THE RELEVANCE OF BRAIN NATRIURETIC PEPTIDES INVESTIGATION IN VARIOUS CARDIOVASCULAR DISEASES

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Background: Brain natriuretic peptides are relevant markers of heart impairment.

Aim: We investigated the relevance of investigating brain natriuretic peptides (NT-proBNP, BNP) in monitoring different types of cardiovascular disease (chronic heart failure due to coronary artery disease, cardiomyopathy, acquired valve disease, congenital heart diseases).

Methods: The NT-proBNP assay (Roche) was performed on 280 patients (mean age 49 years; range 20–89 years) and 48 healthy controls (mean age 43 years; range 13–65 years) and BNP assay (Bayer Shionoria) was performed in a subgroup of 42 patients (mean age 50 years; range 20–79 years). Patients were divided into four groups characterized by severity of heart failure according to the New York Heart Association classification.

Results: NT-proBNP concentrations differed in patients with cardiovascular diseases from controls (median 371 ng/l versus 41.5 ng/l, $p < 0.0001$). The cut off value of NT-proBNP determined in 280 patients with cardiovascular diseases was at 130 ng/l (AUC–area under curve = 0.93; sensitivity 98 %; specificity 79 %). Comparison of NT-proBNP and BNP values in patients showed significant correlation ($r = 0.93$; $p < 0.0001$). NT-proBNP showed significant differences between groups.

Conclusions: Measurement of brain natriuretic peptides is useful and relevant in various types of heart diseases including congenital.

INTRODUCTION

Natriuretic peptides BNP and N-terminal-proBNP are well established serum biomarkers for acute and chronic heart failure^{1–5}. BNP is released from the atria and ventricles of the heart in response to volume overload, increased filling pressures and myocardial wall stretching. BNP causes vasodilatation, reduces blood pressure and stimulates sodium and water excretion. The BNP gene is located on chromosome 1 (1p36.2)⁶. The hormone originates from the precursor peptides preproBNP (134 amino acid) and proBNP (108 amino acids; 12 kDa). ProBNP is the *in situ* precursor of both the biologically active BNP hormone (32 amino acids; 4 kDa), and its inactive metabolite, NT-proBNP (76 amino acids; 8 kDa). Both forms circulate in the blood of normal subjects and in those with heart failure. ProBNP also circulates in blood in monomeric and trimeric (approximately 36 kDa) forms⁷. The estimated *in vivo* half-life of BNP in blood is 20 minutes and between 1 and 2 hours for NT-proBNP and its related forms⁸. BNP is cleared by a combination of receptors,

neural endopeptidases, proteolytic enzymes and renal clearance⁹.

The study was designed to assess the relevance of brain natriuretic peptides (NT-proBNP, BNP) in monitoring various types of cardiovascular diseases (chronic heart failure due to coronary artery disease, cardiomyopathy, acquired and congenital heart disease) using commercially available automated NT-proBNP assay (Roche Diagnostic) and BNP Assay (Bayer Shionoria).

MATERIALS AND METHODS

1. Subjects

Two hundred eighty samples of patients with various types of cardiovascular disease and 48 healthy controls (30 males with a mean age of 43 years, range 18–65 years; 18 females with a mean age of 45 years, range 19–65 years) were recruited for an investigation of serum NT-proBNP levels. Subgroup of 42 randomly selected patients (24 males with mean age of 51 years, range 20–79 years;

