Abstract

Objectives: We examined whether measurement of the plasma BNP concentrations might be useful for the early diagnosis of the existence and severity of disease in patients with heart disease in daily clinical practice. Methods and Results: The plasma BNP and ANP concentrations in 415 patients with heart disease and hypertension and 65 control subjects were measured. Patients with heart disease had higher plasma BNP and ANP concentrations than did those with hypertension or control subjects. Among the etiology of cardiac diseases, specifically dilated cardiomyopathy and hypertrophic cardiomyopathy, was associated with the highest plasma BNP concentrations, whereas dilated cardiomyopathy was associated with the highest plasma ANP concentrations. Plasma BNP concentrations showed an increase as the severity of the heart disease, as graded according to the NYHA classification of cardiac function, increased. In both patients with heart disease and hypertension, the plasma BNP values were higher in those who had abnormalities in their echocardiogram and electrocardiogram as compared to those without any abnormalities. The plasma BNP levels also showed a significant correlation with left ventricular wall thickness and left ventricular mass. On the other hand, the plasma ANP levels showed significant correlations with left ventricular dimension. Receiver operative characteristic analysis revealed that plasma BNP levels showed substantially high sensitivity and specificity to detect the existence of heart diseases. Conclusion: Measurements of the plasma BNP concentrations is useful to detect the existence of the diseases, and abnormalities of left ventricular function and hypertrophy in patients with heart disease in daily clinical practice. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Heart failure; Hypertension; Hypertrophy; Natriuretic peptide

1. Introduction

Brain natriuretic peptide (BNP), a hormone consisting of 32 amino acids, was isolated/identified from the brains of pigs in 1988 [1]. Despite the BNP gene and atrial natriuretic peptide (ANP) gene being located adjacent to each other [2], their secretory kinetics are often dissociated. ANP secretion is stimulated by atrial dilatation. Plasma ANP levels therefore reflect the severity of heart failure [3], while being poorly specific for the causal disease. ANP is primarily produced by myocardial cells in the atrium, whereas BNP is primarily secreted in the ventricles [4]; BNP may thus be a specific index reflecting the early stage of heart disease or impaired cardiac function. In fact, it is known that the plasma BNP concentrations are significantly higher than those of ANP in various heart diseases, particularly severe heart failure [5], hypertrophic cardiomyopathy [6] and acute myocardial infarction [7]. These findings have, however, been reported

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from controlled studies focusing on hospitalized patients, that is, studies in which the morbidity status of the patients was well defined.

Presently, it is often difficult to diagnose patients with mildly symptomatic heart disease or impaired cardiac function. In particular, patients with hypertension may develop progressive cardiovascular complications without showing any symptoms. Cowie et al. [8] have shown that plasma BNP is a useful diagnostic tool for patients with heart failure in primary care. We thus examined whether measurement of the plasma BNP concentrations during routine consultations would reflect the morbidity status of the patients. Namely, we attempted to identify patient background factors that may affect the plasma BNP concentrations in patients with cardiovascular diseases, and investigated whether determination of the plasma BNP concentrations might be useful for the early detection of heart diseases and understanding the morbidity status of such diseases, in comparison with that of the plasma ANP concentrations.

2. Methods

2.1. Subjects

The study was conducted on a total of 480 patients with cardiovascular diseases including hypertension and control subjects, who visited the six participating institutions from February 1998 to October 1999. This study conforms with the principles outlined in the Declaration of Helsinki. Each of the patients gave his/her informed consent for participation in the study.

The underlying disease was diagnosed through a routine consultation, physical examinations and clinical laboratory tests. Chest radiography, electrocardiography at rest, echocardiography, and measurements of the plasma BNP and ANP concentrations were performed. In patients undergoing treatment, the drug administration schedules were maintained.

