Left Atrial Blood Stasis and Von Willebrand Factor–ADAMTS13 Homeostasis in Atrial Fibrillation

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Objective—Left atrial blood stasis is associated with increased risk for left atrial appendage thrombus (LAAT) and stroke in atrial fibrillation (AF). Von Willebrand factor (VWF) is associated with thromboembolism in AF. VWF thrombogenic activity is proportional to multimer size, which is regulated by VWF-cleaving protease (ADAMTS13).

Methods and Results—To assess the association between left atrial blood stasis and VWF-ADAMTS13 system, plasma VWF antigen (VWF:Ag), VWF activity (VWF:Act), and ADAMTS13 activity were measured in 414 consecutive patients with nonvalvular AF (age 63±13 years; 25% women) and in 100 patients (age 64±14 years; 39% women) with normal sinus rhythm. Spontaneous echocardiographic contrast (SEC), left atrial appendage emptying velocity, and LAAT were assessed by transesophageal echocardiography. Presence and intensity of SEC varied directly with VWF:Ag and VWF:Act but not with ADAMTS13 activity. AF patients with LAAT had higher VWF:Ag (200±61 versus 155±52, \( P=0.0006 \)) and VWF:Act (179±57 versus 141±51 \( P=0.0026 \)) compared with those without LAAT. VWF:Ag and VWF:Act were independent predictors of LAAT after adjustment for CHADS2 score (\( P=0.0179 \) and \( P=0.0497 \), respectively).

Conclusion—The association between VWF and SEC may explain the thrombotic propensity in AF. Elevated VWF:Ag may help identify AF patients at risk for LAAT. (Arterioscler Thromb Vasc Biol. 2011;31:2760-2766.)

Key Words: stroke ■ thrombosis ■ von Willebrand factor ■ atrial fibrillation

Cardioembolic stroke in the setting of atrial fibrillation (AF) begins with the left atrial appendage thrombus (LAAT) formation.1–3 The risk for LAAT development and stroke increases with left atrium mechanical function deterioration that is reflected by reduction in left atrial appendage emptying velocity (LAAEV), development of spontaneous echocardiographic contrast (SEC), and progression of left atrial distension.4–7 However, the underlying mechanism behind the relationship between AF, atrial stasis, and thrombotic propensity is complex and poorly understood.

Von Willebrand factor (VWF) is a large plasma glycoprotein that mediates platelet adhesion and aggregation,8 has been associated with stroke risk in AF.9–11 Prior studies comparing VWF levels between AF patients and subjects in normal sinus rhythm (NSR) were limited by small sample size.14–16 Assessment of archived plasma samples from Stroke Prevention in Atrial Fibrillation III participants lacked a comparator.9–11 Moreover, a prior association between elevated VWF and stroke became nonsignificant after adjustment for other clinical predictors.11 The association between VWF and AF therefore remains largely unexplored.

The thrombogenic potential of VWF is directly proportional to VWF activity (VWF:Act) determined by both plasma concentration and multimer size,17 which is regulated by VWF-specific protease ADAMTS13.18 Two patients may have similar plasma VWF content, yet thrombotic propensity will vary considerably depending on the proportion of high-molecular-weight multimer content. This nuance would be missed if mere antigen content was assessed.

Atrial distension is associated with overexpression of VWF multimers,19 and blood stasis has been shown to downregulate ADAMTS13 activity.20 Previous studies, however, have not systematically accounted for degrees of atrial stasis or measured complete variables within the VWF system.

We hypothesized that atrial distension and stasis in AF are associated with the higher VWF and increased proportion of large multimers that lead to the development of LAAT. To address this hypothesis, transesophageal echocardiography (TEE) measures of stasis and LAAT and measures of VWF antigen (VWF:Ag), VWF:Act, and ADAMTS13 activity were compared in consecutive patients with nonvalvular AF. To our knowledge, this concept has not previously been tested in these patients.

