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CASE REPORT

Falsely high B-type natriuretic peptide concentration in patients without heart failure attributed to AxSYM assay: case series of eight subjects

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Abstract

We report a case series of eight subjects complaining of non-specific chest pain without heart failure, but with apparent high concentrations of plasma B-type natriuretic peptide (BNP). No positive clinical characteristics were identified in physical examinations, cardiac imaging, laboratory findings, or pulmonary function tests. However, we observed unusually high BNP values when analysing blood samples of the patients using the AxSYM assay, and this was not supported by readings from Triage® or ADVIA Centaur® assays on the same samples, which showed BNP within the normal range. We believe that the possibility for false readings for high BNP levels in healthy individuals measured by AxSYM assay should be taken into account by physicians in clinical practice to avoid medical errors.

Keywords B-type natriuretic peptide; ProBNP N-terminus; Automated-immunoassay comparison

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Introduction

Both the B-type natriuretic peptide (BNP, amino acids 1–32) and the N-terminus of proBNP (NT-proBNP, amino acids 1–76) are synthesized and secreted from the precursor molecule proBNP (108 amino acids) during conditions of ventricular myocyte stretching due to heart volume overloading or ischemia.[1] Both serve as important biomarkers and are commonly used to acquire a diagnosis and prognosis for heart failure patients.[2] However, a previous study has indicated that various factors are associated with high concentrations of BNP and NT-proBNP in non-cardiac disease,[3] and a case report has suggested that the effect of genetic variation should be taken into account regarding the synthesis and secretion of BNP.[4] The underlying causes and implications of extremely high plasma BNP levels in healthy subjects are poorly understood. We previously reported a singular case with extremely elevated BNP levels in a young girl with non-specific chest discomfort and have since identified more patients with similar characteristics. We hereby report our findings for eight subjects who presented with non-specific chest discomfort absence in history of heart disease, who appeared to have extraordinarily high concentrations of BNP. We now believe these initial concentrations to be erroneous readings generated by the AxSYM assay.

Case series

Eight subjects (55 ± 19 years, female, $n = 5$) were identified with non-specific chest pain or discomfort, out of a total of 1780 patients admitted to the Dong-A University Hospital outpatient clinic between January 2008 to October 2011. These patients had no previous history of cardiac disease, stroke, diabetes mellitus, pulmonary disease, renal insufficiency, or specific medication use. Baseline clinical characteristics were unremarkable including physical examination, electrocardiogram, and chest X-ray. All echocardiograms showed normal wall motion and ejection fractions without significant valvular dysfunction. Coronary angiographies for six subjects revealed normal left-ventricular end diastolic pressure, as well as absence of significant stenosis, and spirometry was within normal limits (Tables *1* and *2*).

Table 1. Baseline clinical characteristics

Demographic characteristics	Subjects ($n = 8$)
Age (years)	55 ± 19
Men	3 (37.5)
BMI (kg/m^2)	25.68 ± 3.07
Associated conditions	
Hypertension	3 (37.5)
Diabetes mellitus	0 (0)
Currently smoking	1 (12.5)
Atrial fibrillation	0 (0)
Previous MI	0 (0)
Previous stroke and TIA	0 (0)
COPD	0 (0)
Renal insufficiency	0 (0)
Medication	
ACE inhibitor	0 (0)
β-blocker	2 (25)
CCB	3 (37.5)
Digoxin	0 (0)
Vital status	
SBP (mm Hg)	122.9 ± 9.7
DBP (mm Hg)	78.1 ± 11.6
Heart rate (per minute)	70 ± 12
Laboratory tests	
White blood cells (10^3/μL)	6.95 ± 1.27
Red blood cells (10^6/μL)	4.76 ± 0.30
Haemoglobin (g/dL)	10.7 ± 1.5
Platelet count (10^3/mm^3)	212.0 ± 49.8
Fasting glucose (mg/dL)	105.0 ± 14.6
Creatinine (mg/dL)	0.98 ± 0.24
Total cholesterol (mg/dL)	192.17 ± 60.4
CRP (mg/dL)	0.48 ± 0.21
Echocardiographic data	
LVEF (%)	63.6 ± 6.3
LVEDd (mm)	48.6 ± 4.9
Coronary angiography data	
No. of disease vessel	0 (0)
LVEDP (mm Hg)	10 ± 2
Pulmonary function test	
FEV_1 (% pred)	93.3 ± 11.3
FEV_1/FVC	80.0 ± 8.7

Data are presented as mean ± SD or number (%).
BMI, body mass index; MI, myocardial infarction; TIA, transient ischemic attack; COPD, chronic obstructive pulmonary disease; ACE, angiotensin-converting enzyme; CCB, calcium channel blocker; SBP, systolic blood pressure; DBP, diastolic blood pressure; CRP, C-reactive protein; LVEF, left-ventricular ejection fraction; LVEDd, left-ventricular end diastolic dimension; LVEDP, left-ventricular end diastolic pressure; FEV_1, forced expiratory volume in 1 s; % pred, % of predicted; FVC, forced viral capacity.