We examined 154 patients with heart diseases, 261 hypertensive patients, and 65 control subjects. The underlying heart diseases included old myocardial infarction in 28 patients, angina pectoris in 46 patients, idiopathic dilated cardiomyopathy in 26 patients, hypertrophic cardiomyopathy in 18 patients, valvular heart diseases in 24 patients, and lone atrial fibrillation in 12 patients. According to the New York Heart Association (NYHA) functional classification, 110 patients were in class I and 44 in class II. There were 15 patients with mitral valve disease, four with aortic valve disease, one with tricuspid valve disease and four with combined valvular disease. Control subjects who came to the hospitals for health check or some other complaints, but were eventually diagnosed as being free from cardiovascular diseases were included. They had no symptoms, and no evidence of significant disease was detected by physical examination, chest radiography, resting and exercise electrocardiography, or routine laboratory tests.

We excluded patients with other cardiac diseases. Excluded from the study were patients with aneurysms, heart failure in the NYHA classes III and IV, metabolic diseases, such as hyperthyroidism, severe lung diseases, renal failure and liver failure. We also excluded those who had an implanted pacemaker and hospitalized patients.

The diagnostic criteria for hypertension was a blood pressure of 140 and/or 90 mmHg or higher or a history of treatment with antihypertensive agents. Patients with left ventricular dilatation but normal coronary arteries and a thin left ventricular wall without a known cardiac disease, such as hypertensive heart disease or hypertrophic cardiomyopathy, were diagnosed as having idiopathic dilated cardiomyopathy. Hypertrophic cardiomyopathy was diagnosed mainly by echocardiography: presence of systolic anterior movement of the mitral valve and asymmetric ventricular hypertrophy; the interventricular septum was at least 1.3 times as thick as the posterior wall. They did not present other causes of ventricular hypertrophy, such as hypertension and aortic stenosis.

2.2. Protocols

We examined the correlation between the plasma BNP levels and the patient background factors. Furthermore, we divided the patients into a normal group and an abnormal group based on the electrocardiographic findings and echocardiographic findings, and compared the mean plasma ANP and BNP concentrations between the two groups.

Echocardiographic parameters were measured within 1 month from the plasma BNP and ANP measurement. Left ventricular dimension was measured in a long axis view of the left ventricle taken with the patient in the left lateral position. Left ventricular volume was obtained using Teichholz’s equation: left ventricular volume=(7.0/2.4+dimension)×dimension$^3$.

Left ventricular ejection fraction (LVEF) was obtained by the following formulae: LVEF=(LV end-diastolic volume−end-systolic volume)/LV end-diastolic volume. Left ventricular mass (LVM) was calculated by the method of Devereux et al. [9].

Patients with ejection fraction (EF) <60%, diastolic dimension (Dd) >55 mm, systolic dimension (Ds) >42 mm, interventricular septum thickness (IVSTD) >12 mm, posterior wall thickness (PWTD) >12 mm, and left ventricular fractional shortening (FS) of less than 29% were identified and grouped into an abnormal group, and the BNP and ANP levels between the normal and abnormal groups were compared.
2.3. Measurement of plasma BNP concentration

Blood samples were collected from the patients in the sitting position in syringes containing EDTA (1 mg/ml) and aprotinin (103 KIU/ml). Serum was separated within 6 h and the samples were stored frozen at −20°C until the measurements. The concentrations of BNP and ANP were measured within 1 week after plasma sampling by an immunoradiometric assay (IRMA) method described before [10].

2.4. Statistical analysis

For comparison of the mean values, when significant difference was noted by analysis of variance, Welch’s test was performed for analyzing the differences between two groups. Since distribution of ANP and BNP showed unequal variances, nonparametric Tukey type multiple comparison was used for analyzing the differences among multiple groups. All of the tests were two-tailed, with the level of significance set at 0.05.

The correlations between the plasma BNP or ANP levels and the NYHA class, echocardiographic findings, electrocardiographic results, systolic blood pressure (SBP), diastolic blood pressure (DBP), and pulse rate were analyzed by the least square method. Multiple regression analyses on BNP and ANP were performed with the use of all the variables. ‘Disease’ was classified into three levels, i.e. control, hypertension and heart disease.