Methods

Patient Recruitment
All patients with nonvalvular AF who had TEE (October 4, 2007, to April 27, 2009) were approached for study participation. Exclusion
criteria included (1) acute illness, stroke, myocardial infarction, or surgery within 30 days; (2) more than moderate heart valvular disease; (3) artificial heart valves; (4) prior unprovoked venous or arterial thrombosis; (5) prior major bleeding unrelated to warfarin therapy; (6) liver disease; (7) active malignancy; or (8) hormonal stimulation (estrogen/progesterone therapy or pregnancy). The details related to AF onset, timing, chronicity, underlying etiologies, cardiac anatomy and physiology, relevant comorbidities, and medical and interventional treatment of AF, particularly anticoagulation, were collected prospectively and entered into SAS GEN database, which is an SAS-based system for managing clinical data. Control subjects consisted of patients in NSR with no prior history of AF. These subjects were recruited from the Primary Care Internal Medicine clinic during their annual medical examination.

Evaluation With TEE
TEE was performed as previously described using commercially available ultrasound instruments and a multiplane probe. 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. SEC was defined as a pattern of dynamic “smoke-like,” slowly swirling, intracavitary echo-densities imaged with gain settings adjusted to eliminate background noise. SEC was graded as absent, mild, moderate, or severe according to the published echocardiographic criteria. The LAAEV profiles were measured over 5 consecutive cardiac cycles using pulsing wave Doppler interrogation with the sample volume positioned 1 cm within the orifice of the LAA. The left ventricular ejection fraction was visually estimated. Aortic atherosclerosis severity was defined as simple when atheroma thickness was <4 mm and immobile. Severe atheroma exceeded 4 mm or contained mobile components. Given the known difficulties in measuring left atrial volume by TEE, left atrium volume index (LAVI) was assessed by transthoracic echo performed within 1 month of the TEE study and calculated by the biplane area-length method. All echocardiographic images were analyzed by the study cardiologist (N.A.), who was blinded to clinical and laboratory data.

Study Definitions and Event Adjudication
The CHADS2 score was assigned for each patient. The presence of AF was confirmed by either ECG or Holter monitoring. Congestive heart failure was defined as the presence of clinical symptoms and signs of heart failure within the last 3 months with or without evidence of left ventricular systolic dysfunction by echocardiography. Diabetes mellitus was diagnosed based on the criteria recommended by the American Diabetes Association. Stroke and transient ischemic attack were defined by criteria proposed by the American Heart Association. AF was classified as paroxysmal, persistent, or permanent in accordance with current guidelines.

Sample Collection
For each patient, 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 AF patient scheduled for electric cardioversion or radiofrequency ablation, phlebotomy was uniformly performed before this procedure.

Assays of Plasma VWF:Ag, VWF:Act, and ADAMTS13 Activity
VWF:Ag in plasma was measured using HemosIL von Willebrand Factor Antigen latex immunoassay kits (Instrumentation Laboratory, Lexington, MA) with 2 ACL TOP coagulation system analyzers (Beckman Coulter, Brea, CA), following the manufacturer’s instructions. VWF:Act in plasma was measured using HemosIL von Willebrand Factor Activity latex immunoassay kits (Instrumentation Laboratory) on 2 ACL TOP coagulation analyzers, following the manufacturer’s instructions. Briefly, prediluted patient samples (1:1 ratio of plasma to diluent) and latex particle–enhanced immunoturbidity reagents were added to testing cuvettes. Optical densities were measured at 405 nm, and VWF:Act was extrapolated from standard curves. The lower limit of normal for VWF:Ag is 55 IU/dL, and that of VWF:Act is 55 U/dL. The standard relationship between activity and antigen is greater than 0.7 (ratio of VWF:Act to VWF:Ag). The validated normal range of the VWF:Act/VWF:Ag ratio (n=452 normal donors) is 0.7 to 1.4, with mean at 1.0. ADAMTS13 protease activity (expressed as percentage of normal) was measured by a fluorescence resonance energy transfer based assay using a VWF 73 amino-acid peptide substrate (commercial kit, GTI Diagnostics, Waukesha, WI).

Plasma VWF Multimer Analysis
Plasma VWF multimers were analyzed using an intermediate resolution discontinuous electrophoretic agarose gel system, with in-gel immunostaining, and near-infrared fluorescent images were acquired and processed using an Odyssey imaging system (LI-COR Biosciences, Lincoln, NE) as previously described.

