Noninvasive Surrogate Markers to Predict Thrombogenesis in Patients with Nonvalvular Atrial Fibrillation

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Background: Atrial fibrillation (AF) confers a substantial increase in the risk of stroke. While the CHADS2 score is considered a reliable predictor of stroke/thromboembolism risk in patients with AF, thromboembolism can occur even in AF patients with a low CHADS2 score (CHADS2 score = 0 or 1). We retrospectively assessed the relationships between left atrial appendage thrombus (LAAT) and patient characteristics, echocardiographic variables, and surrogate blood markers in patients with unanticoagulated AF, in order to determine whether thrombogenesis can be predicted in this patient population.

Methods: The study group was comprised of 31 patients with unanticoagulated persistent non-valvular AF (NVAF) who underwent transthoracic echocardiography (TTE) and transesophageal echocardiography. Patients were divided into those with and without LAAT, and study variables were compared between the 2 groups.

Results: LAAT was found in 14 patients. AF duration, left atrial diameter, left ventricular ejection fraction, left atrial appendage flow, β-thromboglobulin, platelet factor 4, prothrombin time, activated prothrombin time, fibrinogen, D-dimer, and antithrombin III levels did not differ between patients with and without LAAT. However, ANP and BNP levels were significantly higher in patients with LAAT (ANP ≥79 pg/ml, P = 0.03, BNP ≥120 pg/ml: P = 0.03). Moreover, patients with BNP ≥120 pg/ml exhibited an increased risk for LAAT (P = 0.03, odds ratio: 6.19), and patients with ANP ≥70 pg/ml in addition to BNP ≥120 pg/ml exhibited a further augmented risk for LAAT (P = 0.055, odds ratio: 8.75).

Conclusions: In patients with unanticoagulated NVAF, a BNP level ≥120 pg/ml or an ANP level ≥70 pg/ml and BNP level ≥120 pg/ml could prove useful in predicting LAAT.

Key words: atrial fibrillation, left atrial thrombus, atrial natriuretic peptide, brain natriuretic peptide

Introduction

Systemic thromboembolism, including that responsible for ischemic stroke or transient ischemic attack, can arise as a serious complication in patients with atrial fibrillation (AF). Several randomized prospective trials have confirmed that warfarin administration significantly reduces the risk of stroke in patients with non-valvular AF (NVAF) and have provided a basis for guidelines promoting the use of warfarin in patients with AF. The congestive heart failure, hypertension, age, diabetes mellitus, and stroke (CHADS2) scoring system, which is easy for physicians to remember and apply, has been widely validated as a means of risk stratification for predicting stroke in patients with NVAF. Current guidelines recommended anticoagulant therapy for patients with a CHADS2 score ≥2, mainly because the risk of ischemic stroke outweighs the risk of bleeding associated with anticoagulant therapy. However, thromboembolism can occur even in patients with a low CHADS2 score (CHADS2 score 0: 1.9%/year; CHADS2 score 1: 2.8%/year). It is well known that the left atrial appendage (LAA) is a major cardioembolic source in patients with AF who have suffered a stroke. We conducted a retrospective study of the relation between LAA thrombus (LAAT) and patient characteristics, transthoracic and transesophageal echocardiographic variables, and blood markers in patients with persistent AF to determine whether thrombogenesis can be predicted in patients with NVAF.

Methods

Study patients

We identified, for the study, 31 patients (22 men and 9 women, aged 62 ± 8 years) with unanticoagulated persistent NVAF who had undergone transthoracic echocardiography (TTE) and transesophageal echocardiography (TEE). No patient with structural heart disease such as ischemic cardiomyopathy, dilated cardiomyopathy, hypertrophic cardiomyopathy,
Echocardiography

TTE and TEE were performed with an ACUSON Sequoia C256 echocardiography system (Siemens Medical Solutions, Inc., Malvern, PA, USA). Left atrial dimension (LAD) was measured in the parasternal long axis view at end-systole, and left ventricular ejection fraction (LVEF) was assessed by means of M-mode echocardiography (Teichholtz method). A 5-MHz phased-array probe was used for TEE. LAA flow velocity was obtained at the outlet of the LAA by the pulsed Doppler method. A thrombus was defined as a circumscribed and uniformly echodense intracavitary mass that was distinct from the underlying left atrium or LAA endocardium, and the pectinate muscles in more than 1 image plane, and also disappearance of LAAT was confirmed by a repeat TEE after 6–8 weeks of strict anticoagulation therapy.

