Ability of NT-pro-BNP to Diagnose Cardioembolic Etiology in Patients with Acute Ischemic Stroke

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Ability of NT-pro-BNP to Diagnose Cardioembolic Etiology in Patients with Acute Ischemic Stroke

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Abstract

Background

Cardioembolic stroke (CE) is usually associated with a larger ischemic area leading to higher morbidity and mortality rates. No biomarkers for CE are available, which causes difficulty in differential diagnosis of CE from other subtypes of acute ischemic stroke.

Methods

We prospectively evaluated consecutive patients with acute ischemic stroke to identify biomarkers that could distinguish between CE and other subtypes of acute ischemic stroke. Etiological diagnoses were identified according to the National Institute of Neurological Disorders and Stroke (NINDS) Ⅲ classification using clinical examinations, computed tomography (CT), magnetic resonance imaging (MRI), cardiac evaluations, and other tests. The biomarkers N-terminal pro-brain natriuretic peptide (NT-pro-BNP), Thrombin-Antithrombin Ⅲ Complex (TAT), and D-dimer were determined in blood samples collected within 48 hours of onset and compared between groups with and without CE. Non-CE consisted of atherothrombotic brain infarction (ATBI), lacunar infarction (LI), and other stroke subtypes of unknown cause (other).

Results

This study included 279 patients diagnosed with acute ischemic stroke. Serum levels of NT-pro-BNP were significantly higher in those with than in those without CE stroke ($p<0.0001$). Analysis of receiver operating characteristics (ROC) curves indicated that an NT-pro-BNP cutoff of 332 pg/mL provided optimal sensitivity (98.3%) and specificity (75.8%) for distinguishing CE from non-CE.

Conclusions

Serum levels of NT-pro-BNP may help in diagnosis of CE during the acute phase and thus allow appropriate therapy to prevent subsequent cardiogenic stroke.

Key Words: Cardioembolic stroke; Acute ischemic stroke; Biomarker; NT-pro-BNP

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Introduction

Stroke is the third leading cause of mortality and the most common cause of morbidity in industrialized countries[1]. Acute ischemic stroke accounts for approximately 70% of all strokes and is generally classified into several subtypes, including cardioembolic stroke (CE), atherothrombotic brain infarction (ATBI), lacunar infarction (LI), and other stroke subtype of unknown cause, according to the classification of the National Institute of Neurological Disorders and Stroke (NINDS) Ⅲ in 1990 in the United States[2]. Therapeutic strategies for ischemic stroke and long-term management to prevent subsequent stroke events differ depending on the stroke subtype. Therefore, it is critical to define the stroke subtype to provide patients with appropriate therapies.

Cardioembolic stroke is defined as ischemic stroke caused by thrombus due to left ventricular dysfunction, or paradoxical brain embolism delivered through shunt flow from the right to left ventricle. It is usually associated with a larger ischemic area than other subtypes of ischemic stroke, which leads to higher morbidity and mortality rates. Cardioembolic stroke is usually diagnosed based on clinical manifestations as well as the presence of atrial fibrillation (Af) on admission or a history of certain conditions, such as cardiac valve surgery, cardiomyopathy, rheumatoid cardiac disease, and acute myocardial infarction. However, diagnosis of CE can be challenging, particularly when electrocardiograms (ECG) of patients with paroxysmal Af appear normal on admission. Therefore, CE is often confused with other subtypes of acute ischemic stroke.

Several biomarkers, such as hemostatic markers, platelet aggregation factors, D-dimer, and high sensitive C-reactive protein (CRP), were shown to be useful to identify the etiology of acute ischemic stroke[3,4]. For example, D-dimer, a product of fibrin degradation, appears in the circulation when the coagulation system has been activated and red fibrin-rich thrombi have been formed. These red clots typically originate in diseased cardiac chambers, and are therefore associated with the etiology of CE stroke[5-7]. Accordingly, elevated D-dimer may suggest CE even in patients with normal ECG findings on admission.