We also examined to what extent measurement of the plasma BNP and ANP concentrations might be useful in detecting the existence of heart diseases using receiver operating characteristic (ROC) analysis. The sensitivity and specificity of determination of the plasma BNP concentrations were calculated, and an ROC curve was drawn. The sensitivity was defined as the percentage of patients who showed higher BNP and ANP values than a certain concentration among the total number of patients with heart disease and controls. On the other hand, the specificity was defined as the percentage of patients showing lower BNP levels than a certain concentration among the total number of patients with heart disease and controls. Differences between areas under ROC curves for ANP and BNP were compared with a studentized range test [11].

3. Results

3.1. Distribution of plasma BNP and ANP concentrations according to patient background factors

Patients with heart disease had higher plasma BNP and ANP concentrations than did those with hypertension or control subjects (Fig. 1). Among the etiology of cardiac diseases, specifically hypertrophic cardiomyopathy, was associated with the highest plasma BNP concentrations, whereas dilated cardiomyopathy was associated with the highest plasma ANP concentrations.

Plasma BNP and ANP concentrations showed an in-

![Graph](image-url)

Fig. 1. Distribution of plasma BNP concentrations and ANP concentrations by the underlying disease. *P<0.05, †P<0.01, ‡P<0.001, vs. control.
crease as the severity of the heart disease according to the NYHA functional classification increased. Moreover, the plasma BNP values, but not ANP values, were higher even in patients with NYHA class I than in control subjects (Fig. 2). Although the plasma ANP levels also increased with the severity of the heart disease in the same manner as the plasma BNP levels, the extent of elevation was larger in the case of the plasma BNP levels.

3.2. Correlation of plasma BNP and ANP levels with the cardiac findings and laboratory test values

Significantly higher BNP values were noted in the group of patients with abnormal electrocardiographic findings, such as atrial fibrillation, left ventricular high voltage, ST changes, and T wave change. Plasma ANP levels were also higher in the abnormal atrial fibrillation and ST changes (Fig. 3).

Higher BNP values were found in all the patients in the abnormal EF, Ds, IVTD, PWTD, and FS group, and the differences between the normal and abnormal groups were significant (Fig. 4). Plasma ANP levels also showed a tendency towards being higher in the abnormal group, however no significant differences in EF, IVSTD, PWTD, FS were noted between those with abnormal and those with normal echocardiographic findings. Similar results were obtained in the group of patients with hypertension.

As indicated in Table 1, the plasma BNP levels as a whole showed a negative correlation with the EF and positive correlation with the Ds, IVSTD, PWTD, and LVM (r=0.34, P<0.0001, Fig. 5). On the other hand, the plasma ANP levels showed a negative correlation with the EF and positive correlation with the Dd, Ds, PWTD and LVM. Correlations of BNP with age (P<0.05), disease (P<0.02), left ventricular mass (P<0.0001) and atrial fibrillation (P<0.0001) were statistically significant by multiple regression analysis. Age (P<0.0001), left ventricular mass (P<0.001) and atrial fibrillation (P<0.01) were significant determinants of ANP.

In patients with hypertension also, the plasma BNP levels showed a negative correlation with the EF and positive correlation with the Ds, IVSTD, PWTD, LVM, DBP, and pulse rate, while plasma ANP levels showed a positive correlation with only SBP and DBP.

3.3. Sensitivity and specificity of plasma BNP levels to detect the existence of heart diseases

In order to assess the usefulness of measurement of the plasma BNP and ANP levels for the purposes of diagnosis,
The results of this study suggest that determination of the plasma BNP levels for detecting the morbidity status of patients with heart disease may be more valuable than other measurements already in use, since it reflects the morbidity of heart disease in patients undergoing therapy primarily on an out-patient basis. As reported in previous studies, the plasma BNP concentrations in patients with heart disease were elevated in this study also. The present study is thus consistent with findings that measurement of the plasma BNP levels may be useful for the screening of latent heart diseases [12].

