may predict clinical outcome in aortic stenosis.^{172,173,207} In a landmark study, Otto et al.²⁶ demonstrated that baseline peak aortic velocity was a good predictor of subsequent referral for aortic valve replacement or death. Peak aortic velocity also predicted outcome in the current study but not as strongly as N-BNP. A limitation of relying on peak aortic velocity alone is the wide individual variation in the severity of aortic stenosis associated with the onset of symptoms. Previous studies suggest aortic valve calcification, which was not assessed in the current study, is an independent predictor of outcome.²⁰⁸ It is possible aortic valve calcification predicts greater progression of stenosis severity, while increased N-BNP predicts LV decompensation which may occur over time even without further progression of stenosis severity. Exercise testing has been proposed as a method of identifying patients with aortic stenosis who are at increased risk²⁵ but was not performed in this study.

The lack of a close relation between hemodynamic severity of aortic stenosis and symptoms emphasizes the importance of obtaining an accurate history to determine the patient's symptom status. Most patients with aortic stenosis present with decreased exercise tolerance and milder symptoms of heart failure earlier in the course of the disease rather than classic end-stage symptoms of heart failure, angina and syncope.²⁶ The development of symptoms may be insidious and both patients and doctors may find it difficult to determine whether symptoms are due to aortic stenosis. Measurement of plasma N-BNP levels, which increases with symptoms of

heart failure, may be particularly useful when evaluation of symptoms is difficult.²⁰⁹ While only one patient in the current study developed angina, it is noteworthy that the N-BNP level remained within normal limits. Compared with the development of symptoms of heart failure, angina has a different pathophysiology¹⁹⁸ and may not be associated with increased N-BNP levels.²⁰⁹

Because the median duration of follow-up was only 18 months it is likely some asymptomatic patients with elevated plasma levels of N-BNP would become symptomatic with further follow-up. This limitation would underestimate the predictive value of an elevated N-BNP level in current analysis. Plasma levels of N-BNP increased from below to above 50 pmol/L in only 3 subjects during follow-up. A larger study is needed to determine how frequently N-BNP should be measured during follow-up, how to combine interpretation of N-BNP with clinical and echocardiographic information, and whether an elevated plasma level of N-BNP should be an indication for surgery in asymptomatic patients with severe aortic stenosis. Further studies are required to determine whether measurement of other natriuretic peptides alone or in combination, such as BNP, ANP, and N-ANP, provide similar results. Further study is needed to determine whether changes in plasma levels of N-BNP within the normal range are predictive, and whether higher levels of N-BNP predict earlier symptomatic deterioration. Results from the current study provide strong evidence to support the rationale for measuring N-BNP to plan follow-up of asymptomatic patients, and for undertaking further larger prospective studies.

In conclusion, in this study patients with aortic stenosis who have higher plasma levels of N-BNP were much more likely to develop symptoms of heart failure during follow-up than patients with plasma levels of N-BNP in the normal range. This suggests asymptomatic patients with plasma levels of N-BNP above the normal

range should be followed more closely for onset of symptoms. The following chapter describes the effect of aortic valve replacement on N-BNP levels and the association with symptoms.

University of Cape Town

3.3 Plasma Levels of Amino-terminal B-type Natriuretic Peptide Before and After Aortic Valve Replacement for Severe Aortic Stenosis

INTRODUCTION

While some clinicians recommend AVR for asymptomatic patients with severe aortic stenosis due to the risk of sudden death or an inferior late post-AVR outcome,^{210,211} this approach may expose many patients earlier to the risks of AVR and the potential complications of a prosthetic valve.¹² Although there is uniform agreement that AVR should not be delayed after the development of definite symptoms, the symptoms of fatigue and breathlessness may not be due to the aortic stenosis. The study presented in Chapter 3.1 found that in patients with severe aortic stenosis N-BNP plasma levels had a greater diagnostic accuracy for the presence of symptoms of heart failure than echocardiographic measures of aortic stenosis severity and LV function. Patients with plasma levels of N-BNP above the normal range were much more likely to be symptomatic, but some symptomatic patients had plasma levels of N-BNP within the normal range. It is not known whether these patients derive a similar improvement of symptoms as symptomatic patients with elevated N-BNP levels. The aim of this study is to determine the effects of AVR on N-BNP plasma levels in patients with severe AS and the association with symptoms.

METHODS

Study Design

Thirty-seven consecutive patients with severe aortic stenosis scheduled for AVR at Green Lane Hospital were enrolled in the study. Four patients were excluded from the analysis for the following reasons: There were three deaths early post-AVR (two

due to septicaemia, and one due to a stroke), and one patient developed prosthetic valve thrombosis resulting in severe LV dysfunction. Patients underwent clinical evaluation and collection of plasma for measurement of N-BNP levels pre-AVR (median 7, interquartile range [IQR] 1-43 days pre-AVR), at hospital discharge (median 8 [IQR 7-10] days post-AVR), six weeks post-AVR (median 49 [IQR 44-54] days) and six months post-AVR (median 184 [IQR 175-197] days). Echocardiography was performed pre-AVR and 6 months post-AVR. A coronary angiogram was performed pre-AVR and a coronary artery stenosis $\geq 50\%$ was used to signify the presence of coronary artery disease. A detailed description of the methods including clinical assessment, exclusion criteria, normal controls, echocardiography and measurement of N-BNP is described in chapter 2. All patients had an electrocardiogram on the day of discharge from hospital. The following operative data were documented: The prosthetic valve size and type (bioprosthetic or mechanical), concomitant coronary artery bypass graft (CABG) surgery, number of bypass grafts, cross-clamp time, bypass time, Troponin T measured 36 hours post-AVR and post-AVR complications.

Statistical Analysis

Differences in echocardiographic characteristics over time were compared using a paired t-test except LV diastolic function which used the Sign test. Group comparisons were made using a t-test. Associations between N-BNP change and continuous variables were evaluated using Spearman correlation coefficients. A chi-square test was used to compare the surgery and pre and 6 months post-AVR symptom class proportions within pre-AVR N-BNP groups (≤ 50 pmol/L vs. >50 pmol/L).

RESULTS

Clinical and Operative Characteristics

Clinical and operative characteristics are shown in Table 3.3.1. The majority (30/33) of patients were symptomatic (NYHA class \geq II) and 20 patients had mild symptoms (NYHA class II). Seventeen patients had angina (13 had coronary artery disease) and 11 patients had exertional dizziness or syncope. A similar number of patients had a bicuspid and a 'degenerative calcific' cause for aortic stenosis. Most patients (76%) received a tissue aortic valve. The post-AVR troponin T level was elevated, mean 0.56 ± 40 mg/L but there were no q waves on any discharge electrocardiogram. Pre-discharge postoperative complications included new onset atrial fibrillation (n=8), transient ischaemic attack or stroke (n=2) complete heart block requiring a permanent pacemaker (n=1), and a pericardial effusion requiring pericardiocentesis for cardiac tamponade (n=2). Fewer patients were prescribed a loop diuretic after compared to before AVR (14 vs. 10 respectively), but more patients were prescribed an angiotensin converting enzyme inhibitor (6 vs. 11 respectively).

Change in N-BNP Plasma Levels After Aortic Valve Replacement

N-BNP levels before AVR were higher than the 33 age and gender-matched controls (median 89 [IQR 41-150] pmol/L vs. 18 [11-32] pmol/L) and increased further at hospital discharge (195 [110-271] pmol/L). Plasma N-BNP levels progressively decreased at 6 weeks post-AVR (80 [53-136] pmol/L) and 6 months post-AVR (75 [39-105] pmol/L), but remained significantly higher than control subjects at all times, $P < 0.0001$ (Figure 3.3.1). Patients who underwent combined AVR/CABG (n=14) were more likely to have a plasma level of N-BNP above 50 pmol/L (12/14) than patients having AVR alone (n=19), (10/19), $P = 0.067$.

Predictors of N-BNP Levels Six Months After Aortic Valve Replacement

N-BNP levels 6 months after AVR was similar for patients who received a tissue valve (n=25) and a mechanical valve (n=8), p=0.54. There was no significant correlation between the change in N-BNP level after AVR and valve size, (correlation coefficient, $r = -0.12$, $p=0.89$), cross clamp time ($r = -0.31$, $p=0.08$) or bypass time ($r = -0.26$, $p=0.14$). Increase in N-BNP after AVR was associated with post-AVR troponin T level ($r = -0.43$, $p=0.02$).

Echocardiographic Measures and N-BNP Levels Six Months After Aortic Valve Replacement By Pre-Aortic Valve Replacement N-BNP Level

Echocardiographic measures and N-BNP levels before and 6 months after AVR by pre-AVR N-BNP level within normal limits (≤ 50 pmol/L) and above the upper limit of normal (> 50 pmol/L) are shown in Table 3.3.2 and Figure 3.3.2. The aortic valve gradients decreased in both groups. Measures of diastolic function did not change significantly at 6 months for both groups. On average, LV fractional shortening increased and LV end-systolic dimension decreased 6 months after AVR in patients with an N-BNP level above the normal range before AVR. In patients with plasma levels of N-BNP within the normal range these measures of LV function did not change 6 months after AVR. N-BNP levels significantly decreased in patients with a pre-AVR N-BNP level above the upper limit of normal but did not decrease in patients with a pre-AVR N-BNP level within normal limits.

Symptoms Before and After Aortic Valve Replacement By Pre-Aortic Valve Replacement N-BNP Level (Table 3.3.3)

Pre-AVR N-BNP levels increased with the pre-AVR NYHA class, class I (n=3), median 33 [IQR 12, 53] pmol/L; class II (n=20), 84 [39, 145] pmol/L; class III/IV

(n=10), 147 [85, 298]; p=0.049. Of the three patients in class I pre-AVR, 2 had N-BNP levels within normal limits (11 pmol/L and 33 pmol/L respectively) and 1 patient had an N-BNP level of 53 pmol/L. At 6 months post-AVR, the N-BNP levels increased from 11 pmol/L to 37 pmol/L, 33 pmol/L to 60 pmol/L, and 53 pmol/L to 105 pmol/L, respectively. All 3 patients reported no change in symptoms. Three patients remained in NYHA class II and one patient changed from NYHA class II to class III by 6 months post-AVR. Of these 4 patients whose symptoms did not improve by 6 months post-AVR, 3 had a pre-AVR N-BNP level \leq 50 pmol/L. In contrast, 19/20 patients with a pre-AVR N-BNP level above normal limits reported improved symptoms by 6 months post-AVR. By 6 months post-AVR, all patients who had angina pre-AVR were free of angina while 1 patient had persistent exertional pre-syncope (pre-AVR N-BNP level = 33 pmol/L and 6 months post-AVR N-BNP level = 60 pmol/L).

Table 3.3.1: Clinical and operative characteristics of patients with severe aortic stenosis referred for aortic valve replacement

Male, n (%)	21 (64%)
Age, years	70 ± 9.1
NYHA class, n (%)	
I	3 (9%)
II	20 (61%)
III	9 (27%)
IV	1 (3%)
Systolic blood pressure, mmHg	141 ± 22
Sinus rhythm, n (%)	30 (91%)
Hypertension, n (%)	16 (48%)
Cause of aortic stenosis, n (%)	
Degenerative calcific	17 (52%)
Bicuspid	16 (48%)
Coronary artery bypass grafting, n (%)	
None	19 (58%)
One graft	8 (24%)
Two grafts	6 (18%)
Prosthetic valve	
*Tissue, n (%)	25 (76%)
Mechanical, n (%)	8 (24%)
Aortic cross-clamp time, min	73 [55, 95]
Cardiopulmonary bypass time, min	94 [69, 126]
Troponin T, mg/L	0.52 [0.30, 0.72]

Continuous variables are expressed as mean ± SD, or median and [interquartile range, IQR]

n=number of patients

NYHA = New York Heart Association

*Stentless (n=22), stented (n=3)

Table 3.3.2: Effects of aortic valve replacement on echocardiographic measures and N-BNP levels by preoperative N-BNP level.