Brain natriuretic peptide (BNP) can improve the diagnostic accuracy of CE[8]. BNP is formed as a neurohormone mainly by the heart and brain[9,10] after cleavage of a propeptide into a biologically active BNP and biologically inactive N-terminal pro-brain natriuretic peptide (NT-pro-BNP). Both forms are detectable in the systemic circulation. In fact, a previous study showed that plasma BNP levels >76 pg/mL are closely associated with CE and can be used to diagnose CE with a sensitivity of 72% and specificity of 56%[8]. Measuring plasma levels of BNP and NT-pro-BNP may have different advantages because they have different biochemical and physiological characteristics; NT-pro-BNP and BNP are comprised of 76 and 32 amino acids, respectively. In addition, NT-pro-BNP is released into the circulation as an inactive form, and it has a longer plasma half-life than BNP (60 vs 15-20 min, respectively)[11]. That is, NT-pro-BNP degrades more slowly than BNP both in vivo and in vitro. Accordingly, NT-pro-BNP is considered to be more stable in the systemic circulation with less biological variability and a higher circulating concentration. These characteristics suggest that NT-pro-BNP may be a more sensitive biomarker to predict CE etiology; however, little is known about the significance of measuring plasma NT-pro-BNP levels in stroke patients. Therefore, the present study was performed to determine the diagnostic value of NT-pro-BNP and whether plasma NT-pro-BNP level on admission can serve as a useful biomarker to differentiate CE from other subtypes of acute ischemic stroke.

Methods

Patients

Among patients diagnosed with acute ischemic stroke and treated at our hospital between January 2009 and December 2011, those admitted and treated for acute ischemic stroke within 48 hours of onset were included in this study. Patients admitted ≧48 hours of onset, those with an undefined time of onset, and those with transient ischemic attack (TIA) without evidence of acute ischemic lesions on magnetic resonance (MR) diffusion weighted images (DWI) were excluded.

Sampling blood biomarkers

Blood samples were collected into chilled plastic tubes containing ethylenediaminetetraacetic acid (EDTA) within 24 hours of admission. Then, D-dimer and Thrombin-Antithrombin Ⅲ Complex (TAT) were assayed using an automated latex endpoint method and plasma NT-pro-BNP concentrations were determined using a specific immunoradiometric assay (Roche Diagnostics®, Tokyo, Japan)[12].

Stroke classification

Stroke was classified as CE, ATBI, LI, and other subtypes, including stroke of unknown cause, according to the NINDS-Ⅲ criteria[13]. We defined CE as the presence of potential heart disease or arrhythmia that could comprise an embolic source, a history of sudden onset, and radiological findings of stroke lesion consistent with embolism in the main cerebral artery or findings of scattered stroke lesions based on cognate embolic sources. Stroke subtypes were finally confirmed by stroke specialists blinded to the results of biomarkers, and were based on clinical course, detailed medical history, and MR imaging as well as MR angiographic findings.

Statistical analysis

We used medians for nominal and ordinal variables and means and standard deviation (SD) for continuous variables. The statistical significance of intergroup differences was assessed by Pearson's chi-square test for categorical variables, and Student's *t* test or the Mann-Whitney *U* test for continuous variables. Receiver operating characteristics (ROC) curves were constructed to estimate the optimal cutoff with which to diagnose CE and to calculate sensitivity and specificity. In all analyses, $p<0.05$ was taken to indicate statistical significance. Biomarkers were included as dichotomous variables. We adjusted values <0.5 μg/mL to 0.25 μg/mL because the limit of D-dimer detection was 0.05 μg/mL. The data were similarly adjusted for TAT (<2.0 ng/mL was adjusted to 1.0 ng/mL). All data were analyzed using JMP version 9 software.

Results

Between January 2009 and December 2011, 621 patients were admitted to our hospital for stroke treatment. Among them, 449 were diagnosed as having acute ischemic stroke. Of these, 170 were excluded as follows: unknown time of onset (n=40), admission ≧48 hours after onset (n=75), and final diagnosis of TIA without ischemic findings on DWI despite temporary symptoms (n=55). Therefore, the study population finally consisted of 158 (56.6%) male and 121 (43.4%) female patients (total, n=279) with a mean age of 74.2±15.6 years. Table 1 shows the characteristics of the patients, risk factors, and CE and non-CE subtypes.

The subtypes of the 279 patients were finally diagnosed as CE, ATBI, LI, and others in 60 (21.5%), 77 (27.6%), 126 (45.2%), and 16 (5.7%) cases, respectively. The patients were then categorized as CE (n=60, 21.5%) and non-CE (n=219, 78.5%), consisting of ATBI, LI, and others.