Statistical Methods
Demographic, clinical, and echocardiographic patients characteristics were tested for statistical significance using the non parametric Kruskal-Wallis analysis of variance for continuous variables or Pearson’s χ2 test for categorical variables. Wilcoxon rank-sum tests were used for 2-level group comparisons between continuous variables. The relationship between LAAEV, LAVI, the intensity of SEC and the presence of LAAT was tested using Kruskal-Wallis ANOVA. Pearson’s correlation was calculated to assess the relationship between LAAEV, LAVI, and both VWF:Ag and VWF:Act. Logistic regression models were fit to assess the relationship of the VWF and ADAMTS13 variables with LAAT after adjusting for comorbidities using the CHADS2 score. Results are presented as c-statistics, which are equivalent to the area under the receiver operating characteristic curve. To provide consistency and allow for comparisons between the VWF ADAMTS 13 system variables, the odds ratio for developing LAAT is presented as the odds of a value at the 75th percentile of the distribution relative to a value at the 25th percentile was also calculated. The effect of medications on the VWF ADAMTS13 system was tested using linear regression after adjusted for CHADS2 and the type of AF.

Results
Demographic, Clinical and Echocardiographic Data
Of 1423 AF patients screened, 520 had nonvalvular AF, met inclusion and exclusion criteria, and agreed to participate. Ninety-five patients were excluded because the TEE was canceled, the phlebotomy was not performed, or the phlebotomy was performed postprocedure. Eleven patients were excluded because the only documented rhythm was atrial flutter. The remaining 414 patients with documented AF and TEE assessment constituted the study group. TEE studies were requested to exclude intracardiac thrombi before electrophysiological procedures (69%), cardioversion (17%), or for cardioembolic risk assessment (14%).

There were fewer women in the AF group compared with NSR controls (Table 1). Antiplatelet and statin therapy was similar between groups. Significantly more AF patients were on warfarin therapy compared with controls. Three of 414 patients had no SEC assessment by TEE because of suboptimal left atrium appendage image. A varying degree of SEC was noted in 234 patients (57%), including 20 with LAAT (5%). Gradual intensification of SEC, as noted by TEE, up to the development of LAAT was associated with older age, permanent type of AF, lower left ventricular ejection fraction, and the presence of atherosclerotic plaques in aorta. With the
exception of AF patients without SEC, warfarin use was very high in all other AF subgroups. Although the mean CHADS2 scores were similar in NSR and AF groups, a history of prior stroke/transient ischemic attack was more prevalent in AF patients (Table 2). The prevalence of congestive heart failure, hypertension, diabetes mellitus, and prior stroke/transient ischemic attack was associated with greater intensity of SEC and development of LAAT. The overall distribution of CHADS2 scores was similar between NSR subjects and AF patients. In the aggregate, a direct relationship of CHADS2 score with the development and intensity of SEC was apparent. Although significantly more AF patients with SEC or LAAT had high CHADS2 scores (4–6), the majority of AF patients with LAAT (75%) had relatively low CHADS2 scores (1–3).

LAAEV measurement was obtained in 343 (83%) of AF patients. A strong inverse relationship between LAAEV and both the intensity of SEC and LAAT was evident (Figure 1A, P < 0.0001). Transthoracic echocardiogram with LAVI calculation was available in 346 AF patients (84%). A strong direct association was observed between LAVI and degree of SEC intensity (Figure 1B, P < 0.0001). LAVI did not differ in AF patients with LAAT compared with those with severe SEC (P = 0.99).

**VWF-ADAMTS13 System Evaluation**

Overall, the VWF:Ag concentration did not differ significantly between AF patients and NSR controls (157 ± 53 IU/dL versus 144 ± 48 IU/dL, P = 0.08). AF patients without SEC (n = 177) had VWF:Ag essentially identical to that of NSR controls (143 ± 51 IU/dL versus 144 ± 48 IU/dL). AF patients with SEC, however (n = 214), had significantly higher VWF:Ag compared with NSR controls (164 ± 52 IU/dL versus 144 ± 48 IU/dL, P = 0.006). AF patients with LAAT had significantly higher VWF:Ag compared with those without LAAT (200 ± 61 IU/dL versus 155 ± 52 IU/dL, P < 0.001).