	N-BNP ≤ 50 pmol/L (n=11)				N-BNP > 50 pmol/L (n=22)			
	Pre-AVR	6 months post-AVR	Change Mean (95% CI)	P	Pre-AVR	6 months post-AVR	Change Mean (95% CI)	P
Peak aortic velocity, m/s	4.4 ± 0.3	2.3 ± 0.3	-2.1 (-2.4, 1.8)	<0.001	4.7 ± 0.7	2.3 ± 0.4	-2.6 (-2.9, -2.2)	<0.001
Mean aortic gradient, mmHg	46 ± 6.2	12.7 ± 2.8	-33 (-37, -29)	<0.001	54 ± 17.5	12 ± 4.1	-46 (-53.7, -37.5)	<0.001
LV end systolic diameter, cm	2.8 ± 0.4	2.7 ± 0.2	0.03 (-0.35, 0.40)	0.846	3.6 ± 1.1	2.9 ± 0.8	-0.6 (-0.92, -0.20)	0.007
LV end diastolic diameter, cm	4.9 ± 0.6	4.5 ± 0.5	-0.53 (-1.28, 0.23)	0.112	4.9 ± 0.8	4.6 ± 0.6	-0.6 (-1.2, -0.01)	0.048
LV fractional shortening, %	43 ± 8.6	39 ± 5.5	-7 (-20, 6)	0.188	33 ± 9.9	36 ± 9.0	4.5 (1.61, 7.39)	0.006
LV mass index, g/m ²	176 ± 68	204 ± 67	-37 (-34, 48)	0.775	250 ± 57	199 ± 56	-31 (-62.9, 1.25)	0.056
LV Diastolic function								
Normal	0	1			2	3		
Impaired relaxation	9	8			9	6		
Pseudonormal	1	1		1	1	3		1
N-BNP, pmol/L	33 (22, 40)	37 (20, 45)		0.123	143 (89, 193)	102 (75, 113)		0.003

The upper limits of normal for N-BNP = 50 pmol/L. N-BNP levels expressed as median (interquartile range).

AVR = Aortic valve replacement, LV = Left ventricular.

Table 3.3.3: Symptoms by pre-aortic valve replacement N-BNP level

	N-BNP ≤ 50pmol/L	N-BNP > 50pmol/L	P value
Number of patients	11	22	
Pre AVR symptom class			
NYHA class I	2	1	
NYHA class II	8	12	
NYHA class III or IV	1	9	0.11
6 months post AVR symptoms			
Remained asymptomatic	2	1	
Symptoms improved	5	20	
Symptoms not improved	3	1	0.034

NYHA = New York Heart Association

AVR = Aortic valve replacement

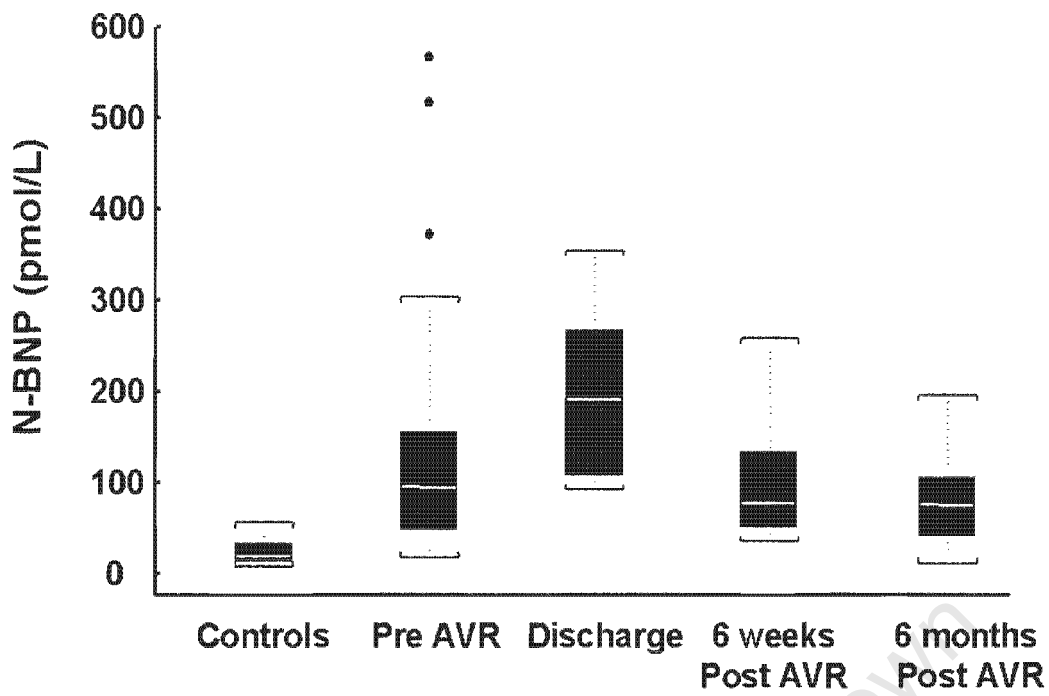


Figure 3.3.1: Box and whisker plot of N-BNP levels before and after aortic valve replacement (AVR). $P < 0.0001$ for N-BNP compared with controls at all time points. $P = 0.03$ for N-BNP 6 months post-AVR compared with N-BNP pre-AVR.

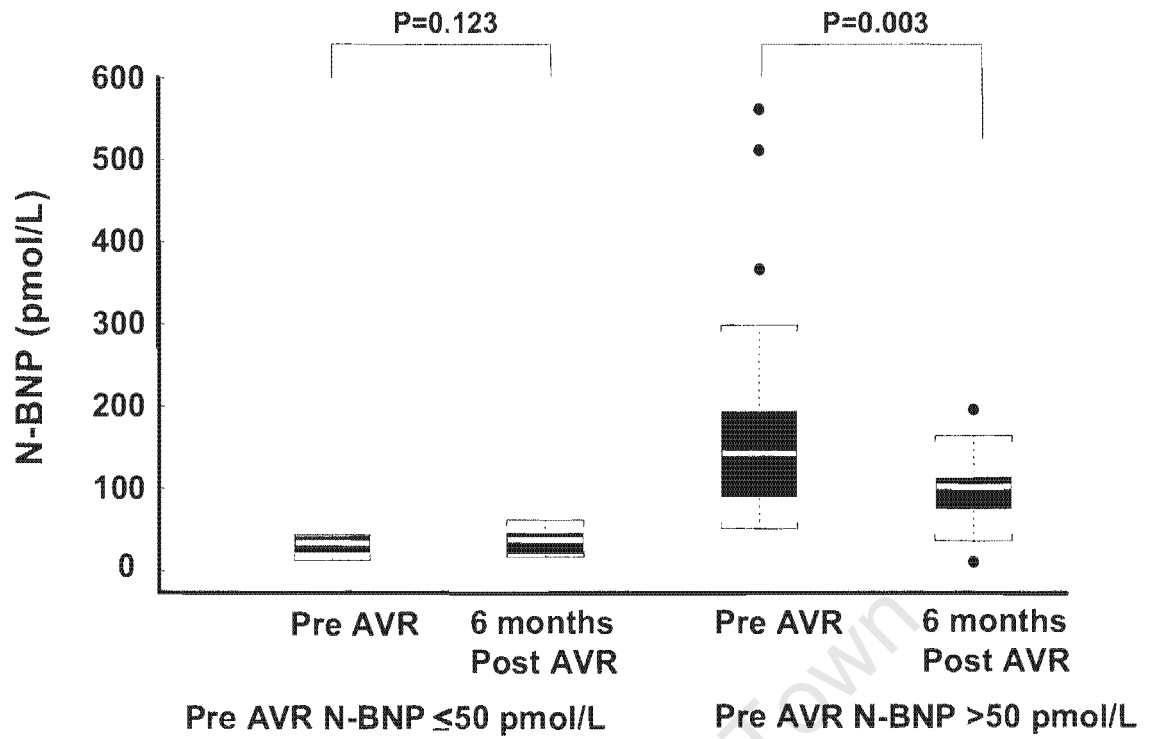


Figure 3.3.2: N-BNP levels before and 6 months after aortic valve replacement (AVR) by pre-AVR N-BNP plasma level.

DISCUSSION

Some cardiologists recommend AVR for asymptomatic patients with severe aortic stenosis to avoid the potential risks of sudden death and irreversible LV dysfunction.²¹¹⁻²¹³ However, while asymptomatic patients with severe aortic stenosis will ultimately require AVR for prognostic benefit, the operative mortality risk is at least 2-3 %, ^{197,214} may be as high as 10% in the elderly²¹⁵ and is even higher when combined with CABG.²¹⁶ A further consideration is the chronic increased risk of prosthetic valve complications and warfarin therapy.²¹⁵ A major limitation of relying on the patient reporting the development of symptoms is that the symptoms of fatigue and breathlessness may not be due to the aortic stenosis, but rather due to other causes such as respiratory disease or deconditioning, especially in the elderly.²¹⁶

In the current study, despite similar improvements in aortic valve gradients, by 6 months post-AVR N-BNP levels significantly decreased and symptoms improved in patients with a preoperative N-BNP level above the upper limit of normal, but N-BNP levels did not decrease and symptoms less reliably improved in patients with a preoperative N-BNP level within normal limits. A previous study that examined the effects of AVR for severe aortic stenosis on plasma levels of N-BNP demonstrated that only patients with an elevated preoperative pulmonary capillary wedge pressure, which correlated strongly with the preoperative N-BNP level, had decreased N-BNP levels at 12 months post-AVR.¹⁶² However, patient symptoms were not reported. Taken together with the results of the current study, these data suggest that LV decompensation associated with increased left atrial pressure may be the stimulus for N-BNP release in symptomatic patients with severe aortic stenosis, whereas compensated LV function associated with normal left atrial pressure is associated

with N-BNP levels within normal limits. Although exercise stress testing has been proposed as a more objective measure of exercise tolerance and hemodynamic response to exercise,^{25,217,218} it is contraindicated in patients who report symptoms of heart failure and therefore was not performed in this study.

Previous studies have shown an increase in natriuretic peptide levels after cardiac bypass surgery.¹²⁰ Consistent with these data, in the current study N-BNP levels increased approximately two-fold by one-week post-AVR, but the increase correlated weakly with the cross-clamp time, bypass time, type and size of the prosthetic valve and correlated modestly with the post-AVR troponin T level. N-BNP levels decreased progressively at 6 weeks post-AVR and at 6 months post-AVR suggesting the initial increase post-AVR may be transient. However, despite decreases in the aortic valve gradients, N-BNP levels remained higher than controls 6 months post-AVR. The persistent elevation of N-BNP levels may be due to adverse effects of cardiac bypass surgery as suggested by an elevation in troponin T,²¹⁹ stenosis of the prosthetic valve or incomplete LV remodelling.²²⁰ Although on average the post-AVR fractional shortening was within normal limits and LV diastolic function was predominantly normal or an impaired relaxation pattern, LV contractility may still have been impaired in the setting of longstanding aortic stenosis and left ventricular hypertrophy.²²⁰ Structural and histological abnormalities of the LV have been demonstrated up to 7 years in patients with aortic stenosis post-AVR. While N-BNP levels remained significantly elevated up to 6 months post-AVR, longer-term studies are needed to determine whether N-BNP levels continue to progressively decrease and whether pre-AVR N-BNP levels predict postoperative clinical outcomes.

In conclusion, on average N-BNP levels are increased in patients with aortic stenosis before valve replacement compared with controls and decrease but remained elevated 6 months after valve replacement despite favourable echocardiographic changes. N-BNP levels decreased by 6 months after aortic valve replacement in patients with elevated preoperative N-BNP levels, but did not decrease in patients with preoperative N-BNP levels within normal limits. While symptoms consistently improved in patients with elevated preoperative N-BNP levels, there was less reliable improvement of symptoms in patients with a preoperative N-BNP level within normal limits. Further larger and longer-term follow-up studies are required to determine whether AVR may be safely deferred in apparently mildly symptomatic patients with severe aortic stenosis and an N-BNP level within normal limits.

CHAPTER 4

Natriuretic Peptides in Aortic Regurgitation

- 4.1 Associations Between Natriuretic Peptide Plasma Levels, Left Ventricular Function and Symptoms in Aortic Regurgitation 94

University of Cape Town

4.1 Associations Between Natriuretic Peptide Levels, Left Ventricular Function and Symptoms in Aortic Regurgitation

INTRODUCTION

The natural history of chronic aortic regurgitation is characterized by a prolonged asymptomatic phase.^{21,221-223} Initially, the LV responds to the increased volume and pressure load with compensatory dilatation and eccentric hypertrophy, but without an increase in filling pressures.²⁸ During this time ejection phase indices such as the ejection fraction remain in the normal range, but there may be progressive impairment of intrinsic myocardial contractility.^{23,224,225} Current AHA/ACC guidelines for aortic valve replacement are based on measures of LV size, LV ejection fraction, and cardiac symptoms,¹² but the optimal timing of surgical intervention remains controversial.²²⁶⁻²²⁸ The clinical challenge is to identify the onset of LV systolic dysfunction early, as postoperative mortality may be increased once patients with severe LV dilatation develop symptoms and/or LV systolic dysfunction.²²⁹⁻²³¹

To date, there is little information available on the response of plasma natriuretic peptides to chronic aortic regurgitation.²³² In this study, natriuretic peptide plasma levels in patients with chronic moderate-severe aortic regurgitation were determined as potential markers of LV systolic function and the presence of cardiac symptoms.