Table 1. The clinical characteristics of the patients

	NYHA I	NYHA II	NYHA III	NYHA IV
N	8	21	10	3
Male	4	12	5	3
Female	4	9	5	0
BNP (median $\pm$ SEM) (ng/l)	106.4 $\pm$ 42.1	102.4 $\pm$ 19.9	344.1 $\pm$ 154.7	586.2 $\pm$ 493.3
NT-proBNP (median $\pm$ SEM) (ng/l)	223.6 $\pm$ 55.6	628.9 $\pm$ 148.2	1642 $\pm$ 459	5653 $\pm$ 5213
History of AIM (%)	0	2 (10)	1 (10)	0
Hypertension (%)	0	2 (10)	1 (10)	0
Systolic pressure [mm Hg] (mean $\pm$ SEM)	124 $\pm$ 5	132 $\pm$ 5	132 $\pm$ 8	120 $\pm$ 10
Diastolic pressure [mm Hg] (mean $\pm$ SEM)	71 $\pm$ 3	85 $\pm$ 7	82 $\pm$ 5	75 $\pm$ 5
ACE inhibitors (%)	3 (38)	6 (29)	5 (50)	2 (66)
Angiotensin inhibitors (%)	0	3 (14)	0	0
Beta blockers (%)	2 (25)	8 (38)	8 (80)	1 (33)
Diuretics (%)	0	10 (48)	6 (60)	1 (33)
Creatinine [μ mol/l] (mean $\pm$ SEM)	82.2 $\pm$ 3.8	89.8 $\pm$ 4.7	92.8 $\pm$ 11.9	130 $\pm$ 70
Coronary artery disease	0	8 (38)	3 (30)	3 (100)
Ebstein anomaly	2 (25)	0	0	0
Ventricular septal defect	0	2 (10)	0	0
Atrial septal defect	2 (25)	2 (10)	4 (40)	0
Acquired aortic or mitral valve disease	2 (25)	2 (10)	0	0
Dilated cardiomyopathy including isolated left ventricular noncompaction	0	2 (10)	1 (10)	0
Tricuspid atresia and complex cyanotic congenital heart disease	0	1 (5)	1 (10)	0
Corrected transposition of the great arteries	0	2 (10)	0	0
Eisenmenger syndrome	0	0	1 (10)	0
Anomalous left coronary artery origin	0	2 (10)	0	0
Transposition of great arteries (Mustard correction)	2 (25)	0	0	0

18 females with mean age of 48 years, range 20–79 years) were selected for the study and their plasma samples were collected for investigation of BNP levels.

Consecutive ambulatory patients were previously examined in a specialized Cardiology Department and were divided into four groups according to the New York Heart Association (NYHA) classification.

Eight patients (19 %) were in the NYHA class I, 20 (48 %) in NYHA class II, 11 (26 %) in NYHA class III and 3 patients (7 %) in NYHA class IV respectively.

2. Methods

Serum and plasma samples were frozen immediately and kept at -20°C until BNP and NT-proBNP were analyzed. Serum levels of NT-proBNP were measured

using commercially available electrochemiluminescence sandwich immunoassay (ECLIA, Roche) on an Elecsys System 2010. Plasma BNP levels were measured using commercially available immunochemiluminescence immunoassay (Shionoria BNP, Bayer) on an Bayer ADVIA Centaur System.

3. Statistical analysis

Differences in NT-pro BNP and BNP concentrations between subgroups were tested for statistical significance either by the nonparametric Mann Whitney U test and Kruskal-Wallis test, if the NT-pro BNP values were not normally distributed, or by the unpaired t-test and One way analysis of variance (ANOVA). A $p < 0.05$ was considered as statistically significant.

AUC = 0.93 (95% CI = 0.90–0.95)

Sensitivity = 98%

Specificity = 79%

Cut off value = 130 ng/l

AUC - Area under curve; CI - confidence interval

Fig. 1. ROC analysis of NT-proBNP

Receiver operator characteristic (ROC) analysis was performed on 280 patients to determine the best cut off value to detect cardiac involvement as well as to assess the discrimination ability (by calculating the area under the ROC curve) of NT-proBNP to recognize patients with various types of heart disease. All data in this paper are expressed as median $\pm$ SEM. Although the distribution of NT-proBNP and BNP levels within the population was skewed, the logarithm (log) of its value was used for statistical analysis.

RESULTS

1. NT - proBNP and cardiovascular diseases

NT-proBNP serum levels in patients with cardiovascular diseases were 371 ± 141.6 ng/l. Patients without heart involvement had significantly lower NT-proBNP levels (41.5 ± 5 ng/l, $P < 0.0001$; Mann Whitney U-test). The area under ROC curve for the detection of heart involvement was high: 0.93 (95 % confidence interval, 0.90–0.95) (Figure 1). The appropriate cut off value for the diagnosis of heart involvement was 130 ng/l. With this cut off, sensitivity was 98 % and specificity was 79 %.