VWF:Act was proportional to antigen concentration, with a VWF:Ag/VWF:Act ratio of 0.9 in AF patients across all SEC subgroups and in NSR controls (Figure 2). Six patients had abnormal VWF:Act/VWF:Ag ratios (< 0.7), and 1 patient had an inverted ratio (1.46). To further evaluate these 7 patients, VWF multimer analyses were performed. Although grossly normal, subtle differences in VWF multimer distribution can
be appreciated (Figure 3). Individuals with reduced activity/antigen ratios are associated with a reduction in the highest molecular weight multimers compared with normal pooled plasma. In contrast, the individual with an inverted ratio displayed a relative abundance of the highest molecular weight multimers. Of interest, this particular patient had displayed a relative abundance of the highest molecular weight multimers compared with normal pooled plasma. In contrast, the individual with an inverted ratio displayed a relative abundance of the highest molecular weight multimers.

**Table 2. CHADS2 Score in Subjects With NSR and Patients With AF in Relation to SEC Intensity and LAAT**

<table>
<thead>
<tr>
<th>Variables (%)</th>
<th>NSR Controls (n=100)</th>
<th>AF Cases (n=414)*</th>
<th>P</th>
<th>No SEC (n=177)</th>
<th>SEC</th>
<th>LAAT (n=20)</th>
<th>P</th>
</tr>
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<tbody>
<tr>
<td>CHF</td>
<td>15</td>
<td>23</td>
<td>0.08</td>
<td>8</td>
<td>24</td>
<td>34</td>
<td>46</td>
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<tr>
<td>Hypertension</td>
<td>50</td>
<td>60</td>
<td>0.07</td>
<td>44</td>
<td>68</td>
<td>71</td>
<td>78</td>
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<tr>
<td>Age &gt;75 y</td>
<td>25</td>
<td>18</td>
<td>0.09</td>
<td>7</td>
<td>21</td>
<td>23</td>
<td>32</td>
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<tr>
<td>Diabetes</td>
<td>21</td>
<td>14</td>
<td>0.06</td>
<td>6</td>
<td>14</td>
<td>22</td>
<td>24</td>
</tr>
<tr>
<td>Stroke/TIA prior</td>
<td>6</td>
<td>14</td>
<td>0.03</td>
<td>9</td>
<td>11</td>
<td>18</td>
<td>30</td>
</tr>
<tr>
<td>CHADS2 (mean ± SD)</td>
<td>1.2 ±1.3</td>
<td>1.4±1.4</td>
<td>0.18</td>
<td>0.8±1.1</td>
<td>1.5±1.2</td>
<td>1.9±1.3</td>
<td>2.4±1.7</td>
</tr>
<tr>
<td>CHADS2 score</td>
<td></td>
<td>0.85</td>
<td></td>
<td></td>
<td></td>
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</table>

NSR indicates normal sinus rhythm; AF, atrial fibrillation; SEC, spontaneous echo contrast; LAAT, left atrial appendage thrombus; CHF, congestive heart failure; TIA, transient ischemic attack.

*Total no. of AF patients also includes 3 cases that had no SEC assessment, only left atrial appendage emptying velocity measurement, because of technical difficulties.

For VWF:Act values, this odds ratio relative to AF was almost identical (1.47).

Because gradual intensification of SEC was associated with permanent type of AF, both VWF:Ag and VWF:Act were higher in patients with permanent AF (174±57 IU/dL and 160±55 IU/dL) compared with paroxysmal AF (148±53 IU/dL and 133±48 IU/dL, P<0.001 and P<0.001, respectively) but not significantly different when compared with patients with persistent AF (160±55 IU/dL and 148±53 IU/dL, P=0.11 and P=0.18, respectively).

The main finding of this study is that AF patients with SEC and particularly with LAAT have significantly higher VWF levels compared with AF patients without SEC or control subjects in NSR. A direct relationship was observed between the degree of left atrial stasis (SEC intensity and LAAT) and elevated VWF. The other 2 TEE measures of left atrium blood stasis, LAAEV and LAVI, were also associated with VWF, although the relationship was less pronounced. SEC generation is complex and influenced not only by blood stasis but also age, left ventricular function, aortic atherosclerosis, plasma fibrinogen, and erythrocytes.5,32 In our patients with AF, VWF levels remained normal until SEC developed. Others have reported conflicting results of VWF levels in AF patients compared with NSR controls.12–16 These findings may reflect differences in accrual rates of AF patients with SEC which was not accounted for in those studies.12–16