METHODS

Study Design

Forty consecutive eligible patients with echocardiographic evidence of chronic moderate-severe aortic regurgitation were enrolled in the study. Patients were considered to have at least moderate aortic regurgitation if they met all three of the following criteria: height of the regurgitant jet >50% of the LV outflow tract from the parasternal long-axis view, LV end-diastolic diameter >5.5 cm and pandiastolic flow reversal in the descending thoracic aorta.²³³ A detailed description of the methods including clinical assessment, exclusion criteria, normal controls, echocardiography and measurements of ANP, BNP and N-BNP is described in chapter 2.

Statistical Analysis

Comparisons between groups for continuous variables were made using the Student *t* test or one-way analyses of variance, and the chi-square test was used for categorical variables. Pearson's correlation coefficient was used to assess the association between natriuretic peptide levels and echocardiographic variables. Analysis of covariance was used to assess natriuretic peptide levels in symptomatic and asymptomatic patients after adjustment for age, gender and LV ejection fraction.

RESULTS

Of the 40 patients with aortic regurgitation, 31 were men and 9 were women. The mean age was 44 ± 17 years (range 18-76 years). The etiology of aortic regurgitation was rheumatic in 16 patients, bicuspid aortic valve in 13 patients, aortic root dilatation in 6 patients, and aortic valve leaflet prolapse in 5 patients. The cardiac rhythm was sinus rhythm in 38, atrial fibrillation in 1, and complete heart

block in 1(not paced). Coronary angiography was performed in 13 patients, and none had flow limiting (>50%) coronary artery stenoses.

Clinical Characteristics and Symptoms

Twenty-seven patients (68%) were asymptomatic (NYHA class 1) and 13 patients (32%) were symptomatic (NYHA class 2, n=10 and NYHA class 3, n=3). One patient had angina (NYHA class 2, normal coronary angiogram). On average, symptomatic patients were older (48 ± 18 years vs. 42 ± 17 years, $p=0.32$), and were more likely to be female (31% vs. 19%, $p=0.44$), but these differences were not statistically significant. Five patients had treated systemic hypertension, all of whom were asymptomatic. Three of the 5 patients with systemic hypertension had an increased left ventricular mass index, while 2 had a normal left ventricular mass index. Of note, all 5 patients with hypertension were treated and only 1 patient had a blood pressure of $> 140/90$ mmHg at the time of the study. Twenty-six patients were prescribed chronic (>6 months) vasodilator therapy, of whom 25 patients were prescribed angiotensin-converting-enzyme inhibitors. There were no significant differences between patients taking vasodilators and those not taking vasodilators with respect to clinical characteristics, symptoms, echocardiographic characteristics or natriuretic peptide levels.

Echocardiographic Measures and Symptoms

Echocardiographic measures for symptomatic and asymptomatic patients are shown in Table 4.1. Symptomatic patients had significantly higher LV systolic wall stress and lower ejection fraction than asymptomatic patients, and a trend towards greater LV volumes and LV diameters. The differences between the two groups were similar when LV measures were indexed to the body surface area.

Natriuretic Peptide Levels and Symptoms

Natriuretic peptide levels were significantly higher in symptomatic compared to asymptomatic patients (Table 4.1) and significantly higher in asymptomatic patients compared to normal controls (Figure 4.1). After adjustment for age, gender and ejection fraction the levels of each natriuretic peptide remained higher in symptomatic compared to asymptomatic patients (N-BNP, 2.4 times higher [95% CI 1.12-5.30], $p=0.027$; BNP, 1.6 times higher [95% CI 0.91-2.97], $p=0.096$; and ANP, 2.2 times higher [95% CI 1.15-4.29], $p=0.019$).

Natriuretic Peptide Levels and Echocardiographic Measures

Correlations between natriuretic peptide levels and echocardiographic measures of LV diameters, volumes, ejection fraction and systolic wall stress were weak (Table 4.2). The scatter plots of the correlations between Ln N-BNP and LV ejection fraction, LV meridional systolic wall stress and LV end-systolic diameter are shown in Figure 4.2. There were weak but significant correlations between the Ln- natriuretic peptides and the diastolic blood pressure (surrogate for left ventricular filling pressure) - correlation coefficients (r) for N-BNP ($r=0.4$), BNP ($r=0.4$) and ANP ($r=0.3$), $p=0.02$ for all.

Natriuretic Peptide Levels and Age

As in normal subjects, natriuretic peptide levels increased with age in patients with aortic regurgitation. In aortic regurgitation patients the correlation coefficients (r) for natriuretic peptides with age were $r=0.44$ for Ln BNP, $r=0.45$ for Ln N-BNP, and $r=0.42$ for Ln ANP ($p < 0.005$ for all).

Table 4.1: Echocardiographic measures and natriuretic peptide levels in asymptomatic and symptomatic patients with aortic regurgitation

Characteristic	Asymptomatic (n = 27)	Symptomatic (n = 13)	P Value*
LV ejection fraction (%)	58 ± 5 (50-66)	54 ± 6 (42-65)	0.03
LV systolic wall stress (g/cm ²)	86 ± 22 (56-127)	108 ± 27 (72-148)	0.03
LV end-systolic diameter (cm)	4.3 ± 0.6 (3.2-6.2)	4.7 ± 0.6 (3.8-5.5)	0.06
LV end-diastolic diameter (cm)	6.6 ± 0.8 (5.6-8.6)	6.9 ± 0.8 (6.0-8.1)	0.23
LV end-systolic volume (ml)	94 ± 38 (47-190)	111 ± 38 (56-161)	0.25
LV end-diastolic volume (ml)	220 ± 67 (114-377)	252 ± 67 (110-375)	0.25
LV mass (g)	276 ± 79 (152-440)	270 ± 75 (164-409)	0.87
Left atrial diameter (cm)	4.2 ± 0.5 (3.2-5.2)	4.4 ± 1.1 (2.9-6.7)	0.72
BNP (pmol/L)	7 (5-13)	12 (7-25)	0.02
N-BNP (pmol/L)	17 (8-34)	44 (23-104)	0.002
ANP (pmol/L)	13 (9-18)	31 (13-57)	0.013

Echocardiographic measures are presented as mean ± SD (range).

Natriuretic peptides expressed as median (interquartile range).

For normal controls, BNP 5 (3-6) pmol/L, N-BNP 9 (6-16) pmol/L, and ANP 6 (4-11) pmol/L, p<0.05 for all natriuretic peptides in controls compared with asymptomatic patients.

LV = Left ventricular

Table 4.2: Correlations between natriuretic peptide levels and echocardiographic measures

Echocardiographic measures	Ln ANP r Value	Ln BNP r Value	Ln N-BNP r Value
LV ejection fraction (%)	-0.18	-0.34*	-0.33*
LV end-systolic wall stress (g/cm ²)	0.26	0.04	0.14
LV end-systolic diameter (cm)	0.01	0.19	0.18
LV end-diastolic diameter (cm)	0.06	0.11	0.10
LV end-systolic volume (ml)	0.01	0.20	0.13
LV end-diastolic volume (ml)	0.13	0.26	0.20
LV mass (g)	0.06	0.26	0.16
Left atrial diameter (cm)	0.07	0.21	0.20

*P=0.04. P>0.05 for all other correlations.

Ln = natural logarithm; r value = correlation coefficient. Lv = Left ventricular

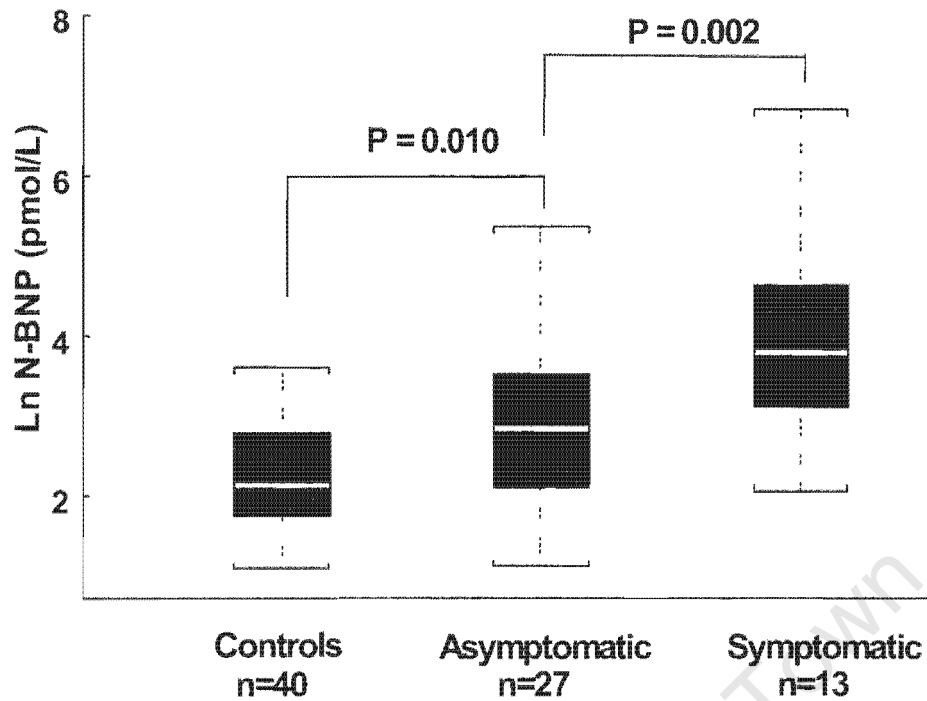


Figure 4.1: Box and whisker plots of Ln N-BNP in controls, asymptomatic patients and symptomatic patients with aortic regurgitation.

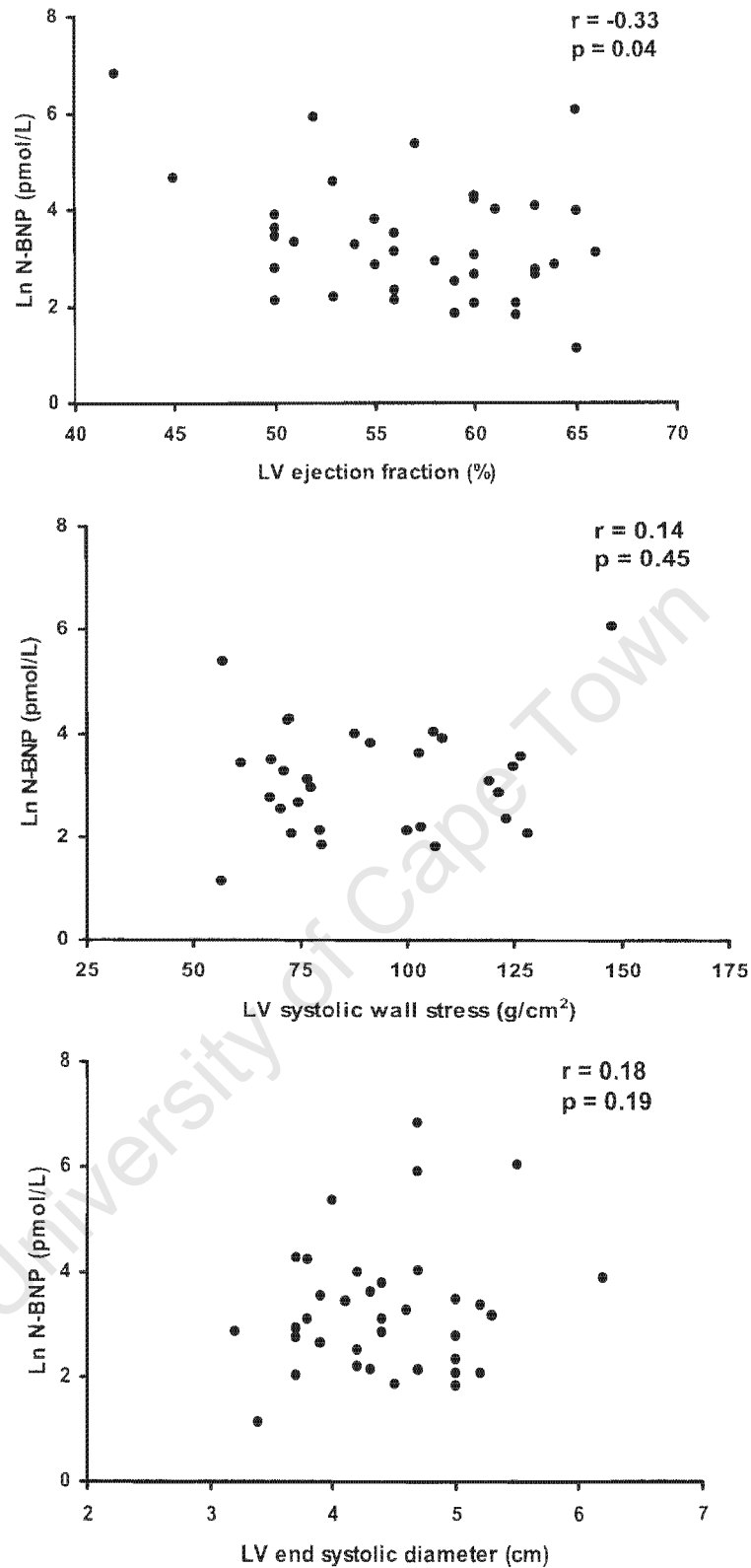


Figure 4.1.2: Correlations between Ln N-BNP levels and the left ventricular (LV) ejection fraction (*top panel*), LV systolic meridional wall stress (*middle panel*), and LV end-systolic diameter (*bottom panel*).