2. Correlation of natriuretic peptides in NYHA classes

Patients were divided by NYHA classification according to severity of the disease. The higher NYHA class, the more significantly NT-proBNP values were increased. Significant differences in medians were found between all NYHA groups ($p = 0.0474$, One way analysis of variance) with the strongest difference between NYHA I and NYHA II groups ($p = 0.0172$, Unpaired t - test) (Figure 2a). Patients in the NYHA IV group were due to small sample size considered together with NYHA III patients.

a: NT-proBNP

Data are presented as box (median, 25th and 75th percentiles) and whiskers (higher and lower values) plots, significant difference between NYHA I and NYHA II groups is marked by * ($p = 0.0172$). Significant differences between patients with less (NYHA I, II) and more (NYHA III, IV) functional involvement are expressed as value of $p < 0.0001$.

Significant differences between all NYHA groups are expressed as $p = 0.0474$.

b: BNP

Data are presented as box (median, 25th and 75th percentiles) and whiskers (higher and lower values) plots, significant difference between NYHA II and NYHA III,IV groups is marked by* ($p = 0.0347$).

The difference between patients with less (NYHA I, II) and more (NYHA III, IV) functional involvement expressed as value of $p = 0.074$ was considered not significant.

Significant differences between all NYHA groups are expressed as $p = 0.0489$.

Fig. 2a, b. Concentration of NT-proBNP and BNP in patients with heart failure according to NYHA classification

The median for BNP values increased with the severity of disease. Patients in the NYHA IV group were included together with NYHA III patients. Significant differences in medians were found between all NYHA groups ($p = 0.0489$, One way analysis of variance) with strongest difference between NYHA II and NYHA III, IV group (unpaired t-test, $p = 0.0347$) (Figure 2b). NT-proBNP showed significantly higher difference between patients belonging to NYHA I and II groups and patients belonging to NYHA III and IV groups (Mann-Whitney U test, $P < 0.0001$). By contrast, the difference of BNP between patients corresponding with NYHA I, II and NYHA III, IV was not significant (Unpaired t-test, $p = 0.074$).

DISCUSSION

Our results confirm the correlation of brain natriuretic peptides with the severity of the diseases according to NYHA classes. The values of NT pro-BNP and BNP in various NYHA categories correlated well ($r = 0.93$, $P < 0.0001$). Nevertheless, greater overlap of values between the various NYHA categories occurred, especially for the lower functional classes (NYHA I, NYHA II) regarding the lower discrimination ability of BNP to distinguish patients with and without heart involvement. Analysis of the area under ROC curve of the current data suggests that a cut-off value of 130 pg/ml for NT-proBNP discriminate well between patients with various cardiovascular diseases and patients with normal hearts. The diagnostic threshold gives a sensitivity of 98 %, specificity of 79 % with area under the ROC curve of 0.93. The NT-pro BNP values increased more strikingly in heart failure than BNP, as is shown in other studies²⁻⁵. Clear separation between normal and abnormal values of NT-proBNP suggests that NT-proBNP is useful and relevant in monitoring cardiovascular diseases. Our results also showed that 78 % patients with congenital heart disease and non-coronary heart disease etiology of heart involvement and 85 % patients with coronary artery disease etiology showed high levels of NT-proBNP. These findings also support the theory that NT-proBNP is clinically useful marker for monitoring various types of heart diseases of different etiology. Nevertheless, our results differ from the conclusions of our previous study¹⁰ showing the similar performance of both brain natriuretic peptides in patients with various forms of left ventricular systolic dysfunction. Various studies have pointed out the role of brain natriuretic peptides as a markers of myocardial dysfunction^{11,12}. From 42 patients included in our study, three patients had a history of myocardial infarction, their levels of BNP and NT-proBNP were elevated above the cut off values, Thus, brain natriuretic peptides remain valid for assessing the monitoring of myocardial infarction. The fact that NT-proBNP seems to be more accurate in identifying patients with heart dysfunction could be attributed to the longer half life of NT-proBNP.

CONCLUSION

Our findings have confirmed, that the measurement of brain natriuretic peptides, especially NT-proBNP is useful and relevant in the diagnosis of various types of cardiovascular diseases including congenital heart diseases.

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