There were no differences in sex, hypertension, diabetes mellitus, hyperlipidemia, smoking habit,

Table 1. Patient characteristics and risk factor profiles

	CE (n=60)	non-CE (n=219)	p-value
age, mean±SD (min-max)	79.6±9.0 (53-96)	72.8±16.8 (60-78)	<0.01
male (%)	28 (46.7)	130 (59.4)	NS
hypertension (%)	43 (71.7)	170 (77.6)	NS
diabetes mellitus (%)	13 (21.7)	60 (27.4)	NS
dyslipidemia (%)	18 (30)	92 (42)	NS
smoking (%)	14 (23.3)	83 (37.9)	NS
alcohol (%)	18 (30.0)	80 (36.5)	NS
Af (%)	52 (87.7)	14(6.4)	<0.01
pulmonary disease (%)	6 (10.0)	17 (7.8)	NS
renal failure (%)	13 (21.6)	42 (19.1)	NS
NIHSS, mean±SD (min-max)	10.8±8.7 (1-30)	4.5±5.0 (1-30)	<0.01

Data are shown as means or numbers (%). CE, cardioembolic stroke; non-CE, non-cardioembolic stroke; and NIHSS, The National Institutes of Health Stroke Scale.

alcohol consumption, pulmonary disease or renal failure, between the CE and non-CE subgroups. On the other hand, age differed significantly between the two groups. At the same time, the clinical National Institute of Health Stroke Scale (NIHSS) on admission in CE subgroup was significantly higher than that in non-CE subgroup.

Levels of the biomarkers, NT-pro-BNP, TAT, and D-dimer, were measured. The amount of plasma NT-pro-BNP was significantly higher in the CE than the non-CE subgroup ($p<0.0001$) (Fig. 1A). Analyses of ROC curves indicated that a cutoff of ≧332 pg/mL provided the best sensitivity and specificity for distinguishing CE from non-CE (98.3% and 75.8%, respectively) (Fig. 2). The positive predictive value (PPV) was 53.6%, and the negative predictive value (NPV) was 99.4%. In contrast, serum levels of neither D-dimer nor TAT differed significantly between the two groups (Figs. 1B and 1C).

Among the 60 patients finally diagnosed as having CE, 52 patients had Af on ECG at the time of admission, whereas the remaining eight patients did not present Af on admission. Four of the eight patients without AF on admission were initially misdiagnosed as after having ATBI and finally diagnosed with CE because continuous ECG monitoring revealed paroxysmal Af after admission. If the presence of Af was used as a biomarker to diagnose CE, sensitivity and specificity were 86.7% and 93.6%, respectively, and PPV and NPV were 78.8% and 96.2%, respectively.

Discussion

We investigated 279 patients with acute ischemic stroke to determine whether plasma level of NT-pro-BNP on admission could be used as a marker to differentiate between stroke subtypes based on etiology, particularly that of CE. Plasma levels of NT-pro-BNP upon admission were significantly higher in patients with than without CE.

Several studies have shown that some blood biomarkers can differentiate etiological subtypes of acute ischemic stroke[7,8,14-16]. Coagulation and fibrinolytic abnormalities may play important roles in the pathogenesis of ischemic stroke. Plasma levels of biomarkers, such as D-dimer produced during fibrin degradation and TAT produced during the generation of thrombin, are related to CE in stroke etiology[6]. Ageno et al reported previously that D-dimer was a useful biomarker of CE stroke in a