DISCUSSION

When determining the optimal timing of surgical treatment for aortic regurgitation, the following need to be considered: First, patients with chronic aortic regurgitation usually do not become symptomatic until after the development of LV dysfunction, and second, surgical treatment does not always restore normal LV function. The clinical challenge is to identify the time point just before the onset of irreversible LV systolic function in the asymptomatic patient with chronic aortic regurgitation.^{28,234} In patients with severe aortic regurgitation and a normal LV ejection fraction, the outcome is worse in symptomatic than in asymptomatic patients.²³¹ With improvement in surgical techniques aortic valve replacement has been performed progressively earlier in the natural history of the disease. However, aortic valve replacement remains controversial for asymptomatic patients with a normal ejection fraction.²³⁴ There is a need for a marker that reflects the onset of LV impairment before an abnormal ejection fraction.

In this study, natriuretic peptide levels were increased in asymptomatic patients with chronic, moderate-severe aortic regurgitation and were higher in symptomatic than in asymptomatic patients, even after adjustment for age, gender and ejection fraction. However, the associations between natriuretic peptide levels and echocardiographic measurements of LV size, systolic function and wall stress were weak. In previous studies of patients with ischemic heart disease or cardiomyopathy and low LV ejection fractions, an increased LV volume has been associated with increased plasma natriuretic peptide levels.^{51,171} In contrast, in the current study, which predominantly included asymptomatic patients with an ejection fraction of >50%, many patients had natriuretic peptide levels similar to those seen in age- and gender-matched controls despite increased LV volumes. This suggests that an

increased LV volume is not the major stimulus for release of natriuretic peptides. These findings support those presented in chapter 3 on the natriuretic peptides in aortic stenosis that increased LV pressure alone is not the major stimulus for the release of natriuretic peptides. Rather, the major stimulus for natriuretic peptide release in patients with aortic valve disease appears to be due to LV decompensation associated with the development of symptoms of heart failure even when the LV ejection fraction is within the normal range.

At present serial clinical evaluation and echocardiography form the cornerstone of clinical decision-making for patients with chronic aortic regurgitation. Although several studies have shown that echocardiographic measures of LV size and systolic function predict symptom onset, there is wide individual variability.^{222,223} Some patients (e.g. women, patients with hypertension, and patients with ischaemic heart disease) develop symptoms or LV systolic dysfunction with less marked LV dilatation.^{12,235} In the current study, the 13 symptomatic patients who underwent aortic valve replacement had on average a smaller LV dimension and a higher ejection fraction than that specified in the current AHA/ACC guidelines.¹² Therefore, natriuretic peptide levels may provide additional information to echocardiography for the evaluation of LV function in aortic regurgitation. A change in LV ejection fraction from rest to exercise has been shown to be predictive for clinical outcome in asymptomatic patients with a normal ejection fraction.²¹ There is a need for further studies comparing the clinical value of exercise testing and natriuretic peptide levels in apparently asymptomatic patients with aortic regurgitation.

In conclusion, this study has demonstrated that in patients with chronic, moderate to severe aortic regurgitation, natriuretic peptide levels are higher in symptomatic

patients than in asymptomatic patients even after adjustment for age, gender and ejection fraction, but the associations between the natriuretic peptides and LV diameters, volumes, wall stress and ejection fraction are weak. These observations suggest natriuretic peptides increase with the transition from the compensated phase to the decompensated phase in patients with chronic aortic regurgitation, a transition that may not be reliably detected by standard echocardiographic measures of LV function.

Further studies are required to determine whether natriuretic peptide levels measured prior to aortic valve replacement predict postoperative LV function and clinical outcome independent of echocardiographic measures of LV function, and whether serial measurement of natriuretic peptide levels in asymptomatic patients predict disease progression and the development of symptoms. These studies have commenced as part of the New Zealand Heart Valve Study (discussed in Chapter 7) and include many patients enrolled in the current study.

CHAPTER 5

Natriuretic Peptides in Mitral Regurgitation

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University of Cape Town

5.1 Association Between Natriuretic Peptide Plasma Levels, Symptoms and Severity of Mitral Regurgitation

INTRODUCTION

Echocardiography is the standard method used to evaluate the severity and cause of mitral regurgitation, and to assess LV systolic function.²³⁶ However, it may be difficult to obtain an accurate quantitative assessment of the severity of regurgitation.^{190,237,238} Quantitative estimates of severity based on measurement of the regurgitant fraction and the regurgitant orifice area are technically demanding. LV function may also be difficult to evaluate in patients with severe mitral regurgitation because the LV ejection fraction can be maintained in the presence of LV dysfunction.²³⁹ The AHA/ACC guidelines recommend surgery for severe mitral regurgitation if symptoms occur or there is evidence of asymptomatic LV dysfunction, defined as a LV end-systolic dimension of >45 mm or an ejection fraction of <60%.¹² However, difficulties in detecting early LV dysfunction, accurately assessing the severity of regurgitation, or recognizing early cardiac symptoms can make it difficult to determine the optimal timing of mitral valve surgery.²⁴⁰

In this study, natriuretic peptide levels were measured in patients with mitral regurgitation as potential markers of the severity of regurgitation, LV dysfunction, and the presence of symptoms.

METHODS

Study Design

Forty-nine consecutive eligible patients with mitral regurgitation and LV ejection fractions of >55% on the screening echocardiogram were enrolled in the study. A detailed description of the methods including clinical assessment, exclusion criteria, normal controls, echocardiography and measurements of ANP, BNP and N-BNP is described in chapter 2. Clinical outcomes including mitral valve surgery, death and hospital admission for heart failure were determined for a median of 16 months following the baseline assessment.

Statistical Analysis

The Student's t-test was used to compare continuous variables between groups. Fisher's exact test was used to compare categorical variables, as more than 25% of the classes had frequencies < 5. Normality was checked for each variable using box and whisker and probability plots and the Shapiro-Wilk normality test. Pearson's correlation coefficient was used to assess the association between the natural logarithm of natriuretic peptide levels and echocardiographic variables. General linear models were used to assess natriuretic peptide levels in symptomatic and asymptomatic patients with adjustment for measures of the severity of mitral regurgitation, LV function, or age and sex (where stated). Ninety-five percent confidence intervals for the ratio of the geometric means between the groups (symptomatic/asymptomatic) are reported for each peptide. The areas under the receiver-operator characteristic (ROC) curves were used to evaluate the diagnostic performance of natriuretic peptide and echocardiographic measurements for the presence of symptoms.

RESULTS

Patient Characteristics

Of the 49 patients with mitral regurgitation, 25 were male and 24 female. The mean age was 55 ± 19 years. There were 100 normal controls, of whom 41 were male and 59 female (mean age 55 ± 11 years). The etiology of mitral regurgitation was rheumatic in 15 patients (4 of whom had mild mitral stenosis [mitral valve area >1.5 cm²]), mitral valve prolapse in 33 patients, and post-endocarditis in one patient. Thirty-three patients were asymptomatic and 16 were symptomatic. The clinical characteristics and echocardiographic measurements of asymptomatic and symptomatic patients are compared in Table 5.1. Symptomatic patients were more likely to be in atrial fibrillation. On average, symptomatic patients had more severe mitral regurgitation as measured by the regurgitant fraction, vena contracta width and mitral regurgitation score. They also had larger left atrial dimensions and a trend towards larger LV end-diastolic and end-systolic dimensions that did not reach statistical significance. The LV ejection fractions were similar in asymptomatic and symptomatic patients, and there were no significant differences in age, sex, smoking, history of hypertension, measured blood pressure, diabetes mellitus, body surface area, or serum creatinine levels.

Relation Between Natriuretic Peptides and Echocardiographic Measures

Correlation coefficients for the associations between each of the natriuretic peptides and echocardiographic measures of the severity of mitral regurgitation, left atrial dimension, and LV size and function are presented in Table 5.2. The plasma levels of BNP, N-BNP and ANP rose with increasing severity of mitral regurgitation and with increasing left atrial dimensions. The correlation coefficients were similar for

BNP, N-BNP and ANP. There was no statistically significant correlation between any of the natriuretic peptides and the LV dimensions or ejection fraction.

Comparison of Asymptomatic and Symptomatic Patients

Table 5.3 shows the natriuretic peptide levels of the symptomatic and asymptomatic patients with mitral regurgitation and the control subjects. After adjustment for age and sex, the levels of each of the natriuretic peptides were on average higher in asymptomatic patients with mitral regurgitation than in normal controls ($p < 0.001$ for all), and higher in symptomatic than in asymptomatic patients with mitral regurgitation ($p < 0.001$ for all). The differences between symptomatic and asymptomatic patients with mitral regurgitation remained after adjustment was also made for the LV end-systolic dimension (BNP 2.06 [1.32-3.23] times higher, N-BNP 2.63 [1.46-4.75] times higher and ANP 2.11 [1.38-3.21] times higher), left atrial dimension (BNP 1.45 [0.95-2.22] times higher, N-BNP 1.57 [1.09-2.70] times higher and ANP 1.79 [1.15-2.81] times higher), mitral regurgitation score (BNP 1.9 [1.1-3.1] times higher, N-BNP 2.5 [1.3-4.9] times higher and ANP 1.8 [1.1-2.9] times higher), and vena contracta width (BNP 1.5 [1.0-2.4] times higher, N-BNP 2.0 [1.1-3.7] times higher and ANP 2.1 [1.3-3.3] times higher). The associations between BNP and the LV end-systolic dimension, vena contracta width and left atrial dimension in asymptomatic and symptomatic patients are shown in Figure 5.1.

The sensitivity, specificity and area under the receiver-operator characteristic curves for symptoms by natriuretic peptide levels and echocardiographic measures are shown in Table 5.4. An ROC curve showing the sensitivity and specificity of BNP, left atrial dimension, vena contracta width and LV end-systolic dimension for the presence of symptoms is shown in Figure 5.2. The area under the ROC curve was

higher for each of the natriuretic peptides than for the echocardiographic measures of the severity of regurgitation, left atrial size and LV dimensions.

Clinical Outcomes

After a median follow-up of 16 (range 6.2 to 23) months, 7 (44%) symptomatic patients had mitral valve surgery (BNP values at baseline: 11 pmol/L; 12 pmol/L; 21 pmol/L; 22 pmol/L; 23 pmol/L; 33 pmol/L and 52 pmol/L). One symptomatic patient had a normal exercise stress echocardiogram and was not referred for surgery (BNP 12 pmol/L). The other 8 symptomatic patients declined surgery or were not referred because of co-morbidity; two died from non-cardiac causes. Four (12%) asymptomatic patients underwent mitral valve surgery; in 3 there was no clinical or echocardiographic change before surgery performed for a flail leaflet (n=2, BNP 6 pmol/L and 7 pmol/L respectively) or co-existent coronary artery disease (n=1, BNP = 7 pmol/L). One asymptomatic patient developed worsening LV function and symptoms, and was referred for surgery after 20 months follow-up (BNP 4 pmol/L). Serial measurements of natriuretic peptides were not obtained during follow-up.