Hematologic measurements

Blood samples were collected from the femoral artery at the time of echocardiography for measurement of baseline plasma atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP) levels. ANP and BNP were measured with the use of chemiluminescent enzyme immunoassay kits (Shionogi Co., Ltd., Tokyo, Japan). Plasma hemostatic markers, i.e., β-thromboglobulin (β-TG), platelet factor-4 (PF-4), fibrinogen, d-dimer, antithrombin III (AT-III), prothrombin time (PT), and activated prothrombin time (APTT), were measure by routine laboratory methods.

Table 1 Clinical, echocardiographic, and hematologic variables in patients with and without LAAT

|                      | LAAT+ group (n = 14) | LAAT− group (n = 17) | P value |
|----------------------|----------------------|----------------------|---------|
| Age (months)         | 61.3 ± 7.5 (14)      | 63.2 ± 9.7 (17)      | 0.55    |
| AF duration (month)  | 61.3 ± 7.5           | 62.3 ± 10.9          | 0.55    |
| LAD (mm)             | 40.8 ± 5.1 (14)      | 41.2 ± 5.5 (17)      | 0.84    |
| LVEF (%)             | 69.9 ± 12.0 (14)     | 68.5 ± 11.8 (17)     | 0.75    |
| LAA flow velocity (cm/seconds) | 37.9 ± 24.7 (13)   | 36.1 ± 25.9 (12)     | 0.86    |
| β-TG (ng/ml)         | 91.8 ± 55.0 (8)      | 84.7 ± 49.0 (11)     | 0.77    |
| PF-4 (ng/ml)         | 41.2 ± 34.5 (8)      | 48.7 ± 39.9 (11)     | 0.67    |
| Fibrinogen (mg/dl)   | 247.5 ± 56.7 (8)     | 267.6 ± 65.5 (11)    | 0.49    |
| d-dimer (ng/ml)      | 153.8 ± 140.4 (8)    | 94.2 ± 57.7 (11)     | 0.22    |
| AT-III (%)           | 96.4 ± 5.2 (8)       | 94.1 ± 11.6 (11)     | 0.61    |
| ANP (pg/ml)          | 82.3 ± 43.9 (13)     | 52.4 ± 20.2 (15)     | 0.03    |
| BNP (pg/ml)          | 165.5 ± 95.9 (13)    | 97.0 ± 66.1 (15)     | 0.03    |
| PT (seconds)         | 12.2 ± 1.3 (8)       | 11.5 ± 0.6 (11)      | 0.13    |
| APTT (seconds)       | 39.4 ± 4.7 (8)       | 36.6 ± 2.9 (11)      | 0.13    |

LAAT: left atrial appendage thrombus, + presence, − absence.
Mean ± SD values are shown. Numbers in parentheses are the numbers of patients for whom the data were available.

Table 2 Prediction of LAAT by ANP and BNP

|          | ANP ≥70 | ANP <70 | BNP ≥120 | BNP <120 | ANP ≥70 plus BNP ≥120 | ANP <70 or BNP <120 |
|----------|---------|---------|----------|----------|-----------------------|---------------------|
| LAAT+    | 6       | 7       | 9        | 4        | 5                     | 8                   |
| LAAT−    | 4       | 11      | 4        | 11       | 1                     | 14                  |

LAAT: left atrial appendage thrombus, + presence, − absence.
Number of patients is shown
ANP ≥70 pg/ml: odds ratio: 2.357, P = 0.25.
BNP ≥120 pg/ml: odds ratio: 6.188, P = 0.03.
ANP ≥70 pg/ml plus BNP ≥120 pg/ml: odds ratio: 8.75, P = 0.05.
Sensitivity: 38.5%.
Specificity: 93.3%.
Positive predictive value: 83.3%.
Negative predictive value: 67.9%.
**Patient groups and comparison of clinical variables**

To clarify the relation between LAAT and clinical, transthoracic and transesophageal echocardiographic variables, and blood markers in patients with persistent AF, we divided the study patients into 2 groups: those in whom LAAT was found upon TTE and TEE and those in whom LAAT was not found upon TTE and TTE. The following variables were compared between the 2 groups: age, duration of AF, LAD, LVEF, LAA flow velocity, β-TG, PF-4, fibrinogen, d-dimer, AT-III, ANP, BNP, PT, and APTT.