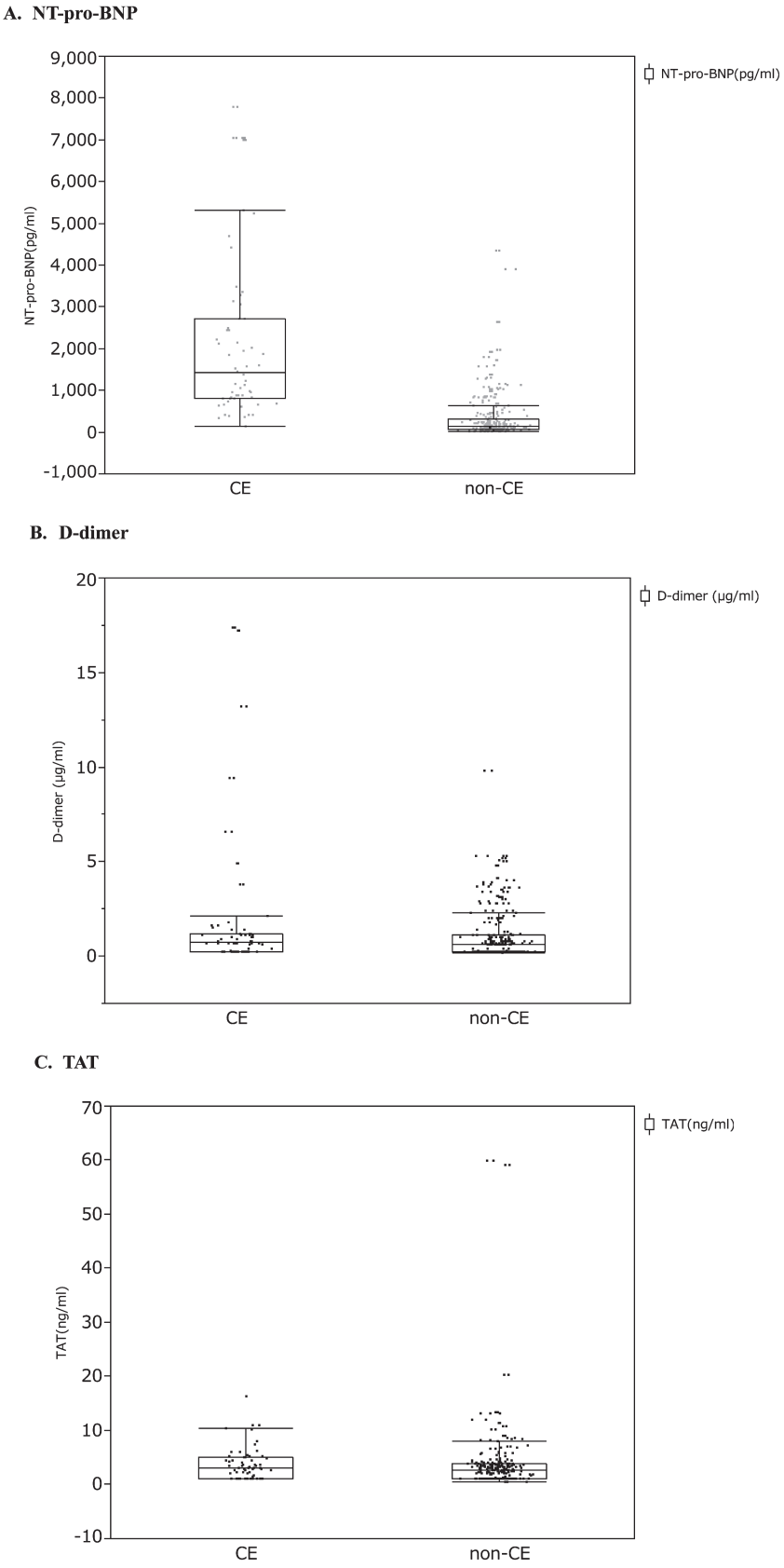

Figure 1. Comparison of biomarkers between cardioembolic stroke (CE) and non-CE groups. Plasma levels of N-terminal pro-brain natriuretic peptide (NT-pro-BNP) were significantly higher in patients with than without CE (A), whereas those of D-dimer (B) and Thrombin-Antithrombin Ⅲ Complex (TAT) (C) were not significantly different between the two groups.

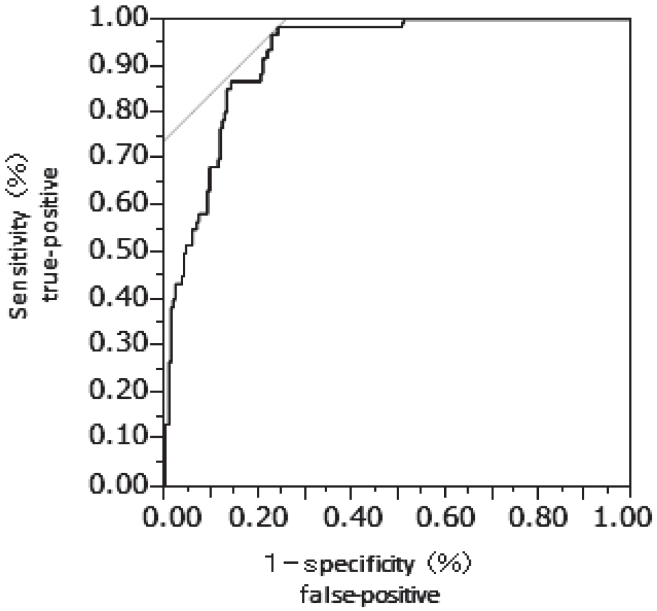

Figure 2. Receiver operating characteristic (ROC) curves showing the ability of N-terminal pro-brain natriuretic peptide (NT-pro-BNP) to distinguish between cardioembolic stroke (CE) and non-CE stroke subtypes.