Table 5.1: Comparison of asymptomatic and symptomatic patients with mitral regurgitation

	Asymptomatic MR (n=33)	Symptomatic MR (n=16)	P
Clinical features, number of patients			
Mitral valve prolapse	23	10	
Rheumatic mitral valve disease	10	5	
Post-endocarditis	0	1	0.34 [†]
Loop diuretic	0	12	<0.0001
ACE inhibitor	6	10	0.003
Atrial fibrillation	2	6	0.01
Echocardiography			
Regurgitant fraction, %	40 ± 14	56 ± 16	0.001
Vena contracta width, mm	3.5 ± 1.0	4.7 ± 0.9	0.0002
MR score	1.4 ± 0.5	2.2 ± 0.4	0.0001
Left atrial dimension, cm	4.2 ± 0.6	5.1 ± 0.8	0.0001
Right ventricular systolic pressure, mm Hg	22 ± 7	32 ± 12	0.01
Left ventricular wall thickness, cm	0.97 ± 0.13	0.96 ± 0.20	0.90
Left ventricular end-systolic dimension, cm	3.1 ± 0.4	3.5 ± 0.8	0.15
Left ventricular end-diastolic dimension, cm	5.3 ± 0.6	5.7 ± 1.0	0.18
Left ventricular ejection fraction, %	63 ± 5	63 ± 6	0.99

Echocardiographic measures are expressed as mean ± SD

[†]Chi-square test used to calculate p-value.

Table 5.2: Correlations between natriuretic peptide levels, age, and echocardiographic measures of the severity of mitral regurgitation and left ventricular function

	Ln N-BNP r (P)	Ln BNP r (P)	Ln ANP r (P)
Age	0.50 (0.0003)	0.46 (0.0009)	0.38 (0.006)
Regurgitant fraction	0.41 (0.003)	0.43 (0.002)	0.40 (0.005)
Vena contracta width	0.54 (<0.0001)	0.60 (<0.0001)	0.37 (0.009)
MR score	0.51 (0.0002)	0.55 (<0.0001)	0.49 (0.0003)
Left atrial dimension	0.66 (<0.0001)	0.65 (<0.0001)	0.49 (0.0004)
Right ventricular systolic pressure	0.33 (0.09)	0.42 (0.03)	0.47 (0.01)
Left ventricular posterior wall thickness	-0.15 (0.30)	-0.13 (0.36)	-0.03 (0.86)
Left ventricular end-systolic dimension	0.16 (0.27)	0.20 (0.16)	0.16 (0.26)
Left ventricular end-diastolic dimension	0.17 (0.26)	0.19 (0.20)	0.13 (0.37)
Left ventricular ejection fraction	0.01 (0.92)	0.003 (0.98)	0.20 (0.17)

Ln = natural logarithm; r = correlation coefficient; P = p value.

Table 5.3: Clinical characteristics and plasma natriuretic peptide levels in normal controls and in asymptomatic and symptomatic patients with mitral regurgitation

	Normal Controls (n=100)	Asymptomatic MR (n=33)	Symptomatic MR (n=16)
Male sex, n(%)	41 (41%)	19 (58%)	6 (37%)
Age, years	55 ± 11	52 ± 18	59 ± 20
BNP, pmol/L*	5.3 (4.8-5.8)	7.1 (6.0-8.4) [†]	16.9 (13.3-21.4) [‡]
N-BNP, pmol/L*	12.3 (10.8 - 14.0)	28.3 (22.5-35.6) [§]	85.4 (61.3-119.0) [‡]
ANP, pmol/L*	9.3 (8.5-10.1)	12.4 (10.7-14.4) [†]	24.1 (19.5-29.9) [‡]

*Geometric mean (95% CI) adjusted for age and sex.

[†] $P < 0.003$ for comparison with normal controls.

[‡] $P < 0.0001$ for comparison with patients with asymptomatic mitral regurgitation.

[§] $P < 0.0001$ for comparison with normal controls.

Table 5.4: Sensitivity and specificity of natriuretic peptide levels and echocardiographic measures for symptoms in patients with mitral regurgitation

Variable	Cut Point	Sensitivity (%)	Specificity (%)	AUC
BNP	>12 pmol/L*	75	85	0.90
N-BNP	>50 pmol/L*	88	79	0.89
ANP	>19 pmol/L*	81	79	0.89
Left atrial dimension	>4 cm	88	30	0.81
MR score	>2	63	91	0.88
Vena contracta width	>4 mm	75	70	0.82
Regurgitant fraction	>50%	69	85	0.78
LV end-systolic dimension	>4.5 cm	13	100	0.63
LV end-diastolic dimension	>6 cm	31	88	0.62
LV ejection fraction	<60%	31	76	0.54

AUC indicates area under receiver-operator characteristic curve.

*Upper limit of normal range.

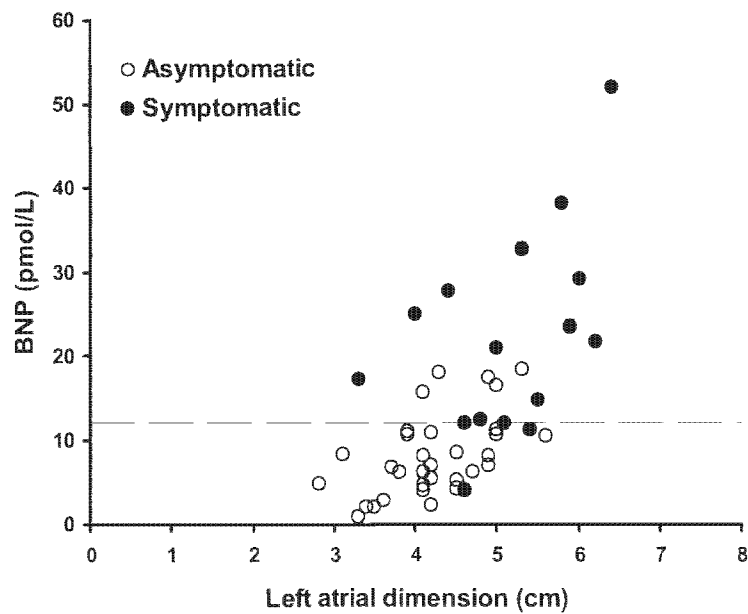
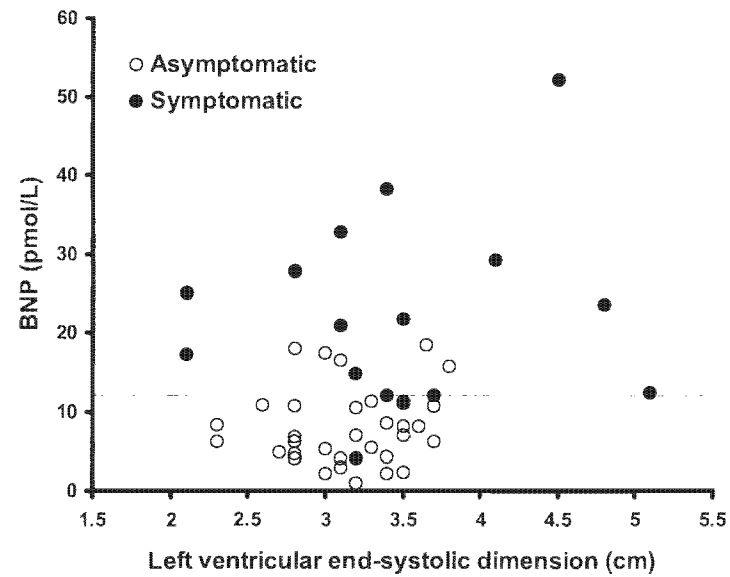
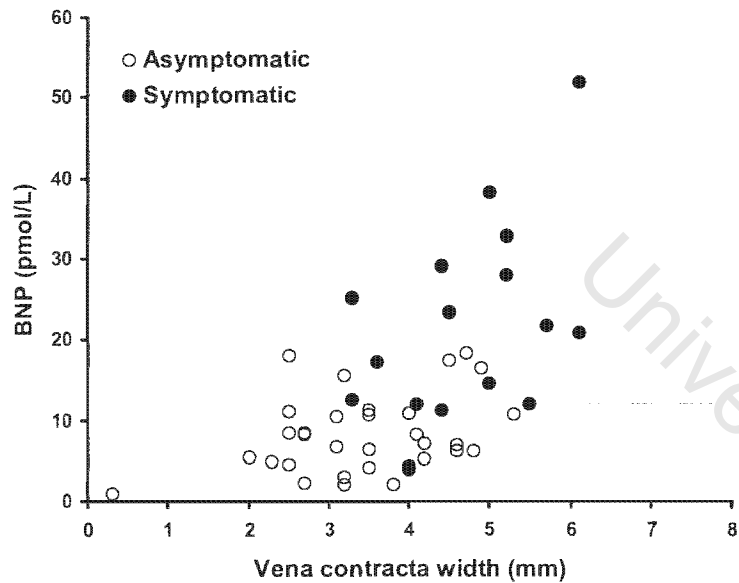


Figure 5.1: Associations between BNP levels and vena contracta width (*top left panel*), left ventricular end-systolic dimension (*top right panel*), and left atrial dimension (*bottom left panel*) in asymptomatic (**open circles**) and symptomatic (**closed circles**) patients with mitral regurgitation. The **dashed lines** indicate the upper limit of the normal laboratory reference range for BNP (12 pmol/L).

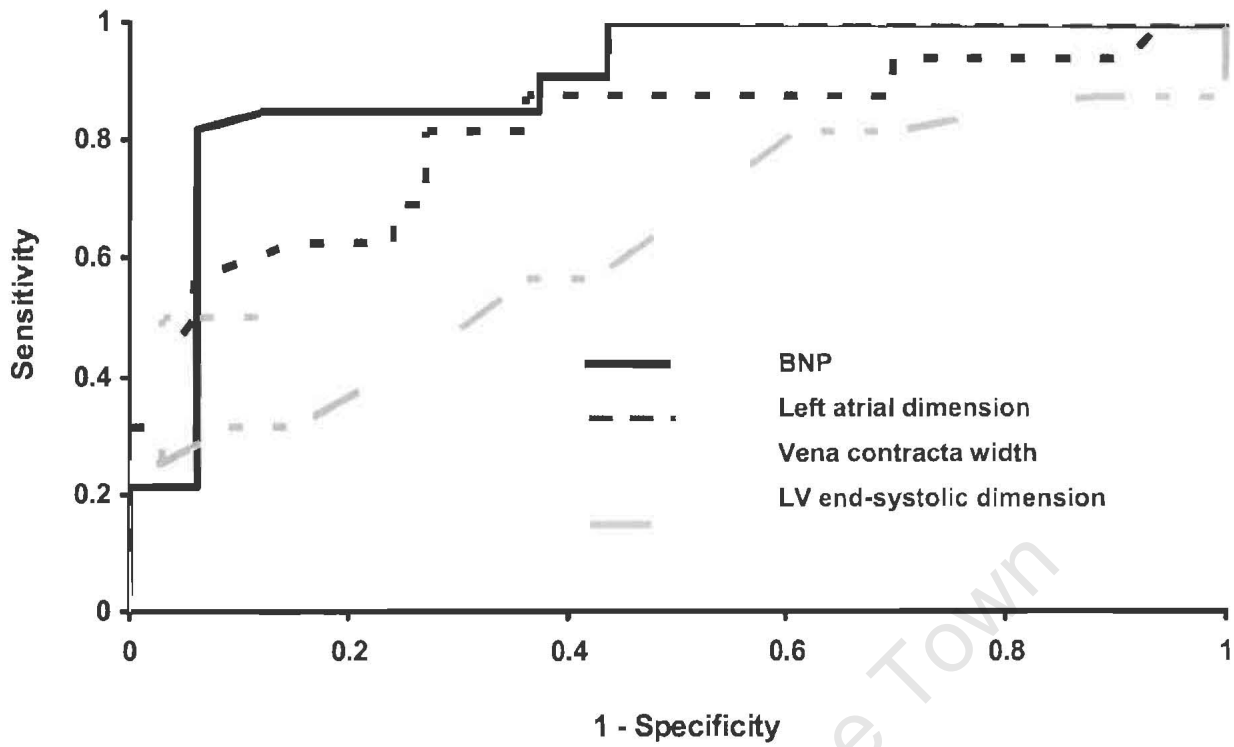


Figure 5.2: Sensitivity and specificity of BNP, left atrial dimension, vena contracta width and left ventricular (LV) end-systolic dimension for symptoms in patients with mitral regurgitation.

