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Full Length Article

Association between P-selectin levels and left atrial blood stasis in patients with nonvalvular atrial fibrillation

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ABSTRACT

Background: P-selectin - a biomarker of platelet and endothelial cell activation is elevated in patients with non-valvular atrial fibrillation (NVAF). However, the association between sP-selectin level and thromboembolic complications in NVAF patients remains controversial. We tested the hypothesis that plasma soluble P-selectin (sPSL) level correlates with the measures of left atrial blood stasis in NVAF.

Methods: Plasma sPSL concentration was measured using solid-phase ELISA in 103 NVAF patients (age 63 ± 14 years; 26% women) and 48 normal sinus rhythm controls (NSR; age 64 ± 14 years; 41% women) who were not on aspirin. Within the group of NVAF cases, 27 had no spontaneous echocardiographic contrast (SEC) detected by transesophageal echocardiography, 31had mild SEC, 15 moderate, 20 severe, and 10 patients had left atrial appendage thrombus (LAAT).

Results: The median soluble sPSL level was higher in NVAF cases compared to NSR controls [(interquartile range) 26 (20 − 32) ng/mL vs 22 (15–29) ng/mL, $p = 0.0045$]. Only NVAF patients with CHA_2DS_2-VASc score ≥ 1 had higher sPSL level compared to NSR controls. Patients with severe SEC had significantly higher sPSL levels [32 (24–38) ng/mL] compared to all other NVAF patients ($p = 0.0042$) and to NSR controls ($p < 0.0001$). Also NVAF patients with LAAT had higher sPSL level compared to NSR controls.

Conclusions: There is a direct correlation between p-selectin level and severe blood stasis in the left atrium. Only NVAF patients with CHA_2DS_2-VASc score ≥ 1 or with LAAT had higher sPSL level compared to NSR controls.

1. Introduction

Atrial fibrillation carries an increased risk of first lifetime stroke and recurrent stroke. Compared to strokes of other etiologies, stroke in atrial fibrillation is associated with worse disability and higher mortality [1–4]. Cardioembolic stroke from non-valvular atrial fibrillation (NVAF) begins with the development of left atrial appendage thrombus (LAAT) [5–7]. This thrombus contain multiple platelet-rich areas [8,9] underscoring the histologic relationship between platelets and stroke in atrial fibrillation. P-selectin is a cellular adhesion molecule that mediates the interaction of activated platelets and endothelial cells with leukocytes and is a recognized biomarker of platelet activation and endothelial dysfunction [10]. Patients with atrial fibrillation have higher levels of soluble P-selectin (sPSL) relative to individuals in normal sinus rhythm (NSR) [11]. Elevated sPSL levels have been implicated as a measure of thrombotic propensity in NVAF by some but not all investigators [10–13].

The primary aim of this study is to determine the relationship between measures of sPSL and transesophageal echocardiographic (TEE) quantification of left atrial blood stagnation including the intensity of spontaneous echocardiographic contrast (SEC) and the presence of LAAT.

2. Materials and methods

2.1. Patients

Study design, patient selection, recruitment, clinical and echocardiographic data collection and assessment have previously been described [14]. This study protocol was approved by the Institutional Review Boards of the Mayo Clinic and Foundation and all research conduct was performed according to the ethical principles of the

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Declaration of Helsinki. All recruited individuals signed a consent form. Briefly, all patients with NVAF (cases) who underwent a clinically indicated TEE (October 4, 2007–April 27, 2009) were approached for study participation. Patients were excluded from participation if they had: acute illness, stroke, myocardial infarction or surgery within 30 days; more than moderate heart valvular disease; artificial heart valves; prior unprovoked venous or arterial thrombosis; prior major bleeding unrelated to warfarin therapy; liver disease; active malignancy; or hormonal stimulation (estrogen/progesterone therapy or pregnancy). Control subjects in normal sinus rhythm with no prior history of atrial fibrillation were recruited from the Primary Care Internal Medicine clinic during their annual medical exam. From our original cohort [14], a subgroup of subjects who were not treated with aspirin was randomly sampled for analysis of this study.

2.2. Echocardiographic data

TEE was performed as previously described [14]. LAAT was defined as an echogenic mass in the appendage or body of the atrium, distinct from the underlying endocardium and pectinate muscles and detected in more than one imaging plane [14,15]. SEC was defined as a pattern of dynamic "smokelike", slowly swirling, intracavitary echo-densities imaged with gain settings adjusted to eliminate background noise. SEC was graded as "absent", "mild", "moderate", or "severe" based on modified Fatkin et al. [16] echocardiographic criteria. LAAEV profiles were measured over 5 consecutive cardiac cycles using pulsed wave Doppler interrogation with the sample volume positioned 1 cm within the orifice of the left atrium appendage [17]. The left ventricular ejection fraction (LVEF) was visually estimated. Aortic atherosclerosis severity was defined as "simple" when atheroma thickness was < 4 mm and immobile. Severe atheroma was defined as atheroma exceeding 4 mm or containing mobile components [14–17]. Left atrial volume index (LAVI) was measured by transthoracic echocardiography performed within 1 month of the TEE study and calculated by the biplane area-length method [17]. All echocardiographic images were analyzed by the study cardiologist (NA) blinded to clinical and laboratory data. Control subjects in NSR did not have evaluations completed with TEE.