cohort of 126 patients, and that the optimal cutoff for predicting CE was 2.00 μg/mL with specificity and sensitivity of 93.2% and 59.3%, respectively[7]. We found that neither serum level of D-dimer nor TAT differed significantly between CE and non-CE groups.

Similarly, neurohormones, such as BNP, pro-BNP, and NT-pro-BNP, are useful biomarkers of CE in patients with acute ischemic stroke of unknown etiology[8,14]. For example, Montaner et al reported that BNP and D-dimer were independent predictors of CE in 707 patients with acute ischemic stroke[8]. Moreover, Shibazaki et al recently reported that the optimal cutoff plasma level of BNP to distinguish CE from other stroke subtypes is 140.0 pg/mL[14]. Rodriguez-Yanez et al found that pro-BNP >360 pg/mL was independently associated with CE in 262 patients with acute ischemic stroke within the first 12 hours of onset[17]. Fonseca et al examined 92 patients with ischemic stroke, including 28 with CE, and reported that mean NT-pro-BNP values were significantly higher for patients with than without CE[18]. We also found significantly higher mean NT-pro-BNP level in patients with than without CE. Analyses of ROC curves showed that an NT-pro-BNP cutoff of 332 pg/mL on admission could identify patients with CE with 98.3% sensitivity and 75.8% specificity. Thus, high NT-pro-BNP levels can indicate CE as the etiology of stroke.

The mechanism by which serum NT-pro-BNP level increases in patients with CE is not fully understood. The NT-pro-BNP level becomes elevated in patients with heart failure, and it is therefore used as a supplementary tool for assessing cardiovascular function[16,19]. Levels of NT-pro-BNP, a neurohormone that is mainly formed by the heart and brain, are also increased in patients with Af[20-22]. In addition, left atrial (LA) diameter increases during Af, including paroxysmal Af, and a large LA diameter leads to heart failure[21,23]. Therefore, the level of NT-pro-BNP should increase in patients when Af is the cause of heart failure[24-26]. Accordingly, NT-pro-BNP could serve as a biomarker to differentiate CE in patients with acute ischemic stroke[18,27].

The advantage of measuring NT-pro-BNP is that its higher molecular weight renders it more biologically stable in the systemic circulation than either BNP or pro-BNP. Thus, the plasma half-life

of NT-pro-BNP is far longer than those of BNP and pro-BNP. This clinically useful feature of NT-pro-BNP makes it convenient as a biomarker in patients with acute ischemic stroke.

In this study, the NT-pro-BNP was shown to be superior in sensitivity as compared to a conventional biomarker, the presence of Af. This finding means that CE should be included in the differential diagnosis when the NT-pro-BNP is high even though Af is absent at the time of admission. Indeed, the four patients who did not show AF on admission and finally diagnosed as having CE had significantly high NT-pro-BNP level. However, using only NT-pro-BNP as a biomarker of CE has some potential limitations. Several factors affect plasma levels of NT-pro-BNP. For example, heart failure, such as Af, left ventricular dysfunction, and chronic renal failure, are correlated with increased NT-pro-BNP levels in elderly patients[24,28,29]. Indeed, 51 patients in non-CE subgroup had plasma NT-pro-BNP levels above the 332 pg/mL cutoff determined from our ROC curves. The patients in CE subgroup were older than those in non-CE subgroup (mean age, 76.9 vs 72.7 years, respectively) and there were 13 cases with chronic renal failure in CE subgroup against to 42 cases in non-CE subgroup. These factors could have affected the NT-pro-BNP level resulting in misdiagnosis of stroke subtype.

In conclusion, serum levels of NT-pro-BNP are significantly higher in patients with than without CE. A cutoff value of 332 pg/mL can distinguish CE from non-CE stroke with acceptable diagnostic accuracy. Despite the small study cohort, our results suggest that measuring serum levels of NT-pro-BNP may help to identify CE among patients with acute ischemic stroke on admission even when CE is not initially suggested.

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