DISCUSSION

Obtaining an accurate quantitative assessment of the severity of mitral regurgitation is technically demanding.^{190,237,238} For this reason a qualitative assessment of severity based on several echocardiographic measures is often used in clinical practice. In this study the natriuretic peptides BNP, N-BNP and ANP increased with increasing mitral regurgitation assessed by three echocardiographic measures, viz. the width of the vena contracta,¹⁸⁹ the regurgitant fraction,¹⁸⁸ and the mitral regurgitation score.¹⁹⁰ On the other hand, there was no statistically significant correlation between the LV end-systolic dimension, LV end-diastolic dimension or LV ejection fraction, and natriuretic peptide levels. The echocardiographic measure most strongly associated with both natriuretic peptide levels and the presence of symptoms was the left atrial dimension.

In addition to associations with echocardiographic measures of the severity of mitral regurgitation, natriuretic peptide levels were higher in symptomatic than in asymptomatic patients. Symptomatic patients were more likely to have more severe mitral regurgitation, but the association between natriuretic peptide levels and symptoms remained statistically significant, although weaker, after adjustment for echocardiographic measures of the severity of regurgitation and LV function. These observations suggest that natriuretic peptide levels provide an additional method for assessing the severity and symptoms of mitral regurgitation when the LV ejection fraction is normal.

As discussed in Chapter 1.3, few published studies have evaluated natriuretic peptide levels in mitral regurgitation. Brookes et al¹⁶³ measured BNP levels in 22 patients with mitral regurgitation, but this study included patients with impaired LV

function and did not assess the relation between the severity of mitral regurgitation and natriuretic peptide levels. As in the current study, BNP levels were higher in symptomatic patients, and there was no significant association between BNP levels and LV dimensions.

In the current study the increase in plasma N-BNP levels seen with increasing severity of mitral regurgitation and with increasing symptoms was greater than for BNP, but the strength of the association was similar. In the current study, natriuretic peptide secretion in patients with mitral regurgitation appeared to be related to increases in left atrial rather than LV dimensions. The relative increases in the plasma levels of BNP and N-BNP were as large as for ANP, and the correlations with the left atrial dimension, severity of mitral regurgitation and symptoms were similar for the three peptides. A possible explanation is that atrial myocytes synthesize BNP as well as ANP in response to the chronic increase in left atrial pressure. This hypothesis is supported by the demonstration of synthesis of BNP by atrial cardiomyocytes and co-storage of ANP and BNP in atrial granules.^{57,241}

The results of this study suggest that natriuretic peptide testing may add to the information obtained by echocardiography in the assessment of mitral regurgitation in clinical practice. When echocardiographic assessment is technically difficult, low N-BNP or BNP levels would suggest that mitral regurgitation is not severe. Measurement of natriuretic peptides may also be useful when it is not clear whether symptoms of dyspnea or fatigue are due to cardiac disease.^{79,81} Prospective follow-up studies are needed to determine whether natriuretic peptides can be used to monitor asymptomatic patients with moderate to severe mitral regurgitation in the primary care setting, or to guide timing of surgery. Limited clinical follow-up in the

current study suggests a single baseline measurement does not reliably predict disease progression. However, as demonstrated in aortic stenosis (Chapter 3.2), patients with an initial N-BNP level within normal limits may develop symptoms associated with increasing N-BNP levels during follow-up, and it is therefore likely that serial testing of natriuretic peptides will be more reliable in predicting clinical outcome than a single measurement alone.

Previous studies have demonstrated a clear inverse relationship between the LV ejection fraction and BNP and N-BNP levels after myocardial infarction or with cardiac failure.^{84,97,242} In the current study there was no clear association between natriuretic peptide levels and the ejection fraction, but patients with an ejection fraction of <55% on the screening echocardiogram were excluded. Further studies are needed to confirm that natriuretic peptide levels increase further when LV function declines in patients with mitral regurgitation. Another consideration is the possible presence of LV dysfunction when the ejection fraction is maintained in mitral regurgitation.

While transthoracic and occasionally transesophageal echocardiography can usually adequately determine the necessary information regarding the cause and severity of the mitral regurgitation, the assessment of LV function may be more complex. The search for an accurate barometer of latent myocardial dysfunction in mitral regurgitation dates to 1922.²⁴³ The need for improved methods for evaluating myocardial mechanics in mitral regurgitation results from some fundamental limitations in using ejection fraction to assess the LV. Ejection fraction measurements are fraught with interobserver error and dependent on heart rate and intravascular volume. If LV function is truly normal, a patient with severe mitral

regurgitation ought to have a hyperdynamic ejection fraction due to the lesion itself. Conversely, patients with severe mitral regurgitation and a low normal ejection fraction may already have significant depression of LV function, which would be unmasked after successful surgical elimination of the leak.²⁴⁴ A "normal" ejection fraction for the patients with mitral regurgitation is estimated to be 65-75%.²⁴⁵ Data from the Mayo Clinic has shown that once the ejection fraction falls to less than 60%, long-term mortality is increased, suggesting that LV dysfunction has already developed at that threshold for ejection fraction.²⁴⁶ Another myocardial factor used in the past to evaluate patients with mitral regurgitation is end-systolic stress.^{19,247} However, some of the simplified calculations are less valid in mitral regurgitation, because up to one-half of the regurgitant volume is ejected into the left atrium before aortic valve opening.²⁴⁸ The noninvasive measurement dp/dt has been postulated to reflect the myocardial factor in patients with mitral regurgitation.²⁴⁹ However, many patients with mitral regurgitation have eccentric jets that cannot be aligned parallel to the transthoracic Doppler beam, so the early systolic velocity envelope is blurred.

The appearance of LV dysfunction after surgical treatment is an adverse prognostic sign that implies the presence of latent dysfunction that was present but not apparent preoperatively.^{20,250} Exercise stress testing may unmask LV dysfunction in patients whose LV is compensated at rest - loss of ability to increase ejection fraction with increasing work (loss of contractile reserve) may be an early marker of LV decompensation and is related to clinical outcomes.^{20,251} Recently, measurement of longitudinal axis function by pulsed-wave tissue Doppler imaging at rest and stress has been shown to be a marker of contractile reserve in the absence of regional LV dysfunction.²² Further studies are required to determine the relative clinical utility of these measures compared with the natriuretic peptides.

In conclusion, plasma levels of BNP, N-BNP and ANP rise with increasing severity of mitral regurgitation and are higher in symptomatic than asymptomatic patients, even after adjustment for echocardiographic measures of the severity of regurgitation and LV ejection fraction. Further studies are required to determine whether preoperative natriuretic peptide levels can predict postoperative LV function as suggested by an abstract presented at the 2003 American College of Cardiology meeting (not yet published in manuscript form),¹⁶⁴ and whether serial measurement of natriuretic peptides will predict disease progression and the development of symptoms, thereby optimising the timing of mitral valve surgery for patients with severe mitral regurgitation. These issues are being addressed by the New Zealand Heart Valve Study, discussed in greater detail in Chapter 7.

CHAPTER 6

High Sensitivity C-Reactive Protein in Aortic Valve Disease

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6.1 High Sensitivity C-Reactive Protein in Aortic Valve Disease

INTRODUCTION

Non-rheumatic aortic stenosis has been regarded as a degenerative condition that increases with prevalence with increasing age. No therapy has been shown to reduce disease progression and the only recommended definitive treatment for symptomatic patients with severe aortic stenosis is aortic valve replacement.¹² Histological evidence that aortic stenosis may be an inflammatory disease with similarities to atherosclerosis was provided by Otto et al.³⁰ who showed the presence of an active inflammatory infiltrate in stenotic trileaflet aortic valves. This finding has subsequently been confirmed by others.^{31,32} In support of this histological evidence of an inflammatory process, increased serum levels of high-sensitivity C-reactive protein (CRP) have recently been demonstrated in patients with non-rheumatic aortic stenosis.²⁵² However, patients with a bicuspid aortic valve were excluded and the effect of aortic valve replacement on CRP levels was not assessed.

The aims of this study are firstly to determine whether CRP levels are elevated in patients with non-rheumatic aortic stenosis, including patients with trileaflet aortic valves and bicuspid aortic valves, compared to patients with non-rheumatic aortic regurgitation and normal controls, and secondly to determine the effect of aortic valve replacement on CRP levels in patients with aortic stenosis.

METHODS

Study Design

Twenty patients with isolated non-rheumatic aortic stenosis (mean aortic valve area $0.77 \pm 0.25 \text{ cm}^2$), 19 patients with isolated non-rheumatic aortic regurgitation and 31 controls were enrolled in the study. Baseline echocardiography and measurement of CRP was performed on all subjects. The inclusion criteria for patients with aortic stenosis was a peak aortic velocity of $\geq 2.5 \text{ m/s}$, and patients with aortic regurgitation required echocardiographic evidence of moderate-severe regurgitation.²³³ The exclusion criteria for all patients and controls were a CRP level of $>15 \text{ mg/L}$,¹⁷⁴ a history of coronary artery disease, cerebrovascular disease, peripheral vascular disease, and a co-existing inflammatory (including endocarditis) or hematological disorder. All 20 patients with aortic stenosis and 9/19 patients with aortic regurgitation underwent coronary angiography within 6 months of the study, and none had a coronary artery stenosis of $>50\%$ luminal diameter. There was no associated additional valve lesion greater than mild in severity and all patients were clinically stable. Control subjects had a normal echocardiogram, electrocardiogram and treadmill test. To assess the effect of aortic valve replacement on CRP levels, the 15 patients with aortic stenosis who underwent aortic valve replacement had a repeat CRP level measurement 6 months after surgery, as did all 31 controls 6 months after enrollment.

Clinical Characteristics

The clinical characteristics documented included age, gender, hypertension, diabetes, height, weight, and smoking and prescribed medications. Patients and controls were defined as hypertensive if they had a blood pressure of $>140/90 \text{ mm Hg}$ or were being treated for hypertension, diabetic if they were being treated with

hypoglycemic drugs or insulin, and smokers if they reported any current cigarette smoking. The body mass index (BMI) was recorded as a measure of obesity.

Definition of Aortic Valve Disease

Echocardiographic data were obtained as described in Chapter 2.3. Aortic valves were considered bicuspid by the echocardiographic findings of two cusps in systole and systolic cusp doming, highly asymmetric thickening or both. A diagnosis of 'degenerative calcific' aortic stenosis was made by the findings of thickening and increased echogenicity of the cusps (excluding the free edges) with reduced systolic opening and calcification of the valve. The valve lesions were considered non-rheumatic by the absence of commissural fusion and mitral valve involvement.

Laboratory Analyses

Plasma stored at -80°C from patients in the natriuretic peptide studies and who were enrolled in the current study were couriered to Auckland Hospital at the completion of the study and thawed for measurement of high-sensitivity CRP using a commercially available latex-enhanced high-sensitivity immunonephelometric assay under the supervision and quality control of Dr Rohan Ameratunga. The detection threshold was 0.15 mg/L and the coefficient of variation was 5% (Dade Behring, Deerfield, Ill). Clinical studies have demonstrated that results with the Dade Behring Inc. CRP assay correlate well with CRP levels on the basis of early research assays.¹⁷⁴

Statistical Analyses

CRP levels are expressed as median [IQR]. As CRP was not normally distributed, a Wilcoxon-signed rank test was used to compare paired data and Spearman's

correlation coefficient was used to assess the association between CRP and echocardiographic variables. One way analysis of variance was used to compare the Ln transformed CRP levels between the controls, aortic stenosis and aortic regurgitation groups. Multiple group comparisons were corrected using a Dunnett test.

RESULTS

Clinical Characteristics

The baseline clinical characteristics are shown in Table 6.1. There was no difference in age between controls and patients with aortic stenosis, but aortic regurgitation patients were significantly younger ($p=0.001$). The cause of aortic stenosis was a bicuspid aortic valve in 8 patients and 'degenerative calcification' of a trileaflet aortic valve in 12 patients. The cause of aortic regurgitation was a bicuspid aortic valve in 10 patients, dilated aortic root in 4 patients and aortic valve leaflet prolapse in 5 patients. The groups were similar with respect to hypertension, diabetes, smoking and body mass index, but aspirin and statins were prescribed more commonly to patients with aortic stenosis than patients with aortic regurgitation and controls.