Extremely high BNP levels were detected using an AxSYM Plus BNP assay (Abbott Diagnostics, IL, USA), with some of the samples repeatedly measured, as shown in Table *2*. However, we obtained readings for normal BNP levels in these same samples, when measured by other natriuretic peptide tests including Triage® BNP Meter (Biosite, San Diego, CA, USA) and ADVIA Centaur® CP (Bayer Diagnostics, Tarrytown, New York, USA). We analysed BNP plasma samples taken again from some of these subjects with a follow-up period of 551 ± 505 days, with values still reading as markedly elevated. There were no significant differences in these values compared with the baseline BNP levels (2828.39 ± 387.42 vs. 2930.95 ± 739.87 pg/mL, $P = 0.812$) as measured using the AxSYM immunoassay.

Discussion

We report mismatched clinical findings for eight subjects with unusually high BNP levels despite re-analysis of the samples (Table *2*). This phenomenon of extremely high values was determined to be unrelated to volume overloading or other conditions associated with elevated BNP levels, such as hypertension or renal insufficiency. Most of the patients involved visited the cardiology outpatient clinic due to non-specific discomfort or pricking sensations. Their coronary angiograms (6/8) as well as left-ventricular end diastolic pressures were within the normal range. These discrepancies led us to investigate further using other measurement tools including proBNP levels, and these values were all later found to be within the normal range. We therefore hypothesized that the microparticle enzyme immunoassay method, which is a biological component of AxSYM, is responsible for the misreading of high BNP concentrations. Previous microparticle enzyme immunoassay method comparison studies have reported that AxSYM readings are in general agreement with Triage and ADVIA Centaur readings, and these measurements have become commercially available for clinical evaluation.[5,6] The variation in BNP levels with regard to intra- and inter-

Table 2. BNP (pg/mL) and NT-proBNP (pg/mL) biomarkers as measured by AxSYM, Triage, and ADVIA automated natriuretic peptide assays

				AxSYM					
No.	Sex	Age (years)	CAG	M 1	M 2	M 3	Triage BNP	ADVIA BNP	ADVIA NT-proBNP
1	M	57	N	1700	2576.87	—	6.8	6.17	21.53
2	M	35	—	2251.30	2654.85	2686.58	8.8	9.26	12.64
3	M	69	N	2995.06	3000	2741.74	23.8	22.26	46.49
4	F	20	N	2982.31	2904.49	4000	9.1	12.71	149
5	F	74	N	3084.87	1679.15	2295.47	5	2	6.57
6	F	67	N	748.92	—	—	27	<2	71.24
7	F	67	N	1959.08	—	—	21.9	12.56	71.37
8	F	47	—	2120.09	—	—	14.8	17.59	50.97

BNP, B-type natriuretic peptide; NT-proBNP, N-terminus of proBNP; CAG, coronary angiography; N, non-significant stenosis; M 1–3, repeated measurements 1–3.

individual findings is a significant issue when considering a large number of healthy subjects.[7] Storti *et al.* proposed that different BNP values were dependent on specific antibodies with different BNP enzyme immunoassay methods.[8] In addition, different epitopes were recognized in these enzyme immunoassays in terms of the antigen–antibody reactions,[9] which can be attributed to variability in the degradation products of BNP in specific samples.

The cause of the false elevated BNP values may be a result of an abnormal immunoassay response with either interference from heterophilic antibodies, human anti-mouse antibodies (stated in the limitations of the AxSYM BNP immunoassay in the package insert), or rheumatoid factors, which can commonly lead to false results on sandwich-type assays,[10,11] as well as BNP results.[12] To exclude possible confounding factors, we analysed rheumatoid factors and heterophilic antibodies including antibodies to Epstein-Barr virus, using the plasma obtained from the whole blood specimens. We also evaluated the hemolytic indices of the samples. All measurements produced negative results. Because the AxSYM assay used EDTA plasma samples, concern remained for high fibrinogen or fibrin levels,[13] and the fibrinogen concentrations of the samples were not measured directly. However, gross examination of the specimens revealed no abnormal findings, and there were no visible fibrin strands.

A previous genetic study demonstrated that specific single nucleotide polymorphisms (rs198389, −381CC genotype) of the natriuretic peptide precursor B gene were associated with elevated BNP levels.[14] However, as these single nucleotide polymorphisms caused abnormal values in all other tests, we do not believe gene variations to be responsible for the findings in our case series.

We therefore suggest that the AxSYM enzyme immunoassay may sometimes produce misleading BNP results, which should be taken into account in clinical settings. In such cases, the Triage or ADVIA Centaur BNP automated-immunoassays are likely to be more accurate methods for use. Further studies are necessary to determine the cause of these false readings.

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Conflict of interest

The authors declare no conflicts of interest.

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