It is well known that the blood ANP concentrations are elevated in atrial fibrillation, particularly in paroxysmal atrial fibrillation, and researchers have suggested this may be one of the factors causing tachycardia-induced polyuria [13,14]. In our study also, patients with atrial fibrillation showed high plasma BNP values. The mechanism by which atrial fibrillation strongly induces the secretion of the primarily ventricle-derived BNP is unknown. However,
since the levels were increased not only in patients of atrial fibrillation with underlying heart disease, such as valvular heart disease, but also in those with lone atrial fibrillation, a BNP secretion-inducing mechanism may be present. Inoue et al. [15] reported that the plasma BNP levels in patients with lone atrial fibrillation were significantly higher than those in controls. They also found higher BNP levels in the coronary sinus than in the anterior interventricular vein, suggesting an increase in atrium-derived BNP. Furthermore, Tuinenburg et al. [16] reported about 70% higher pro-BNP mRNA levels in the right atrial appendage in patients with atrial fibrillation as compared with those in subjects with sinus rhythm. Increased levels were noted in these cases regardless of the existence of underlying valvular disease. These findings suggest that the increased BNP levels in patients with atrial fibrillation may be derived from the atrial tissue. Since atrial fibrillation is one of the significant risk factors of embolism, elevated BNP values could be considered as a warning signal in relevant cases.

In this study, the BNP levels were measured in patients most of whom were taking various drugs. Therefore, the effects of the drugs have undeniably contributed to the results. However, we believe that altered plasma natriuretic peptide concentrations in patients taking drugs for cardiovascular diseases are often more reflective of the altered cardiac function under medication. For example, angiotensin converting enzyme (ACE) inhibitors are known to reduce plasma BNP concentrations in patients with cardiac failure [17] and those with hypertension [18]. However, these may not be because of the direct actions of ACE inhibitors on BNP secretion, but due to reduction of the cardiac afterload and reduction of cardiac hypertrophy effected by them. On the other hand, β-blockers have been reported to elevate the plasma concentrations of ANP and BNP [19,20]. These studies have suggested that this effect of elevating plasma natriuretic peptide concentrations may partially contribute to the overall effects of β-blockers [21]. Our study did not show any clear differences in plasma ANP or BNP concentrations between patients administered β-blockers and those administered other drugs (data not shown). Therefore, the plasma BNP concentrations measured in the study were probably influenced little by the drugs administered, and for the most part, may be reflective of the underlying cardiac overload.

Plasma BNP concentrations have been suggested to be a useful index to determine the prognosis in cases of myocardial infarction [22] and cardiac failure [23]. Plasma norepinephrine concentrations, used also as an index of prognosis in cases of cardiac failure [24] are markedly altered by many factors. Furthermore, the volume of salt intake or the physical position of the patient during the blood sample collection may markedly affect not only the plasma norepinephrine concentrations, but also the renin activity. Plasma BNP concentrations are not subject to these influences, and this may be of advantage for the use of plasma BNP concentrations as an index of the severity of cardiac disease in daily practice.

The plasma BNP concentrations have shown correlation with the measurements of left ventricular hypertrophy, namely IVSTD, PWTD, and LVM. Studies have reported that the prognosis of patients with essential hypertension complicated by cardiac hypertrophy is poor, and that the incidence rate of cardiac accidents in these patients is high [25]. Bettencourt et al. [26] found a significant relationship between BNP and left ventricular mass in community mild to moderate hypertensive patients. Higher BNP levels have been reported to be found in cases with concentric hypertrophy as compared with those with eccentric hypertrophy [27], therefore, the plasma BNP concentration may be an excellent clinical index for determining the risk of cardiac events and the prognosis of the patients. Thus,
especially as a screening test, since it has also been reconfirmed that the plasma BNP concentration is superior to the plasma ANP concentration in reflecting cardiac overload [28], measurement of the plasma BNP concentration could be performed first to determine the cardiac overload in patients with cardiac failure or hypertension.

References