C-Reactive Protein Levels in Subjects

Figure 6.1 compares CRP levels for patients with aortic stenosis, patients with aortic regurgitation and controls. CRP levels were significantly higher in patients with aortic stenosis (median 2.98 mg/L, IQR 1.59 to 5.37 mg/L) than in controls (1.51 mg/L, IQR 0.75 to 2.09 mg/L, $p=0.013$). CRP levels were similar in aortic stenosis patients with bicuspid aortic valves (CRP 3.3 mg/L, IQR 1.6 to 6.2 mg/L) and trileaflet aortic valves (CRP 2.5 mg/L, IQR 1.5 to 4.8 mg/L, $p=0.57$). In contrast, the CRP

levels of the controls did not differ from those of patients with aortic regurgitation (1.00 mg/L, IQR 0.35 to 2.00 mg/L, $p=0.30$) after adjustment for age, and were also similar by cause of aortic regurgitation. The associations between CRP levels and echocardiographic measures of disease severity in patients with aortic stenosis, including peak aortic velocity, aortic valve area and LV ejection fraction, were weak (correlation coefficient <0.21 , $p>0.05$ for all, data not shown).

Effect of Aortic Valve Replacement on C-reactive Protein in Aortic Stenosis

Fifteen patients with aortic stenosis underwent aortic valve replacement. Figure 6.2 compares the change in CRP levels from before aortic valve replacement to 6 months after aortic valve replacement with the change in CRP levels for controls at baseline and after 6 months of follow-up. CRP levels decreased after aortic valve replacement for aortic stenosis (median -38%, IQR -56% to -5%, $p=0.06$), but did not change in normal controls (median -4%, IQR -29% to +42%, $p=0.86$). The decrease in CRP levels after aortic valve replacement was similar for patients with a bicuspid aortic valve (-38%, IQR -61% to +1%) and a trileaflet aortic valve (-33%, IQR -44% to -15%). One patient not previously on a statin was prescribed a statin after aortic valve replacement and 3 patients not previously on aspirin were prescribed aspirin after aortic valve replacement. The results remained similar after these patients were excluded from the analysis.

Table 6.1: Clinical Characteristics of controls, patients with aortic regurgitation and patients with aortic stenosis

	Controls n=31	Aortic Regurgitation n=19	Aortic Stenosis n=20
Male, n (%)	14 (45%)	17 (89%)	13 (65%)
Age, years	66 ± 4.0	45 ± 13.9	67 ± 10.5
Body mass index, kg/m ²	25.8 ± 3.8	27.7 ± 4.9	28.5 ± 4.2
Hypertension, n	0	2	3
Diabetes, n	0	0	0
Smoker, n	1	3	1
Aspirin use, n	0	1	7
Statin use, n	0	0	4

Continuous variables are expressed as mean ± SD

n = number

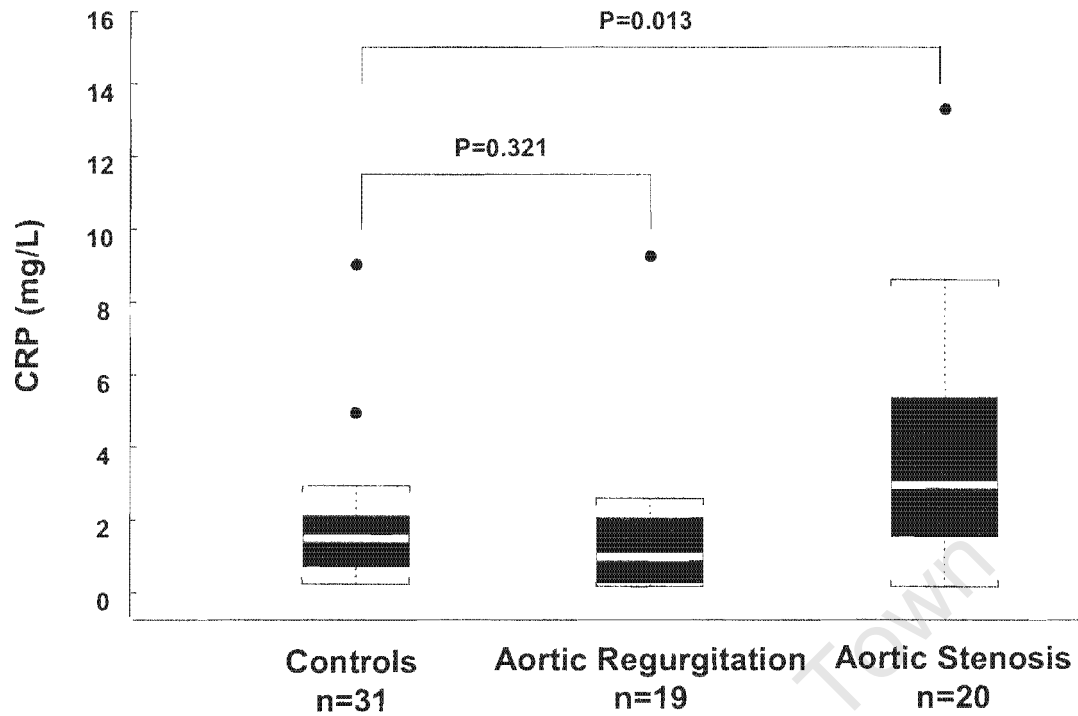


Figure 6.1: Box and whisker plots for the comparison of CRP levels between patients with aortic stenosis, aortic regurgitation, and controls.

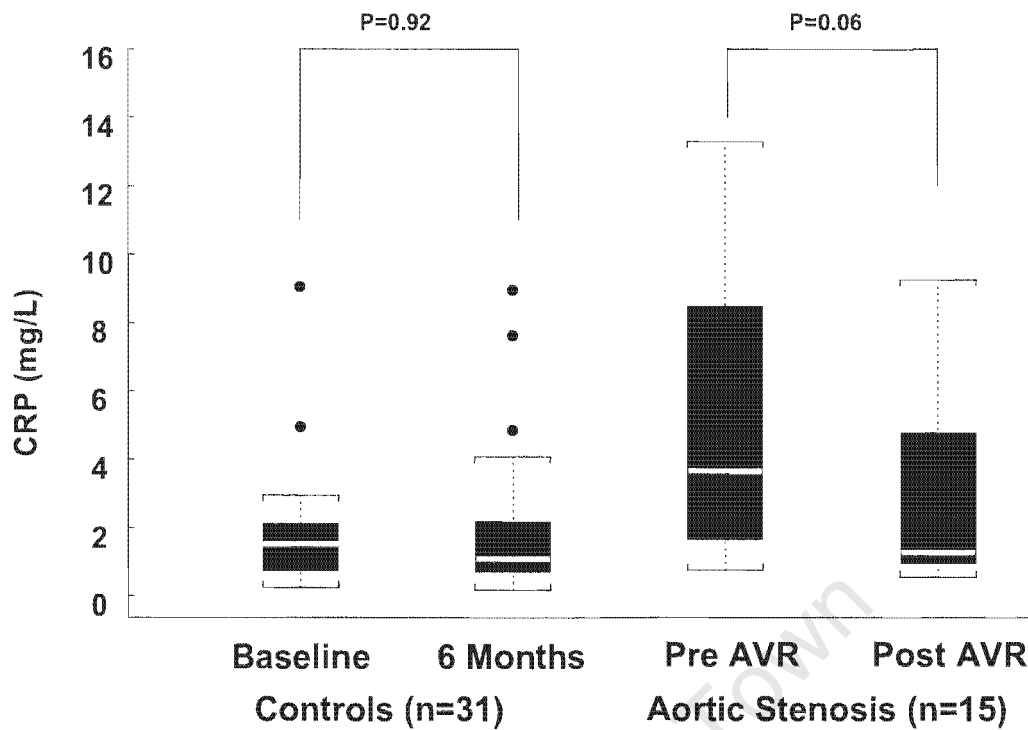


Figure 6.2: Box and whisker plots for the comparison of the change in CRP levels from before to 6 months after aortic valve replacement (AVR) for aortic stenosis and at baseline and after 6 months follow-up for controls.

DISCUSSION

A bicuspid aortic valve is present in 1% to 2% of the population and many of these subjects will eventually develop aortic stenosis requiring valve surgery.²⁵³ Sclerosis of trileaflet aortic valves occurs more commonly (~25% of adults over the age of 65 years of age) and although remains clinically and hemodynamically silent in most subjects, in an appreciable number there is progression to clinically manifest aortic stenosis.²⁵⁴ An improved understanding of the underlying mechanism(s) that results in aortic stenosis may lead to effective treatment strategies that will delay or prevent the progression to symptomatic severe aortic stenosis.

This study demonstrates that CRP plasma levels are elevated in patients with non-rheumatic aortic stenosis with trileaflet or bicuspid aortic valves. Furthermore, the decrease in CRP levels after aortic valve replacement in patients with aortic stenosis is consistent with histological evidence that the aortic valve is the site of active inflammation^{31,32,255} and is the likely cause of the elevation of CRP. Although the decrease in CRP after aortic valve replacement was of borderline significance, this most likely reflected the small study size as the magnitude of reduction of CRP was very similar to that achieved when a statin agent is used for dyslipidaemia.¹⁸¹⁻¹⁸³ The finding that CRP is not elevated in patients with non-rheumatic aortic regurgitation is supported by the lack of inflammatory histological changes in non-rheumatic aortic regurgitation.²⁵⁶

CRP was not elevated in all patients with aortic stenosis and did not decrease in all patients after aortic valve replacement. This suggests that although inflammation of the aortic valve is commonly present in patients with aortic stenosis it is not an inevitable phenomenon, at least as detected by increased CRP plasma levels. Since

this was a cross-sectional study and predominantly included patients with moderate and severe stenosis, it is uncertain whether CRP is increased in aortic sclerosis and mild aortic stenosis. A recent study suggested that CRP is increased in patients with 'moderate to severe' aortic sclerosis and not 'mild aortic sclerosis' compared to patients with a normal aortic valve, suggesting CRP levels may increase with disease severity.²⁵⁷

Many questions remain as to the initiating factor(s) for the changes seen in some aortic valves. Some investigators propose the increased mechanical stress on the aortic valve leaflets by loss of elasticity of the aortic wall may be the most likely cause for the initiation and progression of aortic stenosis.²⁵⁸ However, 70% of patients 65 years of age or older have normal aortic valves assessed by echocardiography.²⁵⁴ Others have suggested clinical factors associated with atherosclerosis are associated with 'calcific degenerative' aortic stenosis, including age, gender, hypertension, smoking, diabetes and raised serum cholesterol,^{259,260} but the specific factors that initiate this disease process have not been well defined. There is conflicting evidence for the role of infectious agents in the pathophysiology of aortic stenosis. Some studies suggest infectious agents such as Chlamydia Pneumoniae and Cytomegalovirus may be involved in the inflammatory process in aortic valves of patients with aortic stenosis,^{261,262} but others do not.²⁶³⁻²⁶⁵ Despite the uncertainties regarding the initiating factors for aortic stenosis, there is now considerable histological evidence for the presence of an active inflammatory process in the early and late stages of aortic stenosis in trileaflet aortic valves^{31,32,255} and bicuspid aortic valves,²⁵³ without any differences in extent or localization of the inflammatory infiltrate.

In conclusion, CRP plasma levels is increased in patients with isolated non-rheumatic aortic stenosis with both bicuspid and trileaflet valves, but is not increased in patients with aortic regurgitation. In addition, CRP decreases after aortic valve replacement for aortic stenosis. These results provide further evidence that non-rheumatic aortic stenosis is an inflammatory disease. Large longitudinal studies that include patients with a wide range of aortic stenosis severity are required to determine whether CRP levels correlate with the severity of aortic stenosis and whether increasing serial CRP levels predict disease progression and clinical outcome.