**Statistical analyses**

Values are shown as mean ± standard deviation (SD). Between-group differences in continuous variables were analyzed by Mann-Whitney U test. Odds ratios were computed for ANP levels (≥70 pg/ml and <70 pg/ml) and BNP levels (≥120 pg/ml and <120 pg/ml), and Fisher’s exact probability test was used to test association between these markers and risk of LAAT. All statistical analyses were performed with StatView 5.0 (SAS Institute Inc., Cary, NC, USA). P < 0.05 was considered statistically significant.

**Results**

No LAAT was detected by TTE. Our examination of the TEE images yielded the following 2 study groups: LAAT-positive group (n = 14) and LAAT-negative group (n = 17). Study variables are shown per patient group in Table 1. Neither age nor duration of AF differed significantly between these 2 groups. LAD, LVEF, and LAA flow velocity also did not differ significantly between the 2 groups. No significant between-group differences were found in β-TG, PF-4, fibrinogen, d-dimer, or AT-III levels or in PT or APTT. Plasma ANP and BNP levels, however, were both significantly higher in the LAAT-positive group than in the LAAT-negative group.

Distribution of ANP and BNP levels and associated odds ratios are shown on Table 2. Both BNP ≥120 pg/ml and ANP ≥70 pg/ml plus BNP ≥120 pg/ml conferred a risk of LAAT at odds ratios of 6.188 and 8.750, respectively.

We thus computed sensitivity and specificity of ANP (≥70 pg/ml vs. <70 pg/ml) and BNP (≥120 pg/ml vs. <120 pg/ml) for prediction of the presence or absence of LAAT. ANP ≥70 pg/ml plus BNP ≥120 pg/ml was of low sensitivity (38.5%) for prediction of LAAT, whereas ANP <70 pg/ml plus BNP <120 pg/ml was of high specificity (93.3%) for prediction of absence of LAAT.

In contrast, the ANP level alone could not differentiate between patients with and without LAAT (Table 2).

**Discussion**

We found that (1) LAD, LVEF, and LAA flow velocity did not differ significantly between patients with and without LAAT; (2) thrombogenesis (assessed on the basis of β-TG, fibrinogen, d-dimer, and AT-III) and platelet activation (assessed on the basis of PF-4) did not differ significantly between patients with and without LAAT; (3) plasma ANP and BNP levels were significantly higher in patients with LAAT than in those without LAAT; and (4) BNP ≥120 pg/ml alone and ANP ≥70 pg/ml plus BNP ≥120 pg/ml differentiated between patients with and without LAAT.

ANP is released from the atria in response to increased atrial pressure. Previous studies have shown that the plasma ANP concentration is useful to stratify patients with AF into a high risk group or a low risk group, and to separate the patients with acute cardioembolic stroke from lacunar stroke. A high plasma ANP level is a noninvasive surrogate indicator of left atrial thrombogenesis in patients with paroxysmal AF.

BNP is secreted from the ventricles, and BNP elevation is associated with left ventricular (LV) systolic and diastolic dysfunction. In addition, previous studies have shown that the BNP level correlates with the LV filling pressure. In the present study, the BNP level was higher in the LAAT-positive group than in the LAAT-negative group, but the BNP level in the LAAT-group was still higher than that of the normal population (18.4–40 pg/ml). The precise mechanism explaining this BNP elevation has not been elucidated, but we showed previously that the BNP level was significantly higher in patients with AF than in those without AF, despite similar LVEFs. LV diastolic dysfunction seems to increase secretion of BNP from the atria.

In the present study, plasma ANP and BNP levels were higher in patients with LAAT than in those without LAAT. Furthermore, BNP ≥120 pg/ml and ANP ≥70 pg/ml plus BNP ≥120 pg/ml could be shown to differentiate between patients with and without LAAT. In contrast, the ANP level alone did not differentiate between patients with and without LAAT. Van Den Berg et al. reported that ANP levels were lower in patients with long-standing AF, suggesting that ANP production is restricted as a result of degenerative changes in the atria inherent to chronic AF. This might explain why ANP is not a sensitive marker for predicting LAAT.

**Study limitations**

Our study was limited by its size and by its retrospective nature. Our data must be interpreted in light
of the inherent limitations of any single-center retrospective investigation. In addition, with the exception of age, patients’ characteristics were not included in the analysis.

Conclusions
Plasma BNP and ANP plus BNP might prove to be useful for predicting the presence of LAAT in AF patients without overt heart failure.

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