2.3. Study definitions and event adjudication

Congestive heart failure (CHF) was defined as the presence of clinical symptoms and signs of heart failure within the last three months with or without evidence of LV systolic dysfunction by echocardiography [18]. Diabetes mellitus was diagnosed based on the criteria recommended by the American Diabetes Association [19]. Stroke, TIA, and systemic embolization were defined by criteria proposed by the American Heart Association [20]. For cases, the presence of atrial fibrillation was confirmed by either electrocardiogram or Holter monitoring. For study analysis, atrial fibrillation was classified as either "non-permanent" (paroxysmal or persistent) or "permanent" in accordance with current guidelines [21]. The $CHADS_2$ and CHA_2DS_2-VASc score was assigned for each case and control [18,22].

2.4. Sample collection

For each subject, 20 mL of citrate blood was collected by antecubital venipuncture using a 19 gauge thin-wall "butterfly" needle with a short plastic tube extension. For NVAF patient (cases) scheduled for electric cardioversion or radiofrequency ablation, phlebotomy was uniformly collected prior to the procedure.

2.5. Assays of plasma soluble P-selectin

Plasma soluble P-selectin was measure in plasma by Human sP-Selectin/CD62P Immunoassay that is a 1.25 h solid phase ELISA utilizing human sP-Selectin and antibodies raised against the recombinant factor (R&D Systems, Inc. 614 McKinley Place NE, Minneapolis, MN 55413, USA). The minimum detectable dose of human sP-Selectin was < 0.5 ng/mL, intra-assay precision ranged CV: 4.9%–5.6% and inter-assay precision CV: 7.9%–9.9%.

2.6. Statistical analysis

Continuous variables (means ± standard deviation) were compared between groups using a two-sample *t*-test. Categorical variables were presented as counts (%) and compared using Pearson's Chi-square test for independence. Ordinal variables (%; median with quartiles) were compared using Wilcoxon rank-sum test. Pairwise correlations between clinical and echocardiographic characteristics by sPSL level also were performed. Cox regression was employed to analyze the association between circulating sPSL levels amongst cases with SEC and LAAT compared to controls. Statistical testing used the two-tailed alpha level of 0.05 for significance.

3. Results

3.1. Patients

Demographic characteristics of 103 NVAF cases and 48 NSR controls are presented in Table 1. There were no differences in age, gender, body mass index between the two groups. Apart from hypertension which was more prevalent amongst NVAF cases, other elements of the CHA_2DS_2Vasc risk tool did not differ. Accordingly, the mean score and the profile of $CHADS_2$ and CHA_2DS_2-VASc scores did not differ between NVAF cases and NSR controls. Not surprisingly, NVAF cases were significantly more often treated with warfarin. Amongst NVAF patients, demographic characteristics did not differ by duration of atrial fibrillation (data not shown).

Duration, type of atrial fibrillation and echocardiographic characteristics of NVAF cases are summarized in Table 2. Atrial fibrillation exceeded 1 month for the majority (80%) of patients and nearly 60% had documented dysrhythmia for more than one year. The majority (79%) of patients had "non-permanent" atrial fibrillation. Few patients (11%) had reduced ejection fraction. Simple atheroma of the thoracic aorta was noted in 60% with only a small minority (4%) with evidence of complex atheroma. Moderate to severe left atrium enlargement as

Table 1
Demographic and clinical variables.

Variable	NVAF[a] cases (N = 103)	NSR[b] controls (N = 48)	P
Female, n (%)	31 (30)	21 (44)	0.1030
Age, years (mean ± SD[c])	61.9 ± 14.8	62.3 ± 14.7	0.8749
65–74 years, n (%)	23 (22)	10 (21)	0.8358
≥75, n (%)	19 (19)	11 (23)	0.5215
Body mass index (mean ± SD)	30.9 ± 6.9	30.1 ± 7.2	0.4689
Congestive heart failure, n (%)	30 (29)	8 (17)	0.0918
Hypertension, n (%)	59 (57)	17 (35)	0.0119
Diabetes mellitus, n (%)	15 (15)	10 (21)	0.3419
Stroke/TIA prior, n (%)	12 (12)	4 (8)	0.5296
Vascular diseases, n (%)	19 (19)	5 (10)	0.1946
$CHADS_2$, mean ± SD	1.43 ± 1.4	1.12 ± 1.5	0.2329
score 0, n (%)	35 (34)	25 (52)	
score 1, n (%)	26 (25)	8 (17)	
score ≥ 2, n (%)	42 (41)	15 (31)	
CHA_2DS_2-VASc, mean ± SD	2.34 ± 2.0	1.87 ± 1.9	0.1791
score 0, n (%)	23 (22)	12 (25)	
score 1, n (%)	19 (19)	13 (27)	
score ≥ 2, n (%)	61 (59)	23 (48)	
Warfarin therapy, n (%)	79 (77)	1 (2)	< 0.0001
Statin therapy, n (%)	27 (26)	9 (19)	0.3088

[a] NVAF – non-valvular atrial fibrillation.
[b] NSR – normal sinus rhythm.
[c] SD – standard deviation.