Chapter 8 addresses the ongoing multicentre New Zealand Heart Valve Study that will determine whether CRP levels correlate with aortic stenosis severity and whether there are greater increases in CRP levels in patients with more rapid progression of aortic stenosis severity. Stimulated by accumulating evidence that aortic stenosis is an inflammatory disease similar to atherosclerosis, non-randomised retrospective studies have been performed and suggest disease progression is slower in patients receiving statin treatment³³⁻³⁵ consistent with the reported anti-inflammatory effects of these agents.³⁶ These studies potentially have major clinical implications for the medical management of aortic stenosis, but randomised placebo controlled studies confirming these effects are currently lacking.³⁵

CHAPTER 7

Ongoing Studies

7.1	New Zealand Heart Valve Study	135
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7.1 New Zealand Heart Valve Study

Following on the studies presented in this thesis, a New Zealand based multi-centre study led by Dr Ralph Stewart has commenced to determine in a large cohort of patients whether 1) serial measurements of natriuretic peptides can reliably detect disease progression and predict the development of symptoms or decrease in LV function in patients with moderate or severe valvular heart disease, and 2) whether natriuretic peptides measured before aortic or mitral valve surgery predict LV function assessed 6 months after cardiac surgery. It is intended to recruit ≥ 150 patients with aortic stenosis, ≥ 150 patients with aortic regurgitation, ≥ 150 patients with mitral regurgitation, ≥ 50 patients with mitral stenosis, and ≥ 100 patients with mixed aortic and/or mitral valve disease.

Although the studies presented in this thesis strongly suggest that the natriuretic peptides provide important additional information to clinical and echocardiographic evaluation of patients with valvular heart disease, the number of subjects in each study was relatively small. Therefore, a cohort study of sufficient size and with a sufficient number of end point events is needed to determine the sensitivity, specificity, positive and negative predictive value of different thresholds of natriuretic peptides and/or change in natriuretic peptides for predicting disease progression. Furthermore, this study will be able to compare the relative predictive value of ANP, BNP and N-BNP as well as a combination of the natriuretic peptides.

The methodology of the New Zealand Heart Valve Study is very similar to the studies presented in this thesis and also will include patients with mitral stenosis and mixed valvular heart disease. In addition to measurement of the natriuretic peptides, other biomarkers including CRP as well as genetic markers will also be assessed.

Mitral Stenosis

Mitral stenosis develops in approximately 40% of patients with rheumatic heart disease.²⁶⁶ It causes chronic pressure load on the left atrium and reduces cardiac output. Over time exercise capacity is reduced, there is progressive left atrial dilation, and an increased risk of atrial fibrillation, thromboembolic stroke and pulmonary hypertension. There is limited data on the natriuretic peptides in mitral stenosis. In previous studies both ANP and BNP^{267,268} were elevated in patients with severe mitral stenosis undergoing balloon valvuloplasty. While there was a decrease in ANP within 24 hours, BNP remained elevated early after valvuloplasty. No studies have assessed associations between levels of different natriuretic peptides and echocardiographic measures of disease severity or symptom status in patients with mild, moderate and severe mitral stenosis. The New Zealand Heart Valve Study will assess several questions relevant to the potential use of natriuretic peptides for clinical assessment and monitoring of disease progression. These include the relation between ANP, BNP and N-BNP levels with severity of mitral stenosis, left atrial size, pulmonary hypertension and right ventricular function and whether higher levels of these natriuretic peptides predict symptom status and/or complications such as atrial fibrillation and pulmonary hypertension.

Mixed Valvular Heart Diseases

Rheumatic heart disease may involve the mitral, aortic or tricuspid valves and patients with chronic rheumatic heart disease often have mixed stenosis and regurgitation of one or more valves. Assessment of ventricular function is made more difficult by a combination of volume and pressure loads on the left and/or right ventricle. For example, an increase in LV dimensions occurs with compensated aortic regurgitation, but indicates LV dysfunction with isolated aortic stenosis. The

AHA/ACC guidelines on the management of mixed valvular heart disease provides no specific management guideline, rather recommending that each case be considered individually.²⁶⁹ Management decisions are in general based on the presence of symptoms, echocardiographic assessment of left or right ventricular systolic function or occurrence of complications such as atrial fibrillation and pulmonary hypertension. However many patients with complex chronic rheumatic heart disease have continued morbidity after surgery related to persistent left or right ventricular dysfunction, or atrial fibrillation. A rational basis for identifying those patients likely to benefit from earlier surgery and for targeting medical treatments is needed.

In the studies on the natriuretic peptides in patients with aortic stenosis, aortic regurgitation and mitral regurgitation presented in this thesis, echocardiographic measures of LV function varied by valve disease and by symptoms. However raised natriuretic peptide levels predicted symptoms in a similar way for all valve lesions. This suggests that measurement of natriuretic peptides may be useful for assessing more complex mixed aortic and mitral valve disease. The New Zealand Heart Valve Study will test this hypothesis.

Inflammatory Markers

In Chapter 6, CRP is shown to be increased in patients with moderate and severe aortic stenosis and decreases after aortic valve replacement. The New Zealand Heart Valve Study will be adequately powered to determine whether CRP levels correlate with the severity of aortic stenosis and whether the severity of aortic stenosis is more likely to progress in patients with higher levels of CRP.

Genetic Markers

It is not known whether certain individuals or populations are genetically predisposed to develop valvular heart disease. While the prevalence of aortic sclerosis and aortic stenosis increase with age, these conditions afflict the minority of the population and are not an inevitable consequence of ageing. It is therefore evident that there may be certain predisposing genetic factors to the development of these conditions. Proposed mechanisms for the development of aortic stenosis include genetic polymorphisms of the vitamin D receptor,²⁷⁰ leading to altered tissue calcification, and apolipoprotein A1, B1, and E polymorphisms,²⁷¹ leading to increased lipid accumulation in the valve leaflets. Rheumatic heart disease remains a common cause of morbidity and mortality due to valvular heart disease, and a genetic predisposition is also considered likely to the development of acute rheumatic fever and rheumatic heart disease. Candidate genes may be those for the structural components of the valve tissue targeted by antibodies to the Group A streptococcus.²⁷² Improved understanding of the genetic basis of valvular heart disease may allow development and targeting of novel preventive treatments. Polymorphisms of genes coding for structural proteins of the extracellular matrix, such as fibrillin, may be associated with aortic root dilation and aortic regurgitation.²⁷³ Identifying genetic markers would allow family screening and targeting of preventive treatments. The response of the LV to the chronic haemodynamic load of mitral and aortic regurgitation in particular may also be influenced by genetic factors, such as those that influence the renin-angiotensin-aldosterone systems.²⁷⁴ Ethics approval has been obtained for the storage of genetic material of patients enrolled in the New Zealand Heart Valve Study, and the analysis of specific, but yet to be determined

genetic markers will be performed by the Department of Genetics at Auckland Hospital.

In conclusion, the New Zealand Heart Valve Study is the largest known prospective study in patients with valvular heart disease and will determine whether serial natriuretic peptide levels can reliably predict the development of symptoms and early LV dysfunction in patients with valvular heart disease. If confirmed, measuring natriuretic peptides may improve the reliability and convenience of long-term management of these conditions by providing a simple low cost way to monitor patients which can be used both in the primary and tertiary care settings. In addition, this study will determine whether natriuretic peptides improve the accuracy of assessment of the valve lesion when echocardiographic or clinical data are equivocal, allowing appropriate identification of patients who should be referred for surgery, as well as those who can be safely observed while still asymptomatic. These issues are particularly relevant in countries where many patients live in remote areas making regular attendance at a specialist cardiology clinic more difficult. In addition the waiting list for cardiac surgery is long in many countries and currently relatively few patients receive surgery when still asymptomatic. In this situation it is particularly important to identify patients at higher risk for worsening LV function and/or sudden death so that urgent surgery can be arranged.

CHAPTER 8

Summary and Conclusions

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SUMMARY AND CONCLUSIONS

The principal findings of the studies presented in this thesis are:

- 1) Plasma levels of the natriuretic peptides increase with increasing severity of aortic stenosis and mitral regurgitation, and are higher in symptomatic patients compared to asymptomatic patients after adjusting for echocardiographic measures of disease severity and left ventricular function.
- 2) A major stimulus for increased natriuretic peptide levels in valvular heart disease appears to be left ventricular decompensation associated with symptoms of heart failure, although other factors such as increased left ventricular pressure and increased left ventricular volume also contribute.
- 3) In asymptomatic patients with severe aortic stenosis, an elevated N-BNP level predicts an increased risk of symptomatic deterioration during follow-up more reliably than echocardiographic measures of disease severity and left ventricular function.
- 4) In symptomatic patients with severe aortic stenosis and an N-BNP level within normal limits, aortic valve replacement results in less reliable improvement of symptoms than in symptomatic patients with an elevated N-BNP level.
- 5) Taken together, these novel data suggest that measurement of natriuretic peptide plasma levels may complement clinical and echocardiographic evaluation of patients with valvular heart disease and may improve the timing of surgery.
- 6) High sensitivity C-reactive protein plasma levels are elevated in non-rheumatic aortic stenosis and decrease after aortic valve replacement. These novel observations are consistent with histological evidence that the aortic valve is the site of active inflammation, and provides stimulus for studies to determine whether agents with anti-inflammatory actions, such as statins may have a role in delaying disease progression in patients with aortic stenosis.

Although the natriuretic peptides have been extensively studied as diagnostic and prognostic markers in many cardiac and non-cardiac conditions with data suggesting a role for the detection of latent left ventricular dysfunction, it is surprising that there is limited data on the potential clinical utility for measuring natriuretic peptide plasma levels in patients with valvular heart disease. However, there are several notable consistent themes which taken together strongly suggest that measurement of natriuretic peptide levels may complement clinical and echocardiographic evaluation of patients with valvular heart disease and may be particularly useful when symptoms or left ventricular function are uncertain.

Whereas echocardiographic assessment of valvular heart disease requires trained and experienced sonographers with meticulous attention to the technical details of imaging and Doppler flow recording, the introduction of automated assays means that measurement of plasma levels of natriuretic peptides is simple, not operator-dependent, relatively inexpensive and reproducible. Assays for measurement of BNP and N-BNP are currently available for diagnostic and prognostic purposes, especially in patients with cardiac failure and acute coronary syndromes, but neither ANP nor N-ANP are in routine clinical use. However, most studies, including the ones presented in this thesis were not adequately powered to determine the relative accuracy of each natriuretic peptide and do not consider the potential for the combined measurement of the natriuretic peptides. These issues are the focus of ongoing studies, including the New Zealand Heart Vale Study.

There are important limitations to consider when evaluating natriuretic peptide levels. Although natriuretic peptide levels may be readily measured, they need to be considered together with clinical, echocardiographic and other available data. Many

conditions may affect natriuretic peptide levels, including age, gender, renal function, severe pulmonary or hepatic disease and cardioactive medications. These factors need to be carefully considered when evaluating a natriuretic peptide level in research or clinical practice, and similar to the limitations of clinical evaluation and echocardiography, a natriuretic peptide level needs to be considered in the context in which it is measured. An important example is the derivation of cut-points to determine the sensitivity and specificity of natriuretic peptide levels for the diagnosis of a particular condition, such as heart failure and acute pulmonary embolism. There is very limited data on whether cut-points should take into account age, gender, renal function, and medication. For these reasons, perhaps the greatest clinical utility for the measurement of natriuretic peptide levels will be the serial assessment of patients with conditions such as valvular heart disease where the many variables that may influence natriuretic peptide levels will be less influential and the patient is used as his/her own control for comparison of serial levels. Further studies are needed to determine intraindividual variation of natriuretic peptide levels in patients with cardiac disease, in particular to determine what absolute or percentage change in natriuretic peptide level should be regarded as a clinically meaningful change.

Finally, the second theme of this thesis was to provide novel data that further supports the growing literature on the “inflammatory hypothesis” of aortic stenosis. In contrast to the remarkable advances in medical therapy for cardiac failure and acute coronary syndromes, cardiac surgical techniques and cardiac anaesthesia, there have been very few advances in the medical management of valvular heart disease. Aortic stenosis is the most common indication for valve surgery in the western world, and the prevalence will most likely increase with the ageing population. Whereas surgical repair for mitral regurgitation is frequently possible, the

only definitive procedure for symptomatic patients with severe aortic stenosis is aortic valve replacement with the associated risks of a prosthetic valve and often the need for lifelong anticoagulation. The data presented in this thesis is consistent with histological evidence that aortic stenosis is characterised by an active inflammatory process of the aortic valve and further studies are therefore required to determine whether the disease process may be modified by agents with anti-inflammatory actions.

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CHAPTER 9

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