Furthermore, VWF:Ag and VWF:Act are independent predictors for LAAT after adjustment for CHADS2 score.
effectively separating those with LAAT. In a large cohort from the Stroke Prevention in Atrial Fibrillation III trial, after adjustment for other clinical predictors, the relationship between VWF and stroke became nonsignificant. However, nearly half of strokes occurring in AF patients are due to noncardioembolic mechanisms, and in this trial, stroke subtypes were not analyzed. Whereas VWF is integral in both the initiation and propagation of thrombus formation, these findings may help explain the association between atrial stasis and thrombotic propensity in AF. Atrial distension both injures local endothelium and stimulates endothelial release of ultralarge VWF multimers. Under laminar blood flow conditions, ultralarge VWF is rapidly degraded by ADAMTS13. Under static conditions, proteolysis is retarded 1000-fold. Therefore, within the fibrillating left atrial appendage, excess VWF secretion and inadequate ADAMTS13 cleavage result in elevated local concentrations of ultralarge VWF multimers, thus favoring thrombus formation. Within the flow conditions of the fibrillating atrium, laminar flow is abolished, velocity (implying both direction and speed) is chaotic, and one might envision undulating unfolding, refolding, and chaotic bending and twisting of the released ultralarge multimer, in turn activating the protein while simultaneously inducing and then inhibiting ADAMTS13 cleavage. Combining variable degrees of atrial endothelial injury further complicates the localized propensity for thrombus formation.

As demonstrated by others, VWF measures may improve thrombosis risk stratification in AF patients. We now provide evidence that measures of atrial stasis are strongly associated with VWF levels. This benefit of adding VWF measures to the risk assessment was illustrated in 20 AF patients with LAAT in our analysis. Among these patients, 40% had low CHADS2 scores, and 10% of patients had scores of 1. This latter group might not have received warfarin anticoagulation if the risk assessment was limited to the CHADS2 system.

Left atrial appendage velocity and VWF concentrations were independently associated with LAAT in a previous study of 109 patients with AF. Moreover, a significant correlation has been observed between the endocardial VWF expression, platelet adhesion, and thrombus formation in the left atrial appendage. These combined data corroborate the relationships between AF, atrial whole blood stasis, VWF levels, and thrombotic propensity found in our study.

There was no evidence of excessive release of large VWF multimers into the general circulation. Only one patient with severe SEC had an inverted ratio of VWF:Act to VWF:Ag (ratio 1.4). In contrast, measures of ADAMTS13 activity for subgroups of patients with nonvalvular atrial fibrillation (AF) and control subjects with normal sinus rhythm (NSR). Measures are presented as mean±standard deviation.
pressure and serum cholesterol.\textsuperscript{38} This suggests that VWF is sufficiently stable for potential use in the long-term prediction of cardiovascular disease. Moreover, VWF levels are not influenced by warfarin or aspirin, common therapies in AF.\textsuperscript{10}

Several study limitations deserve comment. First, SEC is a dynamic process that is subjectively quantified. To improve reproducibility of this measure, assessment was limited to one cardiologist blinded to both clinical and laboratory data. Furthermore, although the TEE was often not repeated sequentially, the measures obtained may not adequately capture the severity of atrial stasis over time. Second, we cannot exclude referral bias for the AF patients enrolled. Only AF patients who had a TEE requested by their primary health care provider were approached for recruitment. The clinical characteristics of these patients, however, did not differ from those of typical AF patients at our institution\textsuperscript{21,22} or participants of large multicenter registries.\textsuperscript{3,4,9} Third, the blood type was not measured. Blood type O is associated with lower than normal values of circulating VWF.

In summary, we now provide evidence supporting a direct relationship between VWF and measures of atrial whole blood stasis. The direct correlation between VWF levels and the severity of left atrial whole blood stasis may help to explain the thrombotic propensity among AF patients. Elevated VWF may identify AF patients at risk for LAAT. There was a temptation to provide a cutoff value for VWF levels discriminating stroke risk or lack thereof. Whereas stroke in AF is a complex and poorly understood phenomenon, defining critical values of one of the pieces of this puzzle represents a willingness to overly simply a complex process. Similar paradigms have been proposed for hypertension and cholesterol. Although undisputed in the risk of coronary disease, the risk and treatment targets are indistinct and constantly changing. Thrombosis in AF is no less complex.

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**Disclosures**

